UNDERSTANDING HOW CHROMOSOME STRUCTURE INFLUENCES RECOMBINATION

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Samantha Ann Mainiero

May 2015

ABSTRACT

Meiotic recombination results in the exchange of DNA between homologous chromosomes, creating allelic diversity while at the same time ensuring correct segregation of chromosomes during the meiotic divisions. Most maize COs are found in the distal regions of chromosomes, whereas the highly repetitive centromeric and pericentromeric regions are often devoid of COs. However double-strand breaks (DSBs), whose formation initiates the recombination pathway, do not follow the same pattern of CO distribution. Instead, maize DSB hotspots occur along the entire length of chromosomes, with no preference for distal or proximal regions. In this study, I tracked the dynamics of the DSB repair protein RAD51 between euchromatin and heterochromatin through the stages of early prophase I. By comparing the spatiotemporal localization of RAD51 between inbred lines with difference CO patterns, I found that the eventual CO pattern in each inbred likely reflects the distribution of early DSB repair.

BIOGRAPHICAL SKETCH

Samantha Mainiero was raised in Zionsville, Pennsylvania. For her undergraduate education, she attended James Madison University where she researched the control of starch degradation in Arabidopsis. She received at Bachelor's degree in Biology in May 2011; she graduated with Honors and magna cum laude. Samantha first came to Cornell University as a summer undergraduate intern at the Boyce Thompson Institute where her research focused on determining the virulence of recently isolated strains of bacteria that cause bacterial speck disease in tomato. She then came back to Cornell as a graduate student in Plant Biology in August 2011, where she has been studying meiotic chromosome restructuring in maize.

This thesis is dedicated to Bonnie and Tony Mainiero, for the time they pulled off the road in Yosemite while they had cellphone service until I was able to call them back. And to Penelope, for keeping me grounded and keeping me going.

ACKNOWLEDGMENTS

I would like to acknowledge and thank my committee, Wojtek Pawlowski, Paula Cohen, and Klaas van Wijk for advice, comments, and motivation. I would also like to thank current and former members of the Pawlowski lab. A big thank you to Yan He, for so many discussions, looking out for me when I was starting in the lab, and always being available cross and plant for me. Thanks to Mischa Olson and Jonathan Gonzalez for much appreciated help with statistics. Thanks to Erin Mattoon, for being the best undergrad around. Thanks to Choon Lin Tiang for answering all my questions about antibodies, fixation, imaging, and microscopes. And thanks to the rest of the Pawlowski lab: Adele Zhou, Minghui Wang, Richie Chen, Gagan Sidhu, Marilyn Wang, Celestia Fang, Angelica Schreyer, and Joy Li for all the help and support provided over the past four years. Additional thanks to Steffi Dukowic-Schulze, Changbin Chen, Inna Golubovskaya, and Rachel Wang for advice and insights into my project. To Ljuda Timofejeva, James Higgins, and Liudmila Chelysheva for cytology tips. Thanks to the Cornell Greenhouse Staff and Growth Chamber facilities for taking care of my plants. And to the Cornell Graduate School for their continued support. A big thank you to the School of Integrative Plant Sciences graduate students, especially my fellow plant biologists, for providing so many enjoyable and memorable experiences over the last four years. And to Sam, Natalie, Jeff, Erin, and Zach for always providing me with hugs, chocolates, and belays. Funding was provided by Cornell University TAship and NSF Plant Genome Grant IOS-1025881.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

MEITOTIC CHROMOSOME STRUCTURE AND FUNCTION IN PLANTS*

Chromosome remodeling through meiosis

Higher-order chromosome structure involves packaging of chromatin above the nucleosome level. During both mitosis and meiosis, chromosomes restructure into highly condensed forms, which are essential for their correct segregation. Recent studies on human mitotic chromosomes at metaphase show that they exhibit two levels of compaction; consecutive arrays of chromatin loops are anchored to axes, with each loop being about 80 kb in size, followed by axial compression (Naumova *et al*., 2013). Mammalian chromosomes at meiosis share a similar loop size, suggesting that the higher-order structure of chromosomes is conserved between the two types of cell divisions (Kleckner *et al*., 2013). Studies in many groups of species, including plants, point to critical importance of higher-order chromosome structure to cellular processes. One of the intensely examined aspects of how chromosome structure affects their function is its role in controlling chromosome interactions during meiosis.

Meiotic prophase is a time when chromosomes undergo dramatic structural remodeling. Following replication during the pre-meiotic S phase, DNA remains dispersed throughout the nucleus. The entry into meiosis begins the process of chromosome individualization and compaction, which eventually result in highly condensed, rod-shaped metaphase chromosomes. However, organizing chromatin into compact chromosomes is not the only purpose of chromosome remodeling. During this process, chromosomes must also pair, synapse, and recombine with their homologous partners. The structure of chromosomes in early meiotic prophase is associated with the progression of these inter-chromosomal interactions, suggesting that chromosome morphology is an integral part of these processes (Yamada and Ohta, 2013). In

*This chapter is reproduced from Mainiero S, Pawlowski WP: Meiotic chromosome structure and function in plants. Cytogenet and Genome Res 143:6-17 (2014) with permission by Karger Publishers.

plants, chromatin reorganization during meiosis becomes especially critical, as genomes are generally large and complex due to copious amounts of repetitive DNA. Repetitive DNA as well multi-gene families, which are also common in plant genomes (Schnable *et al*., 2009), could lead to ectopic interactions of non-homologous chromosomes. Such interactions could result in chromosomes failing to segregate properly into daughter cells.

The first stage of chromosome remodeling begins in leptotene (Figure 1), when DNA condenses into thin threads and sister chromatids are held tightly together (Remeseiro and Losada, 2013). Leptotene fibers are formed as chromatin is looped and anchored to axial elements at the core of the chromosomes (McNicoll *et al*., 2013). The recombination pathway is also initiated during this stage, when the SPO11 protein introduces double-strand breaks (DSBs) into chromosomal DNA (Keeney, 2008). DNA ends adjacent to these breaks are later bound by RAD51 and DMC1, which catalyze single-end invasion of the broken DNA ends into the

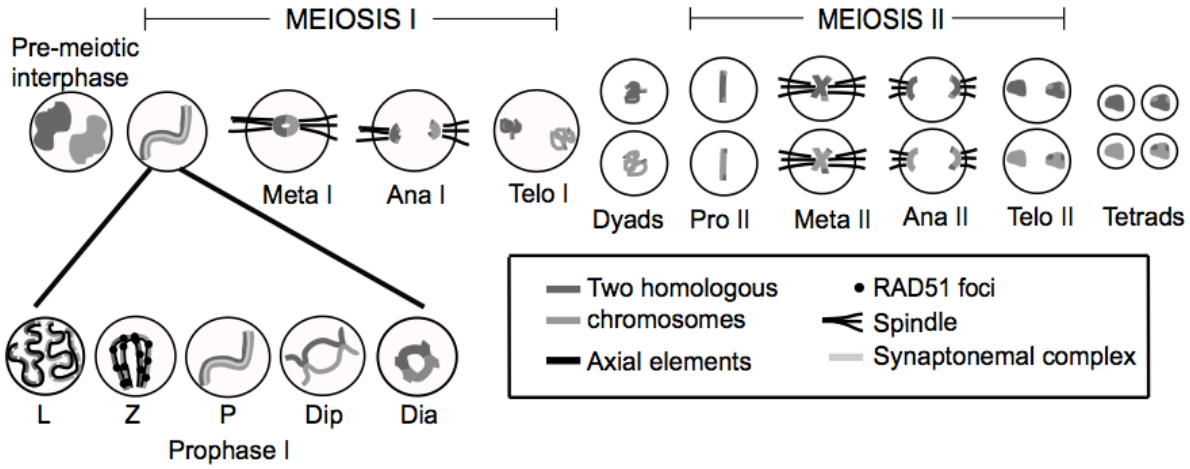

Figure 1: A diagram of meiotic stages showing a single pair of homologous chromosomes. Following DNA replication in pre-meiotic interphase, cells enter meiosis starting in prophase I. Prophase I consists of 5 substages: leptotene (L), zygotene (Z), pachytene (P), diplotene (Dip), and diakinesis (Dia). It is during leptotene, zygotene, and pachytene that homologs pair, synapse, and recombine.

homologous chromosome (San Filippo *et al*., 2008). In most species, this process is thought to be a key feature in homology recognition.

As prophase continues into early zygotene, chromatin fibers expand and their surface increases in complexity (Dawe *et al*., 1994). Heterochromatic knobs elongate and telomeres cluster at the nuclear envelope into a cytological structure termed the bouquet (Tiang *et al*., 2012). As homologs find each other, their axial elements, now called lateral elements, become connected by the installation of the central element of the synaptonemal complex (SC) (Fraune *et al*., 2012). During late zygotene, recombination events become resolved in either crossovers (COs) or non-crossovers (NCOs) (Muyt *et al*., 2009). The shortening of chromosomes continues through diplotene as the SC dissolves and the homologous chromosomes remain held together as bivalents through CO sites, which form cytological linkages termed chiasmata.

Various levels of chromosome structure are important for meiotic processes in plants. Histone modifications and epigenetic reprogramming during meiosis affect recombination progression and distribution of COs (Rosa *et al*., 2013; She *et al*., 2013; Choi *et al*., 2013). The installation of the SC affects recombination as well as pairing (Miao *et al*., 2013; Stronghill *et al*., 2010; Schommer *et al*., 2003; Kerzendorfer *et al*., 2006). Most important however, are the determinants of higher order chromosome structure, which are responsible for the compaction and reorganization of chromosomes. These factors are the focus of this chapter. The main higher order chromosome structure determinants are cohesins and condensins. Cohesins have been well studied in multiple plant systems during both mitosis and meiosis. Their action is needed for chromatid organization and establishing the meiotic chromosome structure that is a prerequisite for pairing and recombination, as well as for providing cohesion of sister chromatids through meiosis I (Bai *et al*., 1999; Bhatt *et al*., 1999; Cai *et al*., 2003; Golubovskaya *et al*.,

2006; Shao *et al*., 2011). Condensins are responsible for compacting chromatin to ensure its proper segregation to daughter cells. Plant condensins are known to be critical during development (Siddiqui *et al*., 2003; Siddiqui *et al*., 2006; Lui and Meninke, 1998; Tzafrir *et al*., 2004; Schubert *et al*., 2013), but little is known about how their establishment of chromosome organization affects meiotic processes. This chapter will combine the limited information about plant condensins with what is known about their roles in animals and fungi to make inferences about how they may function during plant meiosis. In addition to cohesins and condensins, there are other determinants of chromosome structure that have more specialized functions in meiotic processes. One of the best studied is chromatin conformation controlled by the *Ph1* locus, which plays a role in proper chromosome pairing in polyploid wheat (Prieto *et al*., 2004; Prieto *et al*., 2005; Knight *et al*., 2010; Greer *et al*., 2012).

Cohesins

An important player in determining chromosome morphology is the cohesin complex, which forms a ring-like structure holding sister chromatids together during cell divisions. Meiotic sister chromatid cohesion (SCC) is needed for both the proper alignment of chromosomes at the metaphase plate and to create tension across the centromeres, which counteracts the pull of microtubules to allow for correct monopolar attachment to the spindle during anaphase I (Wassmann, 2013). In addition, cohesins have been implicated in determining meiotic chromosome structure that is required for proper chromosome pairing and recombination (Qiao *et al*., 2012; Golubovskaya *et al*., 2006). In prophase I of meiosis, plant cohesins are present at the centromeres as well as over the length of chromosome arms (Lam *et al*., 2005; Qiao *et al*., 2011; Golubovskaya *et al*., 2006; Shao *et al*., 2011; Wang *et al*., 2009). Cohesins

load dynamically onto chromosomes, as the subunits of the complex show spatial and temporal differences in their localization throughout prophase I (Qiao *et al*., 2012). SCC along chromosome arms during prophase is maintained by AtCTF7, a plant homolog of *ScEco1*, which acetylates cohesins, stabilizing the complex around chromatin (Bolanos-Vilegas *et al*., 2013; Singh *et al*., 2013; Beckouet *et al*., 2010). Prior to anaphase I, cohesins along the arms are released by Separase in order to resolve chiasmata, allowing homologs to segregate (Wassmann, 2013). However, SCC is maintained at the centromeres to ensure correct orientation of centromeres to the kinetochores (Wantanabe, 2005).

Similarly to other species, plant centromeric cohesins are protected from Separase cleavage by SHUGOSHINs (AtSGOL1 and AtSGOL2, ZmSGO1, OsSGO1) (Cromer *et al*., 2013; Hamant *et al*., 2005; Wang *et al*., 2011; Zamariola *et al*., 2014). Plant SHUGOSHINs are unique in that they function only during the meiotic divisions (Cromer *et al*., 2013). AtSGOL1 and AtSGOL2 act redundantly during meiosis I to protects centromeric cohesins, thus preventing precocious separation of sister chromatids (Cromer *et al*., 2013; Zamariola *et al*., 2014). A novel protector of cohesins, AtPANS1, has recently been characterized in Arabidopsis. This protein directly interacts with the APC/C complex to maintain centromeric cohesin during interkinesis (Cromer *et al*., 2013).

The cohesin complex consists of two structural maintenance of chromosome (SMC) proteins, SMC1 and SMC3, and two additional non-SMC subunits, Scc3 and Scc1/Rec8 (Klein, 1999). The two SMC proteins form a V-shaped heterodimer around sister chromatids, which is closed by non-SMC subunits (Figure 2a) (Nasmyth and Haering, 2005). During meiosis, the SMC proteins are found along chromosome arms and at centromeres, holding together sister chromatids along their entire length (Lam *et al*., 2005). SMC1, SMC3, and SCC3 are found in

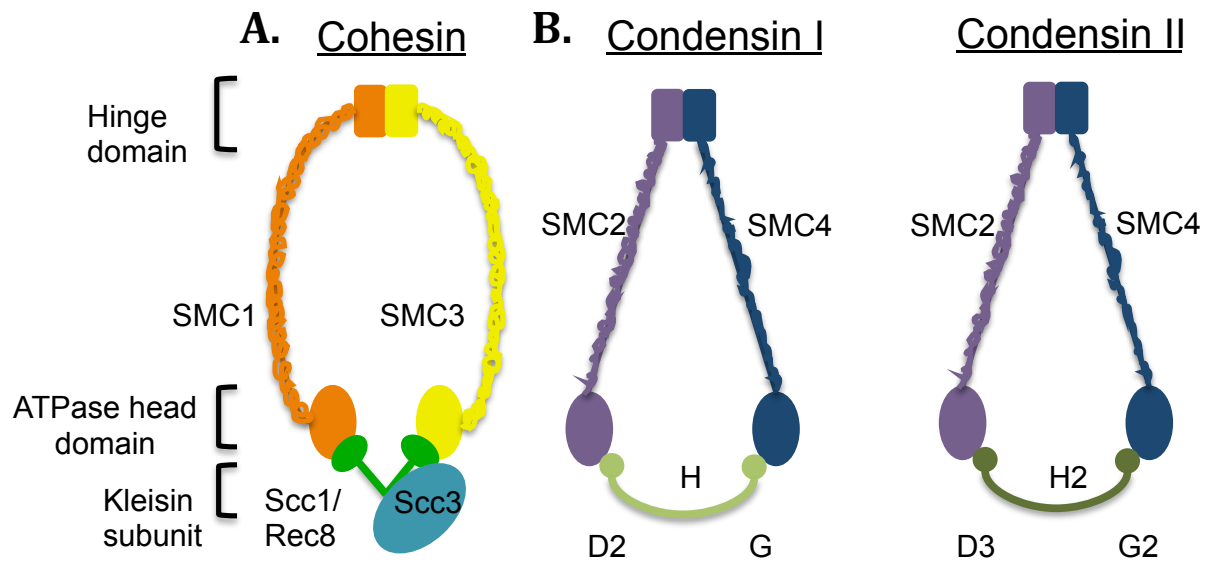

Figure 2: Structure of the cohesin and condensin complexes. Each complex contains two SMC proteins which heterodimerize, forming a ring by binding a kleisin subunit. (A) During mitosis, Scc1 acts as the cohesin kleisin, whereas Rec8 binding is specific to meiotic cohesins. An additional subunit Scc3 is needed for proper function of the complex. (B) Both condensin complexes contain SMC2 and SMC4. Additional CAP subunits bind to the complex and allow for full functionality. CAP-H, CAP-D2, and CAP-G are needed for condensin I activity, while CAP-H2, CAP-D3, and CAP-G2 are required for condensin II.

both mitotic and meiotic cohesin complexes. Knockouts of *SMC1, SMC3,* and *SCC3* in Arabidopsis are seedling lethal (Schubert *et al*., 2009a; Chelysheva *et al*., 2005; Lam *et al*., 2005; Lui *et al*., 2002), demonstrating that they are critical in plant development. Somatic cells defective in cohesin subunits show incorrect alignment of chromatid arms and centromeres (Schubert *et al*., 2009a). These defects then lead to anaphase chromosome bridges and genome instability following mitotic cell divisions (Schubert *et al*., 2009a). As such, the cohesin complex is especially important in actively dividing tissues such as meristems.

Mitotic and meiotic cohesins are distinguished by the α-kleisin that binds the complex. Orthologs of SCC1 are present in mitotic cohesins, while the binding of REC8 orthologs specifies the meiotic cohesin complex. REC8 orthologs, Arabidopsis AtSYN1/DIF1, maize

AFD1, and Rec8 in tomato and rice, are needed to maintain SCC prior to the first meiotic division. (Bai *et al*., 1999; Bhatt *et al*., 1999; Kaszás and Cande 2000; Cai *et al*., 2003; Golubovskaya *et al*., 2006; Qiao *et al*., 2011; Zhang *et al*., 2006; Shao *et al*., 2011).

Meiotic sister chromatid cohesion

The predominant role of the cohesin complex during meiosis is to hold together sister chromatids during the first division. Unlike mutations in other cohesin subunits, plant *rec8* mutant phenotypes are specific to meiosis, resulting in severe fertility defects (Bai *et al*., 1999; Bhatt *et al*., 1999; Golubovskaya *et al*., 2006; Zhang *et al*., 2006; Shao *et al*., 2011). In these mutants, sister chromatids separate precociously during prophase I (Kaszás and Cande, 2000; Cai *et al*., 2003; Shao *et al*., 2011). Cohesin mutants are also defective in chromosome pairing. In maize *afd1* mutants, univalents line up at the metaphase plate (Chan and Cande, 1998) and segregate equationally during meiosis I (Kaszás and Cande, 2000). This phenotype is also seen in Arabidopsis *ssc3* mutants, where tangled bivalents and univalents separate equationally during the first meiotic division (Chelysheva *et al*., 2005). The premature loss of SCC results in bipolar attachment of sister kinetochores to the spindle, as opposed to monopolar attachment. Consequently, a mitotic-like division occurs, in which sisters instead of homologous chromosomes separate during anaphase I (Golubovskaya, 1989; Chelysheva *et al*., 2005).

Cohesins are major determinants of chromosome structure

In early leptotene, sister chromatids begin the process of compaction, by forming loops that are anchored to the axial elements. Cohesins are critical in establishing this meiotic chromosome morphology. Maize AFD1 has been shown to colocalize with ASY1, an axis-

associated protein, starting in leptotene (Golubovskaya *et al*., 2006). The colocalization persists through pachytene as the SC is installed (Golubovskaya *et al*., 2006).

The distinctive chromosome structures of leptotene and zygotene are drastically altered in plant *rec8* mutants (Bai *et al*., 1999; Kaszás and Cande 2000; Zhang *et al*., 2006). Instead of the distinctive chromatin fibers of these stages, the chromatin is intertwined and tangled, hampering individualization of chromosomes (Bai *et al*., 1999; Golubovskaya *et al*., 2006; Zhang *et al*., 2006). In the strongest alleles of *afd1* mutants, *afd1-1* and *afd1-2*, the leptotene and zygotene fibers are completely absent, showing that SCC is critical for proper chromosome organization in maize (Golubovskaya *et al*., 2006) (Figure 3). As the leptotene and zygotene fibers are altered or absent in *rec8* mutants, the processes that rely on these chromatin structures, namely homologous pairing, synapsis, and recombination, are also affected.

In rice and maize cohesin mutants, the telomere bouquet, thought to facilitate chromosome pairing, is not formed (Golubovskaya *et al*., 2006; Shao *et al*., 2011). In addition to pairing being absent, either because of the bouquet defect or because of direct disruption of homologous chromosome interactions, the defects in axial element formation prevent central element installation, impeding synapsis (Bai *et al*., 1999; Bhatt *et al*., 1999; Golubovskaya *et al*., 2006; Zhang *et al*., 2006; Shao *et al*., 2011). Synapsis defects contribute to the presence of univalents at metaphase I and result in chromosomes that are not able to correctly align at the metaphase plate (Bai *et al*., 1999; Bhatt *et al*., 1999; Shao *et al*., 2011).

The correct chromosome structure during the early stages of prophase I is also essential for recombination in plants. Chromosome fragmentation seen in Arabidopsis *syn1* and *scc3* mutants is rescued by the *spo11-1* mutation (Bai *et al*., 1999; Bhatt *et al*., 1999; Chelysheva *et al*., 2005), indicating that cohesin mutants are defective in DSB repair. Additionally, the

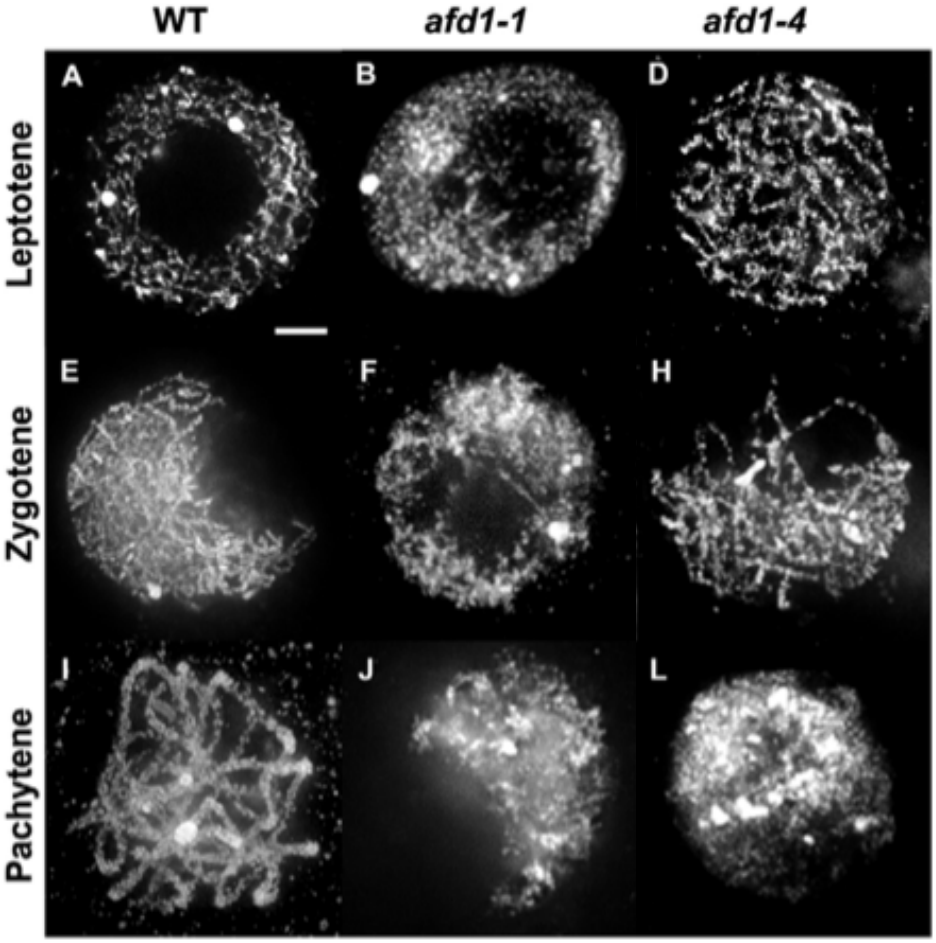

Figure 3: Effects of strong and weak *afd1* alleles on early prophase chromatin structure. In the strong *afd1-1* allele, the leptotene fibers are completely absent, leading to severe defects in later in meiosis. However, leptotene fibers are formed in the weak *afd1-4* allele yet chromatin structure is still affected in later stages. Modified from Golubovskaya *et al*. (2006).

localization of RAD51, a protein facilitating the first step of meiotic DSB repair and a marker of recombination, is abnormal in *afd1* mutants (Golubovskaya *et al*., 2006). In wild-type maize, approximately 500 RAD51 foci are seen on chromosomes during zygotene (Franklin *et al*., 1999). However in the *afd1* mutant, the number of zygotene foci is greatly reduced and those that are present appear as aggregates and patches (Pawlowski *et al*., 2003; Golubovskaya *et al*., 2006). As recombination and homologous pairing are tightly associated in plants (Da Ines *et al*.,

2012; Franklin *et al*., 1999; Franklin *et al*., 2003; Pawlowski *et al*., 2003), the recombination defect in *afd1* must be linked with the pairing defect. Cohesins have differing affects on recombination between Arabidopsis and maize, as evident by normal RAD51 foci formation in Arabidopsis *scc3* mutants (Chelysheva *et al*., 2005). This difference could be related to the fact that the amount of DNA and the fraction of repetitive DNA in maize chromosomes are much higher than those in Arabidopsis, which could result in meiotic chromosomes being differently organized between the two species.

SCC also appears to be needed for repairing DSBs in Arabidopsis somatic cells through homologous recombination. An additional SMC complex, SMC5/6, acts alongside cohesins to establish correct alignment of sister chromatids (Watanabe *et al*., 2009). This alignment then helps to ensure that the correct template is used during homologous recombination (Watanabe *et al*., 2009).

Sex-specific cohesin in Arabidopsis

The genome of Arabidopsis contains three other genes that are closely related to *SYN1* (Dong *et al*., 2001; da Costa-Nunes *et al*., 2006; Jiang *et al*., 2007). *SYN2*, *SYN3*, and *SYN4* are expressed throughout the plant, with highest expression levels in the meristems, initially implicating their protein products in mitotic cohesin complexes (Dong *et al*., 2001; da Costa-Nunes *et al*., 2006). However, further studies point to *SYN3* acting as a significant player during female meiosis (Yuan *et al*., 2012). In *SYN3* RNAi plants, male gametogenesis is only slightly affected, with a marginal reduction in pollen viability (Yuan *et al*., 2012). Yet these plants have significantly reduced fertility, resulting from abortion of megaspore mother cells (Yuan *et al*., 2012). This sex-specific difference on fertility is further apparent at the cytological level. While

male meiocytes show moderate defects and produce wild-type looking microspores, female meiocytes display much more severe defects, including a lack of thin-thread fibers during leptotene/zygotene, presence of univalents, and fragmented chromosomes at metaphase I, and chromosome bridges at anaphase I (Yuan *et al*., 2012). These phenotypes parallel the meiotic aberrations in *syn1* male meiocytes, indicating that SYN1 and SYN3 may have similar functions but in the different sexes.

Chromosome condensation

Condensins are protein complexes that facilitate the compaction of chromosomes into discrete units prior to the cell divisions of mitosis and meiosis. However, their importance stretches beyond packaging DNA into chromosomes prior to cell division (Hirano, 2012). Increasing evidence in animals and yeast points to the importance of the condensin-controlled chromosome structure to the processes of homologous pairing and recombination (Yu and Koshland, 2003; Hartl *et al*., 2008; Mets and Meyer, 2009; Hirano, 2012).

Each condensin complex is made up of five subunits: two SMC proteins and three chromosome-associated proteins (CAPs) (Figure 2b). Both condensin I and II contain SMC2 and SMC4, which dimerize to form a V-shaped structure. This V-shaped structure is closed on its other end by binding of the kleisin proteins CAP-H or CAP-H2 to form a ring that encircles chromatin (Nasmyth and Haering, 2005). CAP-H is the kleisin subunit of condensin I, while CAP-H2 participates in the condensin II complex. The kleisins then serve as scaffolds for the binding of two HEAT repeat-containing proteins, CAP-G and CAP-D2 in condensin I and CAP-G2 and CAP-D3 in condensin II (Nasmyth and Haering, 2005; Hudson *et al*., 2009).

Homologs of condensin I and II are present in plants, including Arabidopsis, maize, and rice. The genome of Arabidopsis contains genes encoding all putative condensin subunits, with two genes encoding SMC2 (*AtCAP-E1* and *AtCAP-E2*) and three genes encoding SMC4 (including *AtCAP-C* and two other putative genes) (Siddiqui *et al*., 2003; Siddiqui *et al*., 2006; Schubert *et al*., 2009b). Although there has been little research on the role of condensins in plant meiosis, several studies have shown their importance in plant development. Both SMC2-encoding genes as well as *AtCAP-C* are expressed throughout the plant, with the highest expression levels in floral organs and tissues with high mitotic indices (Lui *et al*., 2002; Siddiqui *et al*., 2003; Siddiqui *et al*., 2006). Mutations in these genes result in severe developmental defects, such as embryo lethality, developmental delays, defective seed development, abnormal gametogenesis, as well as defective cell patterning and organization of the shoot apical meristem (Siddiqui *et al*., 2003; Siddiqui *et al*., 2006; Lui and Meinke, 1998; Tzafrir *et al*., 2004). Knockout mutations in genes encoding the non-SMC condensin subunits in plants have only been studied for *CAP-D2* and *CAP-D3*, and result in embryo lethality and dwarfism, respectively (Schubert *et al*., 2013). This difference in phenotype indicates that condensin I and II play non-redundant roles during plant development. In the few studies addressing the role of the condensin complexes in plants meiosis, reduced pollen quantity and seed sets have been observed in Arabidopsis condensin mutants (Schubert *et al*., 2013), suggesting that plant condensins are also critical during meiosis.

Condensins and the structure of chromosomes

No studies have been conducted so far to address the role of condensins in establishing the structure of chromosomes specifically during meiosis in plants. However, studies on

condensin functions in animals and fungi, as well as reports on mitotic roles of condensins in plants, allow inferences on the possible functions of these proteins in plant meiosis.

Studies in animals show that condensins I and II load sequentially onto meiotic chromosomes. In mouse, condensin II begins to load on the axes of chromosome arms during metaphase I, whereas condensin I appears at centromeres during this stage and then spreads to chromosome arms during anaphase I (Lee *et al*., 2011; Viera *et al*., 2007). Condensin loading is closely linked to the loading of cohesins. Mouse condensins are interspersed with cohesins along chromosome arms at metaphase I, each occupying their own particular domains (Lee *et al*., 2011). Furthermore, condensins affect the establishment of sister chromatid cohesion and sister chromatid organization in *C. elegans* (Chan *et al*., 2004).

Studies on mitotic functions of plant condensins suggest that they may share similar roles to animal and fungal condensins. Arabidopsis condensin I and II complexes load onto mitotic chromosomes separately, which is similar to the behavior of condensins in animal meiosis. AtCAP-H protein is present in the cytoplasm from interphase up until mitotic prophase, when it begins to load onto the chromosomes (Fujimoto *et al*., 2005). In contrast, condensin II's AtCAP-H2 is in the nucleolus and nucleoplasm during interphase. By metaphase, both proteins are fully localized on the chromosomes, where they remain until cytokinesis is completed (Fujimoto *et al*., 2005). Studies of Arabidopsis CAP-D2 and CAP-D3 in interphase nuclei showed that these condensin subunits are needed for higher-order organization of the nucleus, such as compacting euchromatin, preventing associations between centromeric/pericentromeric repeats, and, in the case of CAP-D3, ensuring proper distribution of cohesins (Schubert *et al*., 2013).

Condensins and chromosome interactions

Chromosome structure is important for facilitating chromosome interactions, so not surprisingly, condensins have been observed to play a role in these processes. Condensins play a role in chromosome pairing in Drosophila males through resolving linkages between chromosomes. In condensin mutants in Drosophila males, paired homologs are not fully individualized before being separated, resulting in chromatin bridges between homologous and non-homologous chromosomes during anaphase I (Hartl *et al*., 2008). As Drosophila males do not undergo recombination, these linkages cannot be due to unresolved recombination events, and are thought to be results of the pairing process. Formation the of anaphase bridges in Drosophila condensin II mutants is suppressed when they are combined with mutations in *teflon*, a gene required for chromosome pairing maintenance (Arya *et al*., 2006; Hartl *et al*., 2008). Therefore, it is hypothesized that the Teflon protein introduces entanglements between chromosomes to facilitate homolog pairing, and condensin II is required to resolve these linkages prior to homolog separation.

Although it is not known if plant condensins play a role in chromosome pairing, their role in resolving linkages between sister chromatids is conserved in Arabidopsis. This is evident by chromosome bridges forming during mitosis in Arabidopsis *cap-e1*, *cap-e2*, and *cap-c* mutants (Siddiqui *et al*., 2003; Siddiqui *et al*., 2006). Interestingly, mitotic bridges are not observed in mutants with disrupted *CAP-D2* or *CAP-D3* (Schubert *et al*., 2013). It is possible that these subunits play a role in resolving chromosome linkages in meiosis rather than mitosis, as the mutants exhibit significant reduction in pollen quantity (Schubert *et al*., 2013).

The effect of condensins on meiotic recombination

Condensins also have a major effect on meiotic recombination. Studies in *C. elegans* indicate that condensin I and II play a role in meiotic DSB formation. Work by Mets and Meyer (2009) showed that condensins influence both the number and distribution of DSBs, and serve as a means of controlling CO formation. These effects are most likely mediated by the influence that condensins exert on chromosome axis formation (Tsai *et al*., 2008). *C. elegans* condensin I and II act independently to affect the DSB number and distribution, with each complex affecting DSB formation by controlling chromosome axis length and structure in its domain of control along the chromosome (Mets and Meyer, 2009). These studies point to an active mechanism that guarantees at least one DSB, and therefore one CO (the obligate CO), is formed on each bivalent (Mets and Meyer, 2009).

Whether the phenomenon of the condensin-controlled chromosome structure influencing recombination patterns is conserved in other species remains uncertain. Yu and Koshland (2003) found that the *S. cerevisiae* condensin does not affect DSB formation. As yeast and *C. elegans* are the only two species in which a connection between condensins and DSB have been investigated, it is unclear whether this role is specific to *C. elegans* or evolved after the divergence of yeast and higher eukaryotes. Since *C. elegans* exhibits a very tight control of the number of COs, condensins may have evolved specifically to help uphold the strict obligate CO requirement in this species. Alternatively, this level of CO control may have evolved with larger and more complex genomes.

There have not been any studies investigating how condensins influence recombination in plants. However, Arabidopsis condensin II has been implicated in playing a role in somatic genome maintenance after genotoxic stress (Sakamoto *et al*., 2011). Mutations in *AtCAP-G2* and

AtCAP-H2 increase the frequency of DSBs following exposure to a DNA break-inducing agent (Sakamoto *et al*., 2011). During interphase, when condensin II is found in the nucleus, the complex is able to reduce DNA damage by either physically protecting the genome or playing a direct role in DSB repair (Sakamoto *et al*., 2011; Fujimoto *et al*., 2005). It is yet to be seen if condensins play a similar role in formation and repair of meiotic DSBs in plants.

At the final steps of recombination, condensins play a role in resolving meiotic COs. Prior to anaphase I, chiasmata between chromosomes must be resolved to allow the homologs to separate to opposite poles. In *S. cerevisiae*, condensin is needed for the separation of chromosomes, by resolving these linkages (Yu and Koshland, 2003). Without condensin, the homologs are held together as they are pulled to separate poles, resulting in chromosome bridges and fragmentation (Yu and Koshland, 2003). In Arabidopsis *smc2* and *smc4* mutants, chromosome bridges have been observed during meiotic anaphase I and II. Therefore, it is likely that plant condensins share with fungal condensins the role in resolving chiasmata prior to homolog separation.

Regulation of homologous chromosome pairing by chromatin structure controlled by the **Ph1** *locus in wheat*

A very strong link between chromosome structure and function has been demonstrated and extensively studied in hexaploid wheat. Hexaploid wheat contains three fairly closely related (homoeologous) chromosome sets as a result of an allopolyploidization event that took place ca. 10,000 years ago (Matsuoka, 2011). For stability of the hexaploid genome, it is critical that during meiosis chromosome pairing and recombination are limited to within-chromosome set (homologous) interactions and that pairing and recombination of chromosomes from different

sets is prevented. To keep its three genomes distinct during meiosis, polyploid wheat has evolved a special mechanism that is controlled by the *Ph1* locus (Riley and Chapman, 1958).

Ph1 has been mapped to a region on chromosome 5B containing a cluster of *cdk*-like genes (Sears, 1977; Roberts et al., 1999; Sidhu et al., 2008; Griffiths et al., 2006; Yousafzai *et al*., 2010b). Additional clusters of *cdk* genes are found in syntenic regions in the other two genomes of polyploid wheat, and *Ph1* activity suppresses the transcription of these *cdk* genes on the homoeologous chromosomes (Griffiths *et al*., 2006; Al-Kaff *et al*., 2008; Yousafzai *et al*., 2010a). The role of *cdk* suppression in ensuring proper chromosome pairing has been confirmed by treatment with the CDK activator okadaic acid (OA). In wheat-rye hybrids containing haploid chromosome sets from wheat and rye, chromosomes do not pair in the presence of *Ph1*, since there are no homologs present. However in the *ph1* mutant, or when treated with OA, some wheat and rye chromosomes undergo homoeologous pairing (Knight *et al*., 2010). It is hypothesized that the increased *cdk* activity in the *ph1* mutant leads to homoeologous pairing through altering chromosome structure (Mikhailova *et al*., 1998; Maestra *et al*., 2002; Greer *et al*., 2012). CDK activity is also important in Arabidopsis meiosis. At high temperatures (23°C) AtCDKG1 is needed for the formation of proper chromosome structure and pairing (Zheng *et al*., 2014), indicating that CDK activity plays a conserved role in pairing of plant chromosomes.

Chromosomes are in an altered conformation during pairing in ph1 mutants

It has been proposed that *Ph1* controls chromosome pairing in wheat by affecting the remodeling of heterochromatic regions of chromosomes (Prieto *et al*., 2004; Prieto *et al*., 2005; Colas *et al*., 2008; Knight *et al*., 2010; Greer *et al*., 2012). In polyploid wheat, chromatin is remodeled and elongated in early meiosis to facilitate the pairing process (Prieto *et al*., 2004;

Maestra *et al*., 2002; Mikhailova *et al*., 1998). In the wheat-rye hybrids, euchromatic regions elongate and decondense, regardless of *Ph1,* to allow a more open conformation during pairing (Prieto *et al*., 2005, Maestra *et al*., 2002). However, subtelomeric heterochromatin in rye chromosomes of the hybrid behaves differently in the presence and absence of *Ph1* (Figure 4a-f). When *Ph1* is present, these regions stay tightly compacted throughout prophase I (Prieto *et al*., 2004). However in *ph1* mutants, the heterochromatin regions also elongate (Prieto *et al*., 2004).

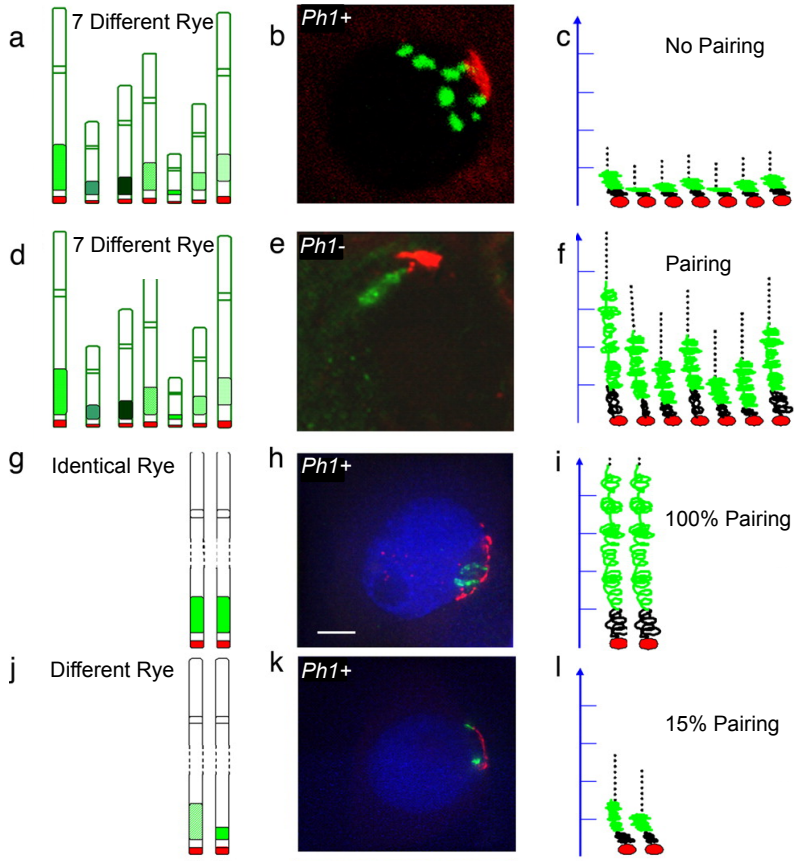

Figure 4: Chromatin remodeling affects pairing efficiency in wheat. Chromosomal interactions were assessed using telomere-specific (red) and rye subtelomeric (green) FISH probes. In wheat-rye hybrids, *Ph1* prevents the rye subtelomeric heterochromatin from elongating (a-c), which precludes pairing between chromosomes bearing the heterochromatic segments. However when *Ph1* is absent, rye heterochromatin elongates (d-f) and pairing occurs. Ph1 affects remodeling of heterochromatin blocks based on their similarity (g-l). When the heterochromatin segments are identical (g-i), heterochromatin elongates and the segments pair. However elongation of the heterochromatin regions does not occur if they are different (j-l). Modified from Colas *et al.* (2008).

The *Ph1*-controlled remodeling of heterochromatin is affected by the degree of sequence similarity between the homoeologous chromosomes undergoing pairing (Figure 4g-l) (Colas *et al*., 2008). In wheat-rye translocation lines containing a pair of identical rye chromosome arm segments, rye heterochromatin elongates, allowing pairing and recombination to take place (Colas *et al*., 2008). However when the rye regions are diverged in sequence and are of different sizes, heterochromatin does not expand and pairing, as well as recombination, is significantly reduced (Colas *et al*., 2008). Possibly, the compaction of heterochromatic domains by *Ph1* excludes these highly repetitive regions from influencing chromosome pairing (Prieto *et al*., 2005).

In addition to controlling chromatin remodeling, *Ph1* also affects the timing of chromosome pairing (Prieto *et al*., 2005; Knight *et al*., 2010). Chromosome pairing in wheat, similar to many other plant species, is thought to begin at the telomeres at the stage of bouquet formation (Prieto *et al*., 2004). In the absence of *Ph1*, chromatin begins to associate and pair while the bouquet is still being formed and chromatin is still being elongated (Prieto *et al*., 2004). Consequently, the chromatin is in a more open state, which may allow repetitive regions of homoeologous chromosomes to associate. The hypothesis that a lack of *Ph1* results in incorrect pairing due to a more open chromosome conformation is further supported by an observation of increased histone H1 phosphorylation in *ph1* mutants (Greer *et al*., 2012). H1 phosphorylation is associated with an open chromatin state (Hale *et al*., 2006). In polyploid wheat, histone H1 is a target of Cdk2 phosphorylation, and the phosphorylation levels of H1 in the presence of *Ph1* can be increased using OA treatment to levels similar to those observed in the *ph1* mutant (Greer *et al*., 2012). Interestingly, this more open chromatin state in *ph1* may

also allow greater access for condensins to bind, possibly providing a link between *cdk* activity and altered chromatin organization.

Without *Ph1* chromosomes are still able to synapse, albeit incorrectly, indicating that *Ph1* affects some part of the homolog recognition process (Gillies, 1987). It is speculated that the altered chromosome state seen in *ph1* mutants affects the choice of template used to repair DSBs, which then allows homoeologous chromosome pairing (Greer *et al*., 2012). When *Ph1* is absent, homoeologous chromosomes could be used as repair templates, whereas in the presence of *Ph1* sister chromatids would be used. It is possible that homoeologous chromosomes synapse even in the presence of *Ph1*, yet DSBs are repaired using sister chromatid as a template, and therefore the homoeologs do not remain connected through chiasmata once the SC disassembles. Arabidopsis CDKG1 may play a similar role in regulate the choice of repair template. The number of CO intermediates is reduced in *cdkg1* mutants, yet DSBs are still repaired, as no chromosome fragmentation is observed (Zheng *et al*., 2014). Likely, incomplete synapsis in the mutant results in DSBs being repaired using the sister chromatid.

Regulation of chromosome structure may also be involved in meiotic adaptation to whole genome duplication in other polyploid species. Analysis of *Arabidopsis arenosa* tetraploids found that numerous genes involved in chromosome structure and synapsis have been targeted by selection following polyploidization (Yant *et al*., 2013; Hollister *et al*., 2012). One of these genes is *SMC3* (Yant *et al*., 2013; Hollister *et al*., 2012). Consequently, *A. arenosa* may represent another polyploid taxon that uses chromosome structure as a means of controlling chromosome interactions.

Outlook

The large sizes of plant genomes make packaging chromatin into chromosomes a major undertaking. The purpose of this process must not only reduce the chromatin volume but also facilitate complicated chromosome functions. Studies in plants show increased transcriptome complexity during meiotic prophase, when chromosomes are already fairly condensed (Chen *et al*., 2010; Yang *et al*., 2011), suggesting that chromosome condensation may specifically act to facilitate gene expression. Chromosomes in meiotic prophase also exhibit dynamic and complex motility (Sheehan and Pawlowski, 2009). These interactions are undoubtedly affected by chromosome structure, which makes it even more imperative to better understand how meiotic processes depend on chromosome structure. Chromosome interactions during pairing and recombination, as documented in this chapter, also require special chromatin conformation. Recent advances such as structured illumination microscopy (Wang, 2013) and chromosomes conformation capture (Naumova *et al*., 2013) methods will allow a more thorough understanding of how plant chromosomes are structured. However, more studies and more sophisticated experimental approaches will be needed to connect chromosome structure to their functions at the mechanistic level.

REFERENCES

Al-Kaff N, Knight E, Bertin I, Foote T, Hart N, et al: Detailed dissection of the chromosomal region containing the *Ph1* locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling. Ann Bot 101:863-872 (2008).

Arya GH, Lodico MJ, Ahmad OI, Amin R, Tomkiel JE: Molecular characterization of *teflon*, a gene required for meiotic autosome segregation in male *Drosophila melanogaster*. Genetics 174:125-134 (2006).

Bai X, Peirson BN, Dong F, Xue C, Makaroff CA: Isolation and characterization of *SYN1*, a *RAD21*-like gene essential for meiosis in Arabidopsis. Plant Cell 11:417-430 (1999).

Bechouet F, Hu B, Roig MB, Sutani T, Komata M, et al: An Smc3 acetylation cycle is essential for establishment of sister chromatid cohesion. Mol Cell 39:689-699 (2010).

Bhatt AM, Lister C, Page T, Fransz P, Findlay K, et al: The *DIF1* gene of *Arabidopsis* is required for meiotic chromosome segregation and belongs to the *REC8/RAD21* cohesion gene family. Plant J 19:463-472 (1999).

Bolanos-Villegas P, Yang X, Wang HJ, Juan CT, Chuang MH, et al: Arabidopsis *CHROMOSOME TRANSMISSION FIDELITY 7* (*AtCTF7/ECO1*) is required for DNA repair, mitosis, and meiosis. Plant J 75:927-940 (2013).

Cai X, Dong F, Edelmann RE, Makaroff CA: The *Arabidopsis* SYN1 cohesion protein is required for sister chromatid cohesion and homologous chromosome pairing. J Cell Sci 116:2999-3007 (2003).

Chan A, Cande WZ: Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. J Cell Sci 111:3507-3515 (1998).

Chan RC, Severson AF, Meyer BJ: Condensin restructures chromosomes in preparation for meiotic divisions. J Cell Biol 167:613-625 (2004).

Chelysheva L, Diallo S, Vezon D, Gendrot G, Vrielynck N, et al: AtRec8 and AtSCC3 are essential to the monopolar orientation of the kinetochores during meiosis. J Cell Sci 118:4621-4632 (2005).

Chen C, Farmer AD, Langley RJ, Mudge J, Crow JA, et al: Meiosis-specific gene discovery in plants: RNA-Seq applied to isolated Arabidopsis male meiocytes. BMC Plant Biol 10:280 (2010).

Choi K, Zhao X, Kelly KA, Venn O, Higgins JD, et al: *Arabidopsis* meiotic crossover hot spots overlap with H2A.Z nucleosomes at gene promoters. Nat Genet 45:1327-1336 (2013).

Colas I, Shaw P, Prieto P, Wanous M, Spielmeyer W, et al: Effective chromosome pairing requires chromatin remodeling at the onset of meiosis. Proc Natl Acad Sci USA 105:6075-6080 (2008).

Cromer L, Jolivet S, Horlow C, Chelysheva L, Heyman J, et al: Centromeric cohesion is protected twice at meiosis, by SHUGOSHINs at anaphase I and by PATRONUS at interkinesis. Curr Biol 23:2090-2099 (2013).

Da Costa-Nunes JA, Bhatt AM, O'Shea S, West CE, Bray CM, et al: Characterization of the three *Arabidopsis thaliana* RAD21 cohesins reveals differential responses to ionizing radiation. J Exp Bot 57:971-983 (2006).

Da Ines O, Abe K, Goubely C, Gallego ME, White CI: Differing requirements for RAD51 and DMC1 in meiotic pairing of centromeres and chromosome arms in *Arabidopsis thaliana*. PLoS Genetics: 8:e1002636 (2012).

Dawe KR, Sedat JW, Agard DA, Cande WZ: Meiotic chromosome pairing in maize is associated with a novel chromosome structure. Cell 76:901-912 (1994).

Dong F, Cai X, Makaroff CA: Cloning and characterization of two Arabidopsis genes that belong to the RAD21/REC8 family of chromosome cohesion proteins. Gene 271:99-108 (2001).

Fraune J, Schramm S, Alsheimer M, Benavente R: The mammalian synaptonemal complex: protein components, assembly and role in meiotic recombination. Exp Cell Res 318:1340-1346 (2012).

Franklin AE, Golubovskaya IN, Bass HW, Cande WZ: Improper chromosome synapsis is associated with elongated RAD51 structures in the maize *desynaptic2* mutant. Chromosoma 112:17-25 (2003).

Franklin AE, McElver J, Sunjevaric I, Rothstein R, Bowen B, et al: Three-dimensional microscopy of the Rad51 recombination protein during meiotic prophase. Plant Cell 11:809-824 (1999).

Fujimoto S, Yonemura M, Matsunaga S, Nakagawa T, Uchiyama S, et al: Characterization and dynamic analysis of *Arabidopsis* condensin subunits, AtCAP-H and AtCAP-H2. Planta 222:293-300 (2005).

Furuno N, den Elzen N, Pines J: Human cyclin A is required for mitosis until mid prophase. J Cell Biol 147:295-306 (1999).

Gillies CB: The effect of the *Ph* gene alleles on synaptonemal complex formation in *Triticum aestivum* x *T. kotshyi* hybrids. Theor Appl Genet 74:430-438 (1987).

Golubovskaya IN, Hamant O, Timofejeva L, Wang CJR, Braun D, et al: Alleles of *afd1* dissect REC8 functions during meiotic prophase I. J Cell Sci 119:3306-3315 (2006).

Golubovskaya IN: Meiosis in maize: *mei* genes and conception of genetic control of meiosis. Advan Genet 26:149-192 (1989).

Greer E, Martin AC, Pendle A, Colas I, Jones AME, et al: The *Ph1* locus suppresses Cdk2-type activity during premeiosis and meiosis in wheat. Plant Cell 24:152-162 (2012).

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, et al: Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploidy wheat. Nature 439:749-752 (2006).

Hale TK, Contreras A, Morrison AJ, Herrera RE: Phosphorylation of the linker histone H1 by CDK regulates its binding to HP1alpha. Mol Cell 22:693-699 (2006).

Hamant O, Golubovskaya I, Meeley R, Fiume E, Timofejeva L, et al: A REC8-dependent plant shugoshin is required for maintenance of centromeric cohesion during meiosis and has no mitotic functions. Curr Biol 15:948-954 (2005).

Harper L, Golubovskaya I, Cande WZ: A bouquet of chromosomes. J Cell Sci 15:4025-4032 (2004).

Hartl TA, Sweeney SJ, Knepler PJ, Bosco G: Condensin II resolves chromosomal associations to enable anaphase I segregation in *Drosophila* male meiosis. PLoS Genetics 4:1-16 (2008).

Hirano T: Condensins: universal organizers of chromosomes with diverse functions. Genes Dev 26:1659-1678 (2012).

Hirano T, Mitchison TJ: A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell 79:449-458 (1994).

Hollister JD, Arnold BJ, Svedin E, Xue KS, Dilkes BP, et al: Genetic adaptation associated with genome-doubling in autotetraploid *Arabidopsis arenosa*. PLoS Genetics 8:e1003093 (2012).

Hudson DF, Marshall KM, Earnshaw WC: Condensin: architect of mitotic chromosomes. Chromosome Res 17:131-144 (2009).

Jiang L, Xia M, Strittmatter LI, Makaroff CA: The Arabidopsis cohesin protein SYN3 localizes to the nucleolous and is essential for gametogenesis. Plant J 50:1020-1034 (2007).

Kaszás E, Cande WZ: Phosphorylation of histone H3 is correlated with changes in the maintenance of sister chromatid cohesion during meiosis in maize, rather than the condensation of chromatin. J Cell Sci 113:3217-3226 (2000).

Keeney S: Spo11 and the formation of DNA double-strand breaks in meiosis. Genome Dyn Stab 2:81-123 (2008).

Kerzendorfer C, Vignard J, Pedrosa-Harand A, Siwiec T, Akimcheva S, et al: The Arabidopsis thaliana MND1 homologue plays a key role in meiotic homologous pairing, synapsis and recombination J Cell Sci 119:2486-2496 (2006).

Kleckner N, Zickler D, Guilaume W: Chromosome capture brings it all together. Science 342:940-941 (2013).

Klein F, Mahr P, Galova M, Bunonomo SB, Michaelis C, et al: A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98:91-103 (1999).

Knight E, Greer E, Draeger T, Thole V, Reader S, et al: Inducing chromosome pairing through premature condensation: analysis of wheat interspecific hybrids. Funct Integr Genomics 10:603-608 (2010).

Lam WS, Yang X, Makaroff CA: Characterization of *Arabidopsis thaliana* SMC1 and SMC3: evidence that AtSMC3 may function beyond chromosome cohesion. J Cell Sci 118:3037-3048 (2005).

Lee J, Ogushi S, Saitou M, Hirano T: Condensins I and II are essential for construction of bivalent chromosomes in mouse oocytes. Mol Biol Cell 22:3465-3477 (2011).

Lui CM, McElver J, Tzafrir I, Joosen R, Wittich P, et al: Condensin and cohesion knockouts in *Arabidopsis* exhibit a *titan* seed phenotype. Plant J 29:405-415 (2002).

Lui CM, Meinke DW: The titan mutants of *Arabidopsis* are disrupted in mitosis and cell cycle control during seed development. Plant J 16:21-31 (1998).

Maestra B, de Jong JH, Shepherd K, Naranjo T: Chromosome arrangement and behavior of two rye homologous telosomes at the onset of meiosis in disomic wheat-5RL addition lines with and without the *Ph1* locus. Chromosome Res 10:655-667 (2002).

Matsuoka Y: Evolution of polyploid triticum wheats under cultivation: the role of domestication, natural hybridization, and allopolyploid speciation in their diversification. Plant Cell Physiol 52:750-764 (2011).

McNicoll F, Stevense M, Jessberger R: Cohesin in gametogenesis. Cur Top Dev Biol 102:1-34 (2013).

Mets DG, Meyer BJ: Condensins regulate meiotic DNA break distributions, thus crossover frequency, by controlling chromosome structure. Cell 139:73-86 (2009).

Miao C, Tang D, Zhang H, Wang M, Li Y, et al: CENTRAL REGION COMPONENT1, a novel synaptonemal complex component, is essential for meiotic recombination initiation in rice. Plant Cell 25:2998-3009 (2013).

Mikhailova EI, Naranjo T, Shepherd K, Wennekes-van Eden J, Heyting C, et al: The effect of the wheat Ph1 locus on chromatin organization and meiotic chromosome pairing analysed by genome painting. Chromosoma 107:339-350 (1998).

Miyazaki WY, Orr-Weaver TL: Sister-chromatid cohesion in mitosis and meiosis. Annu Rev Genet 28:167-187 (1994).

Muyt AD, Mercier R, Mézard C, Grelon M: Meiotic recombination and crossovers in plants. Genome Dyn 5:14-25 (2009).

Naranjo T, Corredo E: Nuclear architecture and chromosome dynamics in the search of the pairing partner in meiosis in plants. Cytogenet Genome Res 120:320-330 (2008).

Nasmyth K, Haering CH: The structure and function of SMC and kleisin complexes. Annu Rev Biochem 74:595-648 (2005).

Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, et al: Organization of the mitotic chromosome. Science 342:948-953 (2013).

Ono T, Losada A, Hirano M, Myers MP, Neuwald AF, et al: Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. Cell 115:109-121 (2003).

Pawlowski WP, Golubovskaya IN, Cande WZ: Altered nuclear distribution of recombination protein RAD51 in maize mutants suggests the involvement of RAD51 in meiotic homology recognition. Plant Cell 15:1807-1816 (2003).

Prieto P, Moore G, Reader S: Control of conformation changes associated with homologue recognition during meiosis. Theor Appl Genet 111:505-510 (2005).

Prieto P, Shaw P, Moore G: Homologue recognition during meiosis is associated with a change in chromatin conformation. Nature Cell Biology 6:906-908 (2004).

Qiao H, Offenberg HH, Anderson LK: Altered distribution of MLH1 foci is associated with changes in cohesins and chromosome axis compaction in an asynaptic mutant of tomato. Chromosoma 121:291-305 (2012).

Qiao H, Lohmiller LD, Anderson LK: Cohesin proteins load sequentially during prophase I in tomato primary microsporocytes. Chromosome Res 19:193-207 (2011).

Remeseiro S, Losada A: Cohesin, a chromatin engagement ring. Curr Opin Cell Biol 25:63-71 (2013).

Riley R, Chapman V: Genetic control of the cytologically diploid behavior of hexaploid wheat. Nature 182:713-715 (1958).

Roberts MA, Reader SM, Dalgliesh C, Miller TE, Foote TN, et al: Induction and characterization of *Ph1* wheat mutants. Genetics 153:1909-1918 (1999).

Rosa M, Harder MV, Cigliano RA, Schlögelhofer P, Scheid OM: The *Arabidopsis* SWR1 chromatin-remodeling complex is important for DNA repair, somatic recombination, and meiosis. Plant Cell 25:1990-2001 (2013).

Sakamoto T, Tsujimoto Y, Uraguchi S, Yoshizumi T, Matsunaga S, et al: Condensin II alleviates DNA damage and is essential for tolerance of boron overload stress in *Arabidopsis*. Plant Cell 23:3533-3546 (2011).

San Filippo J, Sung P, Klein H: Mechanism of eukaryotic homologous recombination. Annu Rev Biochem 77:229-257 (2008).

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, et al: The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112-1115 (2009).

Schommer C, Beven A, Lawrenson T, Shaw P, Sablowski R: *AHP2* is required for bivalent formation and for segregation of homologous chromosomes in *Arabidopsis* meiosis. Plant J 36:1-11 (2003).

Schubert V, Lermontova I, Schubert I: The *Arabidopsis* CAP-D proteins are required for correct chromatin organization, growth, and fertility. Chromosoma 122:517-533 (2013).

Schubert V, Weissleder A, Ali H, Fuchs J, Lermontova I, et al: Cohesin gene defects may impair sister chromatid alignment and genome stability in *Arabidopsis thaliana*. Chromosoma 118:591-605 (2009a).

Schubert V: SMC proteins and their multiple functions in higher plants. Cytogenet Genome Res 124:202-214 (2009b).

Sears ER: An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol *19*:585-593 (1958).

Shao T, Tang D, Wang K, Wang M, Che L, et al: OsREC8 is essential for chromatid cohesion and metaphase I monopolar orientation in rice meiosis. Plant Physiol 156:1386-1396 (2011).

She W, Grimanelli D, Rutowicz K, Whitehead MWJ, Puzio M, et al: Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. Development 140:4008-4019 (2013).

Sheenhan MJ, Pawlowski WP: Live imaging of rapid chromosome movements in meiotic prophase I in maize. Proc Natl Acad Sci USA 106:20989-20994 (2009).

Siddiqui NU, Stronghill PE, Dengler RE, Hasenkampf CA, Riggs CD: Mutations in *Arabidopsis* condensin genes disrupt embryogenesis, meristem organization and segregation of homologous chromosomes during meiosis. Development 130:3283-3295 (2003).

Siddiqui NU, Rusyniak S, Hasenkampf CA, Riggs CD: Distruption of the Arabidopsis SMC4 gene, *AtCAP-C*, compromises gametogenesis and embryogenesis. Planta 223:990-997 (2006).

Sidhu GK, Rustgi S, Shafqat MN, von Wettstein D, Gill KS: Fine structure mapping of a gene-rich region of wheat carrying *Ph1*, a suppressor of crossing over between homoeologous chromosomes. Proc Natl Acad Sci USA 105:5815-5820 (2008).

Singh DK, Andreuzza S, Panoli AP, Siddiqi I: *AtCTF7* is required for establishment of sister chromatid cohesion and association of cohesin with chromatin during meiosis in Arabidopsis. BMC Plant Biol 13:117-126 (2013).

Stronghill P, Pathan N, Ha H, Supijono E, Hassenkampf C: Ahp2 (Hop2) function in *Arabidopsis thaliana* (*Ler*) is required for stablilzation of close alignment and synaptonemal complex formation except for the two short arms that contain nucleolus organizer regions Chromosoma 119:443-458 (2010).

Tiang CL, He Y, Pawlowski WP: Chromosome organization and dynamics during interphase, mitosis, and meiosis in plants. Plant Physiol 158:26-34 (2012).

Tsai CJ, Mets DG, Albrecht MR, Nix P, Chan A, et al: Meiotic crossover number and distribution are regulated by a dosage compensation protein that resembles a condensin subunit. Genes Dev 22:194-211 (2008).

Tzafrir I, Pena-Muralla R, Dickerman A, Berg M, Rogers R, et al: Identification of genes required for embryo development in Arabidopsis. Plant Physiol 135:1206-1220 (2004).

Vazquez J, Belmont AS, Sedat JW: The dynamics of homologous chromosome pairing during male *Drosophila* meiosis. Curr Biol 12:1473-1483 (2002).

Viera A, Gómez R, Parra MT, Schmiesing JA, Yokomori K, et al: Condensin I reveals new insights on mouse meiotic chromosome structure and dynamics. PLoS One 2:e783 (2007).

Wang CJ: Analyzing maize meiotic chromosomes with super-resolution structured illumintation microscopy. Methods Mol Biol 990:67-78 (2013).

Wang M, Tang D, Wang K, Shen Y, Qin B, et al: OsSGO1 maintains synaptonemal complex stabilization in addition to protecting centromeric cohesion during rice meiosis. Plant J 67:583-594 (2011).

Wang CJR, Carlton PM, Golubovskaya IN, Cande WZ: Interlock formation and coiling of meiotic chromosome axes during synapsis. Genetics 183:905-915 (2009).

Wassmann K: Sister chromatid segregation in meiosis II: deprotection through phosphorylation. Cell Cycle 12:1352-1359 (2013).

Watanabe K, Pacher M, Dukowic S, Schubert V, Puchta H, et al: The STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. Plant Cell 21:2688-2699 (2009).

WatanabeY: Sister chromatid cohesion along arms and at centromere. Trends Genet 21:405-412 (2005).

Yamada T, Ohta K: Initiation of meiotic recombination in chromatin structure. J Biochem 154:107-114 (2013).

Yang H, Lu P, Wang Y, Ma H: The transcriptome landscape of Arabidopsis male meiocytes from high-throughput sequencing: the complexity and evolution of the meiotic process. Plant J 65:503-516 (2011).

Yant L, Hollister JD, Wright KM, Arnold BJ, Higgins JD, et al: Meiotic adaptation to genome duplication in *Arabidopsis arenosa*. Curr Biol 23:2151-2156 (2013).

Yeong FM, Hombauer H, Wendt KS, Hirota T, Mudrak I, et al: Identification of a subunit of a novel kleisin-beta/SMC complex as a potential substrate of protein phosphatase 2A. Curr Biol 13:2058-2064 (2003).

Yuan L, Yang X, Ellis JL, Fisher NM, Makaroff CA: The Arabidopsis SYN3 cohesion protein is important for early meiotic events. Plant J 71:147-160 (2012).

Yousafzai FK, Al-Kaff N, Moore G: The molecular features of chromosome pairing at meiosis: the polyploid challenge using wheat as a reference. Funct Integr Genomics 10:147-156 (2010a).

Yousafzai FK, Al-Kaff N, Moore G: Structural and functional relationship between the *Ph1* locus protein 5B2 in wheat and CDK2 in mammals. Funct Integr Genomics 10:157-166 (2010b).

Yu HG, Koshland DE: Meiotic condensin is required for proper chromosome compaction, SC assembly, and resolution of recombination-dependent chromosome linkages. JCB 163:937-947 (2003).

Zamariola L, De Storme N, Vannerum K, Vandepoele K, Armstrong SJ, et al: SHUGOSHINs and PATRONUS protect meiotic centromere cohesion in *Arabidopsis thaliana*. Plant J doi:10.1111 (2014).

Zhang L, Tao J, Wang S, Chong K, Wang T: The rice OsRad21-4, an orthologue of yeast Rec8 protein, is required for efficient meiosis. Plant Mol Biol 60:533-554 (2006).

Zheng T, Nibau C, Phillips DW, Jenkins G, Armstrong SJ, et al: CDKG1 protein kinase is essential for synapsis and male meiosis at high ambient temperature in *Arabidopsis thaliana*. Proc Natl Acad Sci USA doi/10.1073 (2014).

CHAPTER 2

DYNAMICS OF DOUBLE STRAND BREAK REPAIR INFLUENCE CROSSOVER PATTERNS

Introduction

Meiotic recombination results in the exchange of DNA between homologous chromosomes, creating allelic diversity while at the same time ensuring correct segregation of chromosomes during the meiotic divisions. The pathway is initiated by the introduction of double-strand breaks (DSBs) into chromosomal DNA by the SPO11 protein (Keeney *et al*., 1997). These DSBs are then resected by the MRN complex, consisting of MRE11, RAD50, and NSB1 (Keeney, 2008; Mimitou and Symington, 2009). Resection leaves 3' single-stranded DNA (ssDNA) overhangs which RAD51 and DMC1 coat to form nucleoprotein filaments (Forget and Kowalczykowski, 2010). RAD51 and DMC1 catalyze single-end invasion of the ssDNA into the homologous chromosome (San Filippo *et al*., 2008). A subset of the DSBs are then repaired as COs, resulting in exchange of chromosome segments. The remaining DSBs are repaired as non-crossovers (NCOs) using synthesis-dependent strand annealing (Mézard *et al*., 2006).

A vast majority of the known molecular details on meiosis come from studies in yeast. Yet there have been limited studies on the molecular mechanisms of meiosis in plants. Many plants, including major crops such as maize, rice, and wheat, have large genomes and a drastically different genome organization than yeast. For example, it is unlikely that chromosome restructuring or the features that influence CO distribution would be entirely conversed between yeast and maize. Therefore, studies on plant meiosis, and specifically recombination in plants, are needed understand how meiosis proceeds in a complex genome.

COs are not distributed evenly across the genome in most organisms. Most maize COs are found at the distal regions of chromosomes, whereas the centromeric and pericentromeric regions are often devoid of COs (Rodgers-Melnick *et al*., 2015; Gore *et al*., 2009; He *et al*., submitted). However this pattern is not seen at the DSB level. Instead, maize DSBs occur along the entire length of chromosomes, with no preference for distal or proximal regions (He *et al*., submitted).

About 85% of the maize genome consists of repetitive DNA. A large portion of this DNA forms the constitutive heterochromatin that lies in proximal regions of chromosomes (Eichten *et al*., 2012). Interestingly, it is these highly repetitive areas that are largely devoid of COs (He *et al*., submitted). It has been hypothesized that COs are suppressed in areas of highly repetitive elements to preserve genome stability (Peng and Karpen, 2008). COs in these regions could result in chromosome translocations, rearrangements, and fragmentation or insertion/deletion of repetitive arrays. Conversely the distal regions of maize chromosomes, where most COs occur, have low levels of constitutive heterochromatin and high gene density (Schnable *et al*., 2009; Gore *et al*., 2009; Makarevitch *et al*., 2013).

In the study presented here, I tracked the colocalization of RAD51 foci with histone markers associated with different chromatin states to investigate if the spatiotemporal dynamics of RAD51-mediated DSB repair differ between distal-genic and proximal-repetitive regions, and how these repair patterns influence CO distribution. I used the trimethylation of histone 3 lysine 4 (H3K4me3) to track a subset of euchromatic DSBs. This histone modification is an important positive regulator of transcription in plants (Guo *et al*., 2010). Furthermore it is thought to be an important determinant of DSBs in both budding yeast and mouse (Pan *et al*., 2011; Smagulova *et*

al., 2011; Borde *et al*., 2009; Buard *et al*., 2009) and is associated with a subset of maize genic DSB hotspots (He *et al*., submitted).

Histone 3 lysine 9 dimethylation (H3K9me2) was used to mark the repetitive regions of the genome. This histone modification serves as a binding site for chromomethylases to maintain CG, CHG, and a subset of CHH methylation sites (Du *et al*., 2012). Therefore, silencing transposable elements and other repetitive elements requires H3K9me2 to maintain DNA methylation at these sites (Bernatavichute *et al*., 2008). Furthermore, previous studies indicated that H3K9me2 serves as a marker for constitutive heterochromatin in maize (West *et al*., 2014; Eichten *et al*., 2012). Then, to understand how the dynamics of RAD51 influence CO distribution, patterns of heterochromatic RAD51 localization were compared between two inbred lines. Through this analysis, I found that CO patterns are likely a reflection of early DSB distribution.

Materials and Methods

Plant materials

B73 plants were grown in a greenhouse and meiotic anthers were harvested when the plants were about 6 weeks old. CML228 plants were grown in a 1:1 Promix : calcined clay soil mix in a growth chamber, at a 31°C day temperature and 22°C night temperature and 12 hours of light. Meiotic anthers were fixed when plants were about 7 weeks old.

Anther fixation

Staged anthers were dissected from male flowers and fixed in 2% formaldehyde in buffer A (15 mM PIPES pH 7.0, 80 mM KCl, 20 mM NaCl, 2 mM EDTA, 0.5 mM EGTA, 0.2 mM

spermine, 0.5 mM spermidine, 1 mM DTT, and 0.32 M sorbitol) for 30 minutes with gentle shaking. Fixed anthers were rinsed in buffer A for 30 minutes, and then stored in fresh buffer A at 4°C.

Chromosome spread immunofluorescence

Chromosome spreads procedures were modified from Armstrong and Osman (2013). Meiocytes were extruded from fixed anthers onto poly-L-lysine coated glass microscope slides and incubated in a humid chamber at 35°C and digested with 30 μL of 0.4% cytohelicase, 1.5% sucrose, 1% polylvinylpyrolidone for 7 or 10 minutes for CML228 and B73, respectively. The slides were then taken out of the humid chamber and placed directly on a slide warmer at 35°C and chromosomes were spread by the addition of 40 μL of 2% lipsol for 7 or 10 minutes for CML228 and B73, respectively. Slides were then dried for 2 hours at room temperature. Cellular debris was removed from the slides with a 1 minute wash in 1X PBS, 0.1% Triton X-100.

Primary antibodies were diluted in 1X PBS, 3% BSA, 1 mM EDTA, 0.1% Tween-20 to 1:500 and 1:100 for rabbit anti-ZmRAD51A1 (He *et al*., submitted) and mouse anti-HsH3K9me2 (ab1220, Abcam, Cambridge, MA), respectively. Primary incubations were carried out overnight at 4°C in a humid chamber. The slides were then washed twice in 1X PBS, 0.1% Triton X-100 for five minutes. Secondary antibodies were diluted to 1:50 in 1X PBS, 3% BSA, 1 mM EDTA, 0.1% Tween-20. Anti-rabbit TRITC and anti-mouse FITC were used to detect RAD51 and H3K9me2 signal, respectively. Secondary incubations were carried out at 37°C for 30 minutes in a humid chamber. The slides were then washed twice in 1X PBS, 0.1% Triton X-100 for five minutes. Lastly, 15 μg/mL of 4,6-diamidino-2-phenylindole (DAPI) dissolved in

the anti-fade mounting medium Vectashield was applied to each slide. The slides were then closed with a coverslip and sealed with nail polish.

3D immunofluorescence

H3K4me3 was immunocolocalized with RAD51 on meiotic chromosomes as described in Pawlowski *et al*. (2003). Briefly, meiocytes were extruded from fixed anthers and embedded into polyacrylamide pads. The pads were washed twice in 1X PBS for 10 minutes; then the meiocytes were permeabilized with 1X PBS, 1% Triton X-100, and 1 mM EDTA for 1.5 hours. The cells were then blocked for two hours in 1X PBS, 3% BSA, 1 mM EDTA, and 0.1% Tween 20. The pads were then incubated overnight in a humid chamber with 50 μL of a 1:500 dilution of the rabbit anti-ZmRAD51A1 antibody (He *et al*., submitted) and 1:100 dilution of mouse anti-HsH3K4me3 (ab1012, Abcam, Cambridge, MA).

The following day, pad were washed with 1X PBS, 0.1% Tween 20, and 1 mM EDTA eight times for one hour. Pads were left in the wash buffer overnight. The next day, anti-mouse-FITC and anti-rabbit-TRITC, both diluted 1:50, were applied to the pads and incubated for two hours in a humid chamber. The pads were then washed four times for one hour with 1X PBS, 0.1% Tween 20, and 1 mM EDTA. Pads were then washed two times; ten minutes each, in 1X PBS. Next 10 μg/mL of DAPI in 1X PBS was applied for 30 minutes. The pads were then washed three times; ten minutes each, in 1X PBS. Next 1,4-Diazabicyclo-octane (DABCO) anti-fade solution was then applied to the pads three times for one minute each. Pads were then mounted on clean glass microscope slides, topped with a coverslip, and sealed with nail polish.

Imaging and image analysis

Chromosomes were imaged with a DeltaVision imaging widefield fluorescence microscope (Applied Precision, Issaquah, WA). 3-dimensional stacks of the chromosomes were generated by taking a Z section every 0.15 μm through the entire nucleus. SoftWoRx software (Applied Precision, Issaquah, WA) was then used to deconvolve and analyze these 3-D image stacks.

SoftWoRx software was used for the quantification of RAD51 and H3K4me3 foci and to define H3K9me2 domains. For each fluorescent channel, a fluorescence intensity threshold was set based on the signal intensity to eliminate background. Foci were counted automatically when there were more than 50 foci, or by hand for less than 50 foci, in each channel separately. Colocalized foci were defined as sites where foci from both channels overlapped. RAD51 foci colocalized with either histone marker were automatically counted by overlaying the signal from both channels, and counting the number of RAD51 signals that overlapped with the histone signals.

Results

H3K4me3 marks a minor fraction of zygotene DSB repair sites

To examine the dynamics of DSB formation in open chromatin, the RAD51 protein was colocalized with the euchromatin marker H3K4me3. Sidhu *et al*. (submitted) examined the dynamics of H3K4me3 through meiotic prophase in maize and found that the histone marker was visible on chromosomes from leptotene through the end of prophase I. To get a deeper understanding of the role of H3K4me3 during maize meiosis, I quantified the number of H3K4me3 foci over early zygotene, mid zygotene, and late zygotene (Table 1, Figure 5a). It is

during these substages that the majority of DSB-marking RAD51 foci are visible (Franklin *et al*., 1999), and therefore H3K4me3 influence on recombination would be of the greatest relevance during these stages. The most H3K4me3 foci are present during mid zygotene, which is when RAD51 foci on chromosomes reach their peak. To see if the presence of high H3K4me3 foci was related to more RAD51 foci, the number of RAD51 foci were also quantified (Table 1, Figure 5b). Yet there was only a moderate correlation between the number of H3K4me3 foci and the total number of RAD51 per cell.

Table 1: RAD51 colocalized with H3K4me3 during B73 meiosis.

Stage	Number of cells	Mean H3K4me3 foci	Mean total RAD51 foci	Mean number of colocalized RAD51 foci	Mean percentage of colocalized RAD51
Early zygotene	15	753	223	20	10%
Mid zygotene	15	1014	438	42	10%
Late zygotene	15	709	152	25	18%

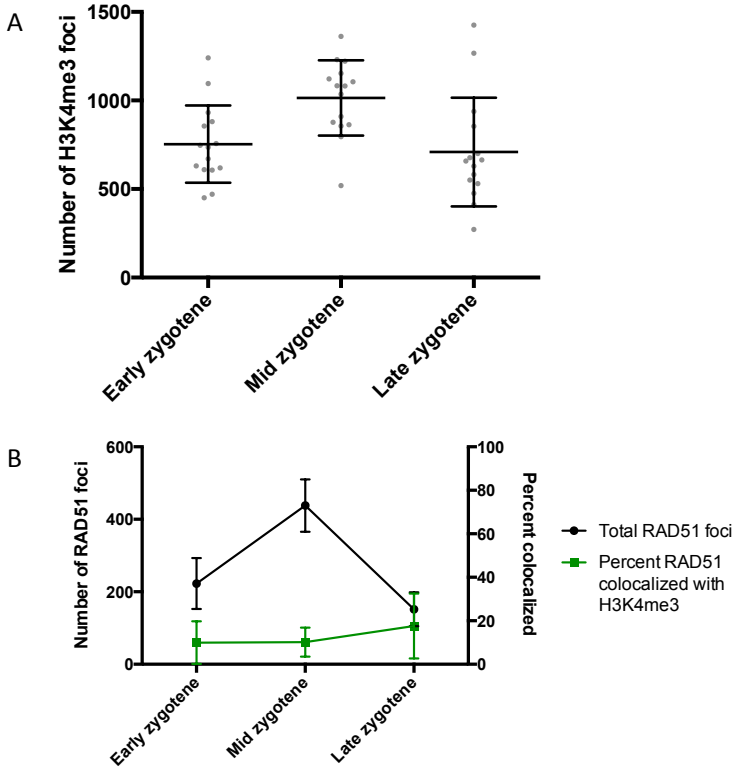

Figure 5: Dynamics of H3K4me3 and RAD51 through early, mid, and late zygotene. A) Quantifications of H3K4me3 foci. B) Total number of RAD51 foci and percent of RAD51 colocalized with H3K4me3. Mean ± one standard deviation is shown for each substage.

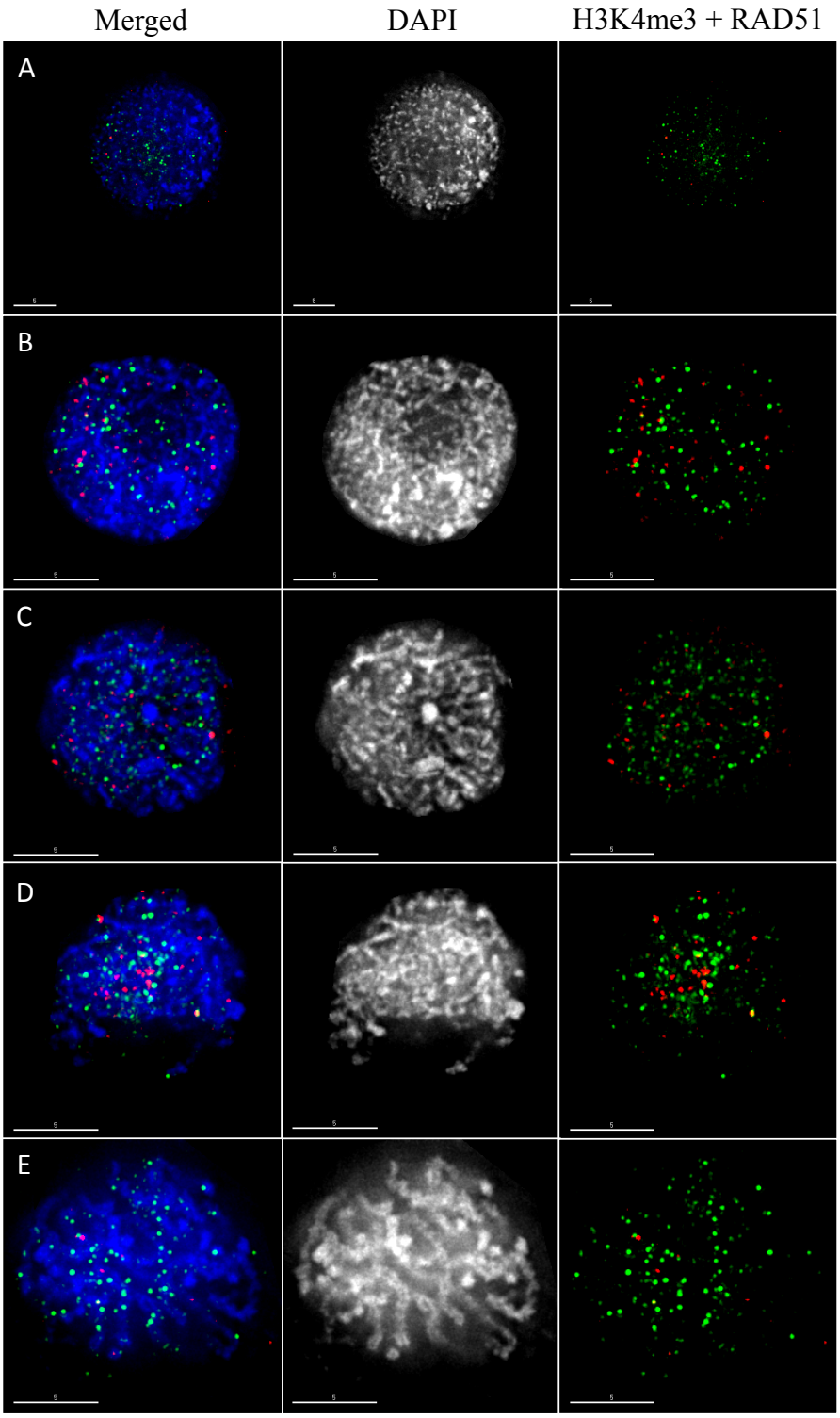

Figure 6: RAD51 colocalization with histone marker H3K4me3 in B73 meiocytes through A) leptotene, B) early zygotene, C) mid zygotene, D) late zygotene, and E) early pachytene. Blue = chromatin, Green = H3K4me3, Red = RAD51. Each image is a projection of 5-10 Z-sections. Scale bar = 5 μm.

I then investigated whether RAD51 colocalization with H3K4me3 varied through the substages of zygotene by quantifying the colocalization of RAD51 foci with H3K4me3 foci (Figure 6, Figure 5b). DSB mapping data indicate that only half of genic DSB hotspots (13% of total DSB hotspots) overlap with H3K4me3 sites (He *et al*., submitted). I found that, consistent with the ChIP-seq data, only 13% of RAD51 foci colocalized with H3K4me3 sites (Table 1). However a similar analysis should be performed in leptotene and early pachytene meiocytes in order to get a complete understanding of the influence of H3K4me3 on RAD51 dynamics.

RAD51-marked DSBs formed in heterochromatin have distinct temporal patterns

To examine the dynamics of constitutive heterochromatin along maize chromosomes, I examined the localization pattern of the histone marker H3K9me2 through prophase I (West *et al*., 2014; Eichten *et al*., 2012). H3K9me2 foci are presenton chromosomes from leptotene to the end of prophase I (Figure 7). However unlike H3K4me3, I was not able to cytologically quantify the number of H3K9me2 foci. Likely because 85% of the maize genome is repetitive, the amount of H3K9me2 signal was so great that individual foci could not be distinguished. Instead, SoftWoRx software was used to define a domain of H3K9me2 signal, discernable as an area containing a large amount of individual H3K9me2 foci along the chromosomes.

Then to investigate the dynamics of RAD51 localization within heterochromatin, I quantified RAD51 localization in relation to the H3K9me2 domain from leptotene through early pachytene (Figure 8, Table 2, Figure 9). I found that there is a strong correlation between the total number of RAD51 foci and the percent of RAD51 foci co-localized with H3K9me2 ($r = 0.638$).

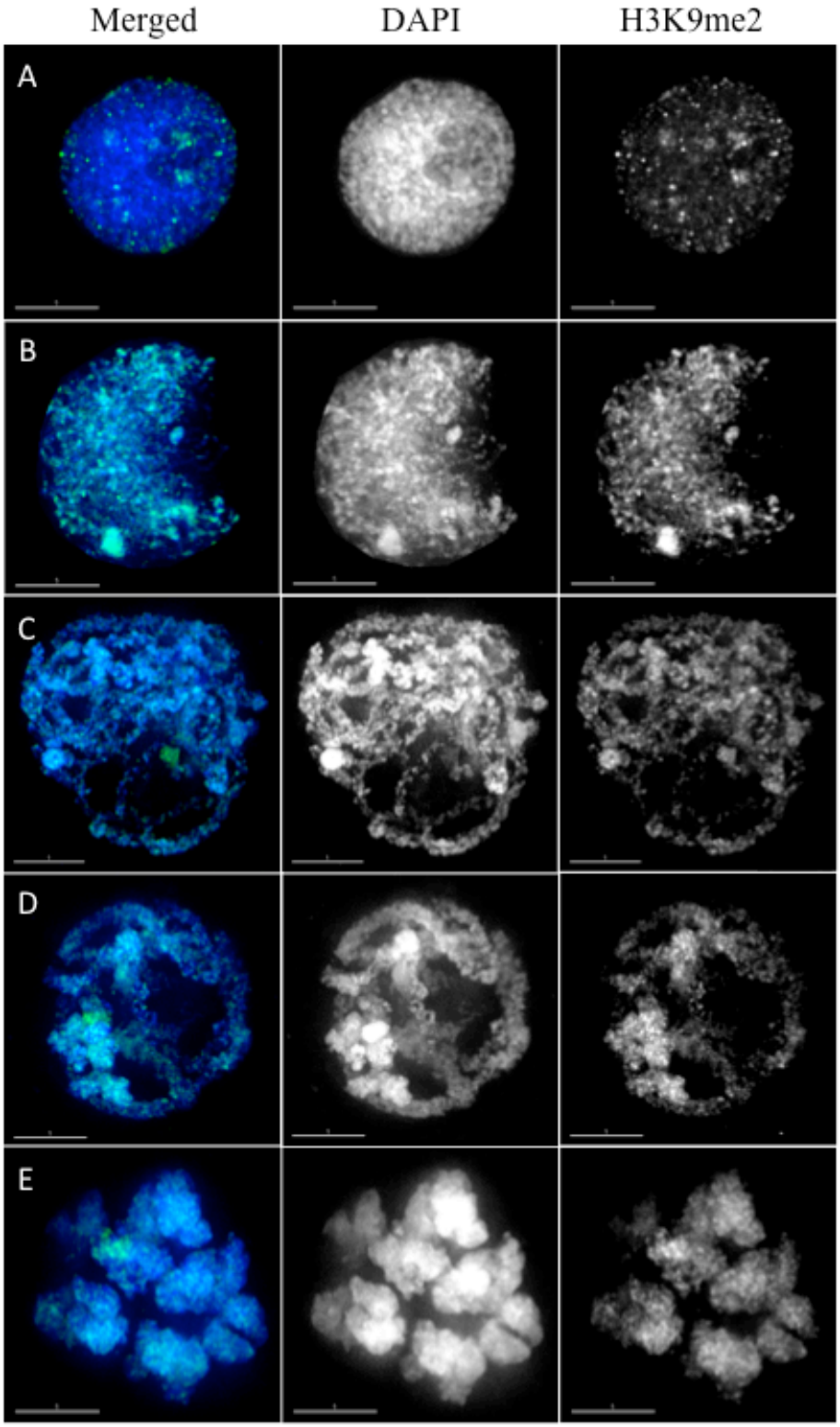

Figure 7: H3K9me2 localization through prophase I. A) leptotene, B) zygotene, C) pachytene, D) diplotene, and E) diakinesis. Blue = chromatin, Green = H3K9me2. Whole nuclei were projected into a single plane for each image. Scale bar = 5 μm.

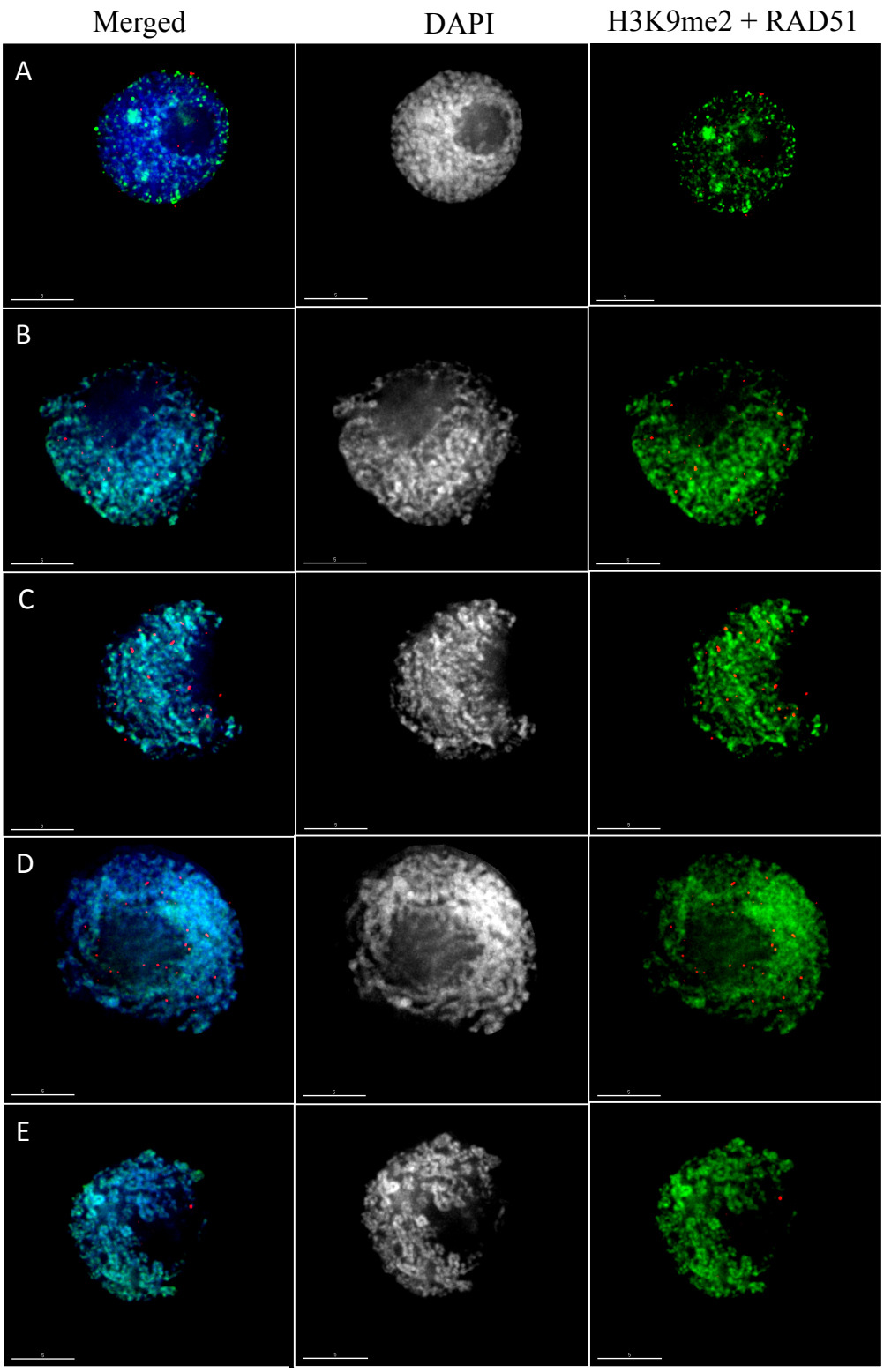

Figure 8: In B73 a portion of RAD51 foci occur within the H3K9me2 domain during A) leptotene, B) early zygotene, C) mid zygotene, D) late zygotene, and E) early pachytene. Blue = chromatin, Green = H3K9me2, Red = RAD51. Each image is a projection of 5-10 Z-sections. Scale bar = 5 μm.

This indicates that during zygotene when RAD51 number are at their peak, RAD51 foci are most likely to occur within the H3K9me2 domain.

RAD51 foci first appear on chromosomes during leptotene, and only about a quarter of these foci occur within the H3K9me2 domain. Similarly, during early pachytene as the numbers of RAD51 foci are drastically reduced as DSBs are repaired, there is also a drop in the percentage of RAD51 foci that occur within the H3K9me2 domain. This indicates that a majority of both the initial and persisting RAD51-associated DSBs occur outside of the constitutive heterochromatin, presumably in euchromatin.

Table 2: RAD51 localization within H3K9me2 domain during B73 meiosis.

Stage	Number of cells	Mean total RAD51 foci	Mean number of colocalized RAD51 foci	Mean percentage of colocalized RAD51
Leptotene	15	14	4	24%
Early zygotene	15	229	153	67%
Mid zygotene	15	418	232	56%
Late zygotene	15	284	191	65%
Early pachytene	15	22	6	21%

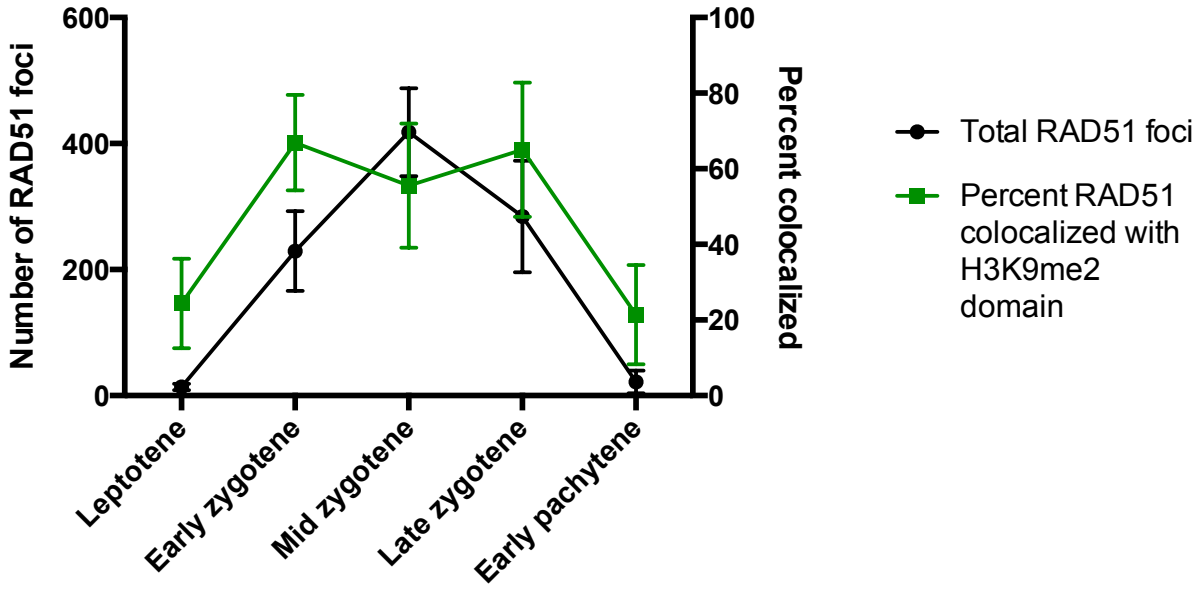

Figure 9: Dynamics of RAD51 during B73 meiosis. Quantifications of total number of RAD51 foci and percent of RAD51 colocalized with H3K9me2 domain. Mean ± one standard deviation is shown for each substage.

Dynamics of RAD51 differ between maize inbred lines that have distinct crossover patterns

To get a deeper understanding of the influence of DSB repair on CO patterns, RAD51 dynamics were analyzed in an inbred line with a drastically different CO distribution than B73. Sidhu *et al*. (submitted) analyzed natural variation in CO patterns by quantifying chiasmata, the cytological linkages of COs, in 14 diverse inbred lines. Their study found B73 has an average of 18 chiasmata, representing 18 COs, per meiocyte. Additionally, they found that B73 homologous chromosomes were predominately connected by two distal chiasmata (Sidhu *et al*., submitted). These data are consistent with what is found mapping maize COs genetically: COs most frequently form in the distal regions of chromosomes (Rodgers-Melnick *et al*., 2015). However Sidhu *et al*.'s study found that the inbred line CML228 has a distinct chiasma distribution, and therefore CO patterns. They found that CML228 has on average 11 chiasmata per meiocyte, indicating a single CO connects most bivalents. Additionally, the chiasma in CML228 were found to be predominately proximal, indicating that in CML228, COs form in chromosomal areas that are excluded from recombination in other inbred lines.

To see if heterochromatic RAD51 localization dynamics could explain the differences in CO distribution between B73 and CML228, I quantified the percentage of RAD51 foci that were localized to the H3K9me2 domain during CML228 meiosis (Table 3, Figure 10, Figure 11). The most striking difference between the two inbred lines occurred during leptotene. Though a

Table 3: RAD51 localization within H3K9me2 domain during CML228 meiosis.

Stage	Number of cells	Mean total RAD51 foci	Mean number of colocalized RAD51 foci	Mean percentage of colocalized RAD51
Leptotene	15	16	8	49%
Early zygotene	15	204	118	59%
Mid zygotene	15	260	136	52%
Late zygotene	15	226	140	61%
Early pachytene	15	66	27	35%

similar number of leptotene RAD51 foci were seen in B73 and CML228, the percentage of RAD51 foci occurring within the H3K9me2 domain varied. Only about a quarter of these early RAD51 foci are found within H3K9me2 domain in B73. However in CML228, 49% of leptotene RAD51 foci were localized to the heterochromatic regions. Two-factor analysis of variance showed that the difference in leptotene RAD51 heterochromatin localization was significantly different between the two inbred lines (Figure 12, Table 4).

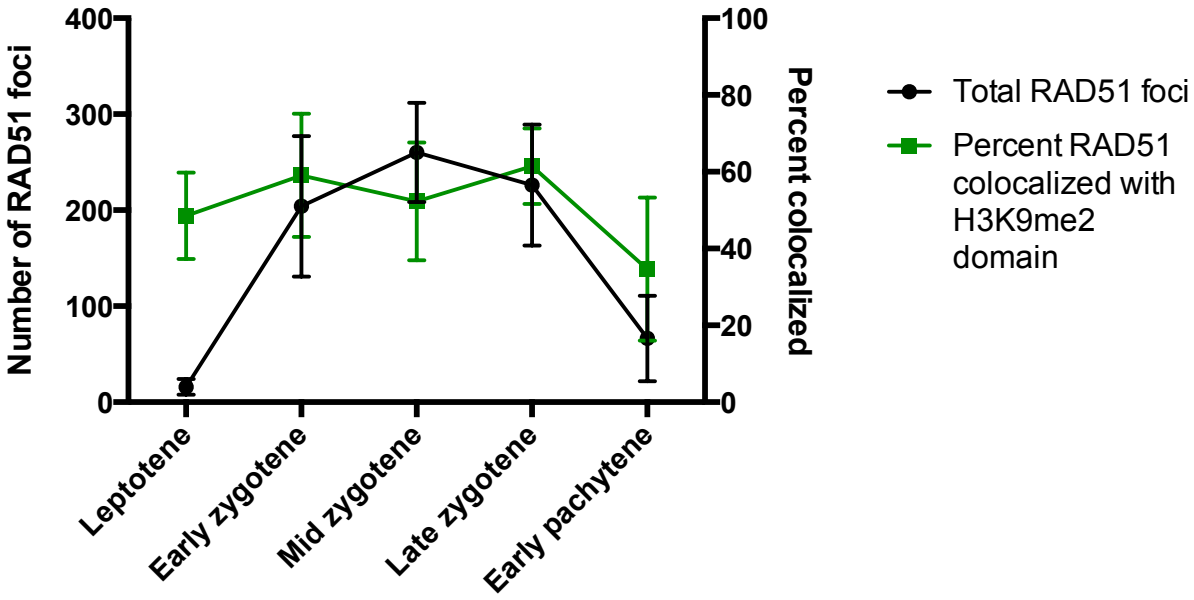

Figure 10: Dynamics of RAD51 in CML228. Quantifications of total number of RAD51 foci and percent of RAD51 colocalized with H3K9me2 domain. Mean ± one standard deviation is shown for each substage.

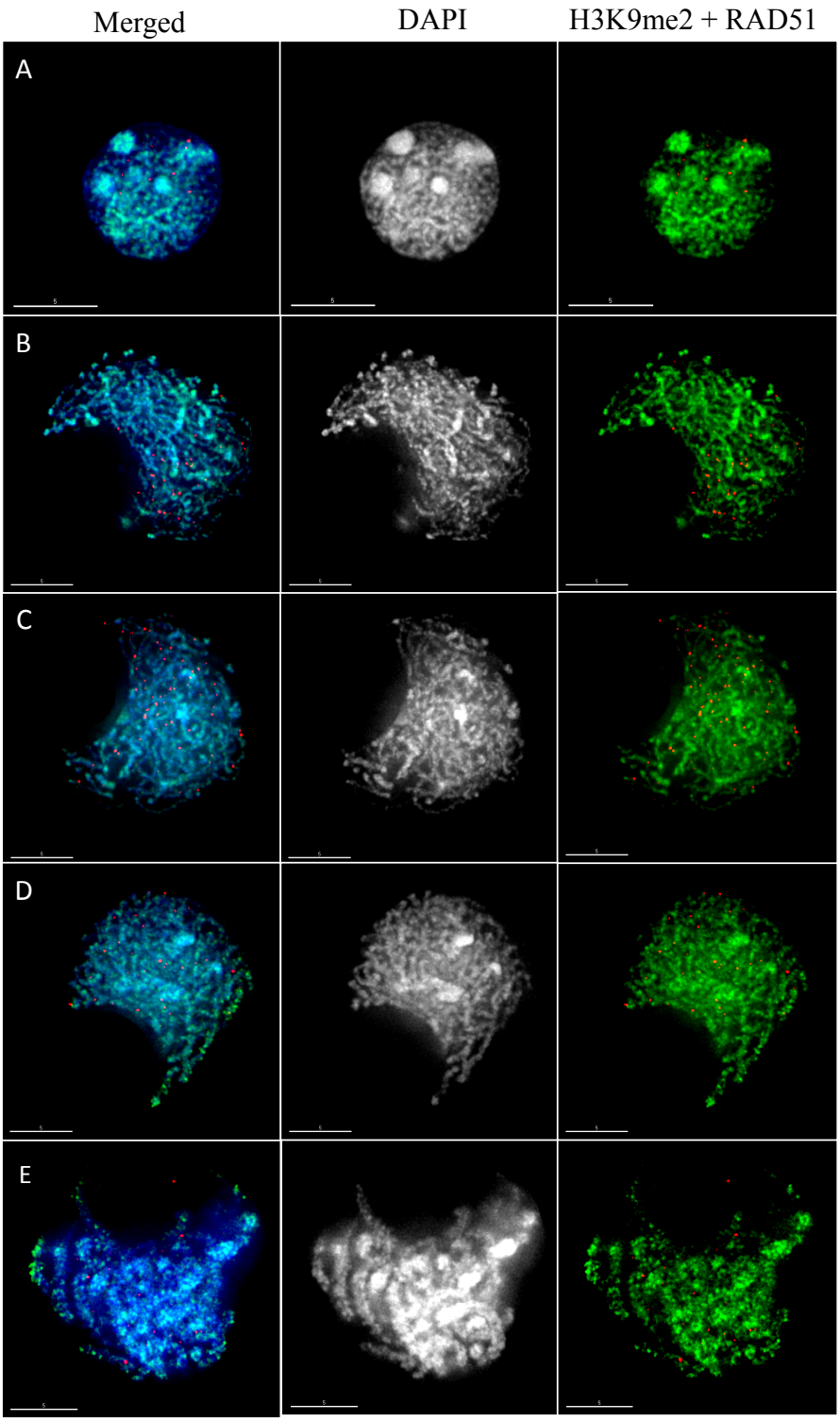

Figure 11: During CML228 meiosis a subset of RAD51 foci colocalize with H3K9me2 during A) leptotene, B) early zygotene, C) mid zygotene, D) late zygotene, and E) early pachytene. Blue = chromatin, Green = H3K9me2, Red = RAD51. Each image is a projection of 5-10 Z-sections. Scale bar = 5 μm.

Table 4: Two-factor nested analysis of variance of the percentage of RAD51 foci occurring within H3K9me2 domain between B73 and CML228 through five substages of prophase I.

	df	Sum of squares	Mean sum of squares	F-value	*p*
Between inbreds	1	0.775	0.07753	3.6613	0.05773
Between stages within inbreds	8	3.6172	0.45216	21.3528	< 2e-16
Error	140	2.9646	0.02118		

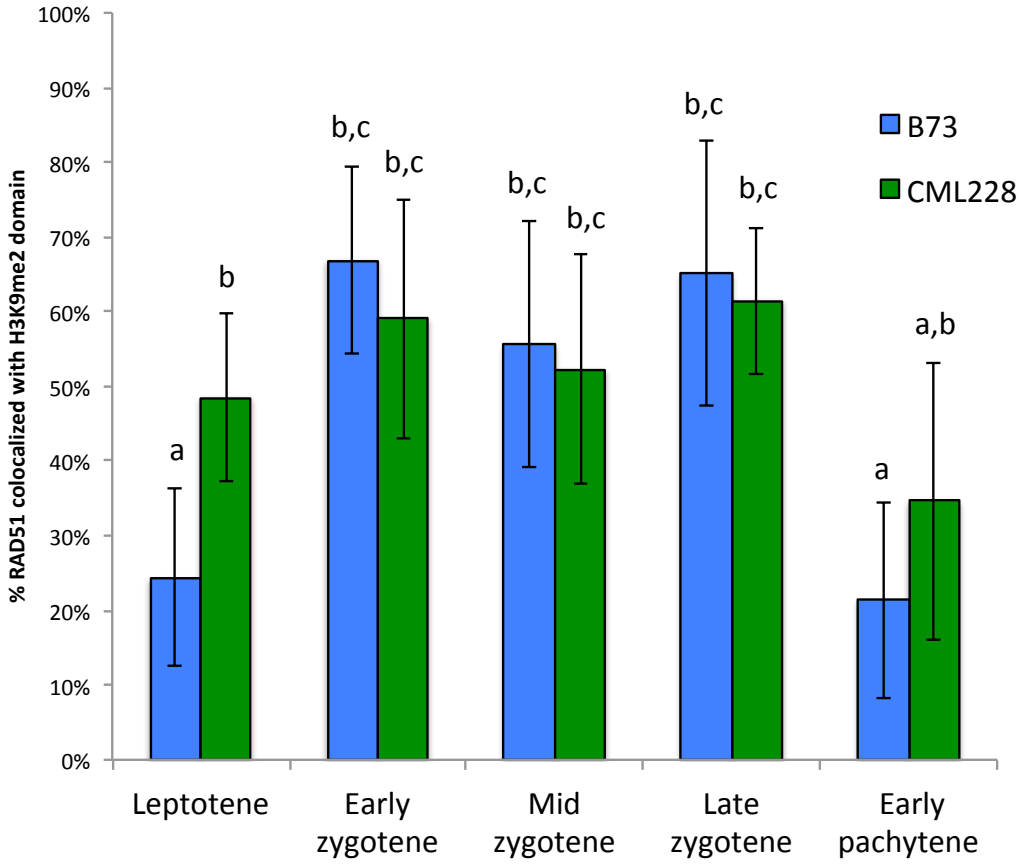

Figure 12: Heterochromatic localization of RAD51 foci is not identical between maize inbreds. Mean ± one standard devication is shown for each substage. Pairs of means grouped by the same letter are not significantly different from each other (Tukey-Kramer method, $p > 0.01$) (Bretz *et al*., 2001).

Discussion

Most DSB hotspots in maize are formed in repetitive regions of the genome (He *et al*., submitted), and the research presented here shows that a majority of RAD51 foci occur within the H3K9me2-marked constitutive heterochromatin. In addition to maize, high-resolution DSB hotspot maps are available for budding yeast and mouse (Pan *et al*., 2011; Smagulova *et al*., 2011). Analyses of yeast DSB hotspots indicate that DSB formation is largely controlled by chromatin organization; in yeast, Spo11 forms breaks where it has the easiest access to chromatin (Pan *et al*., 2011). In both yeast and mouse, DSB hotspots are at sites enriched for H3K4me3 (Borde *et al*., 2009; Buard *et al*., 2009). In yeast, mutations in the methyltransferase Set1, which catalyzes the H3K4me3 modification, result in reduced DSB number and drastic changes in DSB distribution (Borde *et al*., 2009). The dependence of DSBs on H3K4me3 sites is even stronger in mouse, where 94% of mouse DSBs overlap with H3K4me3 sites (Smagulova *et al*., 2011). In mouse, and also humans, DSB formation is driven largely by the histone methyltransferase PRDM9 (Baudat *et al*., 2010). Yet in maize, we see both biochemically and cytologocially, that H3K4me3 is not a major determinant of DSB sites (He *et al*., submitted, Sidhu *et al*., submitted).

The frequent occurrence of maize DSBs within heterochromatin is surprising in terms of maintaining genome stability. It is hypothesized that recombination must be suppressed in repetitive regions of the genome, as recombination in repeats is complicated by the challenge of finding the correct homologous sequence to serve as the repair template when the sequence is repeated potentially thousands of times across the genome. Choosing the incorrect repair template could result in nonallelic recombination and major genome rearrangements (Lichten and de Massy, 2011; Peng and Karpen, 2008). In theory, the easiest way to prevent

recombination in repetitive regions would be to prevent DSB formation in repeats. In Drosophila somatic cells, when DSBs are induced by ionizing radiation, DSBs that are formed within heterochromatin are physically moved outside of the heterochromatic domain prior to DSB repair (Chiolo *et al*., 2011). Yet in maize, a majority of DSBs form within heterochromatin. Though when the distribution of maize DSB hotspots and COs is examined, it becomes clear that while DSBs form within the highly repetitive centromeric and pericentromeric portion of chromosomes, these DSBs are not repaired as COs. Interestingly, CO distribution patterns are matched much more closely when only genic DSB hotspots are considered (He *et al*., submitted).

The results outlined in this chapter indicate that the dynamics of DSB repair differ between genic and repetitive regions. In B73, an inbred line that has COs predominantly occurring in the distal, gene-rich portion of chromosomes (Sidhu *et al*., submitted), nearly 75% of early RAD51 foci localize outside of the H3K9me2-marked heterochromatin. However a strong correlation was found between the total number of RAD51 foci and the percentage occurring within repetitive regions, so that as the number of RAD51 foci increase during zygotene, a majority of foci are localized in heterochromatin. This indicates that in maize, the distribution of DSBs varies temporally. Furthermore, this temporal regulation of DSB may represent a mechanism to prevent heterochromatic COs by delaying DSB formation in these regions.

Large genome grasses such as barley, wheat, rye, and oat share a similar recombination pattern as maize (Higgins *et al*., 2012; Sandhu and Gill, 2002), in which CO are predominantly formed in distal regions of chromosomes and suppressed in pericentromeric regions. In the past several years, numerous studies have demonstrated that the timing of DSB formation influences

the role DSBs play in both recombination and homologous pairing (Joshi *et al*., 2015; Kauppi *et al*., 2013; Higgins *et al*., 2012; Sidhu *et al*., submitted). This timing hypothesis could provide an explanation to why distal DSBs are more likely to be repaired as COs in the grasses. As proposed by Higgins *et al*. (2012), DSBs are formed over a period of time, and those formed earlier are more likely to be repaired as COs. Based on CO distribution data in the grasses, this would mean that distal DSBs form earlier than DSBs in proximal, highly repetitive regions.

In the research presented here, I found that the earliest RAD51 foci that appear during leptotene form predominately outside of repetitive regions, presumably in genic, distal regions. This is consistent with the timing hypothesis: the earliest DSBs form outside of heterochromatin, allowing COs to form outside of heterochromatin. However, from these data it remains unclear whether this pattern reflects the importance of chromosomal location or genomic content in early DSB distribution.

The inbred CML228 has a much higher occurrence of proximal chiasma, indicating that in this line COs occur in areas excluded from recombination in B73 (Sidhu *et al*., submitted). Interestingly in CML228, early RAD51 foci are twice as likely to occur within the H3K9me2-marked heterochromatin domain than in B73. Furthermore, these results indicate that the position of genic regions close to chromosomal ends is not enough to guarantee early DSB formation in these regions. Likely, there are some other components, not just positional cues, which influence DSB formation, and therefore CO patterns.

We propose that in maize early- and late-formed DSBs serve different functions (Figure 13). DSBs formed early are those that are funneled into the CO repair pathway. In B73, most of these early DSBs form in genic regions, whereas more early DSBs form in repetitive regions in CML228. The eventual CO patterns in each inbred reflect the distribution of these early DSBs.

Then during zygotene, additional DSBs are formed, and these late DSBs function in ensuring homologous pairing. Further studies will have to be performed to confirm this model, including quantification of H3K9me2-marked chromatin between the two inbreds. A study by Chia *et al*. (2012) used flow cytometry to investigate the variation in genome size between maize inbred lines. They found that the CML228 genome was 1.028 times the size of the B73 genome. Interestingly, their study further found that variation in the amount of large heterochromatic blocks of repetitive DNA were the primary source of genome size differences between inbreds. Therefore, biochemical analysis of H3K9me2-marked chromatin should be performed in both

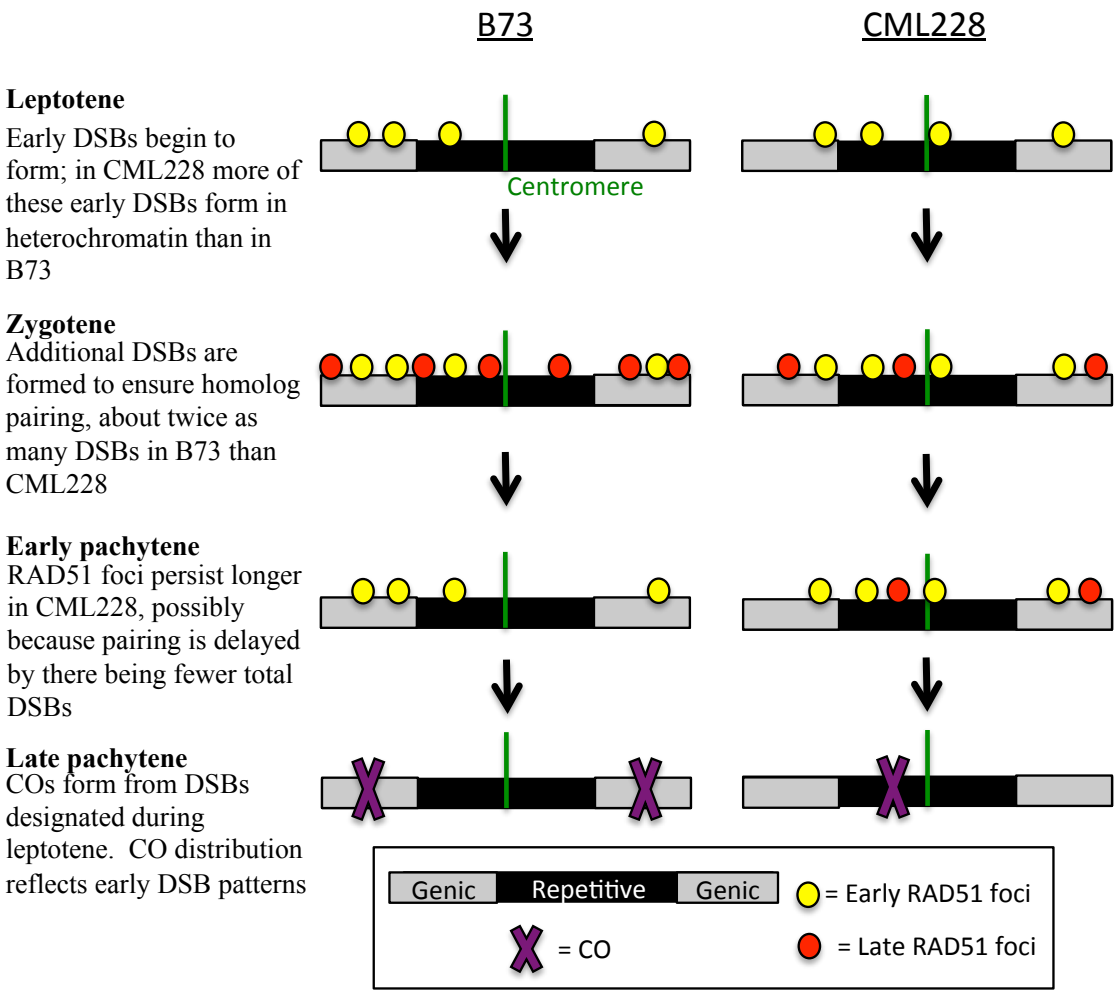

Figure 13: Model for how dynamics of early DSB repair influence CO distribution in different inbred lines.

B73 and CML228 to determine if the variation we see in heterochromatic DSB repair are due to differences in genome organization or differences in the recombination pathway.

It is also unclear from our analyses whether the early RAD51-marked DSBs during leptotene are the same as those DSBs remaining during early pachytene. In B73, the number of the earliest and latest RAD51 foci are similar to the number of COs, and it had previously been proposed that leptotene and pachytene RAD51 foci mark the future CO sites (Franklin *et al*., 1999). It could be that CO repair at the early DSB sites starts immediately after breaks are initiated, and RAD51 at these sites is simply not removed at these sites until later in the repair pathway. To address this question, RAD51 could be colocalized with an intermediate marker in the CO pathway, such as HEI10 (Wang *et al*., 2012; Chelysheva *et al*., 2012). This could then demonstrate when CO-designation occurs and how CO-designation influences RAD51 dynamics.

APPENDIX

GENERATING AN ANTIBODY AGAINST MAIZE CONDENSIN II

Materials and Methods

Plant materials

A344 plants were grown in a greenhouse and meiotic anthers were harvested when the plants were about 6 weeks old.

Phylogenetic analysis

The sequences of genes with the following IDs were used for the maize condensin subunits in the phylogeny analysis: *SMC2*- GRMZM2G006352, *SMC4*- GRMZM2G383623, *CAP-H2a*- GRMZM2G096944, *CAP-H2b*- GRMZM2G180027, *CAP-G2*- GRMZM2G051894. Multiple-sequence alignments were generated using ClustalX (Larkin *et al.*, 2007) and adjusted manually. Bayesian phylogenetic trees were constructed using Mr. Bayses 3.1.2 (Huelsenbeck and Ronquist, 2001). TreeviewPPC (Page, 1996) was used to display phylogenetic trees.

Protein extraction

Whole protein extracts were generated from A344 maize seedlings. Seedlings were frozen in liquid nitrogen and ground with 300 mL of HA buffer (5 mM EDTA, 100 mM Tris, 10% sucrose, 0.19% EGTA) and 5 μL phenylmethylsulfonyl fluoride (PMSF) protease inhibitor. The resulting slurry was centrifuged at 14,000 rpm (18,407 rcf) for 10 minutes at 4°C. A Bradford assay (Bio-Rad Protein Assay) was then performed on the resulting supernatant to determine the concentration of each extract before further use.

SDS-PAGE gels and Western blot analysis

For each protein gel, 450 μg of boiled protein extract was examined on a pre-cast 12% SDS-PAGE gel at 70 V for 10 minutes, then 100 V for 1 hour. Proteins were then transferred to a nitrocellulose membrane via standard transfer methods. Membranes were washed in 1X TBS-T and then blocked in 5% milk, 1X TBS-T, 15 mM EDTA for 1 hour with shaking. Primary antibody incubations were performed for 1 hour at room temperature, with shaking. Prebleeds were diluted 1:50 in blocking buffer; test bleeds were diluted 1:100 in blocking buffer. Secondary antibody incubations were performed for 1 hour at room temperature, with shaking, using a 1:10,000 dilution of anti-guinea pig horseradish peroxidase (HRP) antibody in blocking buffer. Signal was detected applying the SuperSignal West Femto chemiluminescent substrates to the blot, and exposing film to the membrane, and the developing the film.

Results and Discussion

In order to better understand how chromosome condensation and restructuring affect maize meiosis, I set out to develop an antibody against a maize condensin II subunit. I identified the maize condensin II subunits within the maize genome database. Phylogenetic analysis was performed on a subset of the identified maize condensin genes to confirm that they are in fact condensin orthologs (Figure 14).

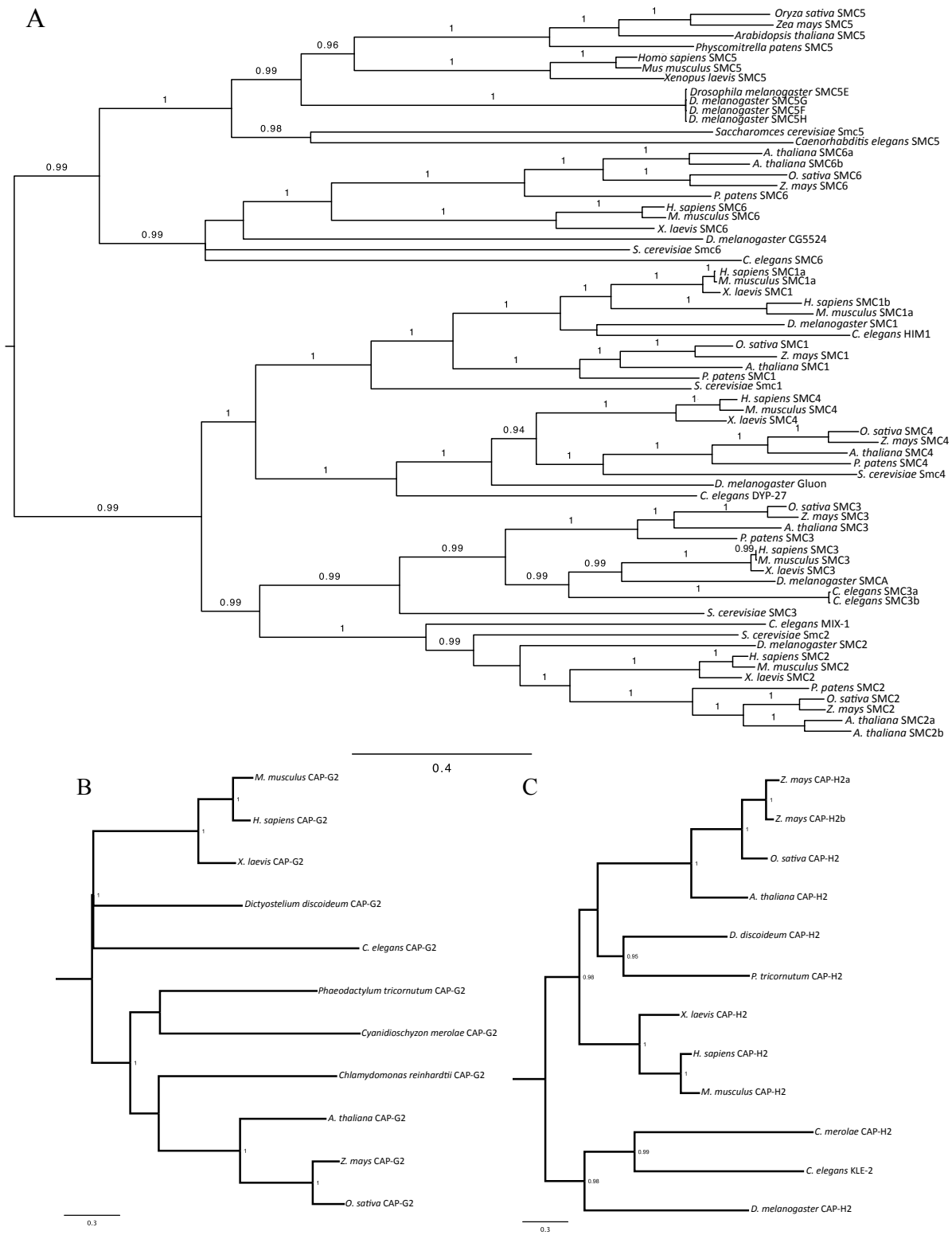

Figure 14: Bayesian phylogeny reconstruction of (A) SMC2 and SMC4, with SMC1 and -3 of cohesin, and SMC5 and -6 of the SMC5/6 complex, (B) CAP-G2, and (C) CAP-H2. Numbers next to branches represent posterior probabilities. All trees are midpoint rooted.

I then designed a peptide epitope against maize CAP-H2 (Figure 14c) based off of commercially available human anti-CAP-H2 antibodies (Abcam ab83848). A peptide antigen based off the amino acid sequence of residues 72-121 was used to generate the human anti-CAP-H2, indicating that this part of HsCAP-H2 is likely exposed to the surface and that this region could provide a good epitope on the ZmCAP-H2 protein. Using a simple BLAST search, we found this region aligned to amino acids 93-122 of maize CAP-H2 protein sequence.

This peptide sequence, found in Table 5, was used as the antigen to generate the maize CAP-H2 antibody (Cocalico Biologicals, Inc, Reamstown, PA) in a guinea pig host. Prebleeds were tested prior to animal inoculation with the peptide to ensure that there was no cross-reactivity between an antigen in the animal's immune system and the maize CAP-H2 protein (74.91 kDa). Western blot analysis with the prebleed did not detect any bands near 75 kDa in a whole seedling protein extract (Figure 15a). Animals were then inoculated with the peptide four times by Cocalico Biologicals. Bleeds after the final inoculation detected a band at 75 kDa in a Western analysis performed on a whole seedling protein extract (Figure 15b).

Table 5: Peptide sequence used to generate maize anti-CAP-H2 antibody.

	Maize CAP-H2 aa93-122
Amino acid sequence	HKKQDQQENVTAQSNQSDPSTIPSEDYIFM

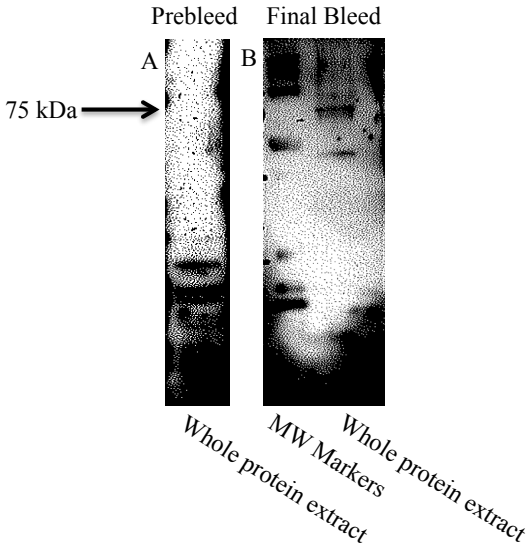

Figure 15: Western blot analysis of anti-ZmCAP-H2. A) Pre-immune sample does not detect any proteins similar in size to ZmCAP-H2 (74.91 kDa). After four inoculations with peptide antigen, the final bleed (B) does detect a protein near 75 kDa marker.

Western blot analysis indicates that the anti-CAP-H2 antibody generated recognizes the maize CAP-H2 protein. Further analyses must be performed to verify this and demonstrate the antibody's specificity. Subsequently this antibody will be a useful tool in further understanding the chromosome restructuring and its influence on meiotic recombination.

REFERENCES

Armstrong S, Osman K: Immunolocalization of meiotic proteins in *Arabidopsis thaliana*: method 2. Methods Mol Biol 990:103-107 (2013).

Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, et al: PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. Science 327:836-840 (2010).

Bernatavichute YV, Zhang X, Cokus S, Pellegrini M, Jacobsen SE: Genome-wide association of histone H3 lysine nine methylation with CHG DNA methylation in *Arabidopsis thaliana*. PLoS One 3:e3156 (2008).

Borde V, Robine N, Lin W, Bonfils S, Géli V, et al: Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. EMBO J 28:99-111 (2009).

Bretz F, Genz A, Hothorn LA: On the numerical availability of multiple comparison procedures. Biometrical J 43:645-656 (2001).

Buard J, Barthès P, Grey C, de Massy B: Distinct histone modifications define initiation and repair of meiotic recombination in the mouse. EMBO J 28:2616-1624 (2009).

Chelysheva L, Vezon D, Chambon A, Gendrot G, Pereia L, et al: The *Arabidopsis* HEI10 is a new ZMM protein related to Zip3. PLoS Genet 8:e1002799 (2012).

Chia JM, Song C, Bradbury PJ, Costich D, de Leon N, et al: Maize HapMap2 identifies extant variation from a genome in flux. Nat Genet 44:803-809 (2012).

Chiolo I, Minoda A, Colmenares SU, Polyzos A, Costes SV, et al: Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell 144:732-744 (2011).

Du J, Zhong X, Bernatavichute YV, Stroud H, Feng S, et al: Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. Cell 151:167-180 (2012).

Eichten SR, Ellis NA, Makarevitch I, Yeh CT, Gent JI, et al: Spreading of heterochromatin is limited to specific families of maize retrotransposons. PLoS Genet 8:e1003127 (2012).

Forget AL, Kowalczykowski SC: Single-molecule imaging brings Rad51 nucleoprotein filaments into focus. Trends Cell Biol 20:269-276 (2010).

Franklin AE, McElver J, Sunjevaric I, Rothstein R, Bowen B, et al: Three-dimensional microscopy of the Rad51 recombination protein during meiotic prophase. Plant Cell 11:809-824 (1999).

Gore MA, Chia JM, Elshire RJ, Sun Q, Ersoz ES, et al: A first generation haplotype map of maize. Science 326:1115-1117 (2009).

Guo, L, Yu Y, Law JA, Zhang X: SET DOMAIN GROUP2 is the major histone H3 lysine 4 tirmethyltransferease in Arabidopsis. Proc Natl Acad Sci USA 107:18557-18562 (2010).

He Y, Wang M, Dukowic-Schulze S, Tiang CL, Bradbury P, et al: The landscape of meiotic recombination in maize (Submitted).

Higgins JD, Perry RM, Barakate A, Ramsay L, Waugh R, et al: Spatiotemporal asymmetry of the meiotic program underlies the predominantly distal distribution of meiotic crossovers in barley. Plant Cell 24:4096-4109 (2012).

Huelsenbeck JP, Ronquist F: MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755 (2001).

Joshi N, Brown MS, Bishop DK, Börner GV: Gradual implementation of the meiotic recombination program via checkpoint pathways controlled by global DSB levels. Mol Cell 57:797-811 (2015).

Kauppi L, Barchi M, Lange J, Baudat F, Jasin M, et al: Numerical constraints and feedback control of double-strand breaks in mouse meiosis. Genes Dev 27:873-886 (2013).

Keeney S: Spo11 and the formation of DNA double-strand breaks in meiosis. Genome Dyn Stab 2:81-123 (2008).

Keeney S, Giroux CN, Klecker N: Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell 88:375-384 (1997).

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al: Clustal W and Clustal X version 2.0. Bioinformatics 23:2947-2948 (2007).

Lichten M, de Massy B: The impressionistic landscape of meiotic recombination. Cell 147:267-270 (2011).

Mézard C, Vignard J, Drouaud J, Mercier R: The road to crossovers: plants have their say. Trends Genet 23:91-99 (2006).

Mimitou EP, Symington LS: DNA end resection: many nucleases make light work. DNA Repair 8:983-995 (2009).

Page RDM: TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications Biosci 12: 357-358 (1996).

Pan J, Sasaki M, Kniewel R, Murakami H, Blitzblau HG: A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. Cell 144:719-731 (2011).

Pawlowski WP, Golubovskaya IN, Cande WZ: Altered nuclear distribution of recombination protein RAD51 in maize mutants suggests the involvement of RAD51 in meiotic homology recognition. Plant Cell 15:1807-1816 (2003).

Peng JC, Karpen GH: Heterochromatic genome stability requires regulators of histone H3 K9 methylation. PLoS Genet 5:e1000435 (2009).

Rodgers-Melnick E, Bradbury PJ, Elshire RJ, Glaubitz JC, Acharya CB, et al: Recombination in diverse maize is stable, predicatable, and associated with genetic load. Proc Natl Acad Sci USA 112:3823-3828 (2015).

Sandhu D, Gill KS: Gene-containing regions of wheat and the other grass genomes. Plant Physiol 128:803-811(2002).

San Filippo J, Sung P, Klein H: Mechanism of eukaryotic homologous recombination. Annu Rev Biochem 77:229-257 (2008).

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, et al: The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112-1115 (2009).

Sidhu GK, Fang C, Olson MA, Falque M, Martin O, et al: Limits to crossover homeostasis (Submitted).

Smagulova F, Gregoretti IV, Brick K, Khil P, Camerini-Otero RD, et al: Genome-wide analysis reveals novel molecular features of mouse recombination hotsopts. Nature 472:375-378 (2011).

Wang K, Wang M, Tang D, Shen Y, Miao C: The role of rice HEI10 in the formation of meiotic crossovers. PLoS Genet 8:e1002809 (2012).

West PT, Li Q, Ji L, Eichten SR, Song J, et al: Genomic distribution of H3K9me2 and DNA methylation in a maize genome. PLoS One 9:e105267 (2014).