Genetic and Epigenetic Regulation of Meiotic Homologous Recombination at Retrotransposons in Fission Yeast

Author: Peter Johansen

Boston College

The Graduate School of Arts and Sciences

Department of Biology

GENETIC AND EPIGENETIC REGULATION OF MEIOTIC HOMOLOGOUS RECOMBINATION AT RETROTRANSPOSONS IN FISSION YEAST

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PETER JOHANSEN

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Abstract

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Author: Peter Johansen

Advisor: Hugh P. Cam, Ph.D.

Meiotic homologous recombination (HR) is not uniform across eukaryotic genomes, creating regions of strong recombination activity dubbed recombination hotspots, and regions of low recombination activity dubbed coldspots. Considerable attention has led to discoveries of a host of factors controlling the formation of hotspots. However, the determinants of coldspots are not as clearly defined. I have previously shown that CENP-B homologs of the fission yeast *Schizosaccharomyces pombe* have a genome surveillance role in regulating the nuclear organization and expression of *Tf2* retrotransposons. Here, I reveal an additional role for CENP-Bs in suppressing meiotic recombination of *Tf2s*. I describe the development of a random sporulation assay to rapidly screen thousands of meiotic progeny for recombination across a locus in a variety of genetic backgrounds. Loss of any CENP-B family members (Abp1, Cbh1, Cbh2), results in increased HR at *Tf2s*. I show that Abp1, which acts as the primary determinant of HR suppression at *Tf2s*, is required to maintain proper recombination exchange of homologous alleles flanking a *Tf2*. In addition, Abp1-mediated suppression of HR at *Tf2s* requires all three of its domains with distinct functions in transcriptional repression and higher-order genome organization. I show that this suppression is likely mediated by Abp1 binding to specific motifs near the 3'end of flanking LTRs. I demonstrate that HR

suppression of *Tf2s* can be robustly maintained despite disruption to chromatin factors essential for transcriptional repression and nuclear organization of *Tf2s*. Intriguingly, I uncover a surprising cooperation between the histone methyltransferase Set1 responsible for histone H3 lysine 4 methylation and the non-homologous end joining pathway in ensuring the suppression of HR at *Tf2s*. Furthermore, I identify a role for the architectural protein condensin involved in 3D chromatin organization and chromosome condensation in restricting HR at *Tf2s*. My study identifies a molecular pathway involving functional cooperation between a transcription factor with epigenetic regulators, DNA repair pathway, and chromosome organizers to regulate meiotic recombination at interspersed repeats.

Dedication

This dissertation is dedicated to the memories of Eleanor Williams and Ann Johansen, for their eternal love and support throughout the years.

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I would like to foremost thank my advisor, Dr. Hugh Cam, for his guidance, insights, and above all else, patience in the production of this dissertation. Working with Hugh has taught me not only how to look at the world as a scientist, but also how to look at myself and see the truth in things.

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I am grateful for the support of all the members of the Cam lab, particularly my fellow grad students, David Layman, Pat Grady, and Lauren Meyer, our postdoctoral fellow Dr. David Lorenz, and our faithful technician Irina Mikheyeva.

Finally, I would not be here today without the unconditional love and support of my family, especially my mother Nancy, who has continued to encourage me all of these years, especially when I needed it most. I kept my promise to you.

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Abbreviations used

TE	Transposable element
HR	Homologous recombination
DSB	Double-stranded break
ChIP	Chromatin immunoprecipitation
H3K9	Lysine residue 9 of histone H3
H3K14	Lysine residue 14 of histone H3
H3K9me	Methylation of H3K9
H3K9me3	Trimethylation of H3K9
LTR	Long terminal repeat
H3K4	Lysine residue 4 of histone H3
ssDNA	single-stranded DNA
HJ	Holliday Junction
NDR	Nucleosome-depleted region
PMSF	Phenylmethylsulfonyl fluoride
ncRNA	non-coding RNA
cM	Centimorgan
DBD	DNA-binding domain (of Abp1)
DDE	Transposase-like domain (of Abp1)
DIM	Dimerization domain (of Abp1)
TBZ	Thiabendazole
Set1C	Set1 complex
COMPASS	Complex of proteins associated with Set1
HDAC	Histone deacetylase
NHEJ	Non-homologous end-joining
EnCODE	Encyclopedia of DNA Elements
NAHR	Non-allelic homologous recombination
RSA	Random spore analysis

Chapter 1. Introduction: The Mechanics and Regulation of Homologous Recombination

Genetic Variability and the Need to Evolve

All known forms of life on Earth, as well those bordering the living/nonliving boundary such as viruses and transposable elements (TEs), are distinguished by their abilities to withstand and accommodate alterations to their genomes. Genetic variability, the capacity for genetic change over time, is essential for populations to adapt and evolve as environments change (Frankham, 2005). For a species to survive, it must be able to maintain the integrity of its genome while permitting adaptations to arise; this pressure from both the environment and other competing organisms is constant (Skoneczna, Kaniak, & Skoneczny, 2015). Many different paths to this goal exist, from simple point mutations to major rearrangements of the genome (Nosil & Feder, 2013). Genetic material can also be transferred laterally from other organisms, particularly in the case of genes encoding for virulence factors (Gyles & Boerlin, 2014).

Meiosis as a Means to Generate Genetic Diversity

For organisms that practice sexual reproduction, genetic diversity is generated at a unique stage of their life cycle called meiosis (Green & Mason, 2013). In meiosis, a diploid progenitor cell undergoes DNA replication and cell division, but then proceeds into another round of cell division, meiosis I and II, respectively. This process reduces the diploid cell into four haploid gametes. In fission yeast, the diploid stage of life is brief, and meiosis restores the cell to its haploid state.

The process of bacterial transformation may be an ancestor of meiosis in eukaryotes, since several genes involved in meiosis are conserved in *Escheria coli*, including the single-strand binding protein RecA (H. Bernstein & Bernstein, 2010). These processes are similar in that they both lead to DNA repair (Michod, Bernstein, & Nedelcu, 2008). Meiosis, however, confers several additional advantages, particularly an increased rate of adaptation to environmental changes (H. Bernstein, Bernstein, & Michod, 2011; Goddard, Godfray, & Burt, 2005). Meiosis is often facultative among protists and unicellular eukaryotes, occurring in response to stress or starvation as a response to endure these conditions (H. Bernstein et al., 2011). Most prominently, the process of homologous recombination (HR) during meiosis is a powerful tool for genetic variability by creating new combinations of alleles (Goddard et al., 2005).

History of Homologous Recombination

The process of meiosis gives rise to haploid gametes whose chromosomes are selected by random segregation from the diploid parent, producing cells with different genomes than the starting diploid genome. The classic second law of independent assortment from the early geneticist Gregor Mendel states that all traits are inherited independently of one another. Mendel's work in pea plants described a diploid sexual system that correctly predicted the behavior of chromosomes during meiosis, despite never having knowledge of what chromosomes or DNA were. However, not all traits segregate randomly; many traits are inherited together at rates higher than predicted by chance.

In 1911, the work of Thomas Hunt Morgan with the fruit fly *Drosophila melanogaster* demonstrated that certain traits are inherited together at rates much greater than would be predicted by chance alone, and that these rates of co-inheritance varied between pairs of genes. This suggested a method by which traits can be inherited together; the traits observed by Mendel likely reside on certain physical identities within the cell, which were later discovered to be chromosomes. Morgan's work led to the concept of genetic linkage, and that the probability of trait co-inheritance was directly proportional to the physical distances of the two traits upon the same chromosome. Morgan first proposed that traits must be able to "cross over" between two paired chromosomes in order to exchange information (Lobo & Shaw, 2008); this hypothesis was later proven by physical observation of chromosomal crossover by Barbara McClintock in 1931 (Creighton & McClintock, 1931). Alfred Sturtevant, a student of Morgan, used this hypothesis to create the first genetic maps, based upon rates of recombination inferred among hundreds of crosses of *Drosophila melanogaster* parents heterozygous for certain traits, decades before genomic sequencing was possible. Sturtevant's work also demonstrated the concept of crossover interference, in which a crossover at one locus can interfere with the creation of other crossover events at nearby loci (Sturtevant, 1913). This further supports the idea that the interaction between co-inherited traits is a physical one; co-inherited traits are physically connected on certain physical structures called chromosomes.

Models and Mechanisms of Homologous Recombination

The double helix structure of DNA discovered by James Watson and Francis Crick in 1953 provided a physical basis for how genetic information may be exchanged in the context of HR, as information from one strand could be "donated" to the other strand, overwriting the existing bases. The first model designed to explain the mechanism of crossing over during meiotic HR was proposed by Robin Holliday in 1964. The Holliday model is composed of two highly-coordinated single-stranded nicks in the DNA, followed by an exchange of strands between homologs. This process creates a heteroduplex of DNA linked together by a chiasmata, or Holliday junction (Figure 1); the junction then migrates to lengthen the heteroduplex region. Resolution of this junction between strands results in either conversion or restoration of the heteroduplex, resulting in the exchange of either a short interval of DNA (gene conversion) or the translocation of an entire chromosome arm (chromosome crossover) (Haber, Ira, Malkova, & Sugawara, 2004). However, as no mechanism to create such coordinated nicks exists, this model has been revised over the years. Instead, Jack Szostak proposed a model based upon the creation of a double-stranded break (DSB) within the DNA that would allow invasion of the homologous strand to facilitate genetic exchange (Szostak, Orr-Weaver, Rothstein, & Stahl, 1983). This model is currently favored in the context of meiosis over other pathways, as it is the most likely to result in a chromosome crossover.

During HR, a complex of three proteins, Mre11 (Rad23 ortholog in fission yeast), Rad50, and Nbs1 (MRN in fission yeast) seeks out DSBs and binds to begin processing

the break for repair (Lamarche, Orazio, & Weitzman, 2010). Helicases unwind the broken double helix, and exonucleases such as Exo1 digest the 5' ends of the DNA to expose a free 3' end (Mimitou & Symington, 2009). The highly-conversed protein Rad51 binds to these 3' ends of DNA to both protect the strand from degradation and to mediate invasion of the partner double helix in search of a sequence of homology (Shinohara, Ogawa, & Ogawa, 1992). Upon binding with a homologous sequence, the native DNA strain is displaced, forming a characteristic displacement loop. DNA polymerase extends the invading 3' end, creating a heteroduplex formed by the donor strand and the homologous template. Likewise, the displacement loop forms a template to repair the original site of the DSB, and any degraded bases are similarly repaired by DNA polymerase. The extension of the damaged strands produces two Holliday junctions, each located just downstream of the newly synthesized DNA where it rejoins its original partner. Nicking endonucleases resolve these junctions, producing either gene conversion or recombinant chromosomes (Blat, Protacio, Hunter, & Kleckner, 2002). (Figure 2)

Meiotic Homologous Recombination Initiation and Hotspots

Unlike DSBs caused by DNA damage, DSBs associated with meiotic HR are deliberately induced by cellular machinery. During prophase I, homologous chromosomes are aligned with one another in a process called synapsis, during which cytoskeletal proteins tether homologous chromosomes to the nuclear envelope and facilitate proper matching of homologous sequences (Scherthan, Bahler, & Kohli, 1994). Chromatin is organized into a structure of loops tethered to a proteineous axis (Borde &

de Massy, 2013; Keeney, Lange, & Mohibullah, 2014). The cell then initiates pre-programmed DSBs across the genome within these loops of chromatin, facilitated by the highly-conserved protein Spo11, which then brings the ends of the DSB to the axis for HR resolution (Cervantes, Farah, & Smith, 2000; Cromie et al., 2007). (Figure 3) The attachment of homologous chromosomes by chiasmata during meiosis I is necessary not only to generate new combinations of alleles within progeny, but also to ensure correct segregation of chromosomes. Failure of cells to undergo HR in meiosis results in high levels of chromosomal nondisjunction, leading to severely aneuploid, inviable gametes (Petronczki, Siomos, & Nasmyth, 2003).

However, genetic distance as determined by rates of HR does not correlate perfectly with physical distance on a chromosome. Physical maps of genomes revealed that the predicted distances between genes were often different than actual physical distances in base pairs, indicating that HR is not randomly distributed across chromosomes (Pan et al., 2011; Petes, 2001; Wahls, 1998). In the fission yeast *Schizosaccharomyces pombe*, the double stranded breaks necessary for initiation of HR are catalyzed by the protein Rec12, a homolog of the human Spo11 (Cervantes et al., 2000). Mapping of Rec12 binding by chromatin immunoprecipitation (ChIP) reveals a distinct pattern of preferential binding sites dubbed "hotspots" that correlate with high levels of meiotic HR, mostly located within intergenic regions (Cromie et al., 2007). Likewise, areas with low Rec12 activity have correspondingly low rates of HR. This pattern of uneven Rec12 binding creates regions of increased or decreased genetic linkage between loci, and suggests that meiotic HR is both non-random and highly

regulated. While a high rate of HR correlates with strong Spo11/Rec12 binding, the converse is not true; not all Rec12 peaks correlate with high HR (Fowler, Sasaki, Milman, Keeney, & Smith, 2014).

Chromatin, Epigenetics, and Regulation of Meiotic Homologous Recombination

Since no consensus binding sequence for Spo11 has been discovered, research has instead turned to epigenetics to understand what governs Spo11 hotspot formation. The organization of DNA into compact chromatin, where DNA is tightly wound around an octamer of histone proteins, offers many means in which biochemical functions can be regulated independent of DNA sequences. Modifications of histone tails that protrude from the core proteins have many functions that can greatly affect chromatin organization and gene function (Bannister & Kouzarides, 2011; Luger, Dechassa, & Tremethick, 2012; Murakami, 2013). One of the most well known of these functions is chromatin compaction. In fission yeast, hypoacetylation of the ninth and fourteenth lysine residues of histone H3 (H3K9 and H3K14) results in less accessibility to underlying DNA sequences, promoting a more restrictive state of chromatin that inhibits binding of RNA polymerase (Litt, Simpson, Recillas-Targa, Prioleau, & Felsenfeld, 2001; Noma, Allis, & Grewal, 2001). Similarly, methylation of H3K9 (H3K9me), which provides binding sites for Swi6, ortholog of human HP1, establishes heterochromatin over chromatin domains such as pericentromeres and subtelomeres. (Nakagawa et al., 2002).

The epigenetic factors and mechanisms that regulate hotspot formation have been highly studied, and vary among different organisms (Brachet, Sommermeyer, & Borde, 2012; Wahls & Davidson, 2012). In mice, Spo11 binding peaks correspond strongly to activity of PRDM9, a meiosis-specific histone methyltransferase that adds a trimethylation mark to the fourth lysine residue of histone H3 (H3K4me3) (Baudat, Imai, & de Massy, 2013; Grey et al., 2011). However, PRDM9 is not essential for all instances of HR. Deletion of PRDM9 does not abolish all HR, but it does deregulate the pattern of Spo11 hotspots(Borde & de Massy, 2013). Likewise, in *S. pombe* deletion of *set1*, which encodes the sole H3K4 methyltransferase, causes genome-wide disruption of Rec12 binding, but does not abolish HR (S. Yamada, Ohta, & Yamada, 2013). Other methods of HR regulation exist both before and after DSB induction, such as the binding of condensins to chromosomes (Li, Jin, & Yu, 2014; Mets & Meyer, 2009), epigenetic modifications of other histone sites (Hirota, Mizuno, Shibata, & Ohta, 2007; Pai et al., 2014), DNA methylation (Wallberg, Glémin, & Webster, 2015), and chromatin remodeling factors (T. Yamada et al., 2004). Openness of chromatin as visualized by DNAse I sensitivity assays correlates with increased DSB frequency (Pan et al., 2011), but it not sufficient in and of itself to promote DSBs (Borde & de Massy, 2013). Likewise, nucleosome-depleted regions near gene promoters are DSB-rich, but again, nucleosome depletion is not the only required factor. Transcription factors, such as Atf1-Pcr1, associated with the well-known *ade6-M26* hotspot in *S. pombe*, can also influence HR (Kon, Krawchuk, Warren, Smith, & Wahls, 1997). Regulation of meiotic HR hotspots is therefore highly multifaceted.

Regulation of Rec12

Current models for Rec12 binding suggest that Rec12 and its associated proteins are stabilized by a heterotrimer of Rec25, Rec27, and Mug20 as part of the linear element complex in *S. pombe* that supplants the synaptonemal complex in other eukaryotes (Fowler, Gutiérrez-Velasco, Martín-Castellanos, & Smith, 2013). A more recent survey of Rec12 binding by high-throughput sequencing of Rec12-bound oligos (ChIP-seq) has shown that binding of Rec12 is more ubiquitous that originally thought, and that crossover within Rec12 hotspots favor inter-sister chromatid repair of double stranded breaks rather than inter-chromosome homolog. Fowler et al. described this phenomenon as "crossover invariance" (Fowler et al., 2014). Likewise, as Sturtevant's work predicted, crossovers exhibit a homeostatic effect on neighboring loci, with a crossover at one locus inhibiting crossovers within several kilobases in either direction (Phadnis, Hyppa, & Smith, 2011). This mechanism favors even distribution of crossovers rather than clustering, most likely to ensure that sufficient chiasmata are created to ensure proper chromosome segregation.

While the mechanics of hotspot formation have been well studied, less is known about the maintenance of areas of low HR. Generally, areas of highly condensed chromatin have been known to be very poor for recombination, most noticeable the centromeres and telomeres (Ludin et al., 2008). For example, Clr4, an *S. pombe* H3K9 methyltransferase, inhibits HR within the centromere (Ellermeier et al., 2010). Among eukaryotic genomes, transposable elements are likewise rich in repetitive sequences,

tightly compacted, and poor in HR events (Ben-Aroya, Mieczkowski, Petes, & Kupiec, 2004; Vader et al., 2011). These elements within the fission yeast genome will be the focus of this study.

Transposable Elements

Transposable elements (TE) are sequences of DNA that may duplicate themselves and mobilize within the genome. Originally discovered by Barbara McClintock in 1953 (McClintock, 1953), TEs fall into two broad categories: DNA transposons, which mobilize by excision of their sequences, and retrotransposons, which create duplicates of themselves via an RNA intermediate. TEs are considered a non-coding DNA portion of the genome, though they may encode genes necessary for their own mobilization, including reverse transcriptase in the case of retrotransposons (Bowen, 2003). The structures of TE genes have high similarity to those of viruses, suggesting that they share a common ancestor, and may be integrated viral genomes that have since become domesticated or rendered inert (Hoff, Levin, & Boeke, 1998).

Since the insertion of a TE into the genome is likely to be mutagenic (Belancio, Hedges, & Deininger, 2008; Tubio et al., 2014), cells have evolved several defenses against these processes. Expression of TE genes is highly regulated epigenetically, and multiple mechanisms exist to silence their expression and prevent mobilization, including chromatin compaction, DNA methylation, genomic imprinting, and RNAi. (Cam et al., 2005; Habibi, Pedram, AmirPhirozy, & Bonyadi, 2015; Hansen et al., 2005). These

methods vary across species, but typically involve epigenetic modifications that promote a more closed chromatin structure to inhibit transcription of TEs (Bucher, Reinders, & Mirouze, 2012; Kim & Workman, 2010; Reichmann et al., 2012). The compact state of chromatin has been implicated in the suppression of meiotic HR hotspots (Ben-Aroya et al., 2004), but again, the compaction of chromatin around a TE is not sufficient to completely exclude HR (Sasaki, Tischfield, van Overbeek, & Keeney, 2013).

Despite the necessity of keeping TEs repressed, TEs have continued to spread throughout the genomes of many, but not all eukaryotes; the genome of *Zea mays* is approximately 90% TEs (SanMiguel et al., 1996). Instead of being excised by selective pressure, TEs may instead become domesticated, and play a role in the regulation of gene expression (Bucher et al., 2012; Cowley & Oakey, 2013; Nowacki et al., 2009). TEs and their associated repeats have also been implicated as being fragile sites within the genome, showing a high probability to result in structural variants and genomic rearrangements when HR is initiated in or nearby (Campbell et al., 2014; Hoang et al., 2010; Li et al., 2014; Vader et al., 2011). Such structural variant mutations are implicated in many human diseases, including chronic leukemia (Lopez, Baumann, & Costa, 2011; Petronczki et al., 2003). Likewise, DNA repair associated with meiotic HR is inherently mutagenic (Rattray, Santoyo, Shafer, & Strathern, 2015). It is likely that cells have evolved mechanisms to prevent such damaging events from occurring by regulating HR within repetitive DNA.

Again, despite being implicated in genomic instability, TEs constitute a significant fraction of large genomes including that of human. To understand how HR may interact with TEs and how cells may regulate this process, we have used fission yeast as our model organism.

Fission Yeast as a Model Organism

The fission yeast *Schizosaccharomyces pombe* is a unicellular fungi of the ascomycota phylum, with a genome consisting of approximately 5,400 genes among 12.57 megabases in three chromosomes, excluding rDNA (Rhind et al., 2011; Wood et al., 2002). Fission yeast is easily grown in the laboratory and is highly amenable to genetic manipulation by transformation with a suitable PCR product (Bahler 1998), making it a popular choice as a model organism.

Works from many laboratories over the years have revealed many similarities between *S. pombe* and mammals (Rhind et al., 2011; Lee & Nurse, 1987). Some of these similarities include cell cycle control, RNA splicing, gene regulation, and HR (Moreno, Klar, & Nurse, 1991; Zhao & Lieberman, 1995). *S. pombe* also contains a high degree of functional similarity to humans in heterochromatin structure, particularly around the large (40-100 kb) centromeres that closely resemble the organization of centromere sequences found in humans(Kinola et al., 2001; Mizuguchi, Barrowman, & Grewal, 2015).

While normally a haploid organism, *S. pombe* is capable of undergoing meiosis under controlled conditions. Upon starvation of nitrogen, cells of opposite mating types undergo fusion, creating a transitory diploid prior to the initiation of meiosis (Smith, 2009). A temperature-sensitive allele of the meiotic repressor gene *pat1* can further be used to synchronize cells at low temperatures to create a highly synchronous meiosis suitable for the study of meiotic recombination intermediates (Hyppa, Cromie, & Smith, 2008; Iino & Yamamoto, 1985; Smith, 2009).

TEs are present within the *S. pombe* genome, most notably the *Tf2* family of retrotransposons. *Tf2* retrotransposons possess similarities to certain classes of retroviruses, encoding genes for viral *gag*-like protein, integrase and reverse transcriptase, and long terminal repeats (LTR) flanking either end of its 4.5 kb sequence (Cam, Noma, Ebina, Levin, & Grewal, 2008; Levin, Weaver, & Boeke, 1990). 13 full length *Tf2* sequences are present within the *S. pombe* genome, along with 35 *Tf2* LTRs and over 100 related LTRs that are believed to be remnants of prior transposition events (Bowen, 2003; Cam et al., 2005). (Figure 4) Most of the 13 full-length TEs are still active, and capable of transposition (Bowen, 2003).

The CENP-B Homologs in *S. pombe*

S. pombe possesses a family of three proteins, Abp1, Cbh1, and Cbh2, that have high homology to the human centromere-binding protein B (CENP-B), and are believed to have been derived from the *pogo* family of transposases (Baum & Clarke, 2000;

Casola, Hucks, & Feshotte, 2007; Halverson, Baum, Stryker, Carbon, & Clarke, 1997; Irelan, Gutkin, & Clarke, 2001; Mateo & Gonzalez, 2014). These three proteins have a high affinity for repetitive DNA, most notably retrotransposon LTRs (Cam et al., 2008). The *S. pombe* CENP-B homologs have partially redundant roles in both maintenance of centromeric heterochromatin (Nakagawa et al., 2002) as well as regulation of the *Tf2* family of retrotransposons (Cam et al., 2008). Loss of *abp1*, the most abundant of the CENP-B homologs, results in a characteristic slow-growth phenotype, aberrant cell morphology, and decreased loading of Swi6, the *S. pombe* homolog of human heterochromatin protein HP1, at pericentromeric heterochromatin (Halverson et al., 1997; Nakagawa et al., 2002). Deletion of *cbh1* likewise reduces Swi6 loading at centromeres, but does not result in deficient growth; however, a double deletion mutant of both *abp1* and *cbh1* has a much more severe phenotype (Nakagawa et al., 2002). Abp1 recruits histone deacetylases Clr3 and Clr6 to their regions (Cam et al., 2008). Loss of either *abp1* or *cbh1* confers sensitivity to the microtubule-destabilizing drug thiabendazole (Irelan et al., 2001). Minichromosome experiments in CENP-B knockout mutants have further confirmed that these proteins play a role in proper chromosome segregation (Baum & Clarke, 2000).

Abp1 and Cbh1 are both highly enriched at *Tf2* retrotransposons as well as solo LTRs across the fission yeast genome (Cam et al., 2008). Abp1 in particular has been shown to inhibit the expression of *Tf2*s; with Cbh1 and Cbh2 acting in a supporting role (Cam et al., 2008). Silencing of *Tf2* expression occurs in part by the recruitment of Clr3

and Clr6, and cooperation with Set1, the sole *S. pombe* H3K4 methyltransferase (Lorenz et al., 2012). Together, these proteins inhibit the transcription of retrotransposon genes.

The *S. pombe* CENP-B homologs also contribute to higher-order structures of chromatin. Visualization of *Tf2s* by fluorescent in situ hybridization (FISH) reveals that these sequences cluster together at the nuclear periphery in "Tf bodies" (Cam et al., 2008). Tf body clustering is mediated by Abp1, Cbh1, and Cbh2 (Cam et al., 2008), as well as Set1 (Lorenz et al., 2012), while association between Tf bodies and centromeres is accomplished by the Ku heterodimer and condensin, which are in turn recruited to Tf bodies by Abp1 (Tanaka et al., 2012). However, Tf body clustering is not required for *Tf2* silencing. Deletion of the dimerization domain of *abp1* has no effect on *Tf2* repression, but causes Tf body declustering (Cam et al., 2008; Lorenz et al., 2012). Conversely, deletion of an RNA-recognition motif RRM2 from *set1* increases *Tf2* expression with a relatively minor effect on declustering (Mikheyeva, Grady, Tamburini, Lorenz, & Cam, 2014). Thus, these two methods of regulation, Tf body clustering and *Tf2* silencing, could be decoupled from one another.

The loci bound by the CENP-B homologs in *S. pombe* are primarily repetitive DNA (Cam et al., 2008; Irelan et al., 2001). Rec12 binding as visualized by ChIP shows little to no activity within *Tf2*s, but it is currently unclear if this is indicative of an HR coldspot. Additionally, *abp1* deletion mutants show impaired recombination intermediates at collapsed replication forks at LTRs during DNA replication (Zaratiegui

et al., 2011). These factors suggest that the CENP-B homologs, particularly Abp1, may play a role in repressing meiotic HR in and around *Tf2*s.

In this thesis, I describe the development of an assay to measure the rate of meiotic HR across various loci. Using this assay, I investigate the rate of HR across *Tf2* retrotransposons, a Rec12 hotspot associated with a long noncoding RNA (lncRNA), and a locus with little activity of Rec12 in both wildtype and CENP-B deletion backgrounds. I then further use this assay to decipher the molecular mechanisms that govern HR at *Tf2*s. In the first section of the results (chapter 2), I describe the development of methods used to obtain meiotic progeny and screen for HR at the loci of interest. In the second part (chapter 3), I describe the results of my recombination assay among the various CENP-B deletion mutants and characterize a selection of isolated recombinant progeny for how loss of *abp1* alters HR between *Tf2* alleles. In chapter 4, I conclude by exploring possible contributions of various chromatin-modifying enzymes and chromosome organizers to CENP-B mediated regulation of HR at *Tf2* retrotransposons, leading to a possible mechanism of how CENP-B regulation affects meiotic HR at *Tf2*s.

Figures

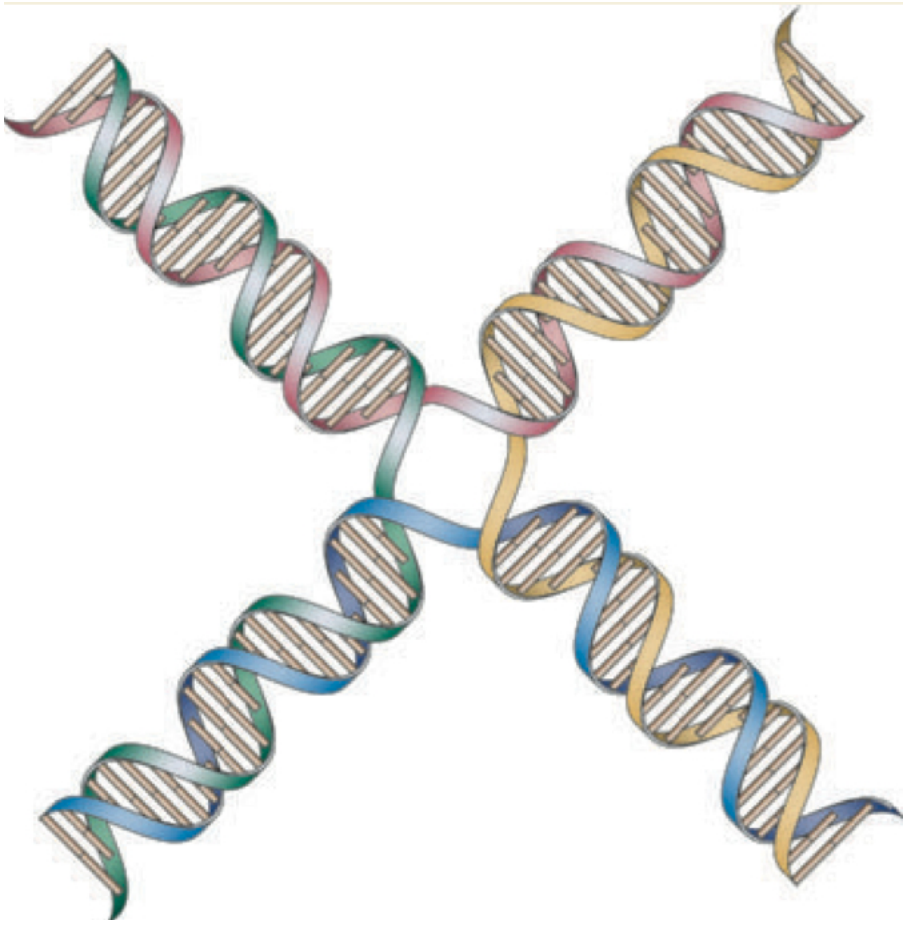

Figure 1: A ribbon diagram of the four-fold unstacked Holliday Junction. Note the exchange of double helix partners between each arm of the junction. (Reprinted by permission from Macmillan Publishers Ltd: Nature Cell Biology (Reynolds), copyright 2004)

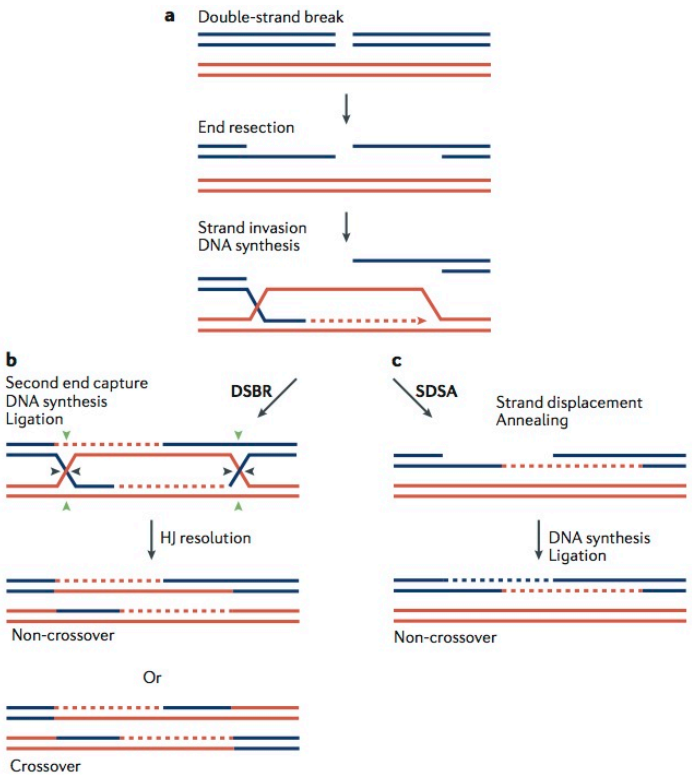

Figure 2: Double-strand breaks (DSBs) can be repaired by several homologous recombination (HR)-mediated pathways, including double-strand break repair (DSBR) and synthesis-dependent strand annealing (SDSA). A) In both pathways, repair is initiated by resection of a DSB to provide 3' single-stranded DNA (ssDNA) overhangs. Strand invasion by these 3' ssDNA overhangs into a homologous sequence is followed by DNA synthesis at the invading end. B) After strand invasion and synthesis, the second DSB end can be captured to form an intermediate with two Holliday junctions (HJs). After gap-repair DNA synthesis and ligation, the structure is resolved at the HJs in a non-crossover (black arrow heads at both HJs) or crossover mode (green arrow heads at one HJ and black arrow heads at the other HJ). (Reprinted by permission from Macmillan Publishers Ltd: Nature Cell Biology (Sung & Klein), copyright 2006)

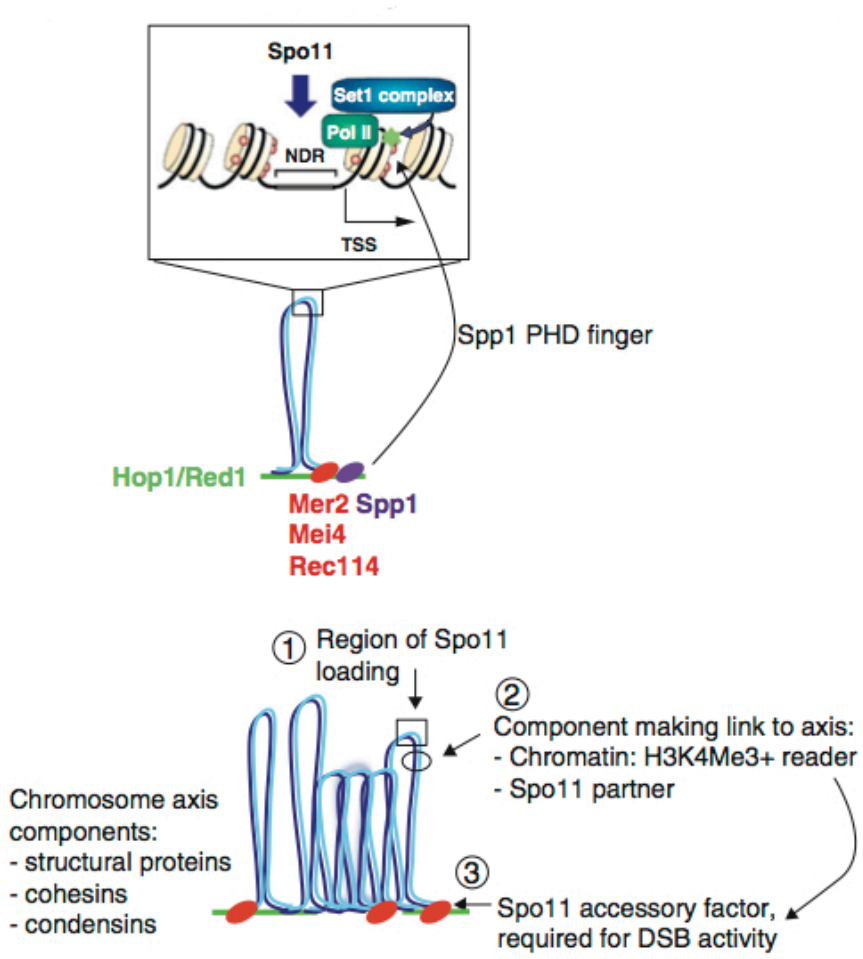

Figure 3: Linking DSB formation and axis-associated DSB repair. In *S. cerevisiae*, Spo11 binds to accessible region of chromatin, often the NDR (nucleosome-depleted regions) at promoters. The Set1 complex is recruited to transcribed genes by interaction with RNA Pol II and promotes H3K4me3 on their first nucleosome (green star, other chromatin modifications shown as orange spheres). Spp1 tethers these regions to the axis through its PHD finger and its interaction with Mer2. DSB formation involves three major steps: first, Spo11 is loaded to DNA either at accessible chromatin regions and/or through recruitment via other partners; second, DSB sites are tethered to the chromosome axis by an axis component that interacts with features linked to DSB sites (chromatin) or

with proteins bound to DSB sites; third, Spo11 is inducing DSB formation. (Reprinted from Current Opinion in Genetics & Development, Vol 23(2), Valerie Borde and Bernard de Massy, Programmed induction of DNA double strand breaks during meiosis: setting up communication between DNA and the chromosome structure, Page 152, with permission from Elsevier.)

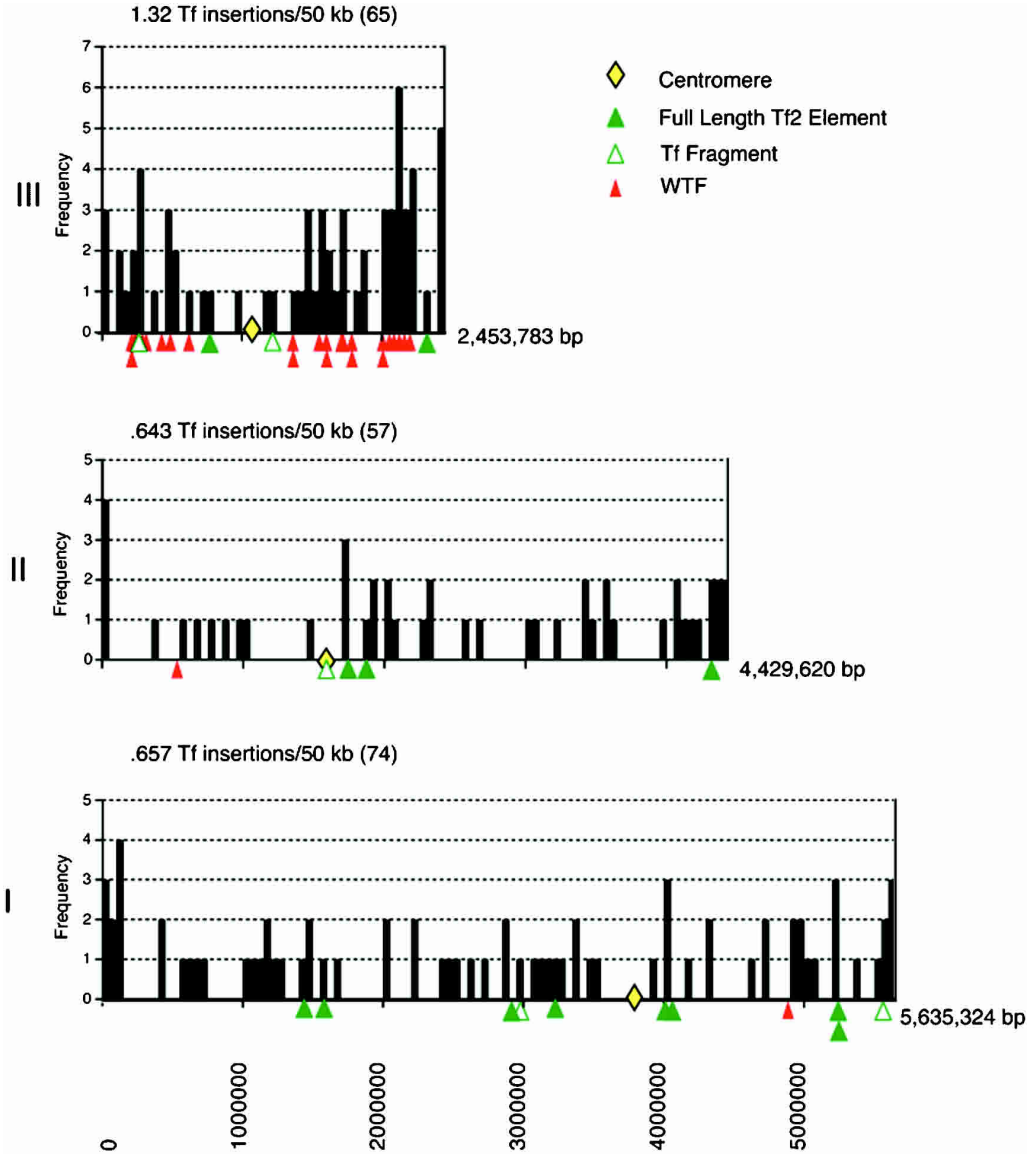

Figure 4: Chromosomal histograms indicating the number of *Tf* LTRs found in bins of 50-kb intervals along the chromosomal arms. The locations of the full-length *Tf2* elements, *Tf* fragments, *wtfs*, and centromeres are also indicated on the axis of each chromosome. The length of each chromosome is shown to the right of the histograms. (taken from Bowen *et al*., 2003. Image licensed under Creative Commons Attribution-NonCommercial 4.0 International License (CC-BY-NC) http://creativecommons.org/licenses/by-nc/4.0/)

Chapter 2. Materials and Methods

Strain construction

Gene deletion or marker insertion for meiotic recombination assay was constructed using heterologous modules as previously described (Bahler, 1998). Double mutants were generated by standard genetic crosses (Moreno, Klar, & Nurse, 1991). Condensin *cut14-206* mutant was obtained from the YGRC center(ATCC). Abp1 domain deletion mutants were constructed as previously described (Lorenz et al., 2012). The *stm1*Δ*::kanMX* and *his7*Δ*::lys2* alleles, as well as strain CHP1053 were provided by Charles Hoffman. All strains were maintained on YEA rich media. The full strain list used in this study can be founded in Table 2.

Meiotic recombination assay

For *Tf2-12* recombination assay, two parental strains were constructed with each containing either a *ura4*$^{+}$ or *kanMX6* cassette upstream or downstream of *Tf2-12*, respectively. For *Tf2-7/8*, a *his7*$^{+}$ or *ura4*$^{+}$ was inserted upstream of *Tf2-7* or downstream of *Tf2-8*, respectively. For the lncRNA *spncRNA111*, a *cloNAT* or *his7*$^{+}$ cassette was inserted upstream or downstream of *spncRNA111*. For the *lys7* locus, a *kanMX6* cassette was inserted within the stm1 gene, 14kb downstream of a *lys7*Δ. Parental strains were crossed on malt extract media and treated with 0.2% glusulase (Perkin-Elmer) overnight at room temperature with gentle agitation. Spores were washed and viewed under a hemocytometer prior to plating on selective media [PGM-Ura-His (*Tf2-7/8*) or PGM-Ura+G418 (*Tf2-12*)]. Approximately 2000-4000 spores were spread to each plate used. A 1:50 dilution of spores was spread onto YEA media to verify total viable spore count.

After 5-7 days incubation at 30°C, colonies growing on selective media were counted. A random sample of 25 colonies were re-cultured on minimal media and grown at 30°C for 3 days to screen for the presence of diploids by light microscopy. Rates of diploid progeny were subtracted from the total putative recombinant colony count. All trails were done in duplicate.

Inverse PCR

Genomic DNA was extracted from putative recombinant colonies with phenol and chloroform as previously described (Mastro & Forsburg, 2014). Genomic DNA was cut with the restriction enzyme NcoI (Thermo), diluted 100-fold and ligated with T4 DNA ligase (Thermo). Ligated products were used to test for putative recombinant strains by PCR using primers positioned inside *his7* and *ura4* reporter genes. A positive control using primers closer to the NcoI cut sites but laid outside of the inserted marker genes verified that circularized ligated constructs were recovered in all cases. A negative control using diploid colonies containing marker genes on separate chromosomes recovered successfully ligated products (using control primers) but no PCR products for the marker genes, verifying that only intramolecular ligation products were present.

Linkage test

Putative *Tf2-7/8* recombinants (*his7*$^{+}$ *ura4*$^{+}$) that failed the inverse PCR assay were crossed with a *his7*Δ*::lys2 ura4*$^{-}$ strain on malt extract, and then treated with

glusulase as above. Approximately 500 spores were plated on YEA media for germination, followed by replica plating to media lacking histidine or uracil to screen for recombinant progeny. Rates of linkage were calculated based upon the number of recombinant colonies {*his7*$^{+}$*/ura4*$^{-}$ or *his7*$^{-}$*/ ura4*$^{+}$} divided by the total screened colonies. Any *abp1* deletions were removed by outcrossing prior to testing for linkage.

Thiabendazole sensitivity assay

Wildtype, *abp1* null and *abp1* domain deletion mutants were cultured in liquid YEA media at 30°C to mid-log phase (~0.5 x 10^7 cells/ml), as detected by an optical density of 0.2 read via spectrophotometer. Cultures were then diluted serially diluted five-fold, and 5 µl was spotted onto plates of either rich YEA media alone or YEA containing 10 µg/ml thiabendazole.

DNA-binding assay

Protein extracts were obtained from *S. pombe* cells (OD_{595}, ~1 to 2), resuspended in HCS buffer (150 mM HEPES, pH 7.2, 250 mM NaCl, 0.1% NP-40, 20 mM each NaF and BGP, 1 mM each EDTA, dithiothreitol, and phenylmethylsulfonyl fluoride [PMSF], and a protein inhibitor tablet [Roche]), and lysed by acid-washed beads in a bead beater (three times for 30 s with a 2-min interval on ice). Protein extracts (0.2 to 1 mg) containing Abp1-FLAG were incubated with ~10 ng of biotinylated LTR fragments prebound to streptavidin beads for 1 h at 4°C. Beads were extensively washed with HCS

buffer and subjected to poly- acrylamide gel electrophoresis (NUPAGE Novex 10% BT; Invitrogen) and Western blot analyses (iBlot; Invitrogen) with anti-FLAG (M2, Sigma) antibodies.

Chapter 3. CENP-B Homologs Regulate Meiotic Homologous Recombination at Retrotransposons

CENP-Bs suppress meiotic HR at solo and tandem *Tf2* retrotransposons

Previous mapping reveals little enrichment of Rec12 at single and tandem *Tf2* elements (Figure 5 and Figure 6) (Cromie et al., 2007; S. Yamada et al., 2013), suggesting that *Tf2s* may be coldspots for meiotic homologous recombination. To detect rare meiotic recombinants at a *Tf2* element, we constructed two parental strains with each containing either a *ura4*$^{+}$ or *kanMX6* gene cassette positioned either upstream or downstream of an active *Tf2-12* element, respectively (Figure 7). We performed a random spore analysis (RSA) assay (Mastro & Forsburg, 2014) of the meiotic products from a cross of the two parents. Surprisingly, the rate of recombination at *Tf2-12* varied from ~0.5-1%, well-within the range of a typical locus (Young, Schreckhise, Steiner, & Smith, 2002). This result indicates that compared to known meiotic HR coldspots such as centromeres (Ellermeier et al., 2010), *Tf2-12* behaves as a neutral spot. We compare the rate of HR at *Tf2-12* with two other loci, *ste11*-associated long ncRNA *SPNCRNA111* that contains a very strong Rec12 peak and *lys7* which has no detectable Rec12 peak (Figure 8 and Figure 9). The rate of HR at *lys7* (0.16% per kb) is comparable to that of *Tf2-12* (0.19% per kb) and two fold less than that of *SPNCRNA111* (0.36% per kb) (Table 1). We next investigated the effects of CENP-B mutations on *Tf2-12* recombination. Loss of *abp1* resulted in 5-fold increase in recombination (Figure 10). This appears to be specific to Abp1-associated *Tf2s* as loss of *abp1* has no significant effect on HR rates at *lys7* or *spncRNA111* (Figures 11-14). Cells lacking either *cbh1* or *cbh2* exhibited a slight increase in recombination, consistent with their auxiliary regulatory roles of *Tf2s* (Cam *et al*., 2008). Chromosome missegration during meiosis could give rise to viable aneuploid spores that could be mistaken for recombinant

progeny. In particular, fission yeast cells with disomy of chromosome III which contains *Tf2-12* are viable. We confirmed by PCR-genotyping that all (16/16) of recombinant progeny had both the *ura4* and *kanMX6* cassette at the expected location (Figure 15).

Whereas most *Tf2s* are spaced apart on the chromosomes, *Tf2-7* and *Tf2-8* exist as tandem retrotransposons. We asked whether the tandem arrangement of retroelements is regulated differently than a solo retroelement. We employed a strategy similar to *Tf2-12* by monitoring the rate of recombination between two reporter genes ($his7^+$ and $ura4^+$) flanking *Tf2-7/8* (Figure 16). The rate of recombination varied ~1-2% (Table 1), a rate that is slightly higher than those at *Tf2-12*, likely due to the larger distance between the reporter genes (~11.5 kb) flanking *Tf2-7/8*. Loss of CENP-Bs resulted in increases of recombination at *Tf2-7/8* similar to those observed at *Tf2-12*, with ~3.5 fold for *abp1*Δ and ~1.5 fold for either *cbh1*Δ or *cbh2*Δ (Figure 17).

To verify that recovered progeny containing both selectable markers were true recombinants, inverse PCR was used to verify the relative position of both markers over a relatively long span (~11.5 kb). We confirmed that the majority (70%) of scored recombinant progeny carry the expected flanking markers (Figure 18). Collectively, our results revealed a characterized role for CENP-Bs in regulating HR at solo and tandem *Tf2* retrotransposons.

Abp1 prevents gene conversion and contributes to the faithful recombination of *Tf2*-associated homologous alleles

HR could result in equal exchange of homologous alleles or gene conversion in which one allele is replaced with its homologous allele (Chen et al. 2007). We investigated whether increased HR at *Tf2*s in *abp1*Δ could affect the incident of gene conversion by crossing a parental strain carrying two dominant markers flanking *Tf2-7/8* with another parent recessive for both (Figure 19). Whereas there were no significant differences in the expected numbers among the various classes of progeny in wildtype, loss of *abp1* resulted in a disproportional number of one recombinant class over the other (Figure 20-21). This result suggests that Abp1 helps prevent gene conversion of homologous alleles flanking *Tf2*s.

We noticed that approximately 30% of recovered progeny from wildtype and *abp1*Δ crosses appeared to be genuine recombinants of *Tf2-7/8* (euploid) but did not pass the inverse PCR test. We reasoned these aberrant recombinants are likely due to either nonallelic recombination between *Tf2-7/8* with another *Tf2* element or improper exchange of the homologous alleles causing one of the markers to be placed on the different side of the restriction site relative to that of the control primers To test these scenarios, we performed backcross of these progeny to determine the linkage between the markers. For the *abp1*Δ aberrant recombinants, *abp1* mutation was first outcrossed with a wildtype prior to linkage analysis. The genetic distance between the two markers is ~1.23 centimorgans (cM) (Figure 22), based upon our previous test (Figure 17). Among the

aberrant recombinants, the *abp1*Δ mutants showed a much higher average linkage compared to wildtype (Figure 23). Deletion of *abp1* also resulted in much greater variance of linkage in all tested strains, ranging from less than 0.4 cM increase to as high as 1.1 cM, with the average net change significantly higher in *abp1*Δ compared to that of wildtype ($p < 0.05$) (Figure 24). Thus, while Abp1 contributes to HR suppression of *Tf2s* that is associated with prevention of gene conversion it also facilitates proper recombination of *Tf2*-associated homologous alleles.

Suppression of HR at *Tf2*s requires all three domains of Abp1

Abp1 consists of three domains: an N-terminal DNA-binding domain (DBD), a transposase-like DDE domain (DDE), and C-terminal dimerization domain (DIM) (Figure 25). We have previously shown that while all three domains are required for the nuclear organization of *Tf2s* into *Tf* bodies, only the DBD and DDE domains are required for the silencing of *Tf2s* (Lorenz et al., 2012).

Strains lacking the *abp1* gene (*abp1*Δ) have been shown to exhibit a slow-growth phenotype and sensitivity to the microtubule inhibitor thiabendazole (TBZ)(Irelan et al., 2001). We found that strains lacking the DNA-binding domain (*abp1*-DBDΔ) but not the dimerization domain (*abp1*-DIMΔ) display growth defects and TBZ sensitivity similar to those of *abp1*Δ, suggesting that the lack of these defects in wild-type cells depends on Abp1 possessing an intact DNA-binding domain (Figure 26). Intriguingly, cells lacking

the transposase (*abp1*-DDEΔ) domain exhibit a slight growth defect and sensitivity to TBZ.

We then assessed the loss of individual domains on the ability of Abp1 to associate with LTR sequences. An *in vitro* pulldown assay showed that whereas full-length Abp1 proteins with the DDE truncation have slightly reduced LTR binding, the lack of either the DBD or DIM domain abrogates Abp1 binding altogether (Figure 27). This contrasts with published data of *in vivo* binding as measured by ChIP, where only deletion of the DNA binding domain completely abrogated Abp1 binding at the 5' LTR of *Tf2-12*, while deletion of the DDE or dimerization domain caused only a slight reduction in enrichment(Lorenz et al., 2012).

To further examine how Abp1-mediated repression of meiotic HR at retrotransposons is related to its roles in transcriptional silencing and organization of *Tf2s*, we investigated the contribution of *abp1* domains to HR suppression of *Tf2s*. Each domain deletion mutant produced a significant increase in HR at *Tf2-7/8*, with the dimerization domain mutant producing the strongest ($p < 0.01$) increase (Figure 28). However, none of these domain mutants produced an increase as high as that of *abp1*Δ, suggesting cooperation among the domains, perhaps requiring both functions of Abp1 in transcriptional silencing and Tf body formation to effectively repress HR of *Tf2s*.

Figures

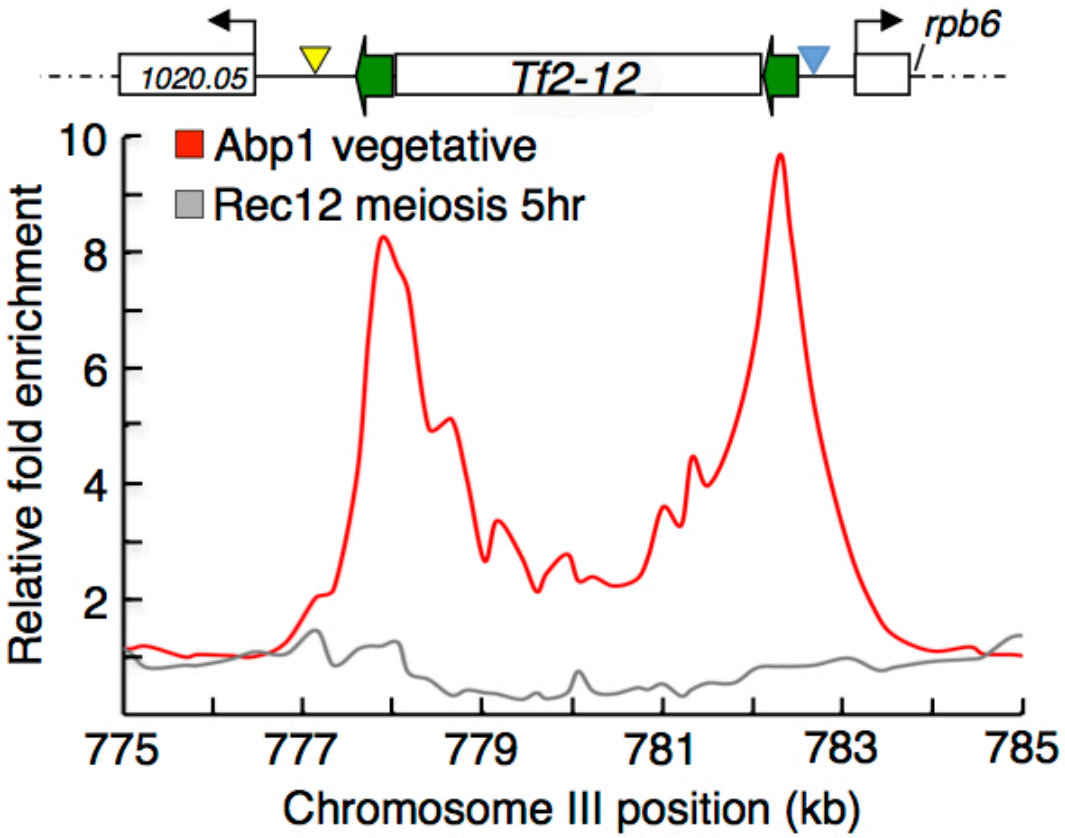

Figure 5: Abp1, but not Rec12, shows enrichment at *Tf2-12* as visualized by two ChIP experiments. Binding of Abp1 and Rec12 were mapped using published ChIP-chip datasets (Abp1: Cam *et al*., 2008; Rec12: Cromie *et al*., 2007). Yellow and blue triangles depict approximate positions of markers used in HR assay.

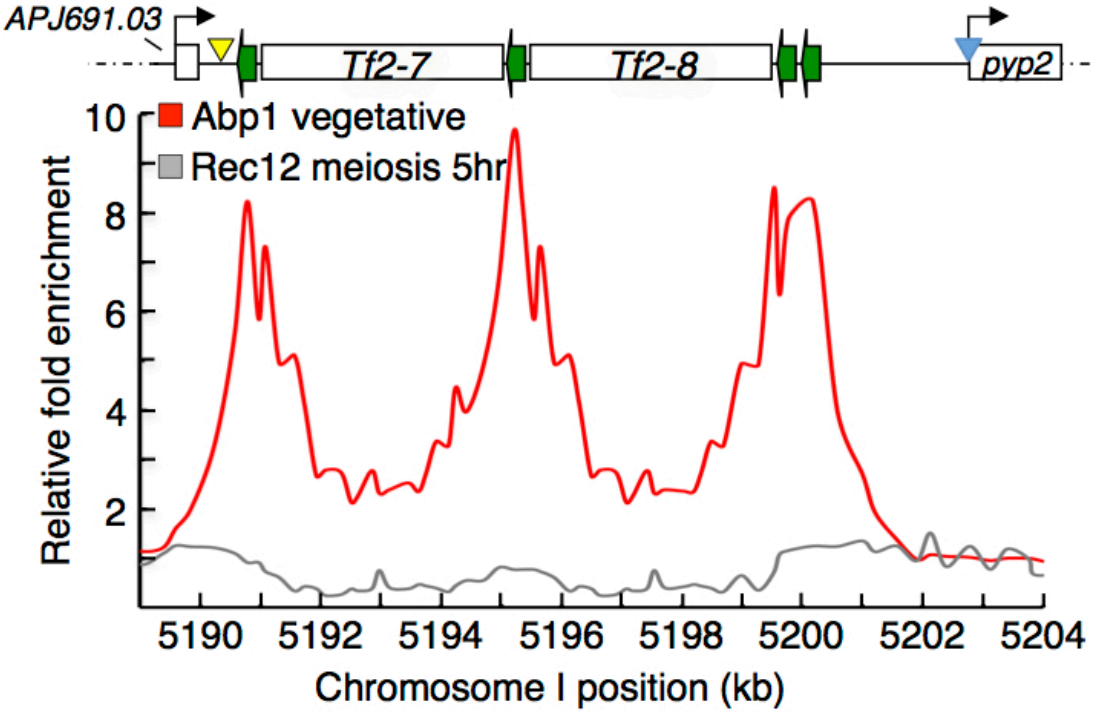

Figure 6: Abp1, but not Rec12, shows enrichment at tandem *Tf2-7/8* as visualized by two ChIP experiments. Binding of Abp1 and Rec12 were mapped using published ChIP-chip datasets (Abp1: Cam *et al*., 2008; Rec12: Cromie *et al*., 2007). Yellow and blue triangles depict approximate positions of markers used in HR assay.

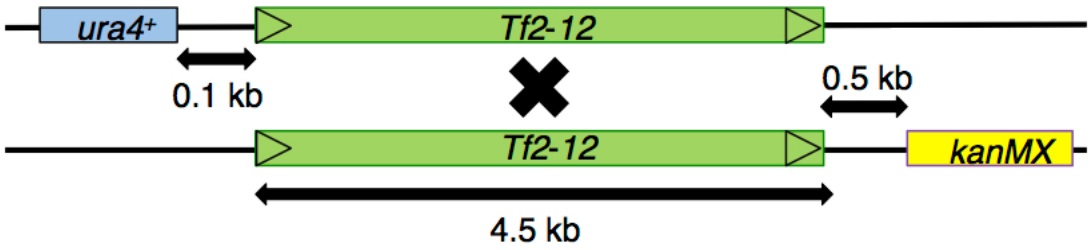

Figure 7: Schematic diagram of marker gene inserts and their distances from *Tf2-12*.

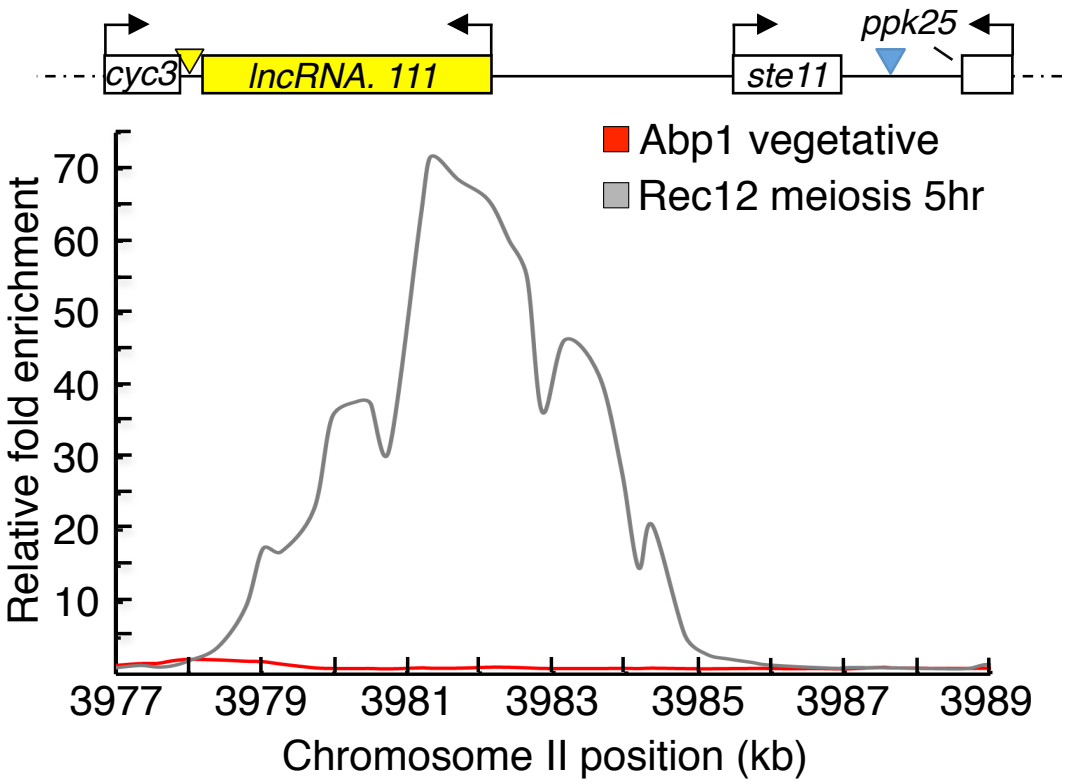

Figure 8: Rec12, but not Abp1, shows enrichment at *ste11*-associated long ncRNA *SPNCRNA111* as visualized by two ChIP experiments. Binding of Abp1 and Rec12 were mapped using published ChIP-chip datasets (Abp1: Cam *et al*., 2008; Rec12: Cromie *et al*., 2007). Yellow and blue triangles depict approximate positions of markers used in HR assay.

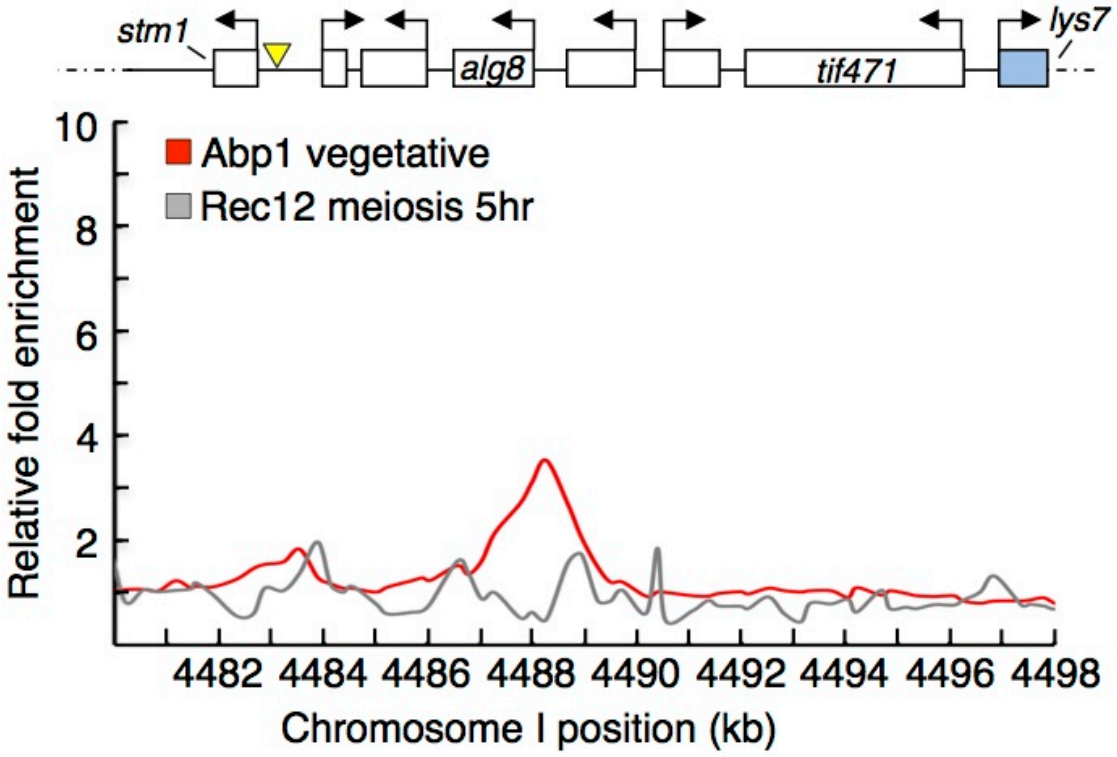

Figure 9: Neither Rec12 or Abp1 shows enrichment at *lys7* as visualized by two ChIP experiments. Binding of Abp1 and Rec12 were mapped using published ChIP-chip datasets (Abp1: Cam *et al*., 2008; Rec12: Cromie *et al*., 2007). Yellow and blue triangles depict approximate positions of markers used in HR assay. For measuring meiotic HR, a parent strain carrying a *KanMX* cassette inserted near *stm1* and a mutant *lys7* allele was crossed with another parent strain carrying a wildtype *lys7*.

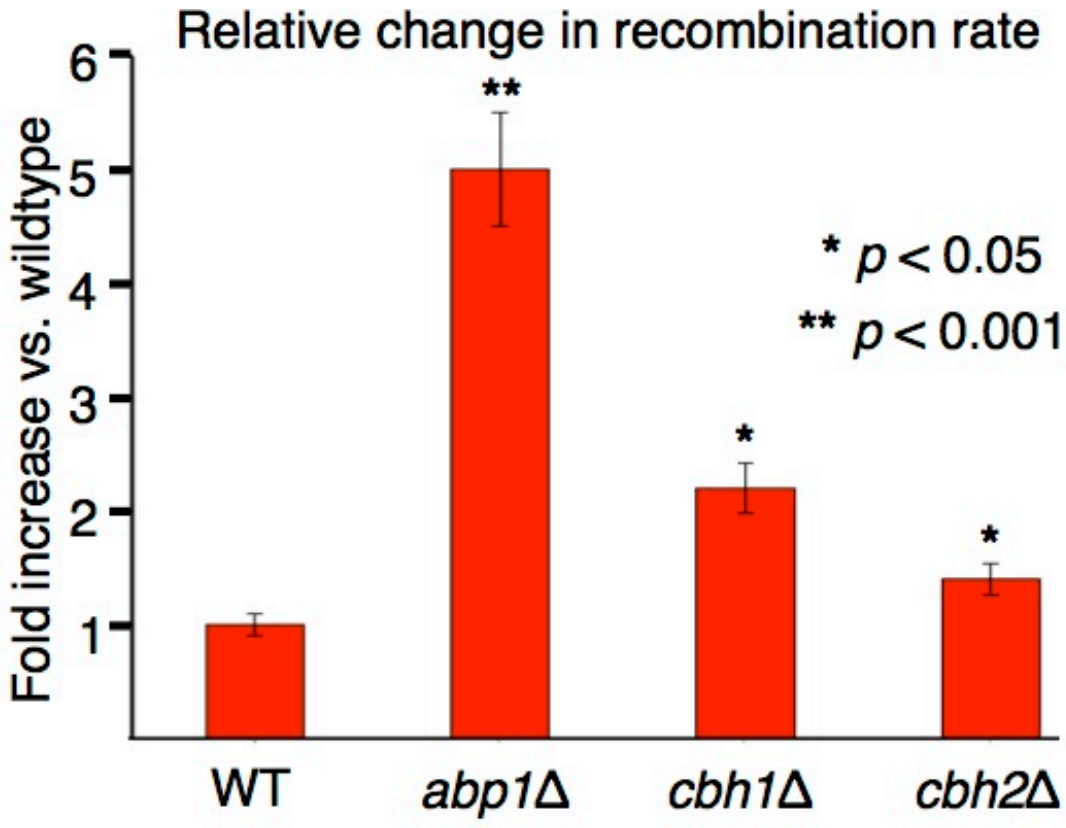

Figure 10: CENP-B mutants show increased rates of meiotic recombination relative to wildtype at *Tf2-12*. Recombinants were recovered from germinated spores grown on media lacking uracil and containing G418 (150 μg/mL). Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

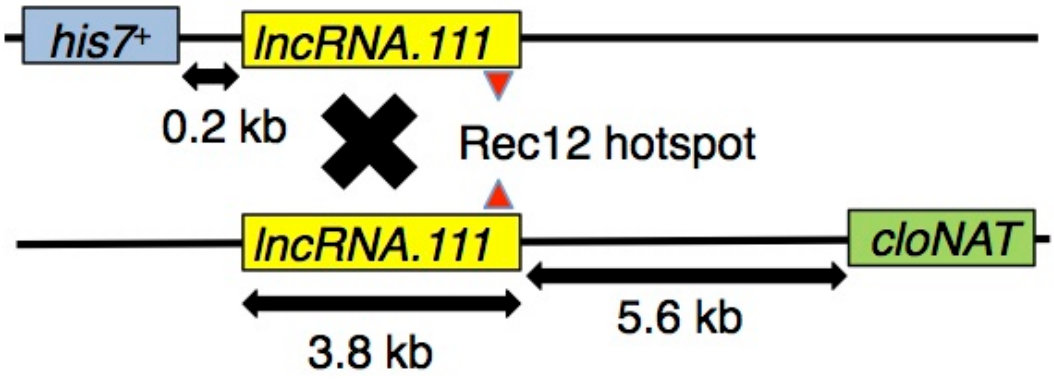

Figure 11: Schematic diagram of marker gene inserts around Rec12-hotsopt (red triangles) near the lncRNA *spncRNA111*.

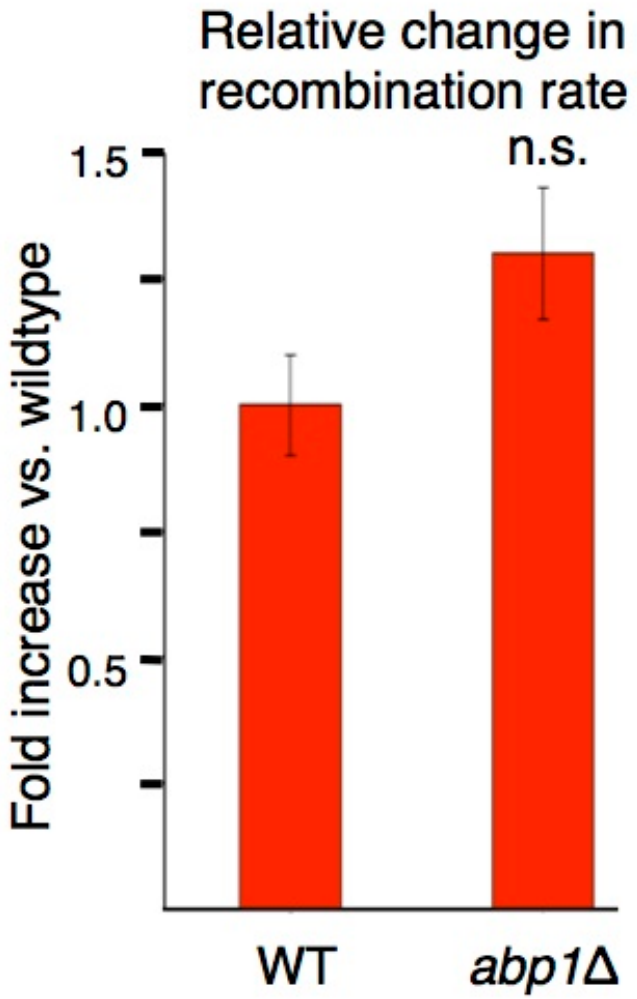

Figure 12: *abp1Δ* shows no significant increase in meiotic recombination relative to wildtype at *spncRNA111*-associated Rec12 hotspot. Recombinants were recovered from germinated spores grown on media containing CloNat (Nourseothricin; 100 μg/mL) minus histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

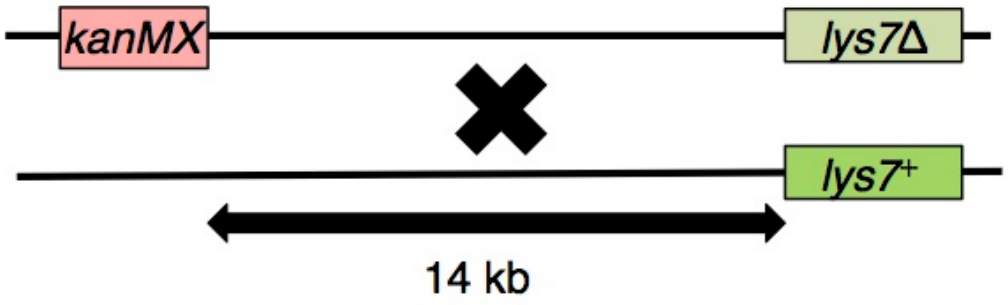

Figure 13: Schematic diagram of marker gene inserts around Rec12 coldspot near *lys7*.

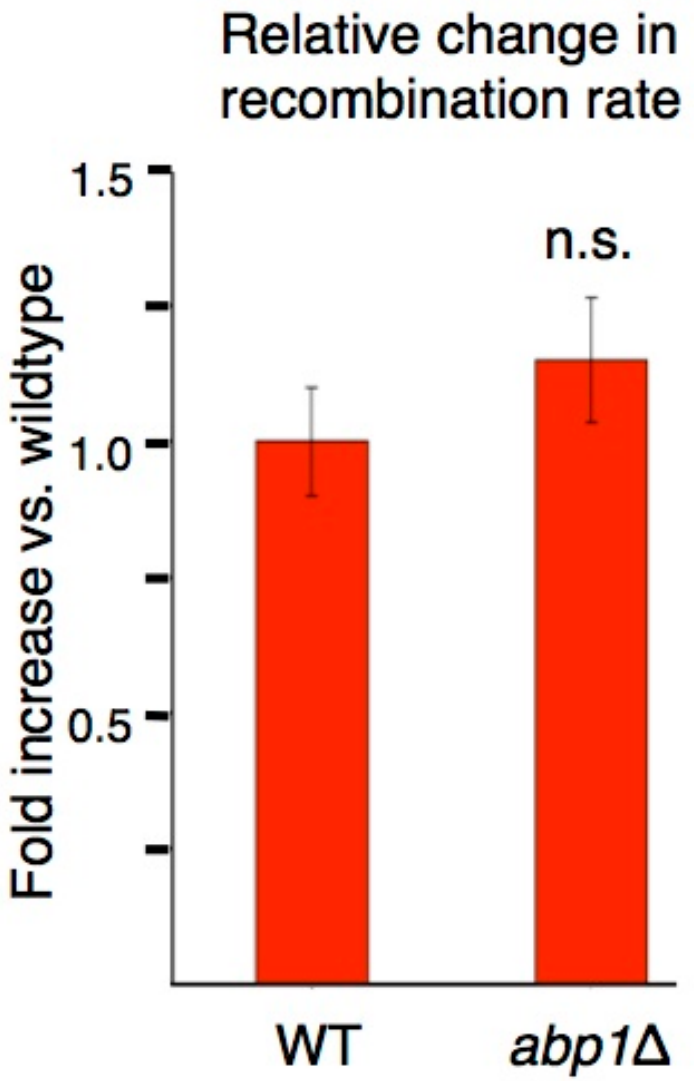

Figure 14: *abp1Δ* shows no significant increase in meiotic recombination relative to wildtype at *spncRNA111*-associated Rec12 hotspot. Recombinants were recovered from germinated spores grown on media containing G418 geneticin (150 µg/mL) minus lysine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

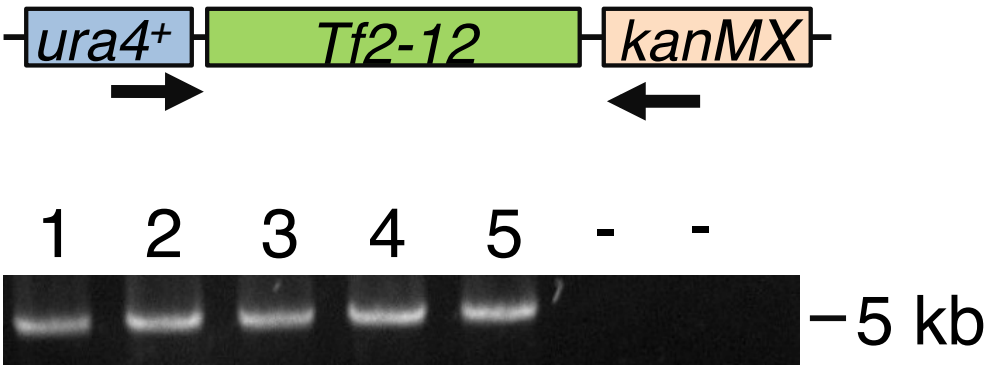

Figure 15: Verification of recombinant progeny by PCR with primers (black arrows) positioned inside the two flanking gene markers. Shown are PCR results of genomic DNA isolated from five independent recombinant progeny and two parental strains as a negative control.

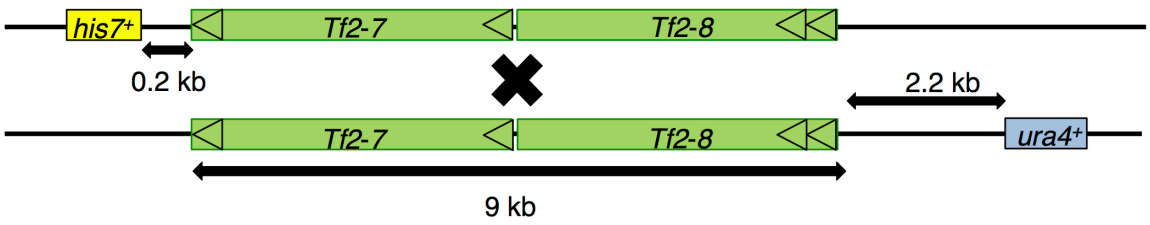

Figure 16: Schematic diagram of marker gene inserts and their distances from the tandem *Tf2-7/8*.

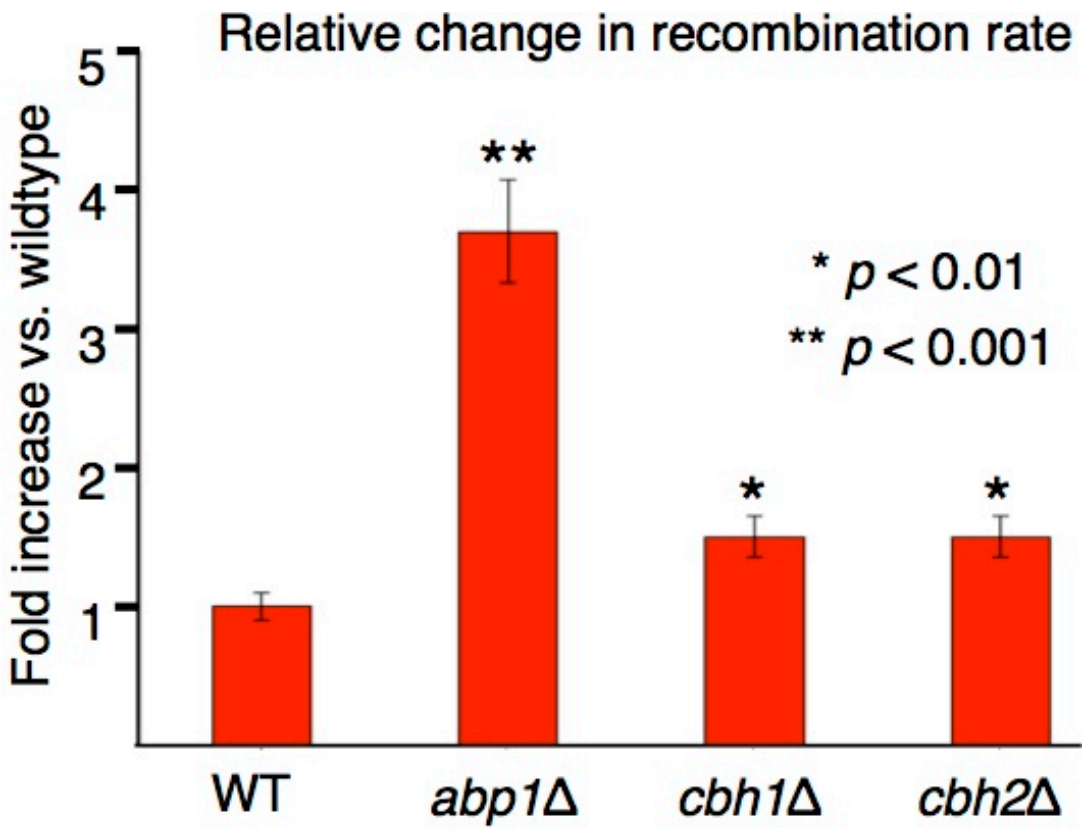

Figure 17: CENP-B mutants show increased rates of meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

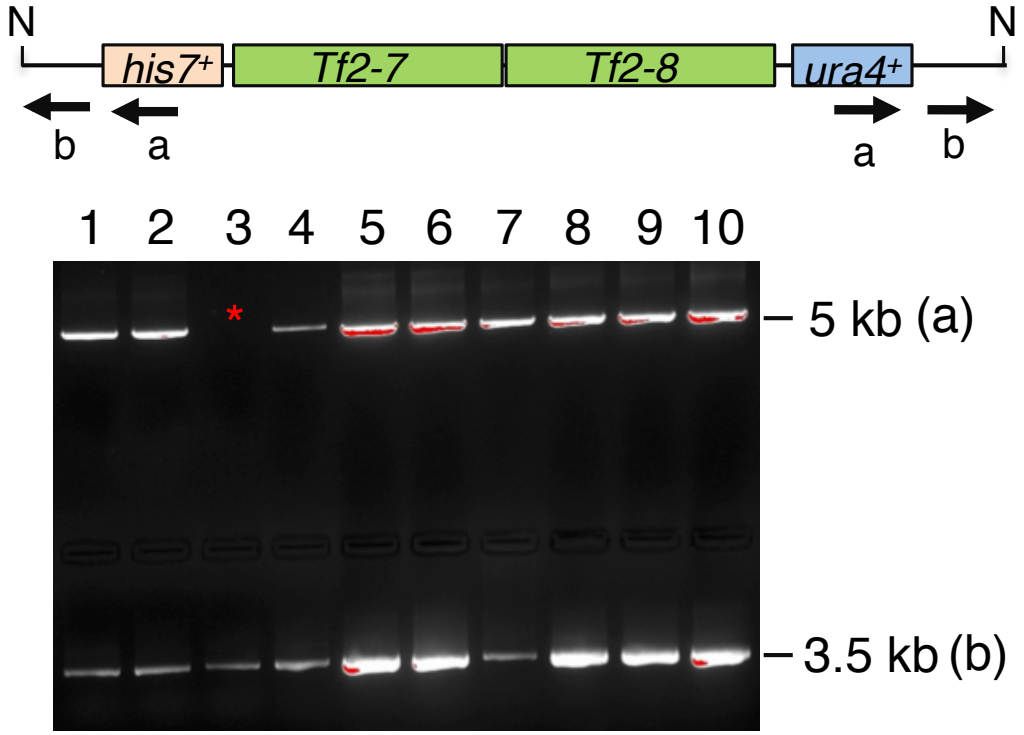

Figure 18: Confirmation of recombinant progeny by inverse PCR. Genomic DNA from 10 independent recombinant progeny was subjected to inverse PCR with primer pairs **a** or **b** (internal control). Upper bands represent amplicons of PCR products with primer pair **a** inside *his7* and *ura4* marker genes; lower bands represent amplicons of PCR products primer pair **b** located outside of marker genes and closer to NcoI (N) cut site. Red asterisk indicates putative nonallelic recombination of marker genes.

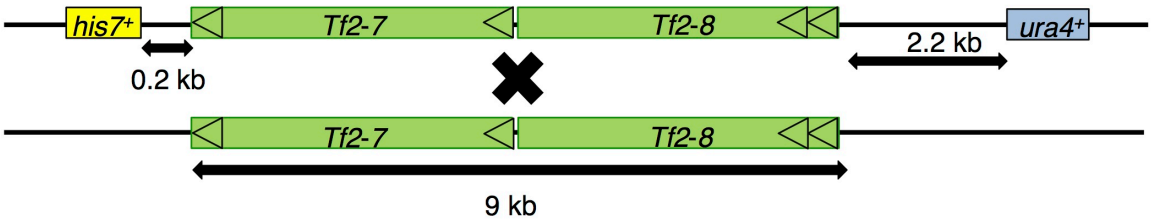

Figure 19: Schematic diagram of a reciprocal cross between a parental strain carrying flanking dominant markers at *Tf2-7/8* and another parent recessive for both markers.

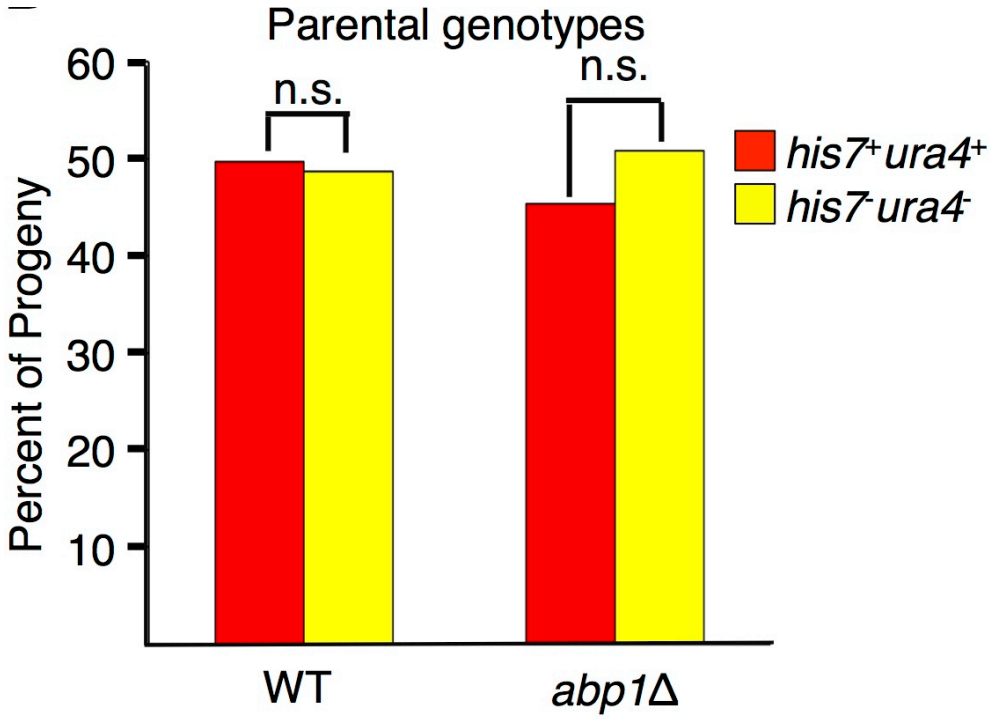

Figure 20: Plot of percent progeny with parental genotypes from reciprocal crosses shown in Figure 19 in wildtype and *abp1*Δ background. *abp1*Δ mutants show slight but not statistically significant preference for *his7*$^{-}$*/ura4*$^{-}$ progeny.

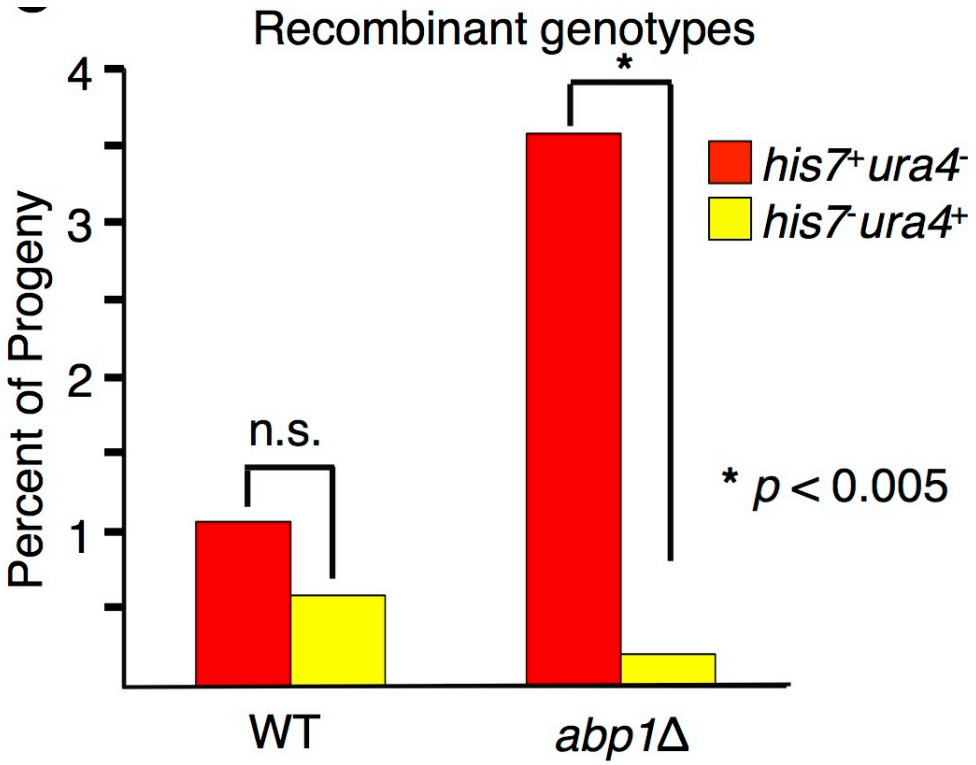

Figure 21: Abp1 prevents gene conversion of *Tf2*-associated marker gene. Plot of percent progeny with recombinant genotypes from reciprocal crosses shown in Figure 19 in wildtype and *abp1*Δ background. *abp1*Δ mutants show strong preference for *his*$^{+}$*/ura*$^{-}$ recombinant progeny.

Background	Genetic Distance (cM)	Net Change
WT	0.96	0.27
WT	1.32	0.09
WT	1.61	0.38
WT	1.26	0.03
abp1Δ	2.38	1.15
abp1Δ	1.62	0.39
abp1Δ	1.96	0.73
abp1Δ	1.47	0.24
abp1Δ	2.07	0.84
abp1Δ	1.65	0.42

Figure 22: Rates of recombination expressed as centimorgan (cM) of putative non-allelic recombinants from both wildtype and *abp1* null (*abp1*Δ) backgrounds. Net change is derived from difference to wildtype control cross (1.23 cM).

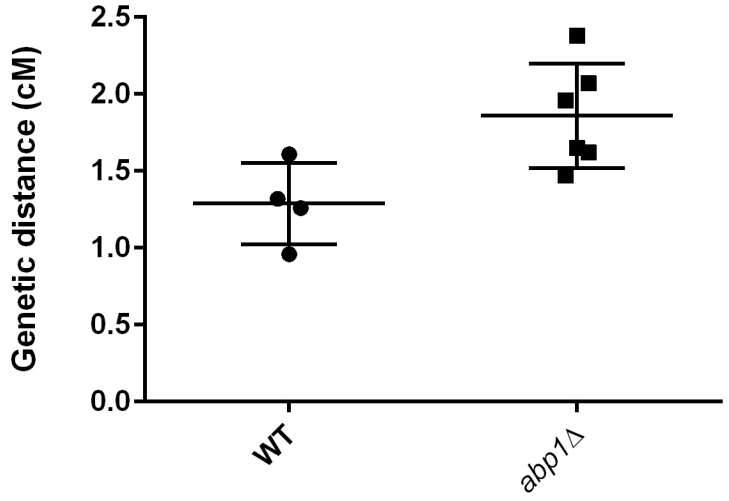

Figure 23: *abp1*Δ putative non-allelic recombinants show greater genetic distance between *his7* and *ura4* marker genes compared to wildtype. Scatter plot shows genetic distances among wildtype (n = 4) and *abp1*Δ mutant (n = 6) progeny that did not produce a 5 kb band using the inverse PCR test. $p < 0.05$ (unpaired *t* test). Middle lines represent mean values; upper and lower lines represent standard deviation.

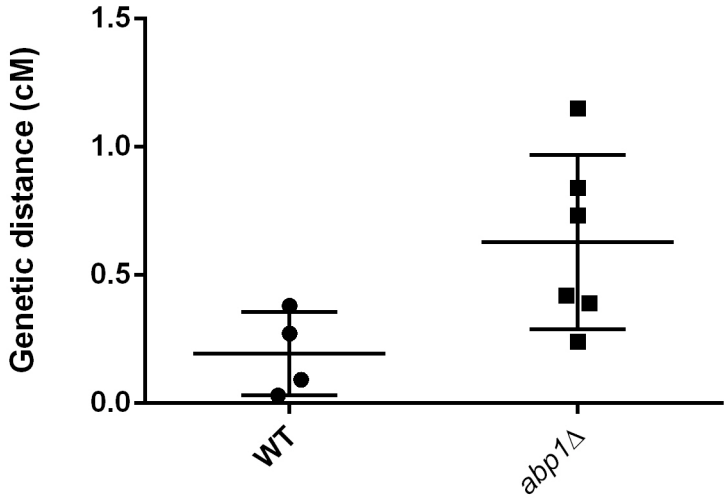

Figure 24: *abp1*Δ putative non-allelic recombinants show greater net change in genetic distance between *his7* and *ura4* marker genes compared to wildtype. Scatter plot shows net changes in genetic distances among wildtype (n = 4) and *abp1*Δ mutant (n = 6) progeny that did not produce a 5 kb band using the inverse PCR test. $p < 0.05$ (unpaired *t* test). Middle lines represent mean values; upper and lower lines represent standard deviation. Net change was derived from wildtype average genetic distance. (1.23 cM)

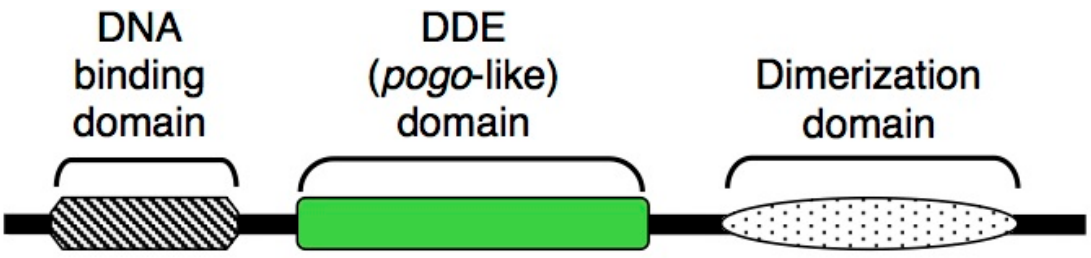

Figure 25: Schematic of the domains of Abp1: DNA-binding (DBD) domain, *pogo*-like (DDE) domain, and dimerization (DIM) domain.

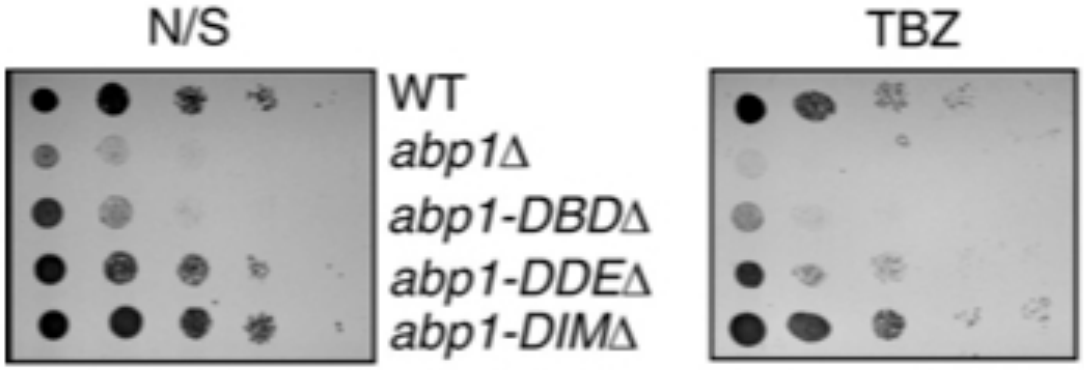

Figure 26: Results of serial dilution analysis of wild-type (WT) and *abp1* null (*abp1*Δ) or domain deletion mutants in nonselective (N/S) media or in the presence of thiabendazole (TBZ) (10 μg/ml). *abp1*Δ and *abp1-DBD*Δ mutants showed increased sensitivity to thiabendazole.

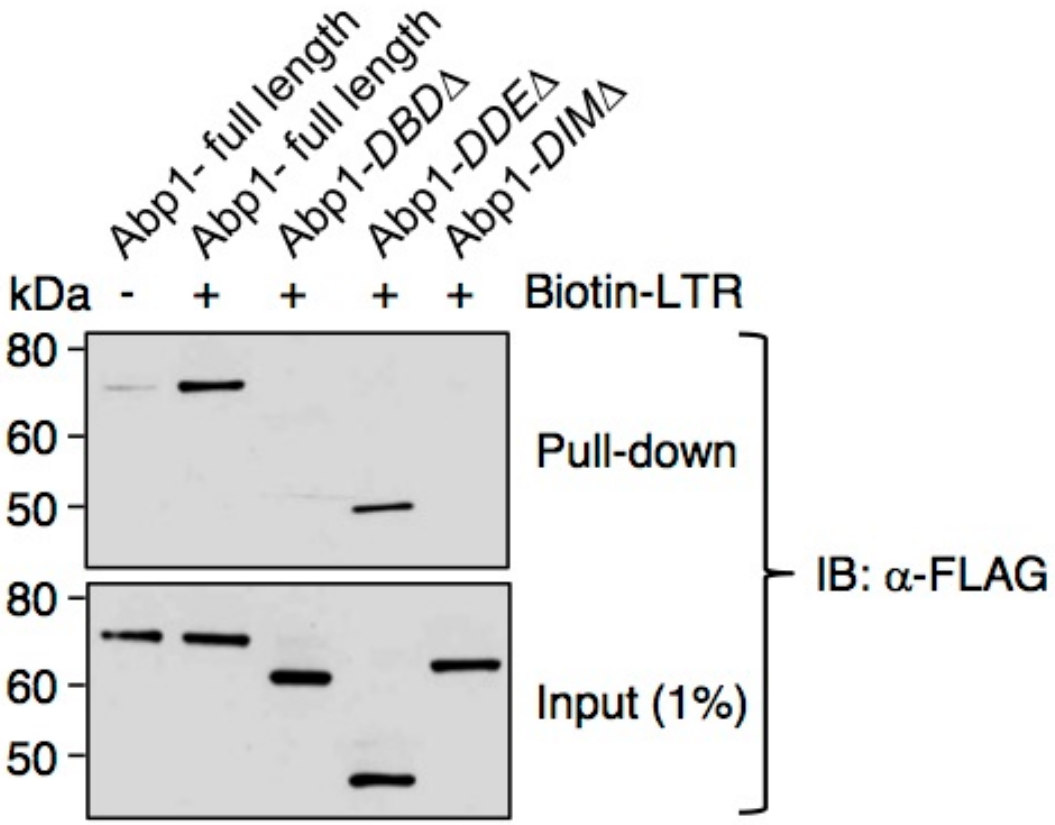

Figure 27: *In vitro* pulldown of DNA fragments containing Abp1 binding sites in *abp1* domain mutants. Biotinylated DNA fragments corresponding to a full-length LTR of *Tf2-6* prebound to streptavidin beads were incubated with protein extracts containing Abp1-FLAG proteins that were either full-length or lacking one of the indicated domains. Bound Abp1-FLAG proteins were detected by immunoblotting with anti-FLAG antibody.

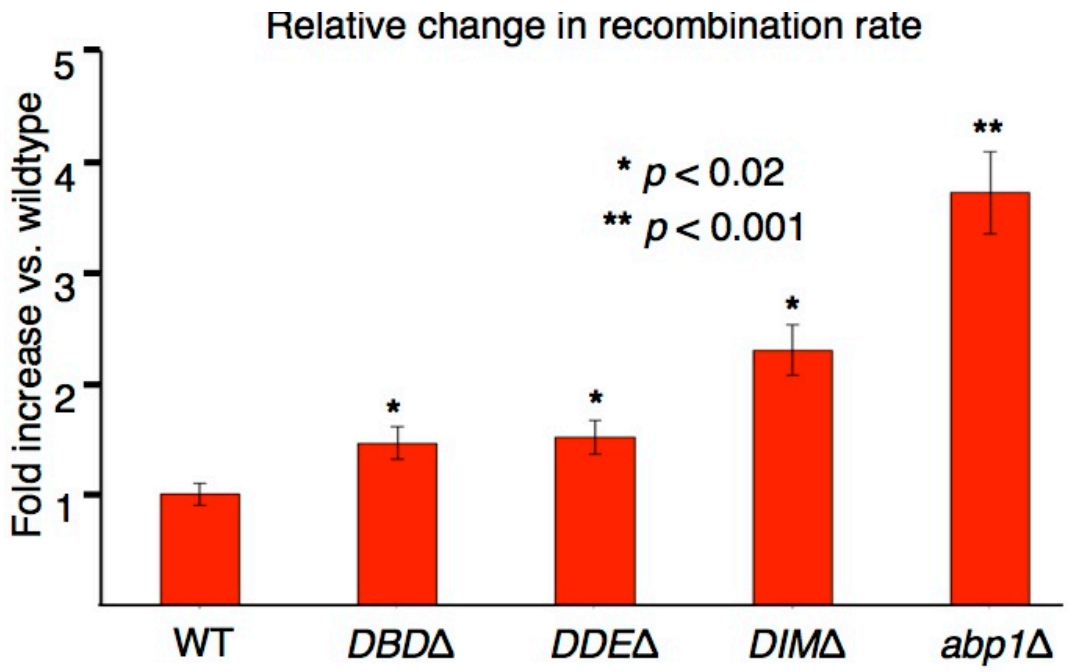

Figure 28: *abp1* domain deletion mutants show increased rates of meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

Chapter 4. Exploring the Regulatory Contributions of Chromatin Modifiers, Nuclear Envelope, DNA Damage Repair, and Architectural Proteins to Meiotic Homologous Recombination at Retrotransposons

Chromatin modifiers are dispensable for HR repression of *Tf2*s

We have recently shown that the histone methyltransferase Set1 silences *Tf2s* independent of H3K4me but relying on H3K4me and the Set1C/COMPASS complex for the nuclear organization of *Tf2s*(Mikheyeva et al., 2014). We assessed the contribution to HR of *Tf2s* by Set1, the catalytic engine of Set1C, and Spp1 whose ortholog has been shown to link meiotic DSBs to chromosome axes in *S. cerevisiae* (Acquaviva et al., 2013; Sommermeyer, Béneut, Chaplais, Serrentino, & Borde, 2012). However, the deletion of these genes produced only slight increases (though not significant; $p > 0.1$; chi-square test) in HR at *Tf2*-7/8, and not to the levels seen in *abp1*Δ (Figure 29).

In addition to Set1C, *Tf2s* are regulated by histone deacetylases (HDACs) (Hansen et al., 2005; Lorenz et al., 2012; Tanaka et al., 2012). We tested Clr3, a class II HDAC, and Hst4, a member of class III HDAC sirtuins. While loss of *hst4* had no noticeable effect, there was a reduction in HR at *Tf2s* in the *clr3*Δ mutant (Figure 30).

Histone chaperone Hip1 and nuclear organizer Lem2 are not required for suppression of HR at *Tf2*s

The histone chaperone HIRA complex exerts strong repression on *Tf2s* (Greenall et al., 2006). We assessed whether loss of Hip1, the founding member of the HIRA complex, could affect HR associated with *Tf2-7/8*. Despite the strong upregulation of *Tf2s* observed in *hip1*Δ (Greenall et al., 2006), it did not result in any appreciable

alteration in HR frequency at *Tf2-7/8* (Figure 31). We also investigated the possibility of nuclear envelope factor such as Lem2 having a role in influencing HR of *Tf2s*. Lem2, an inner nuclear membrane protein, has been shown to help tether telomeres to the nuclear lamina and promote proper organization of chromatin within the nucleus (Y. Gonzalez, Saito, & Sazer, 2012; Hiraoka et al., 2011). We found that similar to *clr3*Δ, there was a reduction in HR at Tf2-7/8 in cells deficient in *lem2* (Figure 32). Collectively, our results suggest that cells can dispense with any single chromatin-modifying factor or nuclear-envelope protein to maintain meiotic repression of recombination at retrotransposons.

Set1 cooperates with the NHEJ pathway to repress HR of *Tf2*s

Abp1 has been shown to recruit the Ku proteins of the nonhomologous end joining (NHEJ) pathway to *Tf2s* to mediate *Tf* body formation and association of *Tf* bodies with centromeres (Tanaka et al., 2012). We investigated whether Abp1-mediated HR suppression of *Tf2s* requires the Ku proteins. Loss of *pku80*, which encodes one of the Ku heterodimer proteins, resulted in 1.6 fold increase of HR (Figure 33), comparable to increases seen in *cbh1*Δ and *cbh2*Δ. Our results suggest that no single known factor shown to be recruited by Abp1 could account fully for the HR increase of *Tf2s* in *abp1*Δ strain.

We explored the possibility that Abp1 recruits multiple factors that act redundantly to restrict HR at *Tf2s*. We tested this idea by examining HR in strain deficient for both *set1* and *pku80.* Whereas loss of *set1* has a negligible effect on HR at

Tf2s, a double mutant of *set1*Δ *pku80*Δ exhibited an appreciable increase of HR relative to *pku80*Δ alone (Figure 33). Together, our results suggest that Abp1 likely suppresses HR of *Tf2s* in part through cooperation between Set1 and NHEJ pathway.

Chromosome architectural protein condensin regulates meiotic HR of retrotransposons

The fission yeast genome is organized within the three dimensional nuclear space with the help of structural chromosomal proteins such as cohesins and condensins (Iwasaki, Tanaka, Tanizawa, Grewal, & Noma, 2010; Mizuguchi et al., 2015). These proteins have also been shown to regulate meiotic recombination coldspots (Li et al., 2014). We examined whether they also affect recombination of *Tf2s*. The meiotic specific cohesin *rec8* is required for sister chromatid cohesion and proper meiotic chromosome pairing (Lin, Larson, Dorer, & Smith, 1992; Molnar, Bahler, Sipiczki, & Kohli, 1995; Watanabe & Nurse, 1999). We could not recover any true *Tf2-7/8* recombinants from a *rec8* mutant cross as analysis of spores (100) germinated on the double selective media (select for reporter genes *his7*$^{+}$ and *ura4*$^{+}$ flanking *Tf2-7/8*) revealed they were all diploids, a likely product of premature segregation of sister chromatids during meiosis I (Ito et al., 2014).

Condensin has been shown to be recruited by Abp1/Ku to *Tf2s* and contributes to *Tf* body formation (Tanaka et al., 2012). We examined whether condensin regulates HR of *Tf2s*. Mutation of the condensin subunit Cut14 results in a moderate increase of HR

around *Tf2-7/8* (Figure 34). Our results reveal a role for higher-order chromatin organization involving architectural protein to suppress HR at interspersed repeats.

Figures

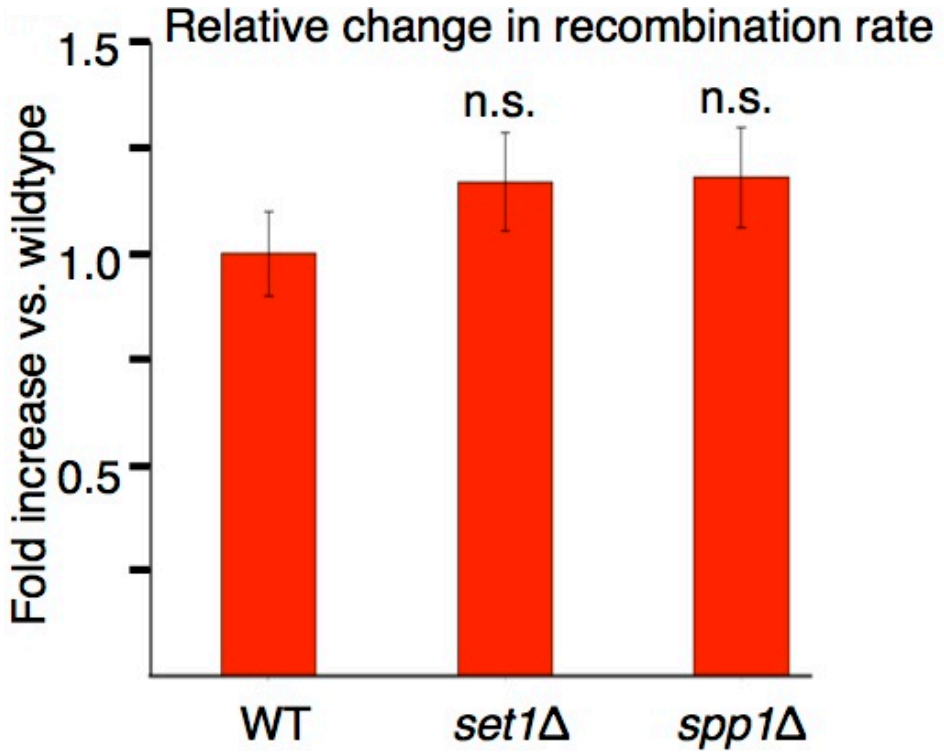

Figure 29: Set1C mutants *set1*Δ and *spp1*Δ show no significant change in meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

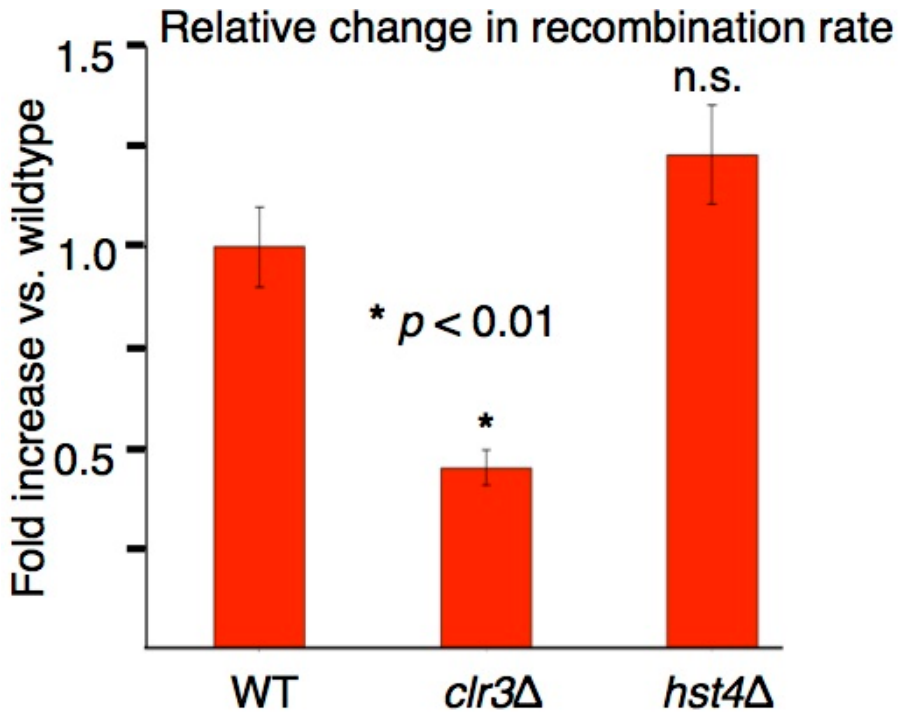

Figure 30: HDAC mutant *clr3*Δ, but not *hst4*Δ, shows reduced meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

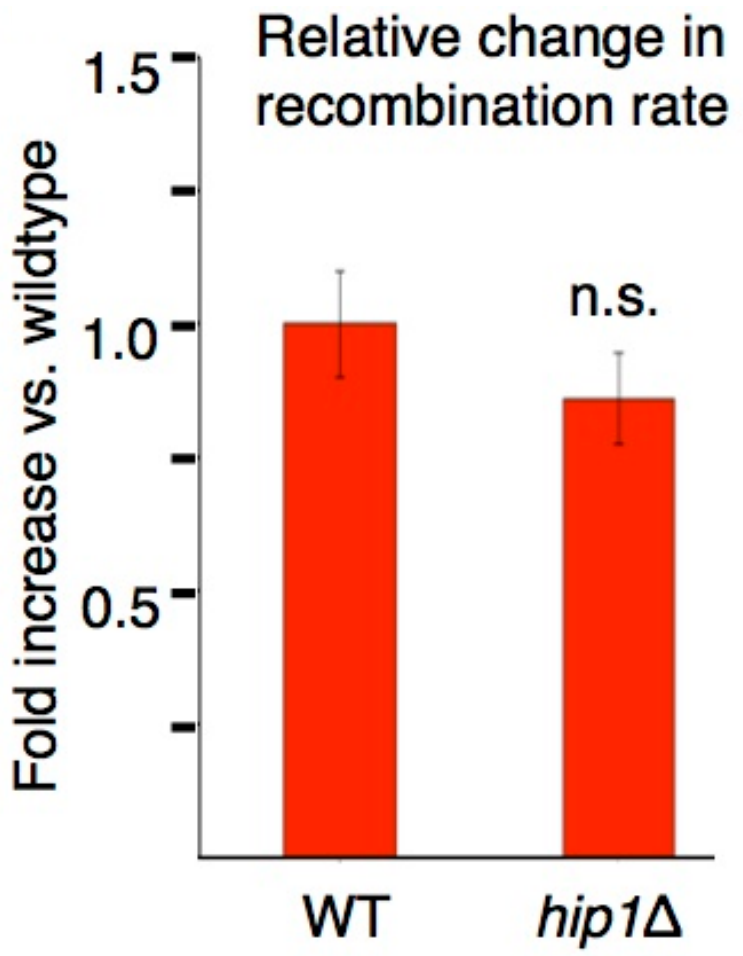

Figure 31: HIRA mutant *hip1*Δ shows no significant change in meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

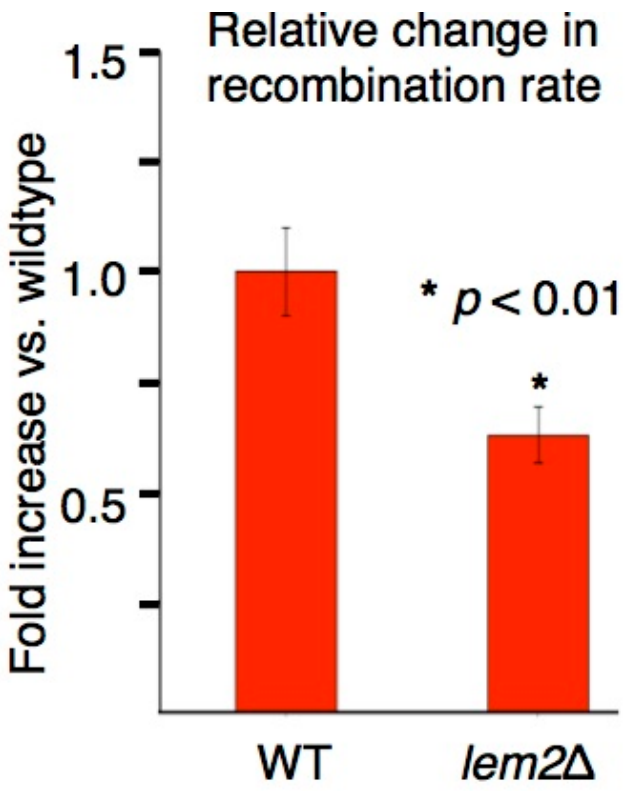

Figure 32: Inner nuclear envelope mutant *lem2*Δ shows reduced meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

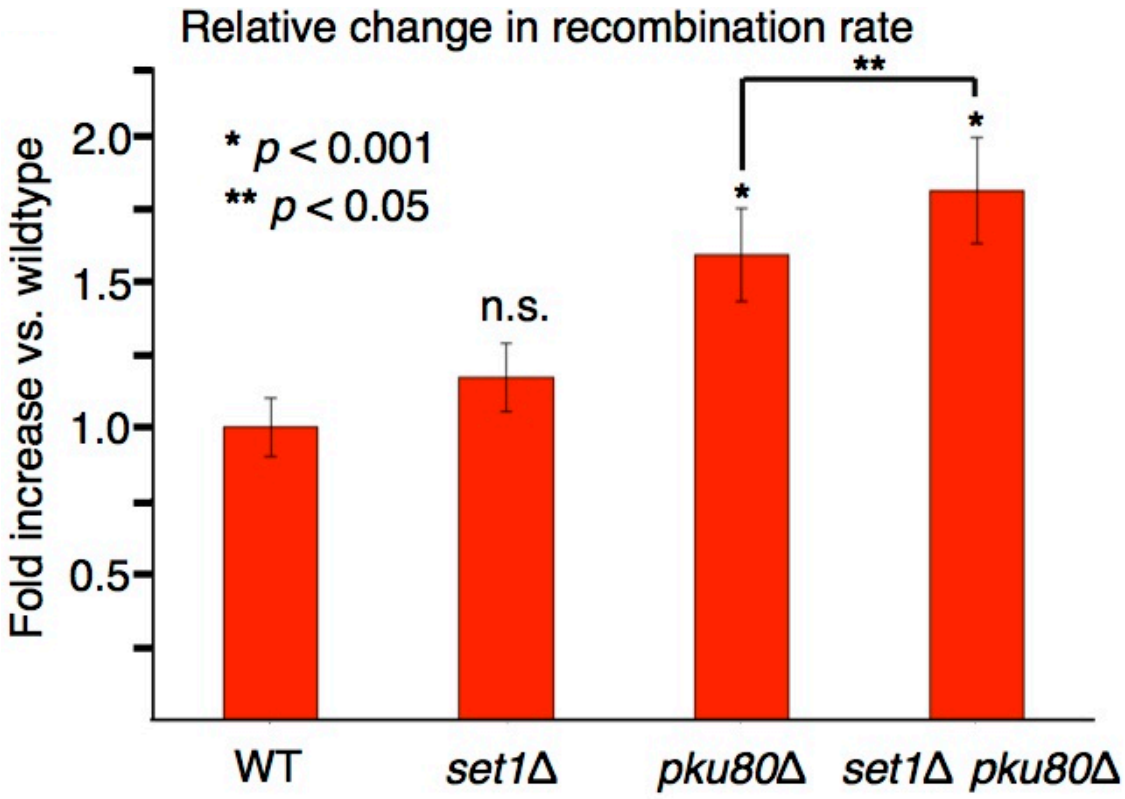

Figure 33: NHEJ mutant *pku80*Δ shows increased meiotic recombination relative to wildtype at tandem *Tf2-7/8,* with *set1*Δ*pku80*Δ double mutant showing further increase in recombination. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

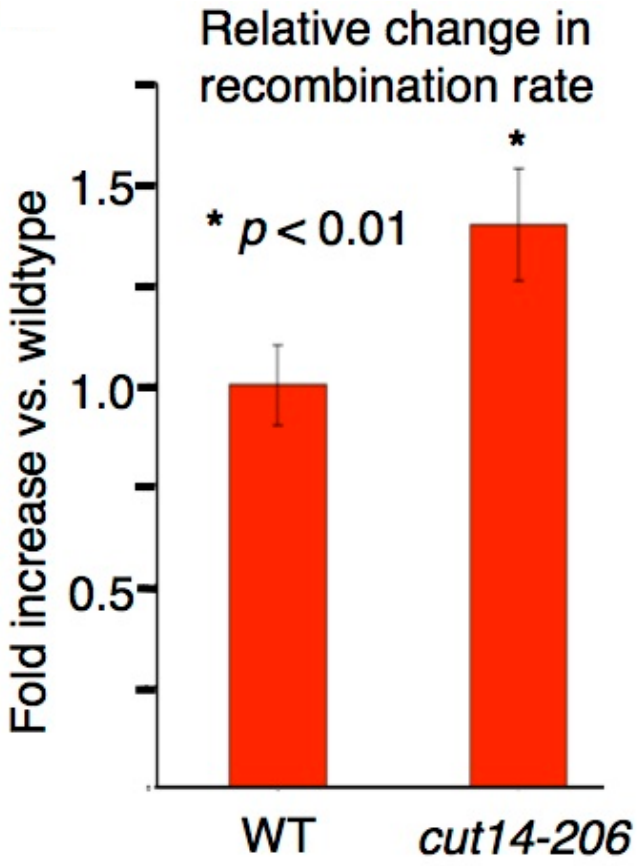

Figure 34: Condensin mutant *cut14-206* shows increased meiotic recombination relative to wildtype at tandem *Tf2-7/8*. Recombinants were recovered from germinated spores grown on media lacking uracil and histidine. Recombination rates were adjusted for diploids. n = 2. Refer to table 1 for actual percentages.

Chapter 5. Summary and Discussion

Summary of Findings

In this thesis, we describe the development of a random sporulation assay used to rapidly screen thousands of spores for recombinant progeny within a locus of interest. This assay was used at multiple loci and in multiple genetic backgrounds to determine how various factors contribute the regulation of meiotic HR. Our findings reveal that the CENP-B homologs, particularly Abp1, in *S. pombe* restrict HR in and around the *Tf2* retrotransposons. Additionally, Abp1 is required to help promote proper exchange of homologous alleles flanking *Tf2s*. Our data further shows that neither of the two properties of Abp1 TE regulation, Tf body clustering and inhibition of expression, are sufficient in and of themselves to restrict HR. Likewise, other factors that have major contributions to repression of *Tf2*s and Tf body formation are dispensable for suppression of meiotic HR at *Tf2*s.

Interestingly, while *Tf2*s are relatively low in Rec12 binding, our findings reveal that these sequences are not necessarily coldspots for HR, as rates of recombination were not significantly lower than the range of a typical locus (Young et al., 2002). Collectively, our findings reveal unexpected insights into the various determinants that govern the regulation of meiotic homologous at interspersed repeats, one of the most ubiquitous elements of giga-base genomes, including that of human.

Discussion

The domestication of *pogo*-like transposases has created CENP-B homologs in many species, including humans, that have high affinity for repetitive DNA (Casola et al., 2007; d'Alençon et al., 2011; Mateo & Gonzalez, 2014). In fission yeast, the family of CENP-B proteins bind to repetitive elements associated with pericentromeric heterochromatin and *Tf2* retrotransposons (Cam et al., 2008). Our findings suggest that in addition to its roles in silencing and nuclear organization of *Tf2s*, CENP-Bs restrict meiotic HR of *Tf2* retrotransposons.

Non-allelic homologous recombination at repetitive DNA can produce gross genome alterations including deletions, duplications, and inversions (Hoang et al., 2010; Rattray et al., 2015; Sasaki, Lange, & Keeney, 2010). These structural variants are implicated in many human diseases such as Gaucher's disease and chronic lymphocytic leukemia (Campbell et al., 2014; Lopez et al., 2011). HR around retrotransposons and their associated LTRs has high potential to cause these damaging events (Sasaki et al., 2010; Vader et al., 2011). Our findings indicate that if HR occurs within the vicinity of a retrotransposon, Abp1 has an important role in ensuring the fidelity of HR by ensuring accurate exchange of homologous alleles that could be linked to its-mediated prevention of gene conversion. Abp1 may carry out this function by promoting the proper alignment of retrotransposon sequences prior to DSB formation and/or help guide the DNA repair machinery during the recombination exchange of homologous *Tf2* alleles. In addition to binding to *Tf2s*, CENP-Bs also bind to solo LTRs that together could contribute novel

regulatory modules to nearby promoters of RNA Polymerase II genes (Cam et al., 2008). In *Drosophila miranda*, gene conversion of transposon-derived regulatory sequences is thought to confer chromosome-wide dosage compensation to the recently evolved male X chromosome (Ellison and Bachtrog, 2015). However, in the case of *S. pombe*, it is possible that Abp1-mediated suppression of gene conversion at *Tf2s* also extends to solo LTRs, allowing these transposon remnant sequences to slowly degrade or integrate into the gene regulatory networks of the transcriptome.

High resolution of Rec12 binding by ChIP-Seq reveals that HR coldspots do not necessarily exclude Rec12 from certain regions such as centromeres (Fowler et al., 2014; Ludin et al., 2008). The maintenance of coldspots may instead be dependent on epigenetic factors and their ability to exclude certain proteins from binding. For example, HR is increased at pericentromeres in the absence of heterochromatin such as the loss of the H3K9 methyltransferase *clr4* (Ellermeier et al., 2010). If this is the case, Rec12 interaction with *Tf2*s may not be occurring in an Abp1-dependant manner.

Similar to hotspots, the identity of coldspots may likewise hinge on binding of transcription factors. The ncRNA *spncRNA111* Rec12 hotspot is located near the *ste11* promoter occupied by Atf1 (Lorenz, Meyer, Grady, Meyer, & Cam, 2014), suggesting that Rec12 hotspot formation at ncRNA *spncRNA111* is likely dependent on Atf1 binding, similar to its role in promoting recombination at the well-characterized *ade6-M26* allele (Kon et al., 1997). In the case of *Tf2* retrotransposons, Abp1 appears to be the

primary determinant in preventing HR of *Tf2*s. The rates of HR surrounding the solo *Tf2-12* (0.7 cM) and tandem *Tf2-7/8* (1.3 cM) are comparable to the genome averages for the expected distances (0.96 cM and 1.24 cM, respectively) (Young et al., 2002). Our results suggest that while *Tf2s* are coldspots for DSBs (Fowler et al., 2014), they are not particularly poor in HR.

In addition to CENP-Bs, the presence of other proteins, including Ku, Set1, and condensin, also restrict recombination, but they likely have overlapping roles in suppressing HR of *Tf2s* (Figure 35). Considering these proteins have distinct functions (i.e., Ku80 in NHEJ and Set1-catalyzed H3K4me Tf body formation and Set1-mediated H3K4me-independent repression of *Tf2s*), it is likely that multiple layers of controls from several distinct pathways act to limit meiotic recombination of *Tf2s* and repetitive elements in general.

While the insertion of a *Ty* retrotransposon reduces DSB activity at a recombination hotspot in the budding yeast *Saccharomyces cerevisiae* (Ben-Aroya et al., 2004), the mechanism of how this occurs is not yet clear. *S. cerevisiae* does not possess CENP-B homologs, and the chromatin structure around *Ty* elements is highly varied (Sasaki et al., 2013). The cause of DSB restriction around TE and other repetitive DNA cannot be absolutely linked to either sequence or chromatin compaction, or not even the presence of genes (Fowler et al., 2014; Sasaki et al., 2013). In *S. pombe*, it remains to be seen if binding of Abp1 is sufficient to inhibit HR in and of itself.

Likewise, it is not yet known if Abp1 binding is sufficient to exclude the binding of Rec12 to a locus, thereby inhibiting DSB formation. However, this seems unlikely, as deletion of *pku80* also increases HR. Since Abp1 recruits the Ku heterodimer to *Tf2*s (Tanaka et al., 2012), Abp1 could suppress HR by promoting Ku-mediated NHEJ repair of DSBs over HR. The presence of condensin may further restrict Rec12 activity at these loci (Li et al., 2014; Mets & Meyer, 2009). A future goal for this study would be the insertion of an Abp1 binding sequence, such as several tandem LTRs, into an existing Rec12 peak to explore if the sequence itself or the presence of Abp1 can inhibit both Rec12 binding and HR.

The genomes of higher eukaryotes are rich in repetitive elements that clearly possess a danger to their host if they are allowed to spread unchecked. However, evolution does not always favor eliminating these sequences, even after they have become domesticated and unable to mobilize further. Is there some benefit to keeping these "selfish genes" within our genomes? The Encyclopedia of DNA Elements (EnCODE) project claims that over 80% of our genome has some detectable biochemical function (Dunham et al., 2012), though not necessarily an essential function. The domestication of transposases that gave rise to the CENP-B homologs were used to control and subsequently rid TEs from the *pombe*/*octosporus* lineage (Rhind et al., 2011). This suggests an interesting scenario: hosts could exploit potential competition between

different classes of TEs (DNA transposons which encodes transposases and retrotransposons) to help control TEs.

In the context of *S. pombe*, deletion of *abp1* disregulates the organization of chromatin within the nucleus (Cam et al., 2008; Mizuguchi et al., 2015). If a cell cannot efficiently rearrange its chromatin in response to environmental cues, then regulation of gene expression and other DNA-based processes such as DNA replication, repair, and recombination may be certainly be compromised. The binding of TEs by Abp1 or similar proteins in other organisms may, therefore, have a role in promoting proper nuclear organization important for the aforementioned DNA-based processes.

Recombination hotspots have not remained constant through evolution. As DNA sequences have changed, so have hotspots arisen and been destroyed over time (Lesecque, Glémin, Lartillot, Mouchiroud, & Duret, 2014). While individual hotspots are rapidly destroyed by gene conversion, others continuously appear to take their place. This creates a paradox: the number of HR hotspots remains relatively constant over time despite the fact that individual HR spots tend to experience high turnover due to changes in underlying DNA sequences (Boulton, Myers, & Redfield, 1997). Since HR is preferentially targeted to intergenic regions, these sequences may act as a reservoir of nascent hotspots, capable of becoming active with additional base pair substitutions (Wahls & Davidson, 2011). HR around the repetitive DNA-rich *Tf2* retrotranposons may lead to uneven reciprocal exchange (Campbell et al., 2014; Uddin, Sturge, Peddle,

O'Rielly, & Rahman, 2011). This too should be selected against, but HR still persists in repetitive DNA (McVean, 2010). Some evolutionary benefit may exist for this paradoxically dangerous relationship between HR and repetitive DNA to be maintained. One proposed suggestion is that some NAHR is necessary to drive the creation of new genes or alleles. Such events tend to favor short (5-50 kb) insertions and deletions in *S. cerevisiae* (Hoang et al., 2010), though the methods of selection for this test obviously cannot recover cells with such severe chromosomal rearrangements that they become inviable. The nature of our RSA limits us to only recovering viable spores, which may mask the more damaging NAHR events that occur. The availability of assays that enable the detection and quantification of inviable progeny with NAHR may help to further shed insights into how cells deal with meiotic HR across various types of repetitive elements.

Figures

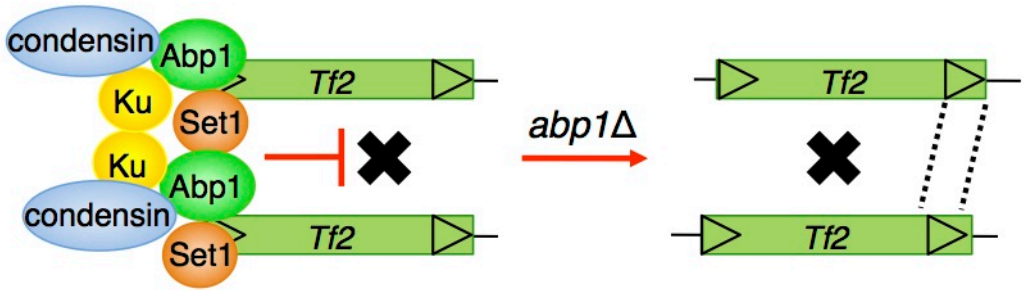

Figure 35: Model of Abp1-mediated suppression of meiotic homologous recombination at retrotransposons in fission yeast. Abp1 binding to specific TA-rich motifs within LTRs (Lorenz et al., 2012)recruits multiple factors that act redundantly to restrict meiotic recombination at *Tf2*s. Abp1 also prevents gene conversion and facilitates proper exchange of homologous alleles if recombination occurs within the vicinity of a *Tf2* element.

Appendix: Tables

Table 1: Recombinant progeny recovered from meiotic crosses expressed as percent of total spores screened

Rate of Recombination at *Tf2*-12		
Strains	**Percent recombinant**	**Total spores screened**
wildtype	0.96%	5525
*abp1*Δ	4.81%	9050
*cbh1*Δ	2.13%	6250
*cbh2*Δ	1.35%	6940
*cbh1*Δ *cbh2*Δ	1.69%	8500
Rate of Recombination at *Tf2*-7/*Tf2*-8		
wildtype	1.24%	7575
*abp1*Δ	4.62%	4050
*cbh1*Δ	1.85%	11400
*cbh2*Δ	1.91%	4275
*cbh1*Δ *cbh2*Δ	1.45%	8925
*abp1-DBD*Δ	1.81%	8050
*abp1-DDE*Δ	1.88%	6900
*abp1-DIM*Δ	2.86%	16530
*hip1*Δ	1.05%	12650
*clr3*Δ	0.56%	19200
*set1*Δ	1.45%	13275
*spp1*Δ	1.46%	7275
*lem2*Δ	0.78%	9525
*hst4*Δ	1.52%	9300
*pku80*Δ	2.18%	10575
*pku80*Δ *set1*Δ	2.49%	17475
cut14-208	1.92%	20250
Rate of Recombination at *lncRNA spncRNA111*-associated Rec12 Hotspot		
wildtype	3.44%	11775
*abp1*Δ	4.51%	10300
Rate of Recombination at *lys7*		
wildtype	2.24%	16900
*abp1*Δ	2.58%	6700

Distribution of Progeny Genotypes in Reciprocal Cross at *Tf2*-7/*Tf2*-8					
Strains	***his7*$^{+}$*ura4*$^{+}$**	***his7*$^{-}$*ura4*$^{-}$**	***his7*$^{+}$*ura4*$^{-}$**	***his7*$^{-}$*ura4*$^{+}$**	**Total**
wildtype	1256	1231	27	15	2529
*abp1*Δ	431	483	34	2	950

Table 2: Strains used in this study

Strain	
PJ26	*h+ ura4-DS/E leu1-32 Tf2-12::ura4$^+$ ade6-M210*
PJ10	*h- ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216*
PJ15	*mat1Mst0 ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216 abp1Δ::LEU2*
PJ42	*h+ ura4-DS/E leu1-32 Tf2-12::ura4$^+$ M ade6- M210 abp1Δ::LEU2*
PJ19	*h+ ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M210 cbh1Δ::LEU2*
PJ36	*mat1Mst0 ura4-DS/E leu1-32 Tf2-12:: ura4$^+$ ade6-M216 cbh1Δ::LEU2*
PJ34	*mat1Mst0 ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216 cbh2Δ::LEU2*
PJ46	*h+ ura4-DS/E leu1-32 Tf2-12::ura4 ade6-M210 cbh2Δ::LEU2*
	mat1Mst0 ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216 cbh1Δ::LEU2
PJ35	*cbh2Δ::LEU2*
	h+ ura4-DS/E leu1-32 Tf2-12::ura4$^+$ ade6-M210 cbh1Δ::LEU2
PJ72	*cbh2Δ::LEU2*
PJ130	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$*
PJ78	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8::ura4$^+$*
PJ94	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ abp1Δ::LEU2*
PJ98	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) abp1Δ::LEU2*
PJ87	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ cbh1Δ::LEU2*
PJ91	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) cbh1Δ::LEU2*
PJ100	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ cbh2Δ::LEU2*
PJ86	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) cbh2Δ::LEU2*
PJ125	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ cbh1Δ::LEU2 cbh2Δ::LEU2*
	h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) cbh1Δ::LEU2
PJ126	*cbh2Δ::LEU2*
	h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ abp1-DBDΔ-
PJ131	*(3X)FLAG::kanMX*
	h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) abp1-DBDΔ-
PJ114	*(3X)FLAG::kanMX*
	h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ abp1-DDEΔ-
PJ120	*(3X)FLAG::kanMX*
	h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) abp1-DDEΔ-
PJ132	*(3X)FLAG::kanMX*
	h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ abp1-DIMΔ-
PJ133	*(3X)FLAG::kanMX*
	h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) abp1- DIMΔ-
PJ116	*(3X)FLAG::kanMX*
PJ136	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ clr3Δ::kanMX*
PJ137	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) clr3Δ::kanMX*
PJ138	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ set1Δ::kanMX*
PJ161	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) set1Δ::kanMX*
PJ157	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) hip1Δ::kanMX*
PJ158	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ hip1Δ::kanMX*
PJ163	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) spp1Δ::kanMX*
PJ164	*h- ura4-DS/E leu1-32 his7Δ Tf2-7::his7$^+$ spp1Δ::kanMX*

PJ174	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ lem2Δ::kanMX*
PJ175	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) lem2Δ::kanMX*
PJ165	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2::ura4$^+$) hst4Δ::kanMX*
PJ176	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ hst4Δ::kanMX*
PJ181	*h- leu1-32 stm1::kanMX lys7Δ*
HC199	*h+ ade6-M216 ura4-DS/E OtrR1::ura4$^+$ leu1-32*
PJ182	*h- leu1-32 stm1::kanMX lys7Δ abp1Δ::LEU2*
HC133	*h+ ade6-M216 ura4-DS/E OtrR1::ura4$^+$ leu1-32 abp1Δ:: LEU2*
MD22	*h- ade6-M216 ura4-D18 leu1-32 his7Δ::lys2$^+$ SPBC26H8.12::his7$^+$*
MD27	*h+ ade6-M210 ura4-D18 leu1-32 his7Δ::lys2$^+$ ste11:cloNAT*
PJ185	*h- leu1-32 his7Δ::lys2$^+$ ste11:cloNAT abp1Δ::LEU2*
PJ186	*h+ leu1-32 his7Δ::lys2$^+$ SPBC26H8.12::his7$^+$ abp1Δ::LEU2*
PJ192	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ pku80Δ::kanMX*
PJ195	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2Δ::ura4$^+$) pku80Δ::kanMX*
	h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ pku80Δ::kanMX
PJ198	*set1Δ::kanMX*
	h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2Δ::ura4$^+$) pku80Δ::kanMX
PJ207	*set1Δ::kanMX*
PJ211	*h- ura4-DS/E leu1-32 his7Δ Tf2-7::his7$^+$ cut14-208(ts)*
PJ212	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-8(pyp2Δ::ura4$^+$) cut14-208(ts)*
	h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ Tf2-8(pyp2Δ::ura4$^+$)
PJ213	*abp1Δ::LEU2*
PJ214	*h- ura4-DS/E leu1-32 his7Δ::lys2$^+$ abp1Δ::LEU2*
PJ215	*h+ ura4-DS/E leu1-32 his7Δ::lys2$^+$ Tf2-7::his7$^+$ Tf2-8(pyp2Δ::ura4$^+$)*
CHP105 3	*h- ade6-M216 ura4-D18 leu1-32 his7Δ::lys2$^+$*

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