Suppression of Meiotic Recombination by CENP-B Homologs in *Schizosaccharomyces pombe*

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ABSTRACT Meiotic homologous recombination (HR) is not uniform across eukaryotic genomes, creating regions of HR hot- and coldspots. Previous study reveals that the Spo11 homolog Rec12 responsible for initiation of meiotic double-strand breaks in the fission yeast *Schizosaccharomyces pombe* is not targeted to *Tf2* retrotransposons. However, whether *Tf2*s are HR coldspots is not known. Here, we show that the rates of HR across *Tf2*s are similar to a genome average but substantially increase in mutants deficient for the CENP-B homologs. Abp1, which is the most prominent of the CENP-B family members and acts as the primary determinant of HR suppression at *Tf2*s, is required to prevent gene conversion and maintain proper recombination exchange of homologous alleles flanking *Tf2*s. In addition, Abp1-mediated suppression of HR at *Tf2*s requires all three of its domains with distinct functions in transcriptional repression and higher-order genome organization. We demonstrate that HR suppression of *Tf2*s can be robustly maintained despite disruption to chromatin factors essential for transcriptional repression and nuclear organization of *Tf2*s. Intriguingly, we uncover a surprising cooperation between the histone methyltransferase Set1 responsible for histone H3 lysine 4 methylation and the nonhomologous end joining pathway in ensuring the suppression of HR at *Tf2*s. Our study identifies a molecular pathway involving functional cooperation between a transcription factor with epigenetic regulators and a DNA repair pathway to regulate meiotic recombination at interspersed repeats.

KEYWORDS meiosis; retrotransposons; homologous recombination; CENP-B; gene conversion; Set1; NHEJ; Condensin

MEIOSIS is a distinct cell division distinguished by the conversion of a diploid cell into haploid gametes during the sexual stages of eukaryotes. Reducing ploidy of a cell containing two different sets of chromosomes requires pairing and subsequent segregation of homologous chromosomes (Hunter *et al.* 2007). In most eukaryotes, pairing of homologous chromosomes is aided by the conserved topoisomerase Spo11, which generates double-strand breaks (DSBs) at preferential sites along the chromosome (Keeney *et al.* 2014). Repair of DSBs by homologous recombination (HR) between homologous chromosomes provides opportunities for interhomolog shuffling of alleles and a platform for proper segregation of homologous chromosomes (Petronczki *et al.* 2003; Keeney and Neale 2006).

Rates of HR vary across chromosomes creating regions of recombination hotspots and coldspots (Cromie *et al.* 2007; Pan and Keeney 2007; Ludin *et al.* 2008; Lichten and de Massy 2011). A variety of determinants govern hotspot formation, including transcriptional promoters, nucleosome-depleted regions, and histone modifications (Wu and Lichten 1994; Pan *et al.* 2011; Smagulova *et al.* 2011; Martin-Castellanos *et al.* 2013; Choi and Henderson 2015). Mapping of Spo11 binding reveals that DSB hotspots associate with trimethylation of histone H3 lysine 4 (H3K4me3) in the budding yeast *Saccharomyces cerevisiae* (Borde *et al.* 2009), mice, and humans (Buard *et al.* 2009; Grey *et al.* 2011; Smagulova *et al.* 2011; Baudat *et al.* 2013). Moreover, deletion of the H3K4me histone methyltransferase *set1* in *S. cerevisiae* reduces meiotic DSB sites and alters the pattern of hotspots across the genome (Borde *et al.* 2009). While not as well-understood as hotspot formation, coldspot regions tend to associate with transcriptional repression and closed chromatin structures such as centromeres and telomeres (Petes 2001).

Structural organization of chromosomes also governs meiotic recombination. In both budding yeast and mice, DSB

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regions appear to organize into alternating domains of low and high DSB activities (Kauppi *et al.* 2011; Pan *et al.* 2011), suggesting a role for higher-order chromatin structures in imposing these patterns. Prior to DSB formation, chromatin becomes organized into a condensed, dynamic structure consisting of chromatin loops bound to an axis enriched for structural proteins such as cohesins, condensins, and meiosis-specific axial-element proteins (Zickler and Kleckner 1999; Borde and de Massy 2013; Keeney *et al.* 2014). Most DSBs appear to restrict to loop regions with a subset of Spo11-associated accessory proteins required for DSB residing on chromosome axes, suggesting that loop sequences are likely brought to chromosome axes prior to DSB formation (Blat *et al.* 2002).

Genome-wide mapping of Rec12, the Spo11 ortholog in the fission yeast *Schizosaccharomyces pombe*, reveals DSB hotspots at long intergenic regions and coldspots at repetitive elements including retrotransposon sequences and heterochromatin domains (Cromie *et al.* 2007; Yamada *et al.* 2013). *S. pombe* has 13 full-length long-terminal repeat (LTR) *Tf2* retrotransposons and >200 solo LTRs (Wood *et al.* 2002; Bowen *et al.* 2003). Similar to *Ty* retrotransposons in *S. cerevisiae* (Pan *et al.* 2011), LTRs and *Tf2*s appear to have relatively low amounts of Rec12-induced DSBs (Fowler *et al.* 2014). The factors contributing to *Tf2*s as DSB coldspots are not known.

We have previously shown that the CENP-B homologs (Abp1, Cbh1, and Cbh2) have a genome surveillance role for retrotransposons by cooperating with histone deacetylases and Set1 to maintain transcriptional repression and nuclear organization of *Tf2*s into *Tf* bodies (Cam *et al.* 2008; Lorenz *et al.* 2012). CENP-Bs also regulate HR at collapsed mitotic DNA replication forks, suggesting a link between CENP-Bs and HR (Zaratiegui *et al.* 2010). In this study, we investigate the roles of CENP-Bs in meiotic recombination. We find that among the CENP-B homologs, Abp1 has the most prominent role in suppressing HR at single and tandem *Tf2*s. However, Abp1 has a constructive role during HR, preventing gene conversion and ensuring faithful recombination exchange of homologous alleles flanking a *Tf2* element. Analysis of *abp1* domain mutants suggests that Abp1 likely utilizes both of its transcriptional silencing and nuclear organization functions to effectively suppress HR of *Tf2*s. Our analysis reveals that cells can dispense with the loss of any single chromatin-modifying factor with a demonstrable role in *Tf2* regulation to maintain HR suppression of *Tf2*s. Finally, we identify a functional cooperation between the histone H3K4 methyltransferase Set1 and Ku proteins of the nonhomologous end joining pathway (NHEJ) in preventing meiotic homologous recombination of *Tf2*s.

Materials and Methods

Strain construction

Gene deletion or marker insertion for meiotic recombination assay was constructed using heterologous modules as previously described (Lorenz *et al.* 2012). Double mutants were generated by standard genetic crosses (Moreno *et al.* 1991).

Condensin *cut14-206* mutant was obtained from the National Bio-Resource Project (NBRP) of the MEXT (Ministry of Education, Culture, Sports, Science and Technology) (Japan). All strains were maintained on yeast extract plus supplements (YES) media. The full strain list used in this study can be found in [Supporting Information, Table S2](#).

Meiotic recombination assay

For *Tf2-12* recombination assay, two parental strains were constructed with each containing either a *ura4⁺* or *kan* 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 *nat* or *his7⁺* cassette was inserted upstream or downstream of *spncRNA111*, respectively. For the *lys7* locus, a *kan* cassette was inserted 14 kb upstream of a *lys7* mutation. 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 [Pombe Minimal Glutamate (PMG) –Ura –His (*Tf2-7/8*) or PMG –Ura +G418 (*Tf2-12*)]. A total of 2000–4000 spores were spread to each selective media plate. A 1:50 dilution of spores was spread onto YES media to verify total viable spore count. After 5–7 days incubation at 30°, colonies growing on selective media were counted. To check for incidence of diploid progeny, a random sample of 25 colonies from the selective media was recultured on Edinburgh Minimal Media (EMM) minimal media and grown at 26° for 3 days to assay for sporulating diploids and with light microscopy examination for nonsporulating diploids based on cell sizes. Rates of diploid progeny were subtracted from the total putative recombinant colony count. All experiments were done in duplicate. A χ^2 test was used to assess the significant changes in HR between a given mutant relative to wild type.

Inverse PCR

Genomic DNA was extracted from putative recombinant colonies with phenol and chloroform as previously described (Forsburg and Rhind 2006). A 100-fold dilution of genomic DNA was cut with the restriction enzyme *NcoI* (Thermo) followed by ligation. 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

Aberrant *Tf2-7/8* recombinants (*his7⁺ ura4⁺*) that failed the inverse PCR assay were crossed with a *his7⁻ ura4⁻* strain on malt extract and then treated with glusulase as above.

Approximately 500 spores were plated on nonselective 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 numbers of screened colonies. Any *abp1* mutations were removed by outcrossing prior to testing for linkage.

Data availability

Strains (Table S2) are available upon request.

Results

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 S1, A and B) (Cromie *et al.* 2007; Yamada *et al.* 2013; Fowler *et al.* 2014), suggesting that *Tf2*s 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 *kan* gene cassette positioned either upstream or downstream of an active *Tf2-12* element, respectively (Figure 1A). We performed a random spore analysis (RSA) assay (Mastro and 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 to 1%, well-within the range of a typical locus (Young *et al.* 2002). This result indicates that compared to known meiotic HR coldspots such as centromeres (Ellermeier *et al.* 2010), *Tf2-12* behaves similarly to a “neutral” spot. We compare the rate of HR at *Tf2-12* with two other loci, *ste11*-associated long noncoding RNA (lncRNA) *SPNCRNA111* that contains a very strong Rec12 peak and *lys7*, which has no detectable Rec12 peak (Figure S1, C and D). The rate of HR at *lys7* (0.16% per kilobase) is comparable to that of *Tf2-12* (0.19% per kilobase) and two-fold less than that of *SPNCRNA111* (0.36% per kilobase) (Table S1). We next investigated the effects of CENP-B mutations on *Tf2-12* recombination. Loss of *abp1* resulted in a five-fold increase in recombination (Figure 1B). This appears to be specific to Abp1-associated *Tf2*s as loss of *abp1* has no significant effect on HR rates at *lys7* or *spncRNA111* (Figure S2). Cells lacking either *cbh1* or *cbh2* exhibited a slight increase in recombination, consistent with their auxiliary regulatory roles of *Tf2*s (Cam *et al.* 2008). Chromosome missegregation during meiosis could give rise to diploids or viable aneuploid spores that could be mistaken for recombinant progeny. In particular, fission yeast cells with disomy of chromosome III, which contain *Tf2-12* are viable. We confirmed by PCR genotyping that all (16/16) of the recombinant progeny have both the *ura4* and *kan* cassette at the expected location (Figure 1C).

Whereas most *Tf2*s are spaced apart on the chromosomes, *Tf2-7* and *Tf2-8* exist as tandem retrotransposons. We asked whether the tandem arrangement of retroelements is regu-

Figure 1 Loss of CENP-B homologs increases meiotic recombination at a retrotransposon. (A) Schematic diagram of marker gene inserts and their distances from *Tf2-12*. (B) Relative fold of meiotic recombination relative to wild type at *Tf2-12* in indicated CENP-B mutants. (C) 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 parental strains (P₁ and P₂) and random recombinant progeny (1–5).

lated 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 2A). The rate of recombination varied ~1–2% (Table S1), a rate that is slightly higher than those at *Tf2-12*, likely due to the larger distance between the reporter genes (~11.4 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 *abp1Δ* and ~1.5-fold for either *cbh1Δ* or *cbh2Δ* (Figure 2B).

To verify that recovered progeny containing both selectable markers were true recombinants, inverse PCR was used to verify the relative position of both markers. We confirmed that the majority (70%) of scored recombinant progeny carry the expected flanking markers (Figure 2C). Collectively, our results revealed a role for CENP-Bs in regulating HR at solo and tandem *Tf2* retrotransposons.

Abp1 prevents gene conversion and contributes to proper 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 *abp1Δ* 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 3A). Whereas there were no significant differences in the expected numbers among the various classes of progeny in wild type, loss of *abp1* resulted in a disproportional number of one recombinant class over the other (Figure 3, B and C). This result suggests that Abp1 helps prevent gene conversion of homologous alleles flanking *Tf2*s.

Figure 2 Increased meiotic recombination at a tandem retrotransposon in CENP-B mutants. (A) Schematic diagram of marker gene inserts and their distances from the tandem *Tf2-7/8*. (B) Relative fold of meiotic recombination relative to wild type in indicated CENP-B mutants at *Tf2-7/8*. (C) 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,” the latter as 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 with primer pair “b” located outside of marker genes and closer to the *NcoI* (N) cut site. Red asterisk indicates possible aberrant recombination of marker genes. Negative controls using DNA from diploid parents confirmed that amplicon “a” was specific to recombinant progeny (data not shown).

We noticed that ~30% of recovered progeny from wild-type and *abp1Δ* crosses appeared to be genuine recombinants of *Tf2-7/8* (euploid) but did not pass the inverse PCR test. We reasoned that 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 (see Figure 2C). To test these scenarios, we performed backcrosses of these progeny to determine the linkage between the markers. For the *abp1Δ* aberrant recombinants, *abp1* mutation was first outcrossed with a wild type prior to linkage analysis. The genetic distance between the two markers is ~1.23 cM (Figure S3A), based upon our previous test (Figure 2A). Among the aberrant recombinants, the *abp1Δ* mutants showed a much higher average linkage compared to wild type (Figure S3B). Deletion of *abp1* also resulted in much greater variance of linkage in all tested strains, ranging from <0.4 cM increase to as high as 1.1 cM, with the average net change significantly higher in *abp1Δ* compared to that of wild type ($P < 0.05$) (Figure S3C). Thus, while Abp1 contributes to HR suppression of *Tf2*s 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 domain containing the

Figure 3 Abp1 prevents gene conversion of *Tf2*-associated marker gene. (A) 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. (B and C) Plot of percentage of progeny with parental (B) or recombinant (C) genotypes from reciprocal crosses in wild-type and *abp1Δ* backgrounds.

conserved residues resembling the DDE motif found in the catalytic site of *pogo* transposases (DDE), and C-terminal dimerization domain (DIM) (Figure 4A). We have previously shown that while all three domains are required for the nuclear organization of *Tf2*s into *Tf* bodies, only the DBD and DDE domains are required for the silencing of *Tf2*s (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 *Tf2*s, we investigated the contribution of Abp1 domains to HR suppression of *Tf2*s.

Each domain deletion mutant produced a significant increase in HR at *Tf2-7/8*, with the dimerization domain mutant producing the strongest increase ($P < 0.01$) (Figure 4B). However, none of these domain mutants produced an increase as high as that of *abp1Δ*, suggesting cooperation among the domains, perhaps requiring both functions of Abp1 in transcriptional silencing and *Tf* body formation to effectively repress HR of *Tf2*s.

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

We have recently shown that the histone methyltransferase Set1 can dispense with H3K4me in the repression of *Tf2*s but depend on H3K4me and the Set1C/COMPASS complex for the clustering of *Tf2*s into *Tf* bodies (Mikheyeva *et al.* 2014). We assessed the contribution to HR of *Tf2*s 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 *et al.* 2013). However, the deletion of these genes produced only slight increases (though not significant) in HR at *Tf2-7/8*, and not to the levels seen in *abp1Δ* (Figure 5A).

In addition to Set1C, *Tf2*s 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,

Figure 4 Suppression of homologous recombination at *Tf2s* requires all three domains of Abp1. (A) Schematic of the domains of Abp1: DNA-binding (DBD) domain, pogo-like (DDE) domain, and dimerization (DIM) domain. (B) Rates of meiotic recombination at *Tf2-7/8* in *abp1* domain deletion mutants and *abp1Δ* relative to wild type.

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 *clr3Δ* mutant ($P < 0.05$) (Figure 5B).

Histone chaperone Hip1 and nuclear organizer Lem2 are not required for HR suppression of *Tf2s*

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 *hip1Δ* (Greenall *et al.* 2006; Cam *et al.* 2008), it did not result in any appreciable alteration in HR frequency at *Tf2-7/8* (Figure 5C). We also investigated the possibility of a 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 (Hiraoka *et al.* 2011; Gonzalez *et al.* 2012). We found that similar to *clr3Δ*, there was a reduction in HR at *Tf2-7/8* in cells deficient in *lem2* (Figure 5D). Collectively, our results suggest that cells can dispense with any single chromatin-modifying factor or nuclear-envelope protein to maintain HR suppression of retrotransposons.

Set1 cooperates with the NHEJ pathway to suppress HR of *Tf2s*

Abp1 has been shown to recruit the Ku proteins of the 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 NHEJ pathway. Loss of *pku80*, which encodes

Figure 5 Effects of histone modification and chromatin organization mutants on meiotic recombination at *Tf2-7/8*. (A–D) Relative rates of meiotic recombination in Set1C/COMPASS mutants (A), HDAC mutants *clr3Δ* and *hst4Δ* (B), the histone chaperone mutant *hip1Δ* (C), and the inner nuclear envelope mutant *lem2Δ* (D).

one of the Ku heterodimer proteins, resulted in 1.6-fold increase of HR (Figure 6A), comparable to increases seen in *cbh1Δ* and *cbh2Δ*. Our results suggest that no single known factor shown to be recruited by Abp1 could account fully for the HR increase of *Tf2s* in *abp1Δ* strain.

We explored the possibility that multiple factors from distinct pathways could act redundantly to restrict HR at *Tf2s*. We tested this idea by examining HR in a strain deficient for both *set1* and *pku80*. Whereas loss of *set1* has a negligible effect on HR at *Tf2s*, a double mutant of *set1Δ pku80Δ* exhibited an appreciable increase of HR relative to *pku80Δ* alone (Figure 6A). Together, our results suggest that Abp1 likely suppresses HR of *Tf2s* in part through cooperation between Set1 and the NHEJ pathway.

Chromosome architectural protein condensin suppresses HR of *Tf2s*

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 *et al.* 2010; Mizuguchi *et al.* 2014). 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 (Ponticelli and Smith 1989; Lin *et al.* 1992; Molnar *et al.* 1995; Watanabe and 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

Figure 6 Cooperation between Set1 and Ku proteins to suppress HR at *Tf2*-7/8. (A and B) Relative rates of meiotic recombination in *set1Δ*, *ku80Δ*, and *set1Δ ku80Δ* (A) and condensin *cut14* mutant (B). (C) 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.

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 *Tf2*s and contributes to *Tf* body formation (Tanaka *et al.* 2012). We examined whether condensin regulates HR of *Tf2*s. Mutation of the condensin subunit *Cut14* results in a moderate increase of HR around *Tf2*-7/8 (Figure 6B). Our results reveal a role for higher-order chromatin organization involving architectural protein to suppress HR at interspersed repeats.

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.* 2008; d'Alencon *et al.* 2011; Mateo and 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 the silencing and nuclear organization of *Tf2*s, CENP-Bs restrict meiotic HR of *Tf2* retrotransposons.

Homologous recombination at repetitive DNA can produce gross genome alterations including deletions, duplications, and inversions (Hoang *et al.* 2010; Sasaki *et al.* 2010; Rattray *et al.* 2015). These structural variants are implicated in many human diseases such as Gaucher's disease and

chronic lymphocytic leukemia (Lopez *et al.* 2012; Campbell *et al.* 2014). 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 is likely 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 alleles. *Tf2* and its related *Tf1* preferentially integrate into the promoters of RNA polymerase II (Pol II) transcribed genes (Bowen *et al.* 2003). In addition to *Tf2*s, CENP-Bs also bind solo LTRs (Cam *et al.* 2008; Zaratiegui *et al.* 2010). CENP-B bound LTRs could contribute novel regulatory modules to nearby promoters of Pol II genes (Cam *et al.* 2008). In *Drosophila miranda*, rapid 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 *Tf2*s 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 reveals that HR coldspots do not necessarily exclude Rec12 from certain regions such as centromeres (Ludin *et al.* 2008; Fowler *et al.* 2014). 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).

The lncRNA *spncRNA111* Rec12 hotspot is located near the *ste11* promoter occupied by Atf1 (Lorenz *et al.* 2014), suggesting that Rec12 hotspot formation at lncRNA *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%) and tandem *Tf2*-7/8 (1.5%) are comparable to the genome averages for the expected distances (0.96% and 1.24%, respectively) (Young *et al.* 2002). Our results suggest that while *Tf2*s 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 *Tf2*s (Figure 6C). Considering these proteins have distinct functions (*i.e.*, Ku80 in NHEJ, Set1-catalyzed H3K4me *Tf* body formation, and Set1-mediated H3K4me-independent repression of *Tf2*s), it is likely that multiple layers of controls

from several distinct pathways act to limit meiotic recombination of *Tf2*s and repetitive elements in general.

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Supporting Information

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Suppression of Meiotic Recombination by CENP-B Homologs in *Schizosaccharomyces pombe*

Peter Johansen and Hugh P. Cam

Figure S1. Distributions of Abp1 and Rec12 binding at studied loci. Binding of Abp1 and Rec12 at (A) *Tf2-12*, (B) tandem *Tf2-7/8*, (C) long noncoding RNA (lncRNA) *SPNCRNA.111* and (D) *lys7* 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 meiotic HR assay. For measuring HR at *lys7*, a parent strain carrying a *kan* cassette inserted near *stm1* and a *lys7* null allele was crossed with another parent strain carrying a wildtype *lys7*⁺.

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Figure S2. Loss of *abp1* does not significantly alter meiotic recombination at Rec12-associated hotspot or coldspot. A) Schematic diagram of marker gene inserts around a Rec12-hotspot (red triangles) near the lncRNA *spncRNA111*. B) Rates of meiotic recombination in *abp1Δ* relative to wildtype at *spncRNA111*-associated hotspot. C) Schematic diagram of marker gene inserts around a Rec12 coldspot near *lys7*. D) Rates of meiotic recombination at *lys7* in *abp1Δ* relative to wildtype. Recombinants were recovered from germinated spores grown on media containing clonNAT (Nourseothricin; 100 ug/mL) minus histidine (B) or G418 (150 ug/mL) minus lysine (D). Recombination rates were adjusted for diploids.

Figure S3. Loss of *abp1* affects the fidelity of allelic recombination flanking *Tf2-7/8*. A) Rates of recombination expressed as centimorgan (cM) of aberrant recombinants from both wildtype and *abp1* null (*abp1*Δ) backgrounds. (B) 1-D scatter plot of genetic distances between the marker genes in aberrant recombinants derived from wildtype and *abp1*Δ background crosses. (C) 1-D scatter plot of net change in genetic distance between the tested strains and that of wildtype (1.23 cM). Upper and lower lines represent the standard deviation. Middle line corresponds to the mean value of the group. Significant differences between the recombinant groups of *abp1*Δ and wildtype (B-C: $p < 0.05$) was assessed using unpaired t-test.

Table S1 Recombinant progeny recovered from meiotic crosses expressed as percent of total spores screened

Rate of Recombination at <i>Tf2-12</i>					
Strains	Percent recombinant	Total spores screened			
wildtype	0.96%	5525			
<i>abp1</i> Δ	4.81%	9050			
<i>cbh1</i> Δ	2.13%	6250			
<i>cbh2</i> Δ	1.35%	6940			
<i>cbh1</i> Δ <i>cbh2</i> Δ	1.69%	8500			
Rate of Recombination at <i>Tf2-7/Tf2-8</i>					
wildtype	1.24%	7575			
<i>abp1</i> Δ	4.62%	4050			
<i>cbh1</i> Δ	1.85%	11400			
<i>cbh2</i> Δ	1.91%	4275			
<i>cbh1</i> Δ <i>cbh2</i> Δ	1.45%	8925			
<i>abp1-DBD</i> Δ	1.81%	8050			
<i>abp1-DDE</i> Δ	1.88%	6900			
<i>abp1-DIM</i> Δ	2.86%	16530			
<i>hip1</i> Δ	1.05%	12650			
<i>clr3</i> Δ	0.56%	19200			
<i>set1</i> Δ	1.45%	13275			
<i>spp1</i> Δ	1.46%	7275			
<i>lem2</i> Δ	0.78%	9525			
<i>hst4</i> Δ	1.52%	9300			
<i>pku80</i> Δ	2.18%	10575			
<i>pku80</i> Δ <i>set1</i> Δ	2.49%	17475			
<i>cut14-208</i>	1.92%	20250			
Rate of Recombination at <i>lncRNA spncRNA111</i> -associated Rec12 Hotspot					
wildtype	3.44%	11775			
<i>abp1</i> Δ	4.51%	10300			
Rate of Recombination at <i>lys7</i>					
wildtype	2.24%	16900			
<i>abp1</i> Δ	2.58%	6700			
Distribution of Progeny Genotypes in Reciprocal Cross at <i>Tf2-7/Tf2-8</i>					
Strains	<i>his7⁺ura4⁺</i>	<i>his7⁻ura4⁻</i>	<i>his7⁺ura4⁻</i>	<i>his7⁻ura4⁺</i>	Total
wildtype	1256	1231	27	15	2529
<i>abp1</i> Δ	431	483	34	2	950

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Table S2 Strains used in this study

Strain	
PJ26	<i>h+ ura4-DS/E leu1-32 Tf2-12::ura4⁺ ade6-M210</i>
PJ10	<i>h- ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216</i>
PJ15	<i>mat1Mst0 ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216 abp1Δ::LEU2</i>
PJ42	<i>h+ ura4-DS/E leu1-32 Tf2-12::ura4⁺ M ade6- M210 abp1Δ::LEU2</i>
PJ19	<i>h+ ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M210 cbh1Δ::LEU2</i>
PJ36	<i>mat1Mst0 ura4-DS/E leu1-32 Tf2-12:: ura4⁺ ade6-M216 cbh1Δ::LEU2</i>
PJ34	<i>mat1Mst0 ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216 cbh2Δ::LEU2</i>
PJ46	<i>h+ ura4-DS/E leu1-32 Tf2-12::ura4 ade6-M210 cbh2Δ::LEU2</i>
PJ35	<i>mat1Mst0 ura4-DS/E leu1-32 Tf2-12::kanMX ade6-M216 cbh1Δ::LEU2 cbh2Δ::LEU2</i>
PJ72	<i>h+ ura4-DS/E leu1-32 Tf2-12::ura4⁺ ade6-M210 cbh1Δ::LEU2 cbh2Δ::LEU2</i>
PJ130	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺</i>
PJ78	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8::ura4⁺</i>
PJ94	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ abp1Δ::LEU2</i>
PJ98	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) abp1Δ::LEU2</i>
PJ87	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ cbh1Δ::LEU2</i>
PJ91	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) cbh1Δ::LEU2</i>
PJ100	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ cbh2Δ::LEU2</i>
PJ86	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) cbh2Δ::LEU2</i>
PJ125	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ cbh1Δ::LEU2 cbh2Δ::LEU2</i>
PJ126	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) cbh1Δ::LEU2 cbh2Δ::LEU2</i>
PJ131	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ abp1-DBDΔ-(3X)FLAG::kanMX</i>
PJ114	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) abp1-DBDΔ-(3X)FLAG::kanMX</i>
PJ120	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ abp1-DDEΔ-(3X)FLAG::kanMX</i>
PJ132	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) abp1-DDEΔ-(3X)FLAG::kanMX</i>
PJ133	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ abp1-DIMΔ-(3X)FLAG::kanMX</i>
PJ116	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) abp1- DIMΔ-(3X)FLAG::kanMX</i>
PJ136	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ clr3Δ::kanMX</i>
PJ137	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) clr3Δ::kanMX</i>
PJ138	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ set1Δ::kanMX</i>
PJ161	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) set1Δ::kanMX</i>
PJ157	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) hip1Δ::kanMX</i>
PJ158	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ hip1Δ::kanMX</i>
PJ163	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) spp1Δ::kanMX</i>
PJ164	<i>h- ura4-DS/E leu1-32 his7Δ Tf2-7::his7⁺ spp1Δ::kanMX</i>
PJ174	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ lem2Δ::kanMX</i>
PJ175	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) lem2Δ::kanMX</i>
PJ165	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2::ura4⁺) hst4Δ::kanMX</i>
PJ176	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ hst4Δ::kanMX</i>
PJ181	<i>h- leu1-32 stm1::kanMX lys7Δ</i>
HC199	<i>h+ ade6-M216 ura4-DS/E OtrR1::ura4⁺ leu1-32</i>
PJ182	<i>h- leu1-32 stm1::kanMX lys7Δ abp1Δ::LEU2</i>
HC133	<i>h+ ade6-M216 ura4-DS/E OtrR1::ura4⁺ leu1-32 abp1Δ:: LEU2</i>
MD22	<i>h- ade6-M216 ura4-D18 leu1-32 his7Δ::lys2⁺ SPBC26H8.12::his7⁺</i>
MD27	<i>h+ ade6-M210 ura4-D18 leu1-32 his7Δ::lys2⁺ stel1::natMX</i>
PJ185	<i>h- leu1-32 his7Δ::lys2⁺ stel1::natMX abp1Δ::LEU2</i>
PJ186	<i>h+ leu1-32 his7Δ::lys2⁺ SPBC26H8.12::his7⁺ abp1Δ::LEU2</i>
PJ192	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ pku80Δ::kanMX</i>
PJ195	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2Δ::ura4⁺) pku80Δ::kanMX</i>
PJ198	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ pku80Δ::kanMX set1Δ::kanMX</i>
PJ207	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2Δ::ura4⁺) pku80Δ::kanMX set1Δ::kanMX</i>
PJ211	<i>h- ura4-DS/E leu1-32 his7Δ Tf2-7::his7⁺ cut14-208(ts)</i>

PJ212	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-8(pyp2Δ::ura4⁺) cut14-208(ts)</i>
PJ213	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ Tf2-8(pyp2Δ::ura4⁺) abp1Δ::LEU2</i>
PJ214	<i>h- ura4-DS/E leu1-32 his7Δ::lys2⁺ abp1Δ::LEU2</i>
PJ215	<i>h+ ura4-DS/E leu1-32 his7Δ::lys2⁺ Tf2-7::his7⁺ Tf2-8(pyp2Δ::ura4⁺)</i>
CHP1053	<i>h- ade6-M216 ura4-D18 leu1-32 his7Δ::lys2⁺</i>

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