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The Compact Chromatin Structure of a Ty Repeated Sequence Suppresses Recombination Hotspot Activity in *Saccharomyces cerevisiae*

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Summary

Recombination between repeated DNA sequences can have drastic consequences on the integrity of the genome. Repeated sequences are abundant in most eukaryotes, yet the mechanism that prevents recombination between them is currently unknown. Ty elements, the main family of dispersed repeats in *Saccharomyces cerevisiae*, exhibit low levels of exchange. Other regions in the genome have relatively high rates of meiotic recombination (hotspots). We show that a Ty element adjacent to the *HIS4* recombination hotspot substantially reduces its activity, eliminating local DSB formation. We demonstrate that the Ty has a closed (nuclease-insensitive) chromatin configuration that is also imposed on the flanking DNA sequences. The compact chromatin structure is determined by sequences at the N terminus of the Ty. Increased binding of the Rap1 protein to the hotspot restores both open chromatin conformation and DSB formation. The chromatin configuration of Ty elements precludes initiation of recombination, thus preventing potentially lethal exchanges between repeated sequences.

Introduction

Homologous recombination is a universal process required for the efficient repair of double-strand DNA breaks (DSBs) and, in most eukaryotes, is needed for the accurate segregation of homologous chromosomes during the first meiotic division. In addition, recombination plays an important role in evolution by generating novel combinations of alleles. In the yeast *Saccharomyces cerevisiae* and many other eukaryotes, meiotic recombination is initiated by DSBs catalyzed by the DNA topoisomerase II-related protein Spo11 (Bergerat et al., 1997; Keeney et al., 1997). Following DSB formation, the broken DNA ends are resected (5′ to 3′), and the resulting 3′-protruding ends invade the homologous chromosome, resulting in the formation of heteroduplex DNA (hDNA). Correction of any mismatches present in the hDNA may result in gene conversion, the nonreciprocal transfer of information between the two interacting alleles. If left uncorrected, hDNA gives rise to postmeiotic segregation (PMS) of the parental alleles. In addition, the joined DNA molecules can also be resolved to generate reciprocal recombination events (reviewed by Merker et al., 2003).

Meiotic recombination occurs more frequently in some regions of the eukaryotic genome than in others. Thus, there is no simple linear relationship between the genetic and physical genome maps (Lichten and Goldman, 1995; Petes, 2001). The regions that show relatively high and relatively low levels of recombination are called hotspots and coldspots, respectively. Global genome-wide analyses, as well as studies of individual hotspots, demonstrate that hotspots have high levels of local DSBs, whereas coldspots often have no detectable local DSBs (Baudat and Nicolas, 1997; Gerton et al., 2000). In addition, since gene conversion and PMS (both non-Mendelian segregation events) originate from heteroduplexes initiated at DSB sites, hotspots exhibit high levels, and coldspots low levels, of non-Mendelian segregation for adjacent markers.

Markers near the 5′ end of *HIS4* and the 3′ end of the neighboring gene *BIK1* have very high levels of non-Mendelian segregation as a consequence of a strong DSB site at the 3′ end of *BIK1* (Detloff et al., 1992; Fan et al., 1995). Formation of the DSB associated with the *HIS4* hotspot requires the binding of the Bas1p, Bas2p, and Rap1p transcription factors (Fan et al., 1995). The requirement for transcription factor binding, however, does not represent a requirement for high levels of transcription, since deletion of the *HIS4* TATA box, which reduces *HIS4* expression at least 20-fold, has no effect on hotspot activity (White et al., 1992). It has been suggested that the binding of transcription factors stimulates recombination either by opening the chromatin (allowing access to the recombination machinery) or by directly tethering the recombination machinery to the chromosome (Fan and Petes, 1996; Koren et al., 2002). A correlation has been observed between preferred sites for DSB formation and nuclease-hypersensitive regions in chromatin (Borde et al., 1999; Fan and Petes, 1996; Mizuno et al., 2001; Ohta et al., 1994; Wu and Lichten, 1994). It appears that nuclease-hypersensitive regions are necessary, although not sufficient, for DSB formation (Fan and Petes, 1996; Wu and Lichten, 1994).

Although hotspots have been extensively studied, less is known about recombination coldspots. Several regions in the genome of *Saccharomyces cerevisiae* with low meiotic recombination have been described, including centromeric regions (Baudat and Nicolas, 1997; Lambie and Roeder, 1988) subtelomeric sequences (Klein et al., 1996), and dispersed Ty elements (Kupiec and Petes, 1988a, 1988b). Ty elements are ~6 kb long retrotransposons flanked by two long terminal repeats (LTRs). Tys are present in 30–40 copies per haploid cell, representing ~3% of the genome (Kim et al., 1998). They are structurally and functionally similar to retroviruses and have a similar (although completely intracellular) life cycle. The spontaneous level of Ty-Ty recombination events is relatively low, and despite their high copy number, most of these events are gene con-

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versions rather than crossovers. For these reasons, chromosome rearrangements mediated by Ty-Ty recombination are rare (Kupiec and Petes, 1988a, 1988b). The mechanism that prevents Ty recombination remains unknown.

Below, we show that a naturally transposed Ty element inserted near the *HIS4* recombination hotspot results in loss of hotspot activity monitored genetically and physically. We show that the Ty element has a closed chromatin configuration, which is also imposed upon its neighboring sequences, leading to the loss of hotspot activity. Our results show that the chromatin configuration of Ty elements precludes initiation of recombination, thus providing a mechanism by which the genomic instability caused by recombination between repeated sequences is prevented.

Results

Heterozygous markers in the *HIS4* and *BIK1* genes have very high rates of non-Mendelian segregation (gene conversion and postmeiotic segregation). These events are initiated at a strong DSB site located near the 3′ end of the *BIK1* gene. The hotspot activity is particularly strong in diploids of the AS4/AS13 genetic background (Stapleton and Petes, 1991). Whereas the rate of non-Mendelian segregation for an average yeast marker is 4% or less (Petes et al., 1991), markers located within the *HIS4* (*his4-lopc* or *his4-IR9*) or *BIK1* (*bik1-IR16*) genes have rates of non-Mendelian segregation as high as 37%–42% (Detloff et al., 1992; Porter et al., 1993). In contrast to the *HIS4* hotspot, the rate of non-Mendelian segregation events involving Ty elements is low, approximately 0.7% (Kupiec and Petes, 1988a). In order to study the properties that determine hotspot/coldspot activity, we created yeast strains in which a naturally transposed Ty element resides in close proximity to the *HIS4* hotspot. As described below, we demonstrate by genetic and physical analyses that the Ty element is capable of greatly reducing the activity of the *HIS4* hotspot.

The Ty917::*URA3* Element Reduces *HIS4* Hotspot Activity

Ty917 was isolated as a spontaneous insertion affecting *HIS4* expression (Roeder and Fink, 1982). This retrotransposon was cloned and marked with a *URA3* gene (Ty917::*URA3*). The marked element was inserted 70 bp upstream of the *HIS4* coding sequence (*his4-917U*) in both copies of chromosome *III*. This insertion does not disrupt the transcription factor binding sites required for hotspot activity (White et al., 1993) and is about 300 bp from the hotspot-associated site of DSB formation (Fan et al., 1995). We then introduced a short palindromic insertion into one of the *his4-917U* alleles, generating the *his4-917u* allele. Palindromic insertions, when present as mismatches in heteroduplexes formed during recombination, are inefficiently repaired, resulting in high rates of postmeiotic segregation, which provide a more accurate estimate of hotspot activity (Nag et al., 1989). Similar short palindromic repeats were also inserted into one of the two copies of *HIS4* (*his4-lopc*) and *BIK1* (*bik1-IR16*). As discussed above, we have previously analyzed

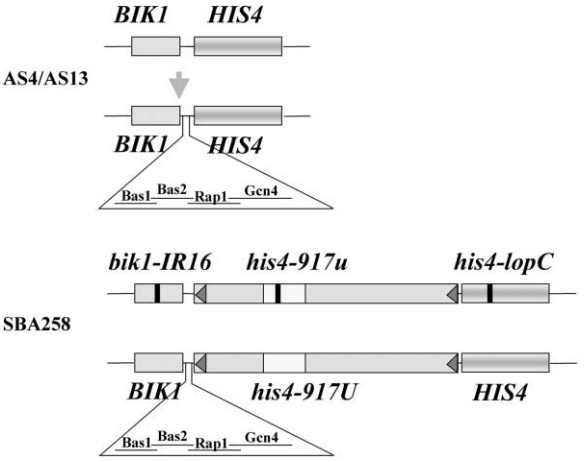

Figure 1. Schematic Representation of the AS4/AS13 Parental Diploid and of SBA258, the Strain Used for Genetic Analysis

SBA258 is homozygous for the insertion of the *URA3*-tagged Ty917 element located upstream of *HIS4* and heterozygous for mutations caused by the insertion of small palindromic insertions within *URA3* (resulting in the *his4-917u* allele), *BIK1* (*bik1-IR16*), and *HIS4* (*his4-lopc*). Triangles indicate the long terminal repeats (LTRs) at the end of Ty917::*URA3*. Arrows represent the site of DSB formation at the recombination hotspot in the control strain.

the non-Mendelian segregation of these two alleles. Thus, the diploid strain used in our genetic studies (SBA258) was heterozygous for three markers near the DSB site (Figure 1). The genotypes of SBA258 and other diploid strains used in our study are shown in Table 1.

The diploid SBA258 was induced to undergo meiosis, and tetrad analysis was carried out. The rates of non-Mendelian segregation for *bik1-IR16*, *his4-917u*, and *his4-lopc* were 21%, 17%, and 18%, respectively. For *bik1-IR16* and *his4-lopc*, these levels of aberrant segregation are approximately half those observed in wild-type strains (Detloff et al., 1992; Porter et al., 1993). Although it could be argued that the reduction in the rate of non-Mendelian segregation of the *his4-lopc* marker reflects its displacement from the DSB site at the 3′ end of *BIK1*, this argument cannot explain the reduction in the non-Mendelian segregation rate of *bik1-IR16*. In addition, markers located at a similar distance from the DSB site as the *his4-917u* allele have been also shown to exhibit 35%–40% non-Mendelian segregation (Detloff et al., 1992). The genetic analysis, therefore, shows that coldspot features affect hotspot activity in a dominant fashion. To examine this phenomenon on a molecular level, we monitored meiosis-specific DSB formation.

Meiosis-specific DSBs are usually examined in *rad50S* diploid strains, since this mutation prevents processing of the DNA ends resulting from the breaks, simplifying their quantitation (Cao et al., 1990). Consequently, we measured hotspot-associated DSBs in a *rad50S* derivative of SBA258 (SBA256) and in an isogenic control lacking the Ty917::*URA3* insertion (FX3). The expected hotspot-associated DSB was clearly seen in the control strain FX3 (representing 6.7% of the total DNA); in contrast, the break was absent in SBA256 ($<$0.5% of the total DNA; Figure 2A). Moreover, no DSBs could be seen within the Ty917::*URA3* insertion. The

Table 1. Relevant Genotypes of Diploid Strains

Strain Name	Alterations Upstream of *HIS4*	Other Alterations
DNY26	None	*his4-lopc/HIS4*
FX3	None	*his4-lopc/HIS4 rad50S::URA3/rad50S::URA3*
SBA258	*his4-917U/his4-917u* (Ty insertion)	*BIK1/bik1-IR16 HIS4/his4-lopc ura3Δ::kanMX/ura3Δ::kanMX*
SBA256	*his4-917U/his4-917u* (Ty insertion)	*BIK1/bik1-IR16 HIS4/his4-lopc ura3Δ::kanMX/ura3Δ::kanMX rad50S::ura3/rad50S::URA3*
SBA286	*his4-CU/his4-Cu* (control non-Ty insertion)	*BIK1/bik1-IR16 HIS4/his4-lopc ura3Δ::kanMX/ura3Δ::kanMX*
SBA322	*his4-CU/his4-CU* (control non-Ty insertion)	*BIK1/bik1-IR16 HIS4/his4-lopc ura3Δ::kanMX/ura3Δ::kanMX rad50S::URA3/rad50S::ura3*
SBA329	*his4-917ULTRΔ his4-917ULTRΔ* (Ty lacking LTRs)	*ura3Δ::kanMX/ura3Δ::kanMX rad50S::URA3/rad50S::ura3*
SBA331	*his4-soloLTR/his4-soloLTR* (single LTR insertion)	*ura3Δ::kanMX/ura3-1 rad50S::URA3/rad50S::URA3*
SBA345	*his4-917UΔN/his4-917UΔN* (Ty lacking N terminus)	*ura3Δ::kanMX/ura3Δ::kanMX rad50S::URA3/rad50S::ura3*
SBA346	*his4-917UΔC/his4-917UΔC* (Ty lacking C terminus)	*ura3-1/ura3-1 rad50S::ura3/rad50S::ura3*
FX2	*his4-51/his4-51* (mutated Rap1p binding site)	*his4-lopc/HIS4 rad50S::URA3/rad50S::URA3*
FX4	*his4-202/his4-202* (extra Rap1p binding sites)	*his4-lopc/HIS4 rad50S::URA3/rad50S::URA3*
SBA321	*his4-202, 917U/his4-202, 917U* (extra Rap1p binding sites and Ty insertion)	*ura3Δ::kanMX/ura3Δ::kanMX rad50S::URA3/rad50S::URA3*

All strains are isogenic except for changes introduced by transformation or by mating with isogenic strains. Only differences from the progenitor genotype of the AS4 × AS13 diploid are shown. The names of the haploid strains used in the construction of the diploids are given in the Strain Construction section of the Supplemental Materials. The genotypes of the haploids are in Supplemental Tables S1 and S2.

absence of DSB formation in the region was confirmed by carrying out Southern blot analysis of DNA digested with several additional restriction enzymes, such as PvuII, PstI and SphI (data not shown). The coldspot effect was specific for the sequences flanking the Ty, since DSBs were detected at comparable levels at a different recombination hotspot, *ARG4*, in both FX3 and SBA256 (2.5% and 2.1% of the total DNA, respectively; Figure 2B). It should be noted that the Ty917::*URA3* insertion eliminates the DSB associated with the *HIS4* hotspot but does not result in very low gene conversion levels for the *HIS4* marker. The relatively high level of *HIS4* conversion, in the absence of an upstream DSB, has been observed previously (Fan et al., 1995) and reflects heteroduplex formation initiated at a DSB located downstream of the *HIS4* locus (Merker et al., 2003).

The Reduced Level of Hotspot Activity Is Specific to the Ty917::*URA3* Insertion

To test whether the observed effects are specific to the Ty element or could be mimicked by any insertion of a similar size, we constructed a strain (SBA322) that had a 7 kb fragment of *URA3* and flanking sequences inserted at the same position as Ty917::*URA3* (*his4-CU*). SBA322 retained the hotspot-associated DSB near the end of *BIK1* (band a, 2.5% of total DNA; Figure 2C). We therefore conclude that the reduction in hotspot activity observed in SBA256 is Ty specific, and not merely due to the presence of a large insertion. In fact, the hotspot activity affected the CU insert, creating, in addition to a DSB at the border of the insert (band b, 2.6% of total DNA), a strong DSB within the CU insert (band c, 6.3% of total DNA; Figure 2C). No such DSB occurs at its regular genomic location on chromosome *V* (Gerton et al., 2000; and data not shown). We also carried out tetrad analysis of a strain homozygous for the CU insertion (SBA286, a *RAD50* version of SBA322). As for SBA258, this strain was heterozygous for palindromic insertions at *BIK1, URA3*, and *HIS4*. As expected from the molecular analysis, the level of non-Mendelian segregation of the *BIK1-HIS4* region in this strain was very high, and similar to that previously described (Detloff et al., 1992; Porter et al., 1993) for strains without any insertion (39%, 37%, and 39% non-Mendelian segregation at *bik1-IR16, his4-Cu*, and *his4-lopc*, respectively) These results confirm that the reduction in hotspot activity is specific for the Ty element.

Interestingly, whereas the Ty insertion had a strong effect on the level of non-Mendelian segregation (which represents formation of heteroduplex DNA), it had a more modest effect on crossing-over. The genetic distance between *bik1-IR16* and *his4-Cu* was 16 cM in SBA286 (carrying the CU insert) and 11 cM in SBA258 (carrying the Ty917 insert). This result is not inconsistent with our conclusion that the Ty917 element reduces the rate of hotspot activity, since many of the events initiated at the *HIS4* hotspot are likely to be resolved as crossovers downstream of the flanking markers (Merker et al., 2003). A relatively high level of crossovers in regions devoid of DSBs has been previously observed (Borde et al., 1999). They may arise from events initiated outside of the interval (Borde et al., 1999).

Determinants of Coldspot Activity within Ty917::*URA3*

Ty elements function as independent transcriptional units and, as such, are able to transpose to new locations in the genome. To localize the region within the Ty responsible for suppressing the *HIS4* hotspot, we constructed strains with deletion derivatives of Ty917::*URA3*. We reasoned that the flanking LTRs may work as insulators or boundary elements (West et al., 2002), defining a Ty-specific chromatin domain that could be responsible for suppressing DSB formation. To test this hypothesis, we analyzed DSB formation in two additional yeast strains. In SBA329, both LTRs of Ty917::*URA3* were deleted (*his4-917ULTR*Δ), whereas in SBA331 all the internal Ty sequences were eliminated, leaving a single LTR in their place (*his4-soloLTR*). If sequences within the LTRs are responsible for the Ty chromatin configuration, then elimination of these sequences might result in reestablishment of the DSB, whereas the LTR may by itself disrupt the hotspot activ-

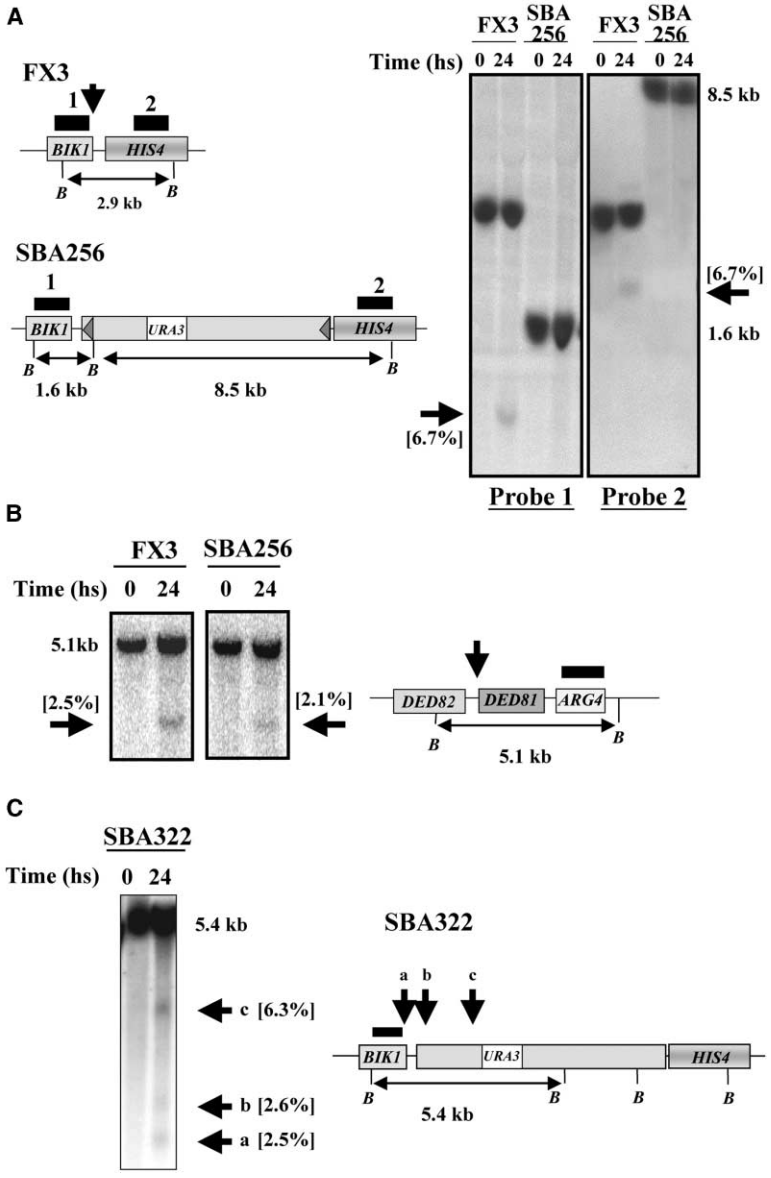

Figure 2. The Ty917::*URA3* Insertion Reduces DSB Formation at the *HIS4* Hotspot

The hybridization probes used are indicated by black rectangles. The position of the meiosis-specific DSB is indicated by an arrow, and the positions of BglII sites are indicated by the letter B. The intensity of the DSBs is shown between brackets, expressed as percentage of total DNA. When no DSB was detected, its level is below 0.5% of the total DNA.

(A) DSB formation at the *HIS4* hotspot in strains FX3 (parental control) and SBA256 (*his4-917U*/*his4-917U*). DNA was isolated from cells of FX3 and SBA256 immediately before (0) and after 24 hr in sporulation medium. The DNA was treated with BglII, and the resulting fragments were examined by Southern analysis. *BIK1* and *HIS4* sequences were used as probes (probes 1 and 2, respectively).

(B) DSB formation at the *ARG4* locus in FX3 and SBA256. To ensure that the strains examined for DSBs at the *HIS4* hotspot had undergone DSB formation at other loci, the filters were stripped of probe and rehybridized to an *ARG4*-specific probe.

(C) DSB formation at the *HIS4* hotspot in a strain (SBA322) homozygous for an insertion of *URA3* (*his4-CU*) of the same size and at the same position of Ty917::*URA3*. Bands labeled a, b, and c correspond the three different DSBs observed. DSB a maps to the same location at the end of *BIK1* as in FX3. DSBs b and c are in the CU insertion.

ity. We found that DSB formation was still suppressed in the *his4-917ULTR*Δ (SBA329) strain (<0.5% of the total DNA; Figures 3A) but not suppressed in the *his4-soloLTR* (SBA331) strain (4.5% of the total DNA; Figure 3B), showing that the recombination-suppressing features of Ty917::*URA3* reside internally within the element.

To further map the sequences responsible for suppressing recombination, we created strains SBA345 (lacking the *HIS4*-proximal end of Ty917::*URA3*; *his4-917*Δ*N*) and SBA346 (lacking the *HIS4*-distal end of Ty917::*URA3*; *his4-917*Δ*C*). The results clearly show that the N terminus of the Ty element carries the sequences responsible for the Ty coldspot behavior. Whereas strain SBA345, in which the Ty N terminus was deleted, showed normal DSB formation (band a, representing 5.7% of the total DNA; Figure 3C), strain SB346, lacking the C-terminal region of the element, exhibited no DSB formation (except for a faint band that mapped to the borders of the *URA3* insert) (Figure 3D; 0.8% of the total DNA). DSBs at the border of similar *URA3* inserts have been previously observed (Borde et al., 1999; Wu and Lichten, 1995). We conclude that the sequences involved in repressing recombination are located within the proximal 4 kb of the element.

Reduced Hotspot Activity Correlates with Reduced DNase I-Hypersensitive Sites

As discussed in the Introduction, DSB sites in *Saccharomyces cerevisiae* are located in nuclease-hypersensitive chromatin, suggesting that DSBs occur at sites where the DNA is particularly accessible. To test whether Ty917::*URA3* affected chromatin structure at the hotspot, we examined the DNase I-sensitivity of chromatin in the hotspot region in strains with and without hotspot activity. These data are shown in Figures 4A–4F. The intensity of the hypersensitive sites was measured and compared to that of the well-characterized *ARG4* re-

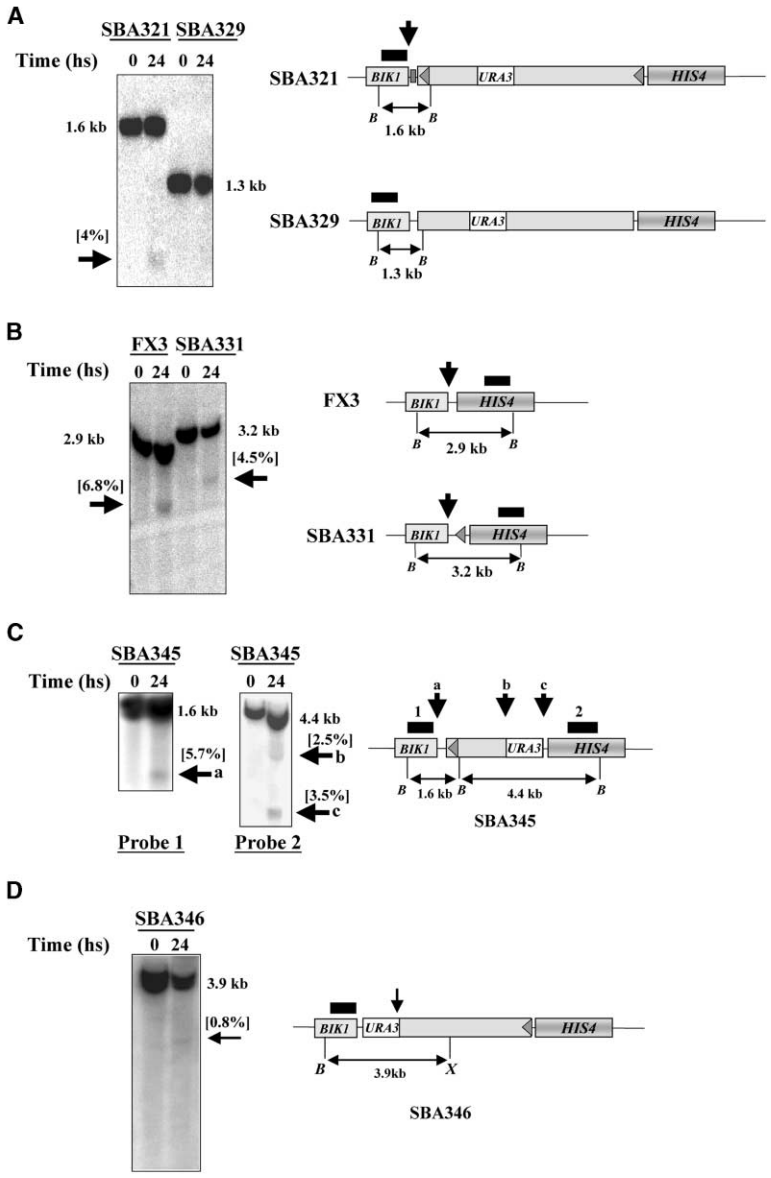

Figure 3. DSBs Associated with the *HIS4* Hotspot in Strains with Deletion Derivatives of Ty917::*URA3*

(A) Southern analysis of a strain (SBA329) that is homozygous for *his4-917ULTR*Δ, lacking both LTRs. Symbols are as in Figure 2. As a positive control in which meiotic DSBs can be observed, we examined DSBs in SBA321, a strain homozygous for *his4-917U*, and a recombination-stimulating insertion of three Rap1p binding sequences (*his4-202*; represented as a gray box).

(B) Southern analysis of DSB formation in FX3 (wild-type diploid) and SBA331 (*his4-soloLTR/his4-soloLTR)*. The *his4-soloLTR* allele represents an insertion of a single LTR at the same position as Ty917::*URA3*.

(C) Southern analysis of DSB formation in SBA345 (*his4-917U*Δ*N/ his4-917U*Δ*N*). This strain is homozygous for a deletion derivative of Ty917::*URA3* that lacks the *HIS4*-proximal end of the Ty element. We analyzed DSB formation using both *BIK1* (probe 1) and *HIS4* (probe 2) sequences.

(D) Southern analysis of DSB formation in SBA346 (*his4-917U*Δ*C/his4-917U*Δ*C*) This strain is homozygous for a deletion derivative of Ty917::*URA3* that lacks the *HIS4*-distal end of the Ty element. We analyzed DSB formation in samples treated with both BglII and XbaI (indicated by the letter X). Only a faint DSB is observed (thin arrow).

combinational hotspot. Quantitative results are presented in Figure 4G. The distribution of DNase I-hypersensitive sites in the hotspot region in the control strain (DNY26) was similar to that previously described (Fan and Petes, 1996). Hypersensitive sites were observed around the Rap1p binding site and near the sites of DSB formation (Figures 4A and 4G). SBA322, the strain with the control (non-Ty) insertion, also showed nuclease-hypersensitive sites around the Rap1p binding site and DSB sites. Furthermore, there were many additional nuclease-hypersensitive sites within the control insertion, consistent with an open, accessible chromatin configuration (Figures 4B and 4G). In striking contrast, the strain bearing the Ty insertion (SBA258) exhibited lower levels and a reduced number of DNase I-hypersensitive sites in the *HIS4* hotspot region (Figures 4C and 4G). Moreover, no nuclease-hypersensitive sites were detected within the 8.5 kb fragment of the Ty917::*URA3* insertion, indicating that this element is present as densely packed chromatin. Analysis of additional Ty elements in the genome revealed a similar closed chromatin configuration (see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/15/2/221/DC1; and data not shown). The absence of nuclease-hypersensitive sites was specific for Ty elements, as the *ARG4* locus in SBA258 exhibited the same levels and pattern of DNaseI hypersensitivity as in all the other strains tested (Figure 4D; and data not shown). These results are consistent with the hypothesis that the compact chromatin configuration of Ty917::*URA3* precludes DSB formation within the element, and this closed configuration "spreads" into the flanking regions, preventing DSB formation at the *HIS4* hotspot.

We also examined nuclease sensitivity of chromatin derived from additional strains. SBA329, lacking LTRs (Figures 4E and 4G), in which the dominant coldspot effect was retained, also exhibited only partial DNase I sensitivity in the region surrounding the Rap1 binding

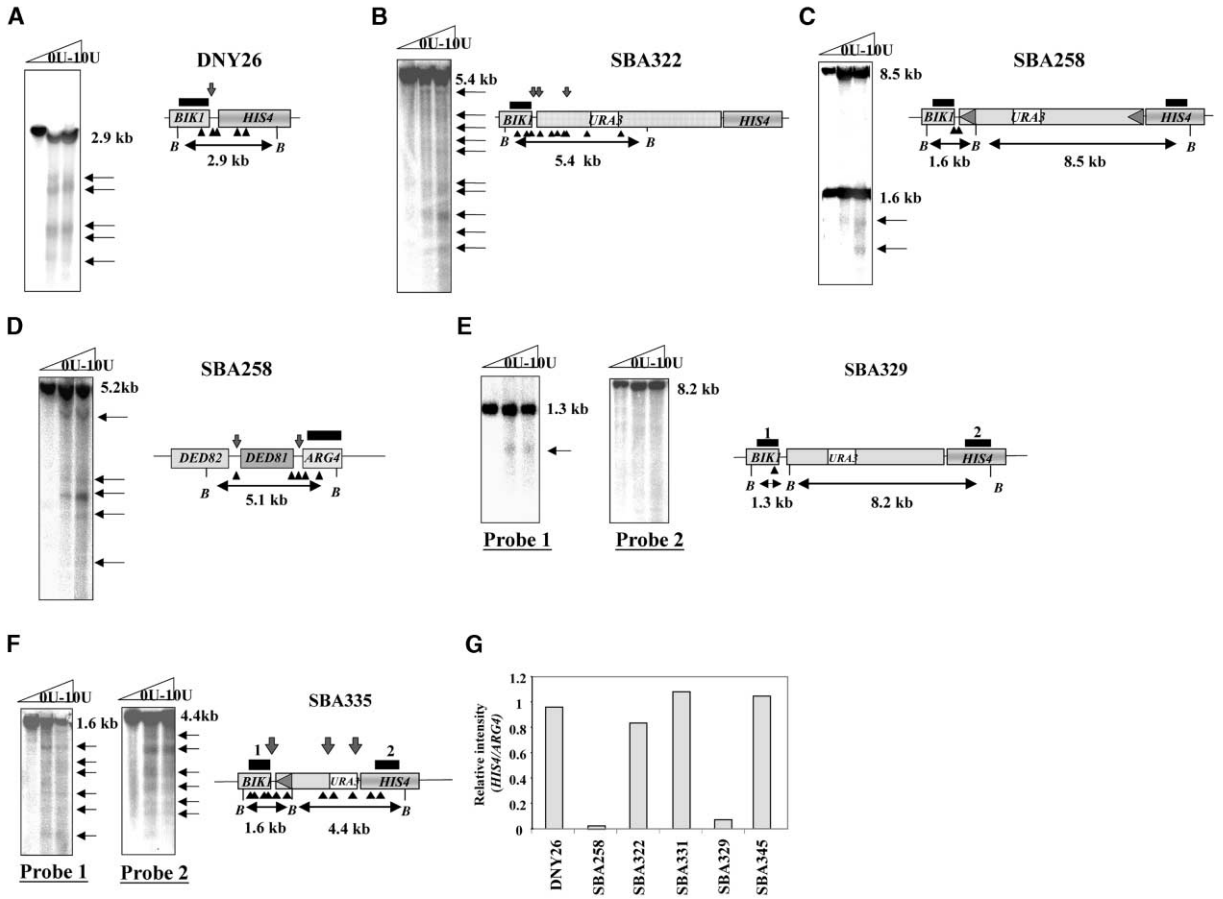

Figure 4. DNase I-Hypersensitive Sites at the *HIS4* Locus in Meiotic Chromatin Derived from Strains with Various Levels of Recombination Hotspot Activity

Chromatin was isolated from cells incubated for 6 hr in sporulation medium and incubated at 0°C with 0–10 units of DNase I. The samples were then treated with BglII and examined by Southern analysis. The hybridization probes used are indicated by black rectangles. Gray arrows represent sites of DSBs, and black triangles are DNase I-hypersensitive sites.

(A) DNY26 (parental control).

(B) SBA322 (*his4-CU/his4-CU*).

(C) SBA258 (*his4-917U/his4-917U*) (*BIK1* and *HIS4* probes).

(D) SBA258 (*his4-917U/his4-917U*) (*ARG4* probe).

(E) SBA329 (*his4-917ULTRΔ/ his4-917ULTRΔ*).

(F) BA345 (*his4-917UΔN/ his4-917UΔN*).

(G) Relative DNase I hypersensitivity of the various strains. After successive hybridization with *HIS4*/*BIK1* and *ARG4* probes, the intensity of the bands representing DNaseI-sensitive DNA was quantitated. The ratio of DNaseI-sensitive DNA in *HIS4* relative to that in *ARG4* is presented for each strain.

site, and no DNase I hypersensitivity within the Ty sequence. In contrast, strains that exhibited high levels of DSB formation, such as SBA331 (solo LTR insert; data not shown) and SBA335 (N terminus deletion of the Ty; Figures 4F and 4G), exhibited sensitivity to DNase I in the whole region. Our results thus define the closed chromatin configuration of Ty elements as a main feature determining the low level of recombination within their sequences and in their surroundings.

The Relationship between Rap1p Binding and *HIS4* Recombination Hotspot Activity

We showed previously that mutating the Rap1p binding site upstream of *HIS4* eliminated hotspot activity and that overproduction of Rap1p in a strain with a wild-type Rap1p binding site elevated *HIS4* recombination activity (White et al., 1991). In addition, a 51 bp insertion of DNA containing three Rap1p binding sites upstream of *HIS4* greatly stimulated recombination (White et al., 1993). Based on these studies, one interpretation of the recombination-suppressing effects of Ty917::*URA3* is that the presence of this element may prevent the binding of Rap1p to its site. To test this hypothesis, we measured Rap1p binding by chromatin immunoprecipitation (ChIP). Rap1-bound DNA sequences were detected by PCR using primers from the *HIS4* hotspot region; PCR-amplified *ACT1* sequences were used as a negative control.

Figure 5A shows that Rap1p binding in a strain with the Ty917::*URA3* insertion (SBA256) is similar to that seen in the control strain (FX3) and higher than what is observed in FX2, a strain that lacks the Rap1p binding

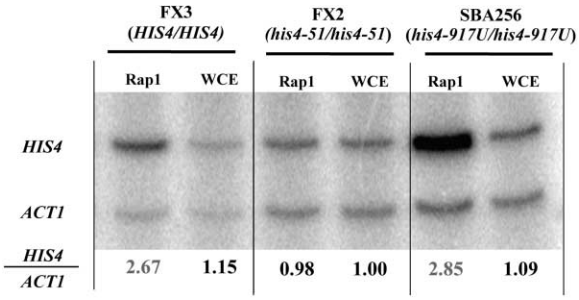

Figure 5. Ty917::*URA3* Does Not Interfere with the Adjacent Binding of Rap1p

We prepared chromatin extracts from FX3 (wild-type *HIS4* upstream region), FX2 (lacking a Rap1p binding site), and SBA256 (Ty-917::*URA3*). Chromatin immunoprecipitation was performed with Rap1p antibody (Rap1) or without antibody (WCE; whole-cell extract). Following the immunoprecipitation and reversal of the cross-links, we performed PCR amplification using primers specific for the *HIS4* hotspot region or the control *ACT1* gene. The ratio between the *HIS4*-specific and the *ACT1*-specific PCR products is shown.

site as a consequence of the *his4-51* mutation (White et al., 1991). We conclude that the reduced level of DSB formation caused by the Ty917::*URA3* insertion is not a consequence of reduced Rap1p binding at the *HIS4* upstream region.

Increased Rap1p Binding Can Restore Hotspot Activity

We have argued that the compact chromatin configuration of Ty917::*URA3* may spread into adjacent regions of the chromosome, suppressing recombination. Rap1p has been shown to act at several genomic locations to separate distinct chromatin domains (Yu et al., 2003). It is possible that the binding of a single Rap1p at *HIS4* may be insufficient to counteract the spreading effect of Ty compact chromatin. Consequently, we constructed a strain (SBA321) in which multiple Rap1p binding sites were inserted adjacent to Ty917::*URA3*. This insertion was previously used to stimulate *HIS4* hotspot activity (*his4-202*) (White et al., 1993). We found that SBA321 exhibited meiosis-specific DSBs (Figure 6A), although the level of DSB formation (4% relative to the parental DNA fragment) was somewhat lower than observed in the FX4 strain with multiple Rap1p binding sites that lacked Ty917::*URA3* (18%). In addition, the chromatin in the hotspot region was more nuclease-hypersensitive in SBA321 than that observed in the isogenic strain lacking the multiple Rap1p binding sites (compare Figures 6B and 4C). The increased Rap1p binding did not alter the chromatin configuration of the Ty element, which remained compact for most of its length (Figure 6B, probe 2). In summary, these results show that increasing levels of Rap1p binding can overcome the recombination-suppressing effects caused by Ty917::*URA3*. Strong binding of Rap1p molecules may act as a barrier to the spreading of closed chromatin conformation from Ty917::*URA3*.

Discussion

Ty elements constitute the main family of large dispersed natural repeats in the *Saccharomyces cerevisiae* genome. Since recombination between Ty elements at nonallelic locations would result in chromosomal aberrations, they represent a potential source of genomic instability. In previous studies, we have shown that Ty elements have low levels of mitotic and meiotic recombination (Kupiec and Petes, 1988a, 1988b). Here, we inserted a Ty element next to a strong meiotic recombination hotspot located upstream of the *HIS4* gene. We found that this Ty element substantially reduced recombination activity at *HIS4* by suppressing the DNA lesion that initiates meiotic recombination. Ty elements thus contain features that determine their low levels of recombination, and these features act in a dominant fashion when located in proximity to a hotspot. We also showed that the Ty chromatin was closed (nuclease-insensitive) and that the presence of the Ty reduced the nuclease-sensitivity of adjacent chromatin.

Several lines of evidence support the conclusion that recombination hotspots are associated with specific types of chromatin structure (reviewed by Petes, 2001). First, the DSBs that initiate meiotic exchange occur between genes rather than within genes, in regions of nuclease-hypersensitive chromatin (Fan and Petes, 1996; Ohta et al., 1994; Wu and Lichten, 1994). It is clear from global studies of hotspot activity, however, that nuclease-hypersensitive chromatin is not sufficient for hotspot activity (Baudat and Nicolas, 1997; Gerton et al., 2000). Second, certain hotspots require the binding of transcription factors for their function (Petes, 2001), presumably to recruit chromatin-remodeling machinery. Third, for some meiotic recombination hotspots, an increase in the nuclease-sensitivity of the hotspot chromatin immediately precedes DSB formation (Ohta et al., 1994).

Certain features of chromatin appear also to be associated with recombination coldspots in *S. cerevisiae* and *S. pombe*. Coldspots in yeast are nonrandomly associated with centromeres and telomeres (Gerton et al., 2000; Klein et al., 1996), regions known to have a chromatin structure that suppresses gene expression (reviewed by Huang, 2002). Meiotic recombination of the mating-type loci of *S. pombe* is suppressed by a mechanism that is dependent on histone deacetylation and histone methylation (Huang, 2002).

One interpretation of the results described above is that the meiotic recombination activity of a region represents an integration of recombination-promoting chromatin modifications (such as hyperacetylated histones) and recombination-suppressing chromatin modifications (possibly hypoacetylated and/or methylated histones). It is possible that recombination activity is regulated by the absolute levels of recombination-stimulating modifications or that there is a code of modifications that is optimal for recombination function (Petes, 2001). In our strains, the Ty917::*URA3* element exhibited a nuclease-insensitive, closed chromatin configuration that spread into the adjacent sequences. In addition, there was a strong correlation between insertions that retained the compact chromatin configuration and those that suppressed DSB formation. These suppressing effects were local; DSB sites located about 5 kb upstream and 3 kb downstream of the *HIS4* DSB were unaffected by Ty-917::*URA3* (data not shown).

Two additional points concerning the recombination-

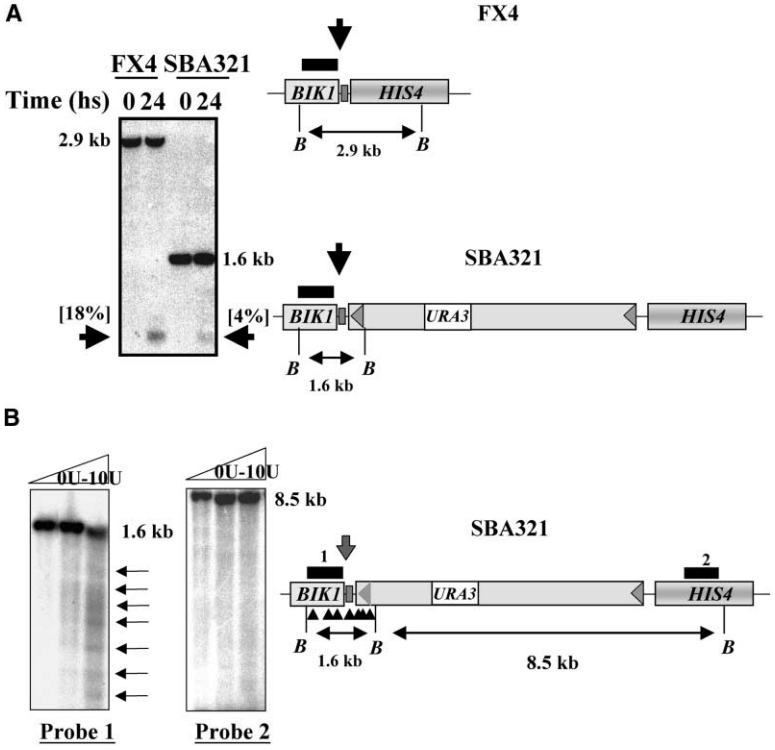

Figure 6. An Insertion with Multiple Rap1p Binding Sites Adjacent to Ty917::*URA3* Stimulates DSB Formation and Increases the Nuclease-Sensitivity of Chromatin at the *HIS4* Hotspot

(A) Strain SBA321 is homozygous for a small insertion containing multiple Rap1p binding sites (*his4-202*) and Ty917::*URA3* (*his4-917U*). Strain FX4 is homozygous for *his4-202*, but lacks Ty917::*URA3*. Gray boxes indicate the insertion. Both strains exhibit meiosis-specific DSBs (indicated by arrows).

(B) DNase I-sensitivity of chromatin isolated from SBA321. Experiments were performed as described in the Figure 4 legend. SBA321 exhibits increased nuclease sensitivity in the region surrounding the Rap1 and the DSB site.

suppression of Ty917::*URA3* should be made. First, Ty transcription does not seem to be required for suppression of recombination. Ty expression (and that of adjacent genes) is reduced in a/α diploid cells (Errede et al., 1980), and accordingly, Ty917::*URA3* is expressed at very low levels (data not shown). In addition, Ty917::*URA3* lacking the LTRs (and thus lacking promoter activity) still suppressed recombination. Second, it has been shown previously that Ty elements can either suppress or elevate expression of adjacent genes depending on their chromosomal context and other factors. The Ty917 insertion suppresses *HIS4* gene expression (Roeder et al., 1980). We find, however, that this suppression of transcription is not directly related to the suppression of *HIS4* hotspot activity. For example, strain SBA346 (*his4-917UΔC*) does not show suppressed gene expression, but it still has suppressed recombination. In contrast, SBA345 (*his4-917UΔN*) is transcriptionally inactive, but exhibits hotspot activity. Thus, transcription of neither the Ty nor the adjacent regions is required for suppression of recombination.

We have preliminarily mapped the region responsible for the closed Ty chromatin configuration to the first 4 kb of Ty917::*URA3*. Deletion analysis was carried out in the past to determine the regions within the Ty elements that affect transcription of adjacent genes (Company and Errede, 1988; Errede et al., 1987; Turkel and Farabaugh, 1993). These studies revealed that both Ty transcription and its effects on neighboring genes are regulated by a complex array of positively and negatively acting sequences, some located within the LTR and some located internally within the Ty element. This region contains the binding sites for multiple proteins including Mcm1, Rap1, Ste12, Gcr1, Gcn4, and Tea1, whose binding influences Ty chromatin configuration (reviewed in Morillon et al., 2002). Ty expression and the effects of the Ty on the expression of adjacent genes are also regulated by a number of chromatin remodeling complexes including SAGA (Pollard and Peterson, 1997), the Swi/Snf complex (Happel et al., 1991), and the Isw1 and Isw2 complexes (Kent et al., 2001). In addition, it has recently been shown that mutations in the Mcm proteins (originally defined as binding to DNA replication origins) also may be involved in the establishment of compact chromatin domains in the vicinity of Ty elements (Dziak et al., 2003). Although it is not clear which of the Ty binding proteins and chromatin remodeling complexes are required for the recombination-suppressing effects of Ty917::*URA3*, an analysis of the effects of mutations in these genes on Ty-mediated suppression of recombination should clarify this issue.

Rap1 is an essential protein needed for optimal levels of *HIS4* expression (Devlin et al., 1991) and required for DSB creation at the *HIS4* recombination hotspot (White et al., 1991). In our experiments, the chromatin around the Rap1p binding site was nuclease hypersensitive in all strains examined, including those that lacked hotspot-associated DSBs. In addition, we found that the insertion of Ty917::*URA3* did not prevent Rap1p binding. An insertion of three Rap1p binding sites relieved the recombination-suppression of the Ty element, restoring DSBs. In addition, the insertion increased the nuclease-sensitivity of the hotspot region. Since Rap1p binding can establish a boundary between open and closed chromatin (Bi and Broach, 1999; Yu et al., 2003), one interpretation of this result is that the binding of multiple Rap1 proteins prevents spreading of the silencing effects of the Ty into adjacent chromosome regions. Alter-

natively, if the recombination activity of a region represents an integration of recombination-activating chromatin modifications and recombination-inactivating modifications, the addition of several Rap1p binding sites might alter the balance toward recombination-promoting changes.

The presence of dispersed repetitive sequences in the genome represents a potential source of genomic instability, since recombination between nonallelic copies can lead to chromosomal aberrations. In some fungal and plant systems, repeated sequences undergo genetic and epigenetic changes that result in their inactivation. For example, in transformed premeiotic *Neurospora* cells containing an integrated trans gene and an identical resident gene, both copies of the repeats are specifically methylated and riddled with point mutations, a phenomenon called repeat-induced point mutation (RIP) (Selker, 1990). In *Ascobolus*, both copies are silenced by methylation, but not mutated, a phenomenon called MIP (methylation induced premeiotically) (Barry et al., 1993). Naturally occurring families of repeated sequences are also methylated in *Ascobolus* (Colot et al., 1996) and in plants (Brutnell and Dellaporta, 1994; Martienssen and Baron, 1994). In *Saccharomyces cerevisiae*, there is no DNA methylation. Nevertheless, Ty activity in yeast is tightly controlled. For example, Ty elements were found to exhibit both copy-number-dependent transcriptional cosuppression (Jiang, 2002) as well as posttranscriptional copy number control (Garfinkel et al., 2003).

Our results show that Tys have a compact chromatin configuration, which precludes initiation of recombination events. Suppression of recombination within Ty elements prevents the creation of potentially lethal chromosome rearrangements by exchanges between members of the repeated family. In addition, recombination-suppression is also advantageous for the Ty element. The chromosome on which recombination is initiated (the one that receives the DSB) usually acts as recipient of information during gene conversion events (reviewed by (Petes, 2001). In diploid strains heterozygous for the insertion of a Ty element near a recombination hotspot, the DSB will usually occur on the chromosome without the element, resulting in a duplication of the Ty element. A meiotic gene conversion bias of this predicted type has been observed (Vincent and Petes, 1989). In this way, Ty-mediated suppression of recombination is a mechanism that leads to a net increase in the number of Ty elements in the genome.

Experimental Procedures

Yeast Strains

All the yeast strains in this study were derived from the haploid strains AS4 (*MATa trp1 arg4-17 tyr7 ade6 ura3*) and AS13 (*MAT*α *leu2-Bst ade6 ura3*) (Stapleton and Petes, 1991) by transformation or crosses with isogenic strains. The details of these constructions are given in the Supplemental Data. Complete genotypes of AS4- and AS13-derived strains are given in Supplementary Tables S1 and S2, respectively. The sequences of oligonucleotides used in the constructions and genetic analysis are in Supplementary Table S3.

Tetrad Analysis

Yeast strains were sporulated at 18°C on solid sporulation medium and dissected onto plates containing rich growth medium (Detloff et al., 1991). The segregation of all the heterozygous markers was examined in 135 full tetrads by replica-plating spore colonies from rich medium to omission media. The segregation of the *bik1-IR16* and *his4-lopc* markers was examined by PCR analysis using primers BIK-F and BIK-R, and HIS-F and HIS-R, respectively (primer sequences in Supplementary Table S4). The sizes of the alleles were 201 bp (*BIK1*), 233 bp (*bik1-IR16*), 140 bp (*HIS4*), and 166 bp (*his4-lopc*).

Southern Analysis of Meiosis-Specific DSBs

Southern analysis was carried out as previously described (Fan et al., 1995). In brief, DNA from samples sporulated for 24 hr in liquid medium at 25°C were treated with BglII, and the resulting fragments were separated by gel electrophoresis. Following transfer to nylon membranes, the samples were hybridized to probes derived from *BIK1* (probe from +338 from the beginning of *BIK1* to −151 relative to the initiating codon of *HIS4*) or *HIS4* (+415 to +1677 of *HIS4*). Quantitation of the DSBs was carried out with the program NIHImage. For many of the experiments, after the data were collected using the PhosphorImager, the hybridizing probes were removed from the membranes, and the membranes were rehybridized to a probe derived from the *ARG4* gene (+260 to +1275). Probes were generated by PCR amplification of yeast genomic DNA. The primer sequences used to generate the probes are given in Supplementary Table S4.

Assays of Nuclease-Sensitivity and Chromatin Immunoprecipitation Assays

DNase I sensitivity of chromatin was analyzed as described (Fan and Petes, 1996; Wu and Lichten, 1994). Chromatin was isolated from cells incubated for 6 hr in sporulation medium. Incubations with DNase I (Roche Applied Science) were carried out for 1–2 min at 0°C with 0–10 units of DNase I. DNase I sensitivity was analyzed for the *HIS4* and *ARG4* regions sequentially, as explained above. The intensity of the bands was measured using the NIHImage program. The local nuclease sensitivity was calculated as the proportion of total signal in the bands generated by DNase I. The ratio of DNaseI-sensitive DNA in *HIS4* relative to that in *ARG4* is presented.

ChIP experiments were performed as described previously (Burke et al., 2000; Mieczkowski et al., 2003). Rap1p-specific antibodies were purchased from Santa Cruz Biotechnology, Inc. The PCR primers used to detect sequences adjacent to the DSB site were BIKIP-F and BIKIP-R (Supplementary Table S4); control fragments for the ChIP experiments were derived from the *ACT1* gene using primers ACT-F and ACT-R.

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