The *Saccharomyces cerevisiae RDN1* Locus Is Sequestered From Interchromosomal Meiotic Ectopic Recombination in a *SIR2*-Dependent Manner

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ABSTRACT

Meiotic ectopic recombination occurs at similar frequencies among many sites in the yeast genome, suggesting that all loci are similarly accessible to homology searching. In contrast, we found that *his3* sequences integrated in the *RDN1* (rDNA) locus were unusually poor participants in meiotic recombination with *his3* sequences at other sites. We show that the low rate of meiotic ectopic recombination resulted from the poor ability of *RDN1::his3* to act as a donor sequence. *SIR2* partially repressed interchromosomal meiotic ectopic recombination at *RDN1*, consistent with its role in regulating recombination, gene expression, and retrotransposition within *RDN1*. We propose that *RDN1* is physically sequestered from meiotic homology searching mechanisms.

DURING meiosis, homologous regions of chromosomes undergo a poorly understood mechanism involving a homology search in order to undergo recombination and proper disjunction. The timing of meiotic recombination events is approximately coincident with chromosome pairing. Double-strand DNA breaks (DSBs), the presumed initiating lesions for meiotic recombination (Nicolas *et al.* 1989; Cao *et al.* 1990; Wu and Lichten 1994; Keeney *et al.* 1997), appear before visible synaptonemal complex (SC), while mature recombination structures are detectable near the time of SC breakdown (Padmore *et al.* 1991).

Most meiotic recombination is allelic (between sites at the same chromosomal position). Eukaryotic genomes contain repetitive DNA in both tandem and dispersed arrangements, creating the potential for ectopic recombination between nonallelic loci. Ectopic recombination includes three classes of events: (1) intrachromosomal (between two different sites on the same chromosome); (2) interhomolog (between two different sites on homologous chromosomes); and (3) interheterolog (between sites on nonhomologous chromosomes). All classes of ectopic recombination have been detected in vegetative and meiotic *Saccharomyces cerevisiae* cells (Klein and Petes 1981; Jinks-Robertson and Petes 1985; Lichten *et al.* 1987; Haber *et al.* 1991; Goldman and Lichten 1996).

Ectopic recombination occurs at high levels in *S. cerevisiae* meiosis, where frequencies are generally only 2- to 17-fold lower than that of allelic recombination (Jinks-Robertson and Petes 1985, 1986; Lichten *et al.* 1987; Haber *et al.* 1991; Goldman and Lichten 1996). Further, the frequencies of ectopic recombination at several sites examined, in which the position of one sequence is held constant, vary over a narrow (2- to 10-fold) range (Haber *et al.* 1991; Goldman and Lichten 1996). These observations are consistent with a proposal that an initiating DSB on one chromosome is sufficient, or rate limiting, to allow meiotic recombination between homologous sequences at any two given locations (Haber *et al.* 1991). In this view, meiotic recombination is driven by an efficient, yet poorly understood, genome-wide homology search mechanism, creating competition between allelic, sister, and ectopic chromosomal interactions (Haber *et al.* 1991).

Ectopic recombination is potentially hazardous in the presence of dispersed repeated sequences. Ectopic crossing over causes chromosomal abnormalities, including deletions, translocations, and acentric and dicentric chromosomes (Jinks-Robertson and Petes 1986; Goldman and Lichten 1996). Thus, eukaryotes might have evolved mechanisms to avoid ectopic recombination in meiosis. Interhomolog recombination is favored in yeast meiosis by three- to sixfold over intersister recombination (Haber *et al.* 1984; Jackson and Fink 1985; Schwacha and Kleckner 1997). Mutations in several genes, including *RED1*, *RAD17*, *RAD24*, *MEK1*, *MEC3*, *DMC1*, *UBR1*, *INP52*, *BUD3*, *PET122*, *ELA1*, *RAD51*, *RAD55*, and *RAD57*, reduce interactions between homologs and/or elevate the frequency of unequal sister chromatid exchange (Schwacha and Kleckner 1997; Thompson and Stahl 1999), suggesting that a distinct meiotic machinery exists for promoting allelic recombination.

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TABLE 1

Yeast strains

Strain	Genotype	Source[a]
Haploids		
DC14	*MAT***a** *his1*	Cold Spring Harbor Laboratory
DC17	*MAT*α *his1*	Cold Spring Harbor Laboratory
JSS104-15B	*MAT***a** *ade2-101 trp1-*Δ*1 ura3-52 his3-*Δ*200 leu2-*Δ*1 lys2-801 cry1*	Mastrangelo *et al.* (1992)
GRY340	JSS104-15B *MAT***a***::URA3*	McGill *et al.* (1990)
GRY818	*MAT*α *ade2-101 trp1-*Δ*1 his3-*Δ*200 lys2-801 cyh2*	
GRY1580	*MAT*α *ade2-101 his3-*Δ*200 trp1-*Δ*1 lys2-801 cyh2*	
GRY1582	*MAT***a** *ade2-101 trp1-*Δ*1 his3-*Δ*200 cyh2 can1 cry1*	
GRY1586	GRY340 *MAT***a***::his3-*Δ*MscI*	
GRF167	*MAT*α *ura3-167 his3-*Δ*200*	G. Fink
DG1252	GRF167 *leu2::hisG* Ty1m*his3-AI-263* Ty1-*588NEO* Ty1 (*tyb::lacZ*)-147	D. Garfinkel
DG1348	GRF167 *trp1::hisG* Ty1m*his3-AI-270* Ty1-*588NEO* Ty1 (*tyb::lacZ*)-147	D. Garfinkel
JC227	GRF167 Ty1m*his3-AI-227*	M. J. Curcio
JC234	GRF167 Ty1m*his3-AI-234*	M. J. Curcio
JC242	GRF167 Ty1m*his3-AI-242*	M. J. Curcio
JC272	GRF167 Ty1m*his3-AI-272* Ty1-*588NEO* Ty1 (*tyb::lacZ*)-147	M. J. Curcio
JC273	GRF167 Ty1m*his3-AI-273* Ty1-*588NEO* Ty1 (*tyb::lacZ*)-147	M. J. Curcio
JC815	GRF167 Ty1m*his3-AI-815*	M. J. Curcio
ED315-93b	*MAT***a** *ura3-*Δ*1 his3-*Δ*200 tyr7-1 cyh2*	
ED315-105c	*MAT*α *ura3-*Δ*1 his3-*Δ*200 tyr7-1*	
ED326-1	GRY309 *sir2::hisG-URA3-hisG*	
ED331-2A	JC234 *sir2::hisG*	
ED332-1A	JC242 *sir2::hisG*	
ED333-2B	JC272 *sir2::hisG*	
ED334-1A	JC273 *sir2::hisG*	
ED361A-1	*MAT***a***::his3-*Δ*MscI ade2-101 trp1*Δ*::hisG ura3-52 his3-*Δ*200 leu2-*Δ*1 tyr7-1 cry1*	
ED374B-13A	*MAT*α *trp1*Δ*::hisG ura3-167 his3-*Δ*200 leu2-K lys2*Δ*::[hisG-leu2-R] tyr7-1*	
ED391-18	ED374B-13A *RDN1::TRP1-his3-621*	
ED392-19	ED374B-13A *RDN1::TRP1-his3-621*	
ED397A	ED374B-13A *CUP1::TRP1-his3-621*	
ED398A	ED374B-13A *CUP1::TRP1-his3-621*	
ED401A	ED374B-13A *arg4::TRP1-his3-621*	
ED402A	ED374B-13A *arg4::TRP1-his3-621*	
ED435-18	ED361-A1 *sir2::hisG*	
ED436-62A	ED391-18 *sir2::hisG*	
ED437-13A	ED392-19 *sir2::hisG*	
ED475-2	JSS104-15B *arg4::TRP1-his3-621*	

(*continued*)

While ectopic recombination occurs frequently, recombination between heterologous chromosomes is still significantly less efficient than allelic recombination (Goldman and Lichten 1996). Further, the efficiency of ectopic recombination between sites on homologous chromosomes decreases with increasing distance between the recombination substrates examined. Thus, some aspect of the pairing and alignment of homologous chromosomes favors allelic over ectopic recombination. Meiotic ectopic recombination between subtelomeric regions occurs with approximately twofold greater efficiency than between subtelomeric and interstitial regions (Goldman and Lichten 1996), consistent with demonstrations of the co-localization of telomeres at the periphery of the nucleus (Klein *et al.* 1992). Further, mutations in the checkpoint genes *MEC1*, *RAD17*, and *RAD24* exhibit elevated intrachromosomal ectopic recombination in meiosis (Grushcow *et al.* 1999), suggesting that the repair of meiotic DSBs is biased toward allelic interactions.

TABLE 1

Continued

Strain	Genotype	Source[a]
Diploids		
D288A	GRY818 × DG1252	
ED289A	GRY818 × DG1348	
ED290B	GRY818 × JC227	
ED291B	GRY818 × JC234	
ED292A	GRY818 × JC242	
ED293A	GRY818 × JC272	
ED294A	GRY818 × JC273	
ED295A	GRY818 × JC815	
ED308A	GRY1586 × JC242	
ED310B	GRY1586 × JC273	
ED339C-1	ED326-1 × ED331-2A	
ED340A-2	ED326-1 × ED332-1A	
ED341E	ED326-1 × ED333-2B	
ED342A-1	ED326-1 × ED334-1A	
ED347C-1	ED339C-1 *sir2::hisG*/*sir2::hisG*	
ED348A-2	ED340A-2 *sir2::hisG*/*sir2::hisG*	
ED349G-1	ED341E *sir2::hisG*/*sir2::hisG*	
ED350A-1	ED342A-1 *sir2::hisG*/*sir2::hisG*	
ED403-18	ED361-A1 × ED391-18	
ED404-19	ED361-A1 × ED392-19	
ED409-1	ED361-A1 × ED397A	
ED410-1	ED361-A1 × ED398A	
ED413-1	ED361-A1 × ED401A	
ED414-1	ED361-A1 × ED402A	
ED479-62	ED435-18 × ED436-62A	
ED480-13	ED435-18 × ED437-13A	
ED485B	ED475-2 × JC234	
ED491A	ED475-2 × JC242	
ED497A	ED475-2 × JC272	
ED503C	ED475-2 × JC273	

[a] All strains not cited were constructed for this work.

We describe another mechanism limiting the tendency of heterologous chromosomes to undergo ectopic recombination. We developed an assay system that surveys the *S. cerevisiae* genome for sites with high and low frequencies of meiotic ectopic recombination. We examined interchromosomal meiotic ectopic recombination between two different versions of the yeast *his3* gene, one of which was placed at different genomic positions. Though most ectopic pairs behaved as expected, the frequency of ectopic recombination involving the yeast ribosomal DNA (*RDN1*) locus was ~100-fold lower than at other sites.

RDN1 undergoes very low levels of interhomolog recombination in meiosis (Petes and Botstein 1977). Such behavior could be due simply to a lack of initiating DSBs, while still retaining the ability to act as an efficient recombination donor. However, we present genetic evidence that *his3* in *RDN1* behaved as an exceptionally poor donor locus in ectopic recombination. We propose that *RDN1* is inaccessible, or sequestered, from a homology search mechanism in meiosis.

The silencing gene *SIR2* acts at *RDN1* by repressing intra- and interhomolog recombination (Gottlieb and Esposito 1989; San-Segundo and Roeder 1999) and RNA polymerase II-mediated transcription (Bryk *et al.* 1997; Fritze *et al.* 1997; Smith and Boeke 1997). We observed that a *sir2* mutation partially elevated the rate of ectopic recombination involving *RDN1*, but not at other sites. Silencing mechanisms play a partial role in controlling the accessibility of donor sequences in ectopic recombination.

MATERIALS AND METHODS

Yeast strains, media, and genetic manipulations: The yeast strains used in this study are described in Table 1. Genomic DNA isolation was performed as described (Rose *et al.* 1990). Synthetic medium in plating experiments, except for that summarized in Table 2, was synthetic minimal medium (Rose *et al.* 1990). For the experiment summarized in Table 2, AA, or synthetic complete medium (Rose *et al.* 1990) containing 86 mg liter^{-1} adenine and 17 mg liter^{-1} *para*-amino benzoic acid was used. Yeast transformations were carried out by the lithium acetate procedure (Gietz *et al.* 1992) or the glusulase-spheroplast procedure (Hinnen *et al.* 1978). Mating type and

TABLE 2

***RDN1::Ty1mhis3-AI* is inaccessible to meiotic ectopic recombination**

Strain	Ty1m*his3-AI* Location[a]	Ty1m*his3-AI* Orientation[b]	Meiotic recombination frequencies × 10^5 His$^+$[c]	Meiotic recombination frequencies × 10^5 Ura$^+$	% His$^+$ linked to *MAT*
ED288A	VII (*G375835*)	Opposite	1.0 ± 0.2	116 ± 12	80
ED289A	X (*J203989*)	Opposite	2.5 ± 0.5	186 ± 58	75
ED290B	IV (*D1518728*)	Same	1.6 ± 0.2	121 ± 17	42
ED292A	XII (*L1060532*)	Opposite	3.0 ± 0.7	204 ± 21	37
ED294A	II (*B326918*)	Opposite	5.9 ± 0.6	218 ± 55	26
ED295A	X (*J354469*)	Same	2.2 ± 0.2	140 ± 20	90
ED291B	XII (*RDN1*)	Same	0.01 ± 0.004	204 ± 15	81
ED293A	XII (*RDN1*)	Same	0.01 ± 0.004	189 ± 14	66

[a] Chromosome and genome position (in parentheses), using numbering system of the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces).
[b] Orientation of *his3-AI* and *his3-621*, relative to centromeres.
[c] Other *his3* allele was *MAT***a***::his3-621*.

mating proficiency were determined by mating to tester strains DC14 and DC17. Disruption of *SIR2* was achieved by transforming haploid strains with plasmid pBST*sir2::URA3* (kindly provided by M. Bryk), which carries the *hisG-URA3-hisG* cassette (Alani *et al.* 1987) inserted in the *Bgl*II site at nucleotide +823 of the protein-coding region. *SIR2* disruptions were confirmed by the inability of cells to mate and by Southern blotting analysis. Homozygous *sir2* disruption diploid strains were constructed by polyethylene glycol (PEG) fusion in which haploid cells were prepared by the glusulase-spheroplast procedure for DNA transformation and mixed in the presence of polyethylene glycol (PEG 3350). Mixtures were plated on synthetic medium that selects for the diploids.

Recombination substrates: The Ty1m*his3-AI* (Curcio and Garfinkel 1991), *trp1-089-his3-621* (McGill *et al.* 1990), and *his3-*Δ*MscI* (Derr and Strathern 1993) constructs are described in previous studies. The *trp1-089-his3-621* and *his3-*Δ*MscI* cassettes were inserted at an *Eco*RI site on chromosome *III* near *MAT***a** at nucleotide C196972, using the numbering system of the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces). Single unspliced (His$^-$) Ty1m*his3-AI* insertions were previously generated in haploid strains (Bryk *et al.* 1997; M. J. Curcio, personal communication). The Ty1m*his3-AI* insertion site junctions in strains JC234, JC242, JC272, and JC815 (haploid parents of ED291B, ED292A, ED293A, and ED295A, respectively) were determined by Bryk *et al.* (1997). The Ty1m*his3-AI* insertion site junctions in strains DG1252, DG1348, and JC227 (haploid parents of ED288A, ED289A, and ED290A, respectively) were determined in this study by recovering plasmids from His$^+$, *MAT*α meiotic segregants of the diploids in *Escherichia coli* strain KC8 (*hsdR leuB600 trpC9830 pyrF::tn5 hisB463 lacD X74 strA galU,K*; kindly provided by K. Struhl via M. Mastrangelo). *S. cerevisiae HIS3* complements the *hisB463* mutation in *E. coli* (Struhl *et al.* 1976); therefore, ampicillin-resistant colonies were replica plated to medium that selects for histidine prototrophy. The Ty1/yeast and yeast/plasmid junctions of recovered His$^+$ plasmids were sequenced using the ABI Prism DNA Sequencing kit (PE-Applied Biosciences, Inc.). The Ty1m*his3-AI* 5′ LTR/yeast junction sequence in JC815 (haploid parent of ED295A) was also determined in this fashion, confirming the results of Bryk *et al.* (1997). The Ty1m*his3-AI* insertion site junction in strain JC273 (haploid parent of ED294A) was determined by vectorette polymerase chain reaction (PCR; Riley *et al.* 1990) on isolated DNA, followed by DNA sequencing.

The *TRP1-his3-621* DNA cassette was constructed as follows: PCR products of *TRP1* (nucleotides −102 to +755) and *HIS3* (nucleotides −189 to +819) were synthesized and cloned as *Apa*I-*Xho*I fragments in pBluescript II KS(+) and pBluescript II SK(−) (Stratagene, La Jolla, CA), respectively. Both constructs were digested with *Apa*I and *Sca*I and joined together. The new plasmid, pESD62, has *TRP1* and *HIS3* in divergent transcriptional orientations, with the pBluescript II multiple cloning site polylinker from *Sac*I to *Xho*I in a palindromic repeat flanking the cassette. The *Asp*718 fill-in mutation of *his3-621* was introduced by replacing the *Nsi*I-*Nde*I fragment of *HIS3* with the corresponding region from cloned *his3-621*, generating pESD64.

Fragments from *RDN1* (with respect to the start of the 35S rRNA gene: nucleotides −1078 to −471 with *Apa*I and *Xho*I ends; nucleotides −470 to +200 with *Xho*I and *Sac*I ends) and *CUP1* (nucleotides −818 to +50 with *Apa*I and *Xho*I ends; nucleotides +51 to +463 with *Xho*I and *Sac*I ends) were synthesized by PCR. PCR products were digested with the appropriate restriction enzymes and joined at their *Xho*I ends by cloning into pBluescript II SK(−). *TRP1-his3-621* was liberated from pESD64 with *Xho*I and cloned into the *Xho*I site of the *RDN1* and *CUP1* plasmids in both orientations. The *TRP1-his3-621* insertion site within an *RDN1* repeat unit in ED403-18 and ED404-19 is identical to that of Ty1m*his3-AI* in ED293A (Bryk *et al.* 1997). For insertion into *ARG4*, a plasmid containing the *ARG4* gene (kindly provided by M. Lichten) was digested with *Acc*I (nucleotide +230) and made blunt ended using Klenow enzyme. *TRP1-his3-621* was liberated from pESD64 with *Sma*I and ligated to the filled-in *Acc*I site of *ARG4* in both orientations. All *TRP1-his3-621* insertions were generated by transformation and selection for a Trp$^+$ phenotype in haploid strains. The location and orientation of *TRP1-his3-621* insertions *in vivo* was confirmed by Southern blot analysis.

Recombination measurements: Three single colonies of each diploid yeast strain were cultured at 30° in 200 ml liquid YEP (1% yeast extract, 2% Bacto-peptone) containing 2% potassium acetate to a density of ~5×10^6 cells ml^{-1}. Just immediately prior to sporulation, ~1×10^8 cells were removed for DNA preparation to be used in Southern blotting analysis. To determine the frequencies of vegetative His$^+$, Ura$^+$, and

Leu$^+$ events, one-half of the remaining culture was pelleted and resuspended in H_2O. Dilutions were plated on YEP containing 2% glucose (YEPD) and synthetic medium lacking either histidine, uracil, or leucine. Frequencies were calculated by dividing the number of prototrophs on selective plates by the number of colonies on YEPD. The other half of the culture was pelleted, resuspended at a density of $\sim 5 \times 10^7$ cells ml^{-1} in minimal sporulation medium (2% potassium acetate, 2.5 mg liter^{-1} phenylanine, 1 mg liter^{-1} each adenine sulfate and uracil, 0.5 mg liter^{-1} each histidine-hydrochloride, leucine, lysine-hydrochloride, tryptophan, methionine, and arginine, 0.4 mg liter^{-1} tyrosine, and 0.2 mg liter^{-1} proline), and agitated at 30° for 3 days, generating spore-containing asci.

Spores were liberated from asci as follows: Incubation in 5.0 ml 2.5% glusulase at 30° for 1 hr, followed by addition of 5.0 ml ice-cold 0.5% Triton / 10 mm EDTA. Asci were pelleted by centrifugation, washed three times in 10 ml ice-cold 0.5% Triton / 10 mm EDTA, and resuspended in 5.0 ml ice-cold 0.5% Triton / 10 mm EDTA. Asci were sonicated on ice using a Vibra-Cell sonicator (Sonics and Materials, Inc.; 80% duty cycle, microprobe output setting ~4.5) 3 × 40 sec, pelleted by centrifugation, and washed twice more in 5.0 ml ice-cold 0.5% Triton / 10 mm EDTA. Spores were resuspended in 1.0 ml ice-cold 0.5% Triton / 10 mm EDTA. Spore concentrations were adjusted to $\sim 1 \times 10^8$ ml^{-1}. The fraction of single spores following this procedure was ~75%. Spores were diluted appropriately in 0.5% Triton / 10 mm EDTA and plated on YEPD and synthetic medium lacking histidine, uracil, or leucine. Frequencies of meiotic recombination to His$^+$, Ura$^+$, and Leu$^+$ were determined by dividing the number of colonies arising on selective media by the number of haploids, as estimated by doubling the number of red colonies on YEPD plates derived from the heterozygous *ade2* marker. The fraction of red colonies on YEPD plates ranged from 42 to 54%, indicating that most colonies arising after the isolation procedure were derived from spores.

Donor/recipient ratios: A total of 100 His$^+$ recombinants from each diploid strain following sporulation were patched to AA-his plates. After overnight growth, patches were replica plated to mating-type testers and to various media to determine genetic markers. All cryptopleurine-sensitive (*CRY1*) *MAT*α His$^+$ recombinants were considered to have their His$^+$ phenotype unlinked to *MAT*, because such recombinants should occur via nonreciprocal gene conversion of Ty1m*his3-AI*. *MAT***a** His$^+$ spore progeny were replica plated to plates containing spreads of strain ED315-105c (relevant genotype *MAT*α *ura3-52 his3-*Δ*200*) and permitted to mate. Diploid patches were selected on synthetic minimal medium lacking histidine, leucine, adenine, tryptophan, and lysine. These patches were sporulated and replica plated to strains GRY1580 and GRY1582 (relevant genotypes *MAT***a** and *MAT*α, respectively, *URA3 his3-*Δ*200*) on YEPD. After overnight incubation allowing mating, the plates were replica plated to synthetic complete medium lacking histidine and uracil. Under these conditions, if His$^+$ was linked to *MAT***a**, then second-generation diploid patches would grow only when spores mated to GRY1580 (*MAT*α). If the His$^+$ phenotype was not linked to *MAT*, patches would grow with similar efficiencies when spores mated with GRY1580 (*MAT*α) or GRY1582 (*MAT***a**). A similar test to confirm that the *HIS3* recombinant gene in *MAT*α *cry1* His$^+$ segregants was linked to *MAT* was performed by mating to the *MAT***a** *ura3-52 his3-*Δ*200* strain ED315-93b.

RESULTS

The *S. cerevisiae* genome contains sites that undergo high ("hotspots") and low ("coldspots") levels of meiotic recombination. Many chromosomal sites that sustain high frequencies of meiotic DSBs undergo high frequencies of meiotic recombination (Sun *et al.* 1989; Wu and Lichten 1994, 1995; Baudat and Nicolas 1997; Borde *et al.* 1999). However, only a small number of hotspots have been characterized in detail (Nicolas *et al.* 1989; Malone *et al.* 1994; Fan *et al.* 1995; Lichten and Goldman 1995). We were interested in making a genome-wide survey for additional hotspots and coldspots to better understand how the effect of genome position can govern the efficiency of meiotic recombination.

To identify additional hotspots and coldspots of meiotic recombination, we designed a system comparing the frequencies of meiotic recombination between a pair of homologous recombination substrates placed in several ectopic positions (Figure 1). A bank of isogenic diploid strains was generated, each carrying two different mutant *his3* genes. All strains carried one mutant *his3* at a constant position, near *MAT***a**, and a second mutant *his3*, the variable, at distinct ectopic sites. Meiotic DSBs, the presumed initiating lesions for meiotic recombination in *S. cerevisiae*, occur at normal levels on chromosomes even in the absence of nonsister homology (Nicolas *et al.* 1989; Gilbertson and Stahl 1994; Wu and Lichten 1995). Therefore, we did not expect that varying the position of one recombination substrate would significantly affect the ability of the constant substrate to sustain initiation events. Thus, in principle, any differences in meiotic recombination rates among the various ectopic pairs were expected to reflect changes in the properties of the variable recombination substrate when moved to different locations.

We used variable *his3* insertions generated by two different methods. In one set of experiments (Figure 2A), *his3-AI* was placed in variable positions by the *S. cerevisiae* retrotransposon Ty1 (Bryk *et al.* 1997; M. J. Curcio, personal communication). Haploid strains previously found to carry single, unspliced (His$^-$) Ty1m*his3-AI* insertions were chosen. Insertion sites were determined subsequently. Each Ty1m*his3-AI*-bearing haploid was mated to a second strain, GRY818, carrying *his3-621* inserted in constant position on chromosome *III* between *CRY1* and *MAT***a**. In the second set of experiments (Figure 2B), *his3-621* was inserted at variable positions in haploids by targeted integration using selection for a linked *TRP1* gene. *TRP1-his3-621* haploids were mated to a second strain, ED361A-1, carrying *his3-*Δ*MscI* near *MAT***a** (same position and orientation as *his3-621* in GRY818). Recombination between the *his3* markers at variable loci and *MAT***a***::his3* can generate His$^+$ colonies, which are visualized in patches or quantitated among random spores. The *MAT-CRY1* interval was chosen for integration of the constant *his3* because, being a known coldspot for meiotic recombination (Oliver *et al.* 1992; Baudat and Nicolas 1997), it was expected to maximize the sensitivity of detecting insertion sites that are hotspots for meiotic recombination.

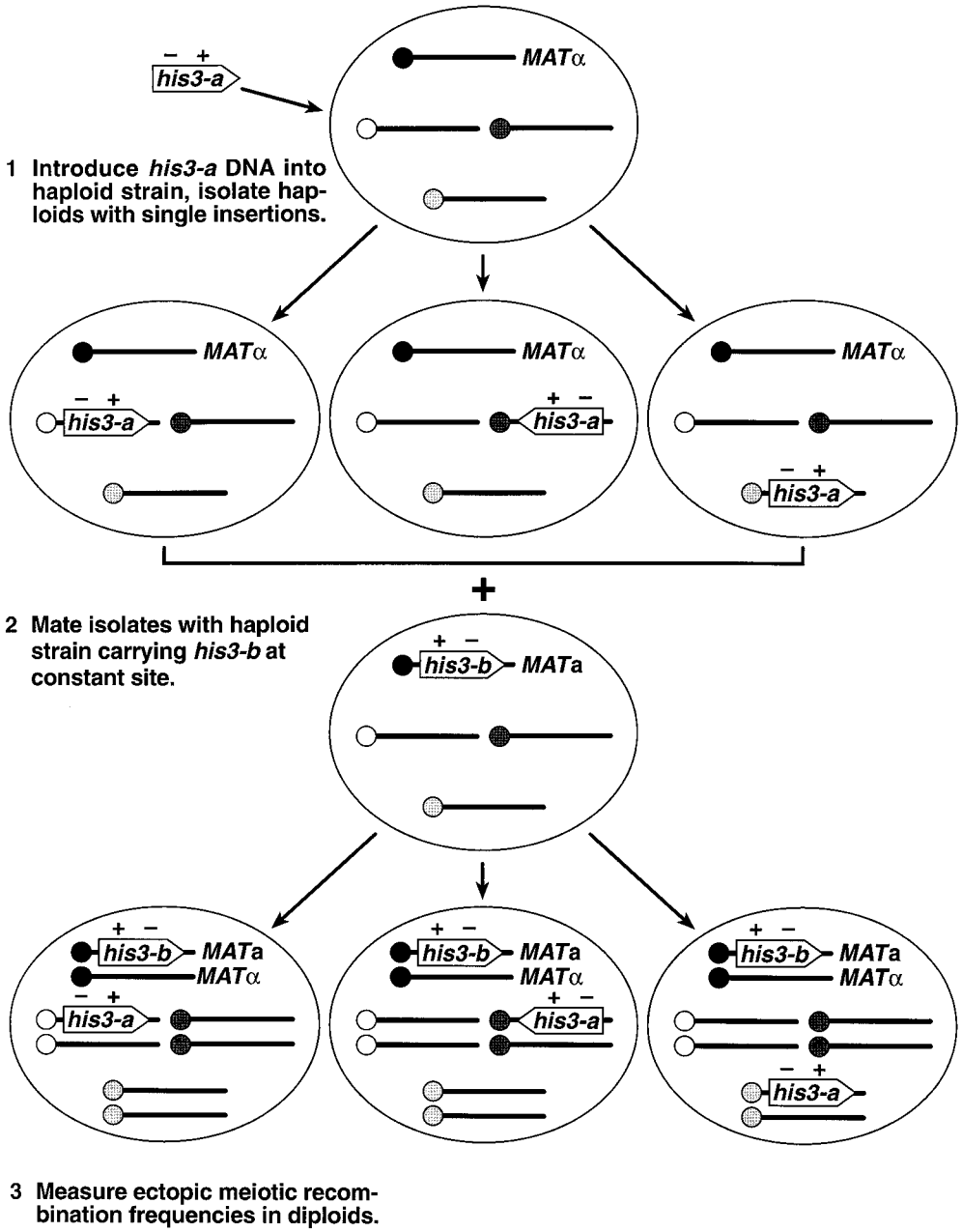

Figure 1.—Strategy for identifying genomic sites with exceptional properties in meiotic recombination. (Top) A nonfunctional *his3* gene (*his3-a*) is introduced into a haploid strain in which the resident *HIS3* was deleted. Individual derivatives carrying *his3-a* at different sites are isolated and mated to another haploid carrying a second mutant *his3*, *his3-b*, placed near *MAT***a**. The resulting isogenic diploids (bottom) are unable to grow on medium lacking histidine (His^-). Diploids are sporulated and allowed to complete meiosis. Ectopic recombination between *his3-a* and *his3-b* can create a fully functional *HIS3* gene, permitting the cell to grow on synthetic medium lacking histidine (His^+).

Each diploid also carried the *ura3-52* and *ura3-167* mutations in allelic positions at *URA3* on chromosome V. Recombination between these can generate Ura^+ spores, which we quantitated as a control for sporulation and overall recombination efficiency.

Initial test of the ectopic recombination system: As described below, we observed efficient meiotic ectopic recombination between the *his3-AI* sequences inserted at several sites by Ty1 and the *his3-621* gene near *MAT***a**. However, the identification of the His^+ prototrophs required verification that those colonies arose from meiotic recombination and not by retrotransposition or RNA-mediated recombination.

The increase in His^+ frequencies upon sporulation is illustrated by the behavior of two diploid strains, ED292A (carrying a Ty1m*his3-AI* on chromosome *XII* at position *L1060532*, using the numbering system of the *Saccharomyces* Genome Database) and ED294A (carrying a Ty1m*his3-AI* on chromosome *II* at position *B326918*). The mitotic levels of His^+ for ED292A and ED294A were 1.0×10^{-7} and 1.3×10^{-7}, respectively. Upon sporulation the His^+ frequencies increased over 100-fold to 3.1×10^{-5} and 5.3×10^{-5}, respectively. An internal control for allelic recombination using mutations in *ura3* showed a 100-fold increase upon sporulation over mitotic levels (from 1.8×10^{-5} to $\sim 2 \times 10^{-3}$). This increase in His^+ frequency is consistent with meiotic ectopic recombination between the Ty1m*his3-AI* element and the *his3-621* sequence at *MAT*. However, Ty1m*his3-AI* can generate His^+ cells via Ty1 transposi-

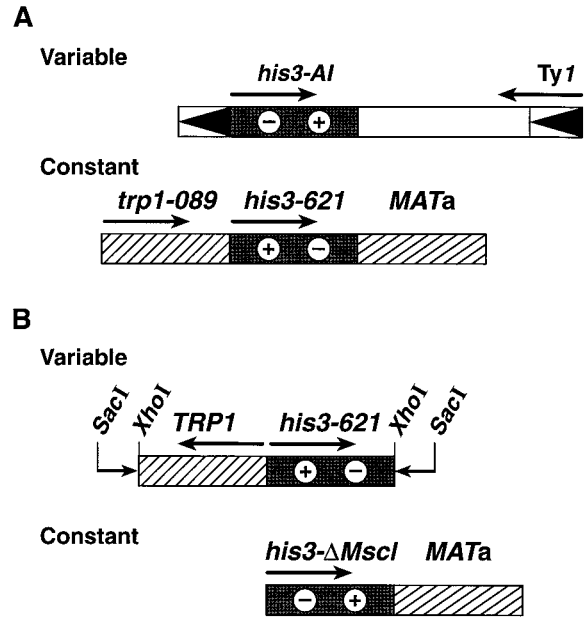

Figure 2.—*his3* recombination substrates. (A) Ty1m*his3-AI* × *MAT***a**::*trp1-089-his3-621*. Ty1m*his3-AI* (variable) is a hybrid Ty1 transposable element (white) carrying a defective *his3* gene (gray) inserted near the 3′ long-terminal repeat (boxes with arrows). *his3-AI* contains a 104-bp artificial intron (AI) inserted at the *Msc*I site. (B) *TRP1-his3-621* × *MAT***a**::*his3* Δ*MscI*. *TRP1-his3-621* (variable) is *his3-621* (gray), a defective *his3* gene containing a fill-in of the *Asp*718 site, linked to a fully functional *TRP1* gene (crosshatched) in divergent transcriptional orientation. *TRP1-his3-621* is flanked by a partial pBluescript II polylinker (Stratagene) in a palindromic repeat. Large arrows represent the direction of transcription. Small arrows represent the direction of the polylinker from the *Sac*I site to the *Xho*I site. *TRP1-his3-621* can be inserted at any locus in the *S. cerevisiae* genome by standard cloning and transformation techniques and selection for the ability to grow on synthetic medium lacking tryptophan.

tion (Curcio and Garfinkel 1991) or RNA-mediated gene conversion (Derr *et al.* 1991). To determine the contribution of these mechanisms to the frequencies of meiotic His^+ events in our ectopic recombination system, we replaced *his3-621* near *MAT***a** in ED292A and ED294A with *his3*-Δ*MscI*. The *his3-AI* and *his3*-Δ*MscI* mutations are at the same position in *HIS3* and hence cannot recombine to generate *HIS3*. A His^+ phenotype can arise in the *his3*-Δ*MscI* × Ty1m*his3-AI* diploids only via Ty1m*his3-AI* transposition to a new site, or RNA-mediated gene conversion between Ty1m*HIS3* cDNA and endogenous Ty1 elements or the *MAT***a**::*his3*-Δ*MscI* sequence.

The frequencies of meiotic His^+ formation in the *MAT***a**::*his3*-Δ*MscI* × Ty1m*his3-AI* derivatives of ED292A and ED294A (ED308A and ED310B, respectively) following sporulation were 1×10^{-7} and 0.2×10^{-7}, about the same as their mitotic levels. Thus meiosis did not strongly increase the level of Ty1m*his3-AI* transposition. In summary, these results support the conclusion that virtually all His^+ events observed following sporulation of *MAT***a**::*his3-621* × Ty1m*his3-AI* diploids were due to meiotic ectopic recombination.

RDN1* is a poor participant in meiotic ectopic recombination:** We determined whether placing Ty1m *his3-AI* in different locations affected the frequency of meiotic ectopic recombination. Eight diploids were examined, each containing *his3-621* near *MATa** and Ty1m*his3-AI* in different places. Six strains (ED288A, ED289A, ED290B, ED292A, ED294A, and ED295A; Table 2) exhibited a frequency of meiotic recombination to His^+ varying over only a sixfold range. A similar range of meiotic recombination frequencies among different ectopic crosses, in which the position of one sequence is held constant, also has been observed previously (Goldman and Lichten 1996).

Two strains, ED291B and ED293A (Table 2), behaved much differently from the other six. The frequencies of meiotic His^+ events in ED291B and ED293A were ~100-fold lower than that of the other six strains of Table 2. In all of the strains of this experiment, the frequencies of allelic recombination at the control locus, *URA3*, varied over less than a twofold range. Thus, the low frequency of meiotic His^+ formation in ED291B and ED293A was not due to a defect in sporulation or the general machinery of meiotic recombination, but rather to the effect of genomic position on the Ty1m*his3-AI* recombination substrate. Interestingly, both ED291B and ED293A contained Ty1m*his3-AI* insertions in the nontranscribed spacer region of the ribosomal DNA (*RDN1*), a cluster of 100–200 9.1-kb transcription units arranged in a direct repeat on chromosome *XII* (Petes and Botstein 1977).

Although meiotic allelic recombination at *RDN1* occurs at very low frequency (Petes and Botstein 1977), the result presented in Table 2 was unexpected, given the proposal that homology searching makes all genomic sites accessible to ectopic recombination. This result suggests that events that initiated at the constant *his3* sequence and that were sufficient to promote recombination with *his3* at most of the ectopic sites we tested were not sufficient to promote recombination with a *his3* insert within *RDN1*. Therefore, we considered several possibilities to account for our observation of such an exceptionally low rate of meiotic ectopic recombination involving *RDN1*.

First, we eliminated the possibility that the Ty1m*his3-AI* sequences in these strains might have been lost before meiosis due to recombination between the tandemly repeated *RDN1* units. In the experiment summarized in Table 2, DNA was extracted just before meiosis from one sample each of diploids ED292A (carrying Ty1m*his3-AI* on chromosome *XII*, outside *RDN1*) and ED294A (carrying Ty1m*his3-AI* on chromosome *II*); three samples each of the diploids carrying Ty1m*his3-AI* inserts in *RDN1* (ED291B and ED293A); one sample each of the haploids JC234, JC242, JC272, and JC273 (parents of ED291B, ED292A, ED293A, and ED294A, respectively);

TABLE 3

TRP1-his3-621* × *MAT*a*::his3-*Δ*MscI

Strain	*TRP1-his3-621* Locus	*TRP1-his3-621* Orientation[a]	Meiotic recombination frequencies × 10^5 His$^+$	Ura$^+$	Leu$^+$
ED413-1	*ARG4*	Same	16.0 ± 1.0[b]	161 ± 3[b]	22.2 ± 1.1[b]
ED414-1	*ARG4*	Opposite	15.5 ± 1.4	163 ± 15	21.0 ± 3.0
ED409-1	*CUP1*	Same	6.7 ± 0.2	140 ± 17	20.3 ± 0.9
ED410-1	*CUP1*	Opposite	5.6 ± 0.8	165 ± 15	20.6 ± 3.0
ED403-18	*RDN1*	Same	0.16 ± 0.03[b]	169 ± 28	23.5 ± 3.8
ED404-19	*RDN1*	Opposite	0.08 ± 0.01	152 ± 9	22.0 ± 1.7
ED479-62[c]	*RDN1* (*sir2*)	Same	0.75 ± 0.32[b]	133 ± 5	11.4 ± 3.3[b]
ED480-13[c]	*RDN1* (*sir2*)	Opposite	0.62 ± 0.12	156 ± 32	13.4 ± 2.2

[a] Orientation of *his3-*Δ*MscI* and *his3-621*, relative to centromeres.
[b] $n = 2$.
[c] ED479-62 and ED480-13 are isogenic with ED403-18 and ED404-19, respectively.

and the other haploid parent, GRY818 (no Ty1m*his3-AI* insert; *his3-621* near *MAT***a**). DNA was digested with *Cla*I, Southern blotted, and probed with *his3* DNA. We observed that the intensity of the 1026-bp *his3-AI* fragment was similar among the eight diploid samples (data not shown), indicating that the low rate of meiotic ectopic recombination involving *RDN1* was not due to large-scale loss of Ty1m*his3-AI* prior to meiosis.

In similar fashion, we asked whether Ty1m*his3-AI* might have been lost from *RDN1* during meiosis, possibly due to unequal sister chromatid recombination. We isolated 44 haploid colonies from ED293A following meiosis from the nonselective (YEPD) plates in the experiment summarized in Table 5. Because the Ty1m*his3-AI* insert is hemizygous in ED293A, a well-maintained insert should be present in ~22 (50%) of the haploid meiotic derivatives. DNA was isolated from each haploid, digested with *Cla*I, Southern blotted, and probed with *his3* DNA. We found that 26 of the 44 haploids (59%) still retained the 1026-bp *his3-AI* insert in *RDN1* following meiosis (data not shown). This result indicates that Ty1m*his3-AI* is well maintained in *RDN1*, yet is nearly unable to participate in meiotic ectopic recombination.

The low rate of ectopic recombination at *RDN1* is independent of Ty1: Another possible explanation for the low rate of meiotic ectopic recombination involving *RDN1* is that the presence of Ty1 sequences in ED291B and ED293A might have created a substrate that was a poor participant in ectopic recombination at *RDN1*, since these insertions are sensitive to rDNA silencing (Bryk *et al.* 1997). A similar phenomenon was also reported by Smith and Boeke (1997), although they also found that a minimal *URA3* promoter is silenced at *RDN1* without Ty1 sequences. To determine whether other substrates without Ty1 sequences were also poor participants in meiotic ectopic recombination when inserted at *RDN1*, we made a new recombination substrate. This construct contained *his3-621*, marked with *TRP1* and lacking Ty1 DNA (Figure 2B). *TRP1-his3-621* can recombine with *his3-*Δ*MscI* (placed in the constant position in the *MAT***a**-*CRY1* interval) to yield His$^+$ prototrophs.

We placed *TRP1-his3-621* in diploids at the *ARG4*, *RDN1*, and *CUP1* loci. The cassette was inserted in both possible orientations, to determine any potential influence of ectopic crossovers that lead to acentric and dicentric chromosomes. In addition to measuring allelic recombination at *URA3* (*ura3-52* × *ura3-167*), we included an internal control for meiotic ectopic recombination to Leu$^+$ (*leu2-K* × *lys2::leu2-R*).

When *TRP1-his3-621* was inserted at *ARG4*, we observed efficient meiotic ectopic recombination to His$^+$ (Table 3). The frequency of meiotic recombination was about 100-fold higher than during vegetative growth (data not shown). Orientation of the cassette did not significantly alter the frequency of meiotic ectopic recombination to His$^+$ (Table 3, compare ED413-1 with ED414-1).

When *TRP1-his3-621* was inserted at *RDN1*, the frequency of meiotic ectopic recombination to His$^+$ was about 100-fold lower than in the strain carrying the variable substrate at *ARG4* (Table 3). Orientation of the cassette did not significantly affect the frequency of ectopic recombination to His$^+$ (Table 3; compare ED403-18 with ED404-19). Meiotic allelic recombination at the control *URA3* locus varied by less than a factor of two among the strains carrying *TRP1-his3-621* at *ARG4* or *RDN1*. Therefore, the low rate of meiotic ectopic recombination involving *RDN1::TRP1-his3-621* was not due to an inability to induce meiotic recombination.

We also determined whether the low rate of meiotic ectopic recombination involving *RDN1* was a common property of naturally occurring direct repeats. We inserted *TRP1-his3-621* at the *CUP1* locus on chromosome *VIII*. *CUP1* is a 2.1-kb gene that, like *RDN1*, exists in a 2- to 30-copy tandemly repeated array (Fogel and Welch

1982). Our strains carried at least three repeat units, as estimated by Southern blotting (E. S. Davis and J. N. Strathern, unpublished data). Although the position of insertion of *TRP1-his3-621* within a *CUP1* repeat unit was confirmed by Southern analysis, the repeat unit within the array carrying the insertion was not identified. The frequency of ectopic recombination at *CUP1* was about 2.5-fold lower than at *ARG4* and at least 35-fold higher than at *RDN1*. As for *ARG4* and *RDN1*, orientation of the *TRP1-his3-621* cassette did not significantly influence the frequency of meiotic ectopic recombination to His^+ (Table 3; compare ED 409-1 with ED410-1).

These results permit the following conclusions: (1) The low rate of meiotic ectopic recombination between *MAT***a***::his3* and *RDN1::his3* did not require the presence of Ty1 sequences; (2) the low rate of meiotic ectopic recombination involving *RDN1* was independent of the orientation in which *TRP1-his3-621* was inserted; (3) *TRP1-his3-621* inserted within *ARG4* underwent efficient meiotic ectopic recombination; and (4) despite being a directly repeated locus itself, *CUP1* was also an efficient participant in meiotic ectopic recombination.

RDN1* is a poor donor locus in meiotic recombination:** One published report provides evidence that meiotic DSBs that initiate recombination do not occur within *RDN1* (Høgset and Øyen 1984). Therefore, another explanation for the low rate of meiotic ectopic recombination involving *RDN1* is that the constant *MATa***::his3* was also unable to initiate meiotic ectopic recombination. Two experiments were conducted to address this possibility.

In the first experiment, we estimated the rate of initiation of meiotic ectopic recombination near *MAT***a***::his3-621*. In meiotic gene conversion in *S. cerevisiae*, the chromosome sustaining DSBs tends to act as the recipient (Nicolas *et al.* 1989). We have observed a similar bias of the initiating chromosome acting as recipient in mitotic recombination events (McGill *et al.* 1993). Therefore, the frequency at which *MAT***a***::his3-621* became His^+ should reflect the rate of initiation of meiotic ectopic recombination at that locus. As the *his3-621* sequence was tightly linked to *MAT***a** in the strains of Table 2, we determined the percentage of His^+ events linked to mating type among spore progeny in the six strains with Ty1m*his3-AI* insertions outside *RDN1*.

Among the six strains with Ty1m*his3-AI* insertions outside *RDN1*, the fraction of His^+ events linked to *MAT***a** ranged from 26 to 90% (Table 2). These data suggest that *MAT***a***::his3-621* was about as likely to serve as a recipient of His^+ information in meiotic recombination as the Ty1m*his3-AI* insertions outside *RDN1*. To estimate the frequency at which *MAT***a***::his3-621* serves as recipient in the six strains with Ty1m*his3-AI* insertion sites outside *RDN1*, we multiplied the percentage of His^+ events linked to *MAT***a** by the overall rate of meiotic ectopic recombination to His^+. These calculations resulted in frequencies that ranged from 0.63×10^{-5} to 2×10^{-5}. The overall rate of meiotic ectopic recombination in the strains carrying Ty1m*his3-AI* at *RDN1* was much lower than this estimated initiation rate, suggesting that *his3* inserted at *RDN1* was a poor donor in meiotic ectopic recombination with *his3* inserted near *MAT***a**.

The *MAT-CRY1* interval on chromosome *III* is a coldspot for meiotic recombination (Oliver *et al.* 1992), sustaining relatively few meiotic DSBs (Baudat and Nicolas 1997). We hypothesized that, if *RDN1::his3* was a poor donor with *MAT***a***::his3*, then *RDN1::his3* also should be a poor donor with a locus that undergoes efficient ectopic recombination with *MAT*. We conducted a second experiment, in which we made *ARG4* the site of the constant *his3* substrate, *TRP1-his3-621*, instead of the *trp1-089-his3-621* insertion near *MAT***a**. We constructed a set of such diploid strains, ED491A, ED503C, ED485B, and ED497A, which were isogenic with ED292A, ED294A, ED291B, and ED293A, respectively, from the experiment summarized in Table 2. However, we note that while the natural *ARG4* locus is a hotspot for allelic recombination in meiosis (Nicolas *et al.* 1989), we do not know whether DSBs characteristic of *ARG4* hotspot activity are occurring in the *arg4::TRP1-his3-621* construct or whether such DSBs outside the region of homology will be sufficient to initiate ectopic recombination.

We compared the frequencies of meiotic ectopic recombination between *arg4::TRP1-his3-621* and the Ty1m*his3-AI* insertions at different sites within this new strain set. A result similar to that presented in Table 2 was obtained (Table 4). Strains containing Ty1m*his3-AI* insertions at *RDN1* were still two orders of magnitude less proficient in meiotic ectopic recombination to His^+ than strains with Ty1m*his3-AI* insertions elsewhere. In addition, *MAT***a***::his3* and *arg4::his3* recombined efficiently with each other (Table 3). Taken together, these results suggest that neither *MAT***a***::his3* nor *arg4::his3* was capable of efficient recombination with *RDN1::his3*.

***SIR2* participates in the sequestration of *RDN1*:** Loss of *SIR2* function elevates the rate of mitotic and meiotic

TABLE 4

arg4::TRP1-his3-621* × Ty1m*his3-AI

Strain	*his3-AI* location[a]	Meiotic recombination frequencies × 10^5	
		His^+	Ura^+
ED491A	XII (*L1060532*)	1.9 ± 0.2	231 ± 24
ED503C	II (*B326918*)	8.1 ± 0.6	202 ± 4
ED485B	XII (*RDN1*)	0.02 ± 0.01	250 ± 27
ED497A	XII (*RDN1*)	0.01 ± 0.01	208 ± 12

[a] Chromosome and genome position (in parentheses), using numbering system of the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces).

TABLE 5

Loss of Sir2 function elevates the frequency of meiotic ectopic recombination involving *RDN1::Ty1mhis3-AI*

Strain	*his3-AI* location[a]	*SIR2* allele	Meiotic recombination frequencies $\times 10^5$	
			His^{+}[b]	Ura^{+}
ED291B	XII (*RDN1-234*)	*SIR2*	0.01 ± 0.002	263 ± 58
ED347C-1	XII (*RDN1-234*)	*sir2::hisG*	0.33 ± 0.14	204 ± 13
ED293A	XII (*RDN1-272*)	*SIR2*	0.01 ± 0.005	271 ± 36
ED349G-1	XII (*RDN1-272*)	*sir2::hisG*	0.14 ± 0.02	187 ± 14
ED292A	XII (*L1060532*)	*SIR2*	3.1 ± 0.1	279 ± 17
ED348A-2	XII (*L1060532*)	*sir2::hisG*	1.5 ± 0.1	207 ± 15
ED294A	II (*B326918*)	*SIR2*	5.3 ± 0.3[c]	244 ± 1[c]
ED350A-1	II (*B326918*)	*sir2::hisG*	1.6 ± 0.3	200 ± 21

[a] Chromosome and genome position (in parentheses), using numbering system of the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces).
[b] Other *his3* allele was *MAT***a***::his3-621*.
[c] $n = 2$.

interhomolog and intrachromosomal recombination at *RDN1* (Gottlieb and Esposito 1989; San-Segundo and Roeder 1999). Therefore, we examined the role *SIR2* plays in meiotic ectopic interchromosomal recombination involving *RDN1* by constructing appropriate diploid strains homozygous for disruption of *SIR2* (Table 5). *SIR2* disruption elevated the rate of meiotic interchromosomal recombination between *MAT***a***::his3-621* and two different Ty1m*his3-AI* insertions in *RDN1* ~15- to 30-fold (compare ED291B with ED347C-1 and ED293A with ED349G-1). The *sir2* disruption did not increase the frequency of ectopic recombination to His^{+} at other loci (compare ED292A with ED348A-2 and ED294A with ED350A-1) or significantly increase the frequency of allelic recombination to Ura^{+}.

We also inactivated *SIR2* in the strains carrying *TRP1-his3-621* at *RDN1*. In these strains, *sir2* disruption caused a reproducible five- to eightfold increase in the frequency of meiotic ectopic recombination involving *RDN1* (Table 3; compare ED479-62 and ED480-13 with ED403-18 and ED404-19, respectively). This relative increase was less than the *sir2*-mediated, 15- to 30-fold elevation of meiotic ectopic recombination involving *RDN1* when Ty1m*his3-AI* was the substrate (compare Table 3, ED479-62 and ED480-13, with Table 5, ED347C-1 and ED349G-1), although the absolute frequencies were comparable.

We demonstrated that the low level of ectopic recombination did not reflect loss of the *TRP1-his3-621* sequences from *RDN1* before or during meiosis by scoring the Trp^{+} phenotype in spore clones. Among the Ade^{-} spore-derived colonies from the strains of Table 3, 60% from ED403-18 (*SIR2*/*SIR2*) and 53% from ED479-62 (*sir2::hisG*/*sir2::hisG*) were Trp^{+} (~50% were expected to still carry the insert). The presence of a full-length *TRP1-his3-621* insert at the onset of sporulation in the *SIR2* and *sir2::hisG* diploids was confirmed by Southern blotting (not shown).

DISCUSSION

Meiotic ectopic recombination between two different *his3* sequences occurred at an exceptionally low rate when one sequence was inserted in the yeast rDNA array (*RDN1*). Two lines of evidence suggest that *RDN1* acted as an unusually poor donor sequence in meiotic ectopic recombination.

Meiotic ectopic recombination between *MAT***a***::his3* and *RDN1::his3* occurred at low frequency, despite evidence that *MAT***a***::his3* acted as an efficient recipient of His^{+} events and presumably was sustaining initiating chromosome breaks (Table 2). The frequency at which *MAT***a***::his3-621* served as recipient in the six strains with Ty1m*his3-AI* insertion sites outside *RDN1* ranged from 0.63×10^{-5} to 2×10^{-5}. If this range of frequencies reflected the rate of initiation of meiotic ectopic recombination at *MAT***a***::his3-621* and if an initiating break on one chromosome is rate limiting, then the frequency of meiotic ectopic recombination between *MAT***a***::his3-621* and any other site should fall within this range of $\sim 1 \times 10^{-5}$. However, the frequency of meiotic His^{+} formation in the strains carrying Ty1m*his3-AI* at *RDN1* was about 100-fold lower than this apparent initiation rate (Table 2). This result suggests that despite efficient initiation of meiotic ectopic recombination at *MAT***a***::his3-621*, *RDN1::*Ty1m*his3-AI* was a poor template for repair of these events.

Second, *RDN1::his3* was a poor participant in meiotic ectopic recombination with *MAT***a***::his3* or *arg4::his3*. In contrast, *MAT***a***::his3* and *arg4::his3* recombined well (1.5×10^{-4}) with each other (Table 3), demonstrating that one or both loci were capable of initiating ectopic

recombination in meiosis. If initiation is rate limiting, either *MAT***a***::his3* and/or *arg4::his3* should undergo efficient ectopic recombination with *RDN1::his3*. In contrast, the rates of ectopic recombination to His^+ in the *RDN1::his3* × *arg4::his3* crosses were not significantly different from those in the *RDN1::his3* × *MAT***a***::his3* crosses. We argue that the *S. cerevisiae RDN1* locus served as a poor donor because it was inaccessible, or sequestered from a genome-wide homology search employed by meiotic cells. This model predicts that an initiating DSB in a *his3* outside *RDN1*, if unable to recombine with *RDN1::his3*, will instead be repaired using the homolog or the sister chromatid as a template.

Our results demonstrate that the initiating event alone is not sufficient for meiotic ectopic recombination. A previous study suggests that all genomic regions can act as donors with similar propensity (Haber *et al.* 1991). However, those experiments examined a small number of donors, and most of the donors were on the same chromosome as the recipient. Among other ectopic crosses, in which the position of one substrate is held constant, the frequencies of interchromosomal meiotic ectopic recombination vary over rather narrow (2- to 10-fold) ranges (Goldman and Lichten 1996). As shown by Goldman and Lichten (1996), however, allelic and ectopic recombination between homologs is more efficient than ectopic recombination between heterologous chromosomes, suggesting some role for homologous chromosome pairing in enhancing the efficiency of meiotic recombination.

RDN1 undergoes much less meiotic crossing over than expected from its size (Petes and Botstein 1977; Zamb and Petes 1982), but experiences frequent meiotic unequal sister chromatid exchange (USCE; Petes 1980). These observations can be explained by the following scenarios: First, *RDN1* lacks initiating DSBs required for crossover (Høgset and Øyen 1984), while USCE occurs by a different mechanism than allelic recombination. Accordingly, mitotic USCE within *RDN1* is *RAD52* independent (Zamb and Petes 1981). Second, the Hop1p protein, which is required for promoting the formation of meiotic allelic recombination intermediates at the expense of USCE (Schwacha and Kleckner 1994), is normally excluded from the nucleolus (San-Segundo and Roeder 1999). Mutations in *SIR2* and *PCH2*, however, cause mis-localization of Hop1p to the nucleolus, and *RDN1* becomes competent for allelic recombination (San-Segundo and Roeder 1999).

A precedent for the idea that not all sequences act as efficient recombination donors has been observed in mating-type interconversion in the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe*. The directionality that mating-type interconversion exhibits, that is, the tendency of *MAT*α cells to switch to *MAT***a** or *mat1-p* cells to switch to *mat1-m*, may reflect a cell-type-dependent sequestration of the donor sequences related to the meiotic sequestration of *RDN1* described here.

Mating-type interconversion is a mitotic gene conversion event, initiated by a site-specific chromosome break at the mating-type locus (*MAT* in *S. cerevisiae*; *mat1* in *S. pombe*). Both yeasts carry transcriptionally silenced copies of mating-type genes distal to the mating-type loci (*HML*α and *HMR***a**, in *S. cerevisiae*; *mat2-P* and *mat3-M* to the right of *mat1* in *S. pombe*). The mating-type loci are flanked by DNA having perfect homology with both silent cassettes, yet during switching haploid cells are selective, because they use only the donor locus normally carrying information of the opposite mating type. Hence, the accessibility of the ectopic donor sequences is regulated by mating type. Similar to our observation of meiotic recombinational sequestration of *RDN1*, when *MAT* is undergoing efficient initiation of recombination stimulated by the Ho-endonuclease, it is unable to participate in ectopic recombination with one of the unlinked, homologous loci.

Cis-acting elements and *trans*-acting factors regulate the nonrandom donor choice during interconversion, but the precise mechanism seems to be different in both yeasts. In both systems, donor choice is associated with repression of recombination within nearby intervals (Thon and Klar 1993; Wu and Haber 1996; Grewal and Klar 1997; Szeto *et al.* 1997; Szeto and Broach 1997). In *S. pombe*, chromatin structure modification is proposed to play a role in regulating donor choice (Grewal and Klar 1997).

How might the role of Sir2p in meiotic sequestration of *RDN1* described here be related to its known roles in silencing and rDNA mitotic stability? *SIR2* was first defined on the basis of its requirement in silencing transcription of *HML*α and *HMR***a** (Klar *et al.* 1979; Ivy *et al.* 1986). Transcriptional silencing at telomeres is also Sir2p dependent (Aparicio *et al.* 1991), and silencing of pol II promoters inserted within the rDNA repeat array was recently also shown to be dependent on Sir2p (Bryk *et al.* 1997; Smith and Boeke 1997). Sir2p has not been shown to have a direct role in the recombination associated with mating-type switching beyond blocking access of the Ho-endonuclease to its potential recognition sites in *HML* and *HMR*. In contrast, Sir2p's activity in the rDNA repeats was first revealed by its role in repressing inter- and intrachromosomal recombination at *RDN1* (Gottlieb and Esposito 1989; San-Segundo and Roeder 1999). Sir2p does not bind DNA, but has both *in vitro* ADP-ribosyltransferase (Tanny *et al.* 1999) and NAD-dependent histone deacetylase (Shin-Ichiro *et al.* 2000) activities. One or both of these activities appear to be essential for silencing *in vivo* (Tanny *et al.* 1999; Shin-Ichiro *et al.* 2000). In addition, *SIR2* overexpression causes histone deacetylation (Braunstein *et al.* 1993).

Mitotic and meiotic recombination occur at higher levels in transcriptionally active DNA (Thomas and Rothstein 1989; Wu and Lichten 1994), although transcription itself is not required for meiotic recombi-

nation (Schultes and Szostak 1991; White *et al.* 1992). These findings suggest that efficient recombination requires the presence of DNA that is accessible to recombination factors. Sir2p probably plays a role in the establishment or maintenance of chromatin structure at *RDN1*. Recombinational sequestration and RNA polymerase II-dependent transcriptional silencing might share some common chromatin structure determinants. Consistent with this idea, transcription of the *RDN1*:: Ty1m*his3-AI* elements in strains ED291B and ED293A is silenced in a *SIR2*-dependent manner in their haploid parents JC234 and JC272, respectively (Bryk *et al.* 1997).

Our results demonstrate that *RDN1* was sequestered from meiotic ectopic recombination in a *SIR2*-dependent manner whether we used Ty1m*his3-AI* or *TRP1-his3-621* as the substrate. *SIR2* disruption resulted in a smaller-fold increase in meiotic ectopic recombination frequency at *RDN1* in the strains carrying *TRP1-his3-621* (5- to 8-fold) compared with Ty1m*his3-AI* (15- to 30-fold). These differences were probably due to the higher His^+ recombination levels in the *SIR2* strains carrying *TRP1-his3-621* at *RDN1* and could reflect dependence on the expression of the *TRP1* gene.

We propose that all recombination events involving *RDN1* (except for intersister exchange) are repressed in a *SIR2*-dependent manner. However, *SIR2* disruption only partially relieved the block of *RDN1* to interchromosomal ectopic recombination. One possible cause of sequestration is the physical localization of *RDN1* in the nucleolus. Therefore, additional barriers that are unique to interchromosomal ectopic recombination might exist.

What might be the evolutionary significance of recombinational sequestration in meiosis? In mating-type switching, donor choice is nonrandom to ensure productive switching and mating following sporulation. In meiosis, one possibility is that natural direct repeat sequences are inhibited from all ectopic recombination to maintain correct copy number and avoid chromosome translocations. However, *CUP1::his3* underwent high levels of interchromosomal meiotic ectopic recombination (Table 3), demonstrating that recombinational sequestration is not a general property of naturally occurring direct repeats. *CUP1* might not be subjected to sequestration because it is much shorter than *RDN1* and is not part of a specialized intranuclear structure such as the nucleolus.

Alternatively, recombinational sequestration might protect some genomic regions from the consequences of invasion by transposable elements. This model has also been proposed by Smith and Boeke (1997). *RDN1* is an efficient target of induced Ty1 transposition (Bryk *et al.* 1997). This phenomenon could result from the large number of repeat units in the array or the tendency of RNA polymerase III-transcribed genes to serve as efficient recipients of Ty1 transposition (Kim *et al.* 1998). If a Ty1 element transposed to *RDN1*, meiotic ectopic recombination with one of the other ~30 genomic Ty1 elements (Cameron *et al.* 1979) could disrupt the structure of the nucleolus, resulting in decreased fitness.

Regions other than the rDNA of the *S. cerevisiae* genome are also sequestered from meiotic ectopic recombination. E. J. Louis (personal communication) has observed that telomeres are poor participants in meiotic ectopic recombination with nontelomeric loci. Unlike *RDN1*, telomeres underwent efficient allelic and interchromosomal ectopic recombination with other telomeres. We surveyed the genome for additional sequestered sites by making insertions of the *TRP1-his3-621* cassette (Figure 2B) using restriction endonuclease-mediated illegitimate recombination (Schiestl and Petes 1991). With that approach we again identified *RDN1* and found an additional site on chromosome *IV*, between Ty1 and Ty2 elements at position *D987072*, as positions sequestered from meiotic ectopic recombination (E. S. Davis and J. N. Strathern, unpublished data). Additional investigation is required to determine how many other such sites exist and what functions are required for their sequestration.

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