Multiple Heterologies Increase Mitotic Double-Strand Break-Induced Allelic Gene Conversion Tract Lengths in Yeast

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Manuscript received May 4, 1999
Accepted for publication June 28, 1999

ABSTRACT

Spontaneous and double-strand break (DSB)-induced allelic recombination in yeast was investigated in crosses between *ura3* heteroalleles inactivated by an HO site and a +1 frameshift mutation, with flanking markers defining a 3.4-kbp interval. In some crosses, nine additional phenotypically silent RFLP mutations were present at ~100-bp intervals. Increasing heterology from 0.2 to 1% in this interval reduced spontaneous, but not DSB-induced, recombination. For DSB-induced events, 75% were continuous tract gene conversions without a crossover in this interval; discontinuous tracts and conversions associated with a crossover each comprised ~7% of events, and 10% also converted markers in unbroken alleles. Loss of heterozygosity was seen for all markers centromere distal to the HO site in 50% of products; such loss could reflect gene conversion, break-induced replication, chromosome loss, or G2 crossovers. Using telomere-marked strains we determined that nearly all allelic DSB repair occurs by gene conversion. We further show that most allelic conversion results from mismatch repair of heteroduplex DNA. Interestingly, markers shared between the sparsely and densely marked interval converted at higher rates in the densely marked interval. Thus, the extra markers increased gene conversion tract lengths, which may reflect mismatch repair-induced recombination, or a shift from restoration- to conversion-type repair.

DNA double-strand breaks (DSBs) can be repaired in yeast by end-joining (Critchlow and Jackson 1998), recombinational repair leading to gene conversion (Nickoloff and Hoekstra 1998), or break-induced replication (BIR; Malkova *et al.* 1996; Morrow *et al.* 1997; Bosco and Haber 1998). Gene conversion and BIR both lead to loss of heterozygosity (LOH), as does chromosome loss and some G2 crossovers (Figure 1). Gene conversion, involving nonreciprocal information transfer from a donor to a recipient allele (Petes *et al.* 1991), is a common genetic outcome of DSB repair in yeast. Recent evidence suggests that gene conversion also plays a significant role in the repair of chromosomal DSBs in mammalian cells (Taghian and Nickoloff 1997; Liang *et al.* 1998). Gene conversion may act to maintain homogeneity of or introduce diversity into gene family members (Keil and Roeder 1984; Klein 1984), and conversion from pseudogene donors has been implicated in human diseases (*e.g.*, Watnick *et al.* 1998). DSBs strongly enhance gene conversion as well as crossovers and deletions mediated by single-strand annealing (Nickoloff and Hoekstra 1998). Meiotic conversion in yeast is associated with crossovers in 30–70% of events, and similarly, crossovers are often associated with conversion (Petes *et al.* 1991); this association can be explained by recombination models that include Holliday junctions (Szostak *et al.* 1983; Sun *et al.* 1991). Gene conversion has several other distinguishing features (Petes *et al.* 1991; Nickoloff and Hoekstra 1998). For DSB-induced conversions, an allele suffering a DSB is nearly always the recipient, although conversions of unbroken alleles during plasmid transformation occur at low frequency (Roitgrund *et al.* 1993). When three or more markers are followed, conversion of flanking markers is almost always associated with conversion of the central marker, *i.e.*, conversion tracts are usually continuous. Although these features can be explained by models invoking conversion via gap repair (Szostak *et al.* 1983), current information indicates that most or all gene conversion in yeast involves mismatch repair of heteroduplex DNA (hDNA; Petes *et al.* 1991; Nickoloff and Hoekstra 1998; Weng and Nickoloff 1998).

One limitation of gene conversion studies is that events can be followed only at heterologous sites (markers). As the number of markers increases, so does the resolution for measuring conversion tract lengths and structures (*i.e.*, continuity, directionality). However, markers themselves have been shown to influence the events under study. For example, in bacteria, yeast, and mammalian cells, sequence divergence strongly inhibits spontaneous recombination, often by 100- to 1000-fold

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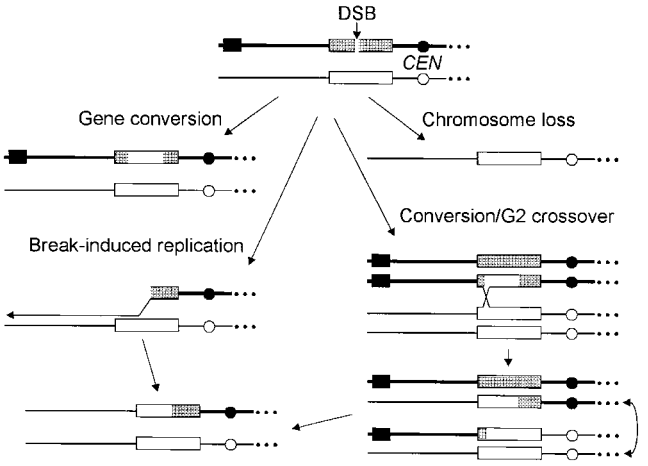

Figure 1.—Fates of broken chromosomes. Gene conversion leads to local LOH, but heterozygosity is retained at a distant, telomeric marker (black box). BIR may lead to partial LOH, but all markers centromere-distal to the DSB are lost. BIR may lead to complete LOH if invasion occurs closer to the centromere (not shown). Chromosome loss leads to complete LOH. Conversion associated with a G2 crossover can yield the same products as BIR if homologs (marked by arrows) cosegregate in mitosis.

(Claverys and Lacks 1986; Waldman and Liskay 1987; Rayssiguier *et al.* 1989; Bailis and Rothstein 1990; Harris *et al.* 1993; de Wind *et al.* 1995; Matic *et al.* 1995; Selva *et al.* 1995; Chambers *et al.* 1996; Datta *et al.* 1996, 1997; Porter *et al.* 1996; Yang and Waldman 1997; Elliott *et al.* 1998; Chen and Jinks-Robertson 1999). In yeast, as little as 1% heterology has been shown to reduce spontaneous ectopic recombination by as much as 8-fold (Datta *et al.* 1997). This inhibition is thought to partly reflect reduced efficiency of strand invasion (DasGupta and Radding 1982), although this may be important only with highly diverged sequences. In large part, inhibition is mediated by the mismatch repair system (de Wind *et al.* 1995; Selva *et al.* 1995; Chambers *et al.* 1996; Datta *et al.* 1996, 1997; Negritto *et al.* 1997), which is thought to scan hybrid DNA and abort recombination when too many mismatches are detected (hDNA rejection). Conversion tract lengths for spontaneous ectopic events were apparently reduced by sequence divergence, an effect that can also be explained by hDNA rejection (Harris *et al.* 1993; Chen and Jinks-Robertson 1998). These reductions in recombination frequencies and tract lengths stand in sharp contrast to several other findings. In meiosis, additional markers decreased crossovers, but increased conversion frequencies (but not tract lengths); these effects were thought to reflect mismatch repair-induced secondary recombination events (Borts and Haber 1987). In a second meiotic study, a single additional marker between an initiating DSB and a distal marker increased conversion of the distal marker (suggesting that the additional marker increased tract lengths); these authors favored the idea that mismatches increased hDNA in a single event rather than stimulating secondary recombination events (Schultes and Szostak 1990). It is difficult to explain increases in gene conversion frequencies and tract lengths in light of hDNA rejection. However, at comparable levels of sequence divergence, DSB-induced recombination is reduced to a lesser extent than spontaneous recombination (Mezard *et al.* 1992; Mezard and Nicolas 1994; Priebe *et al.* 1994); in fact, in two yeast studies, DSB-induced mitotic recombination was not reduced by 15% divergence (Resnick *et al.* 1992; Larionov *et al.* 1994). These results suggest that hDNA rejection may operate to a lesser extent or not at all during DSB-induced recombination.

hDNA rejection has also been invoked to explain polarity gradients, a term that describes the decline in meiotic conversion frequencies along the lengths of genes (reviewed in Petes *et al.* 1991; Nicolas and Petes 1994). Polarity gradients were reasonably explained by the presence of meiosis-specific DSBs at the high conversion ends of genes (Sun *et al.* 1989), and variable degradation of ends that reflected the form of the polarity gradient (Sun *et al.* 1991). However, this view is incomplete since polarity gradients are eliminated in *msh2* (mismatch repair) mutants (Alani *et al.* 1994) and when markers are used that yield poorly repaired mismatches when included in hDNA (Detloff *et al.* 1992), implicating mismatches/mismatch repair in the formation of polarity gradients. Two models have been proposed that incorporate these findings (reviewed in Nicolas and Petes 1994). One draws on the idea of hDNA rejection, with reduced conversion as a function of distance from the DSB reflecting reduced extension of hDNA upon incorporation of mismatched bases; in this view, hDNA rejection must occur when only a single mismatch is detected (Alani *et al.* 1994). The alternative view suggests that hDNA is generally not limiting (*i.e.*, hDNA rejection is weak or absent), but that mismatch repair switches from largely conversion-type repair of mismatches near the initiating DSB to perhaps equal frequencies of conversion-type and restoration-type repair at more distant mismatches (Detloff *et al.* 1992; Kirkpatrick *et al.* 1998). Since meiotic conversion largely reflects events initiated by DSBs (Nickoloff and Hoekstra 1998), the latter view with minimal hDNA rejection is consistent with the minimal effects of sequence divergence on DSB-induced mitotic conversion.

In this article we describe an analysis of allelic gene conversion in yeast stimulated by a specific DSB in a defined 3.4-kbp interval containing either 4 markers, or an additional 9 markers. In the densely marked interval, 12 of the 13 markers were present in a 1.2-kbp region (1% sequence divergence). The extra markers reduced spontaneous recombination severalfold. In contrast, there was no reduction for DSB-induced recombination, indicating minimal hDNA rejection for DSB-induced events. We also report that the average

minimum conversion tract length is twice as long in the densely marked interval as in the sparsely marked interval. We show that the dominant mode of DSB repair involves mismatch repair of hDNA, with BIR/G2 crossover/chromosome loss playing minor roles. The marker-dependent increases in tract lengths are therefore discussed in relation to mismatch formation and repair.

MATERIALS AND METHODS

Plasmid DNA, yeast transformation, and plasmid rescue: Plasmid preparation and manipulation and yeast culture and transformation were described previously (Sambrook *et al.* 1989; Sweetser *et al.* 1994). *ura3* alleles with HO sites at position 432 (a natural *Nco*I site) and with or without nine phenotypically silent restriction fragment length polymorphisms (RFLPs; *ura3*R-HO432 and *ura3*-HO432, respectively) or a *Bss*HII linker insertion (*ura3*-HO432-Bss14-409) were described previously (Nickoloff *et al.* 1986; Sweetser *et al.* 1994; Weng and Nickoloff 1998). *ura3*-X764 is wild type except for a +1 frameshift at position 764 that creates an *Xba*I site (Sweetser *et al.* 1994). Plasmid RscRI is a transplacement vector containing 2.0-kbp and 0.9-kbp regions up- and downstream of *URA3*, plus *LEU2* and pUC19 (see Figure 2A). Derivatives of RscRI were constructed by inserting each of the *ura3*-HO432 alleles (as *Hin*dIII fragments) between pUC19 and *LEU2*, creating plasmids RscRI-ura3-HO432, RscRI-ura3R-HO432, and RscRI-ura3-HO432-Bss14-409. RscBam is identical to RscRI except for two restriction site differences, one in pUC19 and one at the 5′ end of *LEU2* (these create additional silent RFLPs flanking pUC19 and *ura3*). *ura3*-X764 was inserted into RscBam as above, creating plasmid RscBam-ura3-X764. *Spe*I digestion of RscRI and RscBam derivatives allows one-step replacement of *URA3* with pUC19-*ura3-LEU2* (Figure 2A). Plasmids were rescued by *Bsp*DI digestion of yeast genomic DNA as described previously (Cho *et al.* 1998), which releases pUC19, *ura3*, and part of *LEU2*. Plasmids used as mapping controls for rescued products were constructed by *Bsp*DI digestion/religation of RscRI and RscBam derivatives.

Yeast strains: Strain genotypes are given in Table 1. All strains were derived from YPH250 (Sikorski and Hieter 1989). Gross chromosome changes were confirmed by Southern hybridization and all markers were confirmed by restriction mapping of rescued plasmids. To simplify construction of some diploid strains, appropriate haploids were first transformed with *ARS1*/*CEN4* plasmids carrying either *TRP1* or *HIS3*; these plasmids were cured from selected diploids before use in recombination assays. Strain DY3024 (*MAT*α) was created from DY3017 (*MAT***a**; Sweetser *et al.* 1994) by transient expression of *GALHO*. DY3031 and DY3051 are meiotic products of JD1001 and JD1000, respectively. DY3065 and DY3066 are meiotic products of JD1003. DY3065 was transformed to His^+ with a 1.8-kbp *HIS3 Bam*HI fragment to create DY3428. DY3427 and DY3438 were created by transformation of RscBam-ura3-X764 into DY3065 and DY3428, respectively. DY3424 was created by transformation of DY3066 with pHSSGalHOLys, which targets *GALHO* (an inducible source of HO nuclease) to *lys2*; this plasmid is a derivative of pHSS19 (Nickoloff and Reynolds 1991), a kanamycin-resistant vector that does not interfere with rescue of *ura3* alleles linked to pUC19 (ampicillin resistant). DY3439 was created by transformation of DY3424 to Ura^- Leu^+ with RscRI-ura3R-HO432. The diploid product of DY3438 × DY3439 is DY3515-13 (Figure 2B); the 13 heterozygosities are indicated by the "−13" in the strain name and this nomenclature is used for all diploid strains carrying recombination substrates. SW3440 was created by transformation of DY3424 to Ura^- Leu^+ with RscRI-ura3-HO432. SW3516-4 is a diploid product of SW3440 and DY3438. Thus, SW3516-4 is identical to DY3515-13 except that it lacks nine RFLP markers in *ura3*; both strains have identical flanking markers (5′R/5′B and 3′B/—) that define a 3.4-kbp interval, and they also share the two markers that inactivate *ura3* (HO432/*Nco*I, and —/X764), as shown in Figure 2C. HO432 and X764 do not revert at detectable frequencies in the absence of recombination (Sweetser *et al.* 1994).

Strain JC3443 is identical to SW3440 except that the *ura3* allele carries a 14-bp palindromic insertion (Bss14-409) upstream of HO432. JC3519-5 is a diploid product of JC3443 and DY3427, and is thus identical to SW3516-4 except that it carries Bss14-409. The Bss14-409 marker was used to monitor hDNA as described previously (Weng and Nickoloff 1998).

Because *GALHO* can be leaky even when repressed (Sweetser *et al.* 1994), spontaneous recombination was measured in strains identical to DY3515-13 and SW3516-4, but lacking *GALHO* (JC3520-13 and JC3521-4, respectively). JC3444 and JC3445 were constructed by transforming RscRI-ura3R-HO432 and RscRI-ura3-HO432, respectively, into DY3066. JC3520-13 and JC3521-4 are diploid products of JC3444 and JC3445, respectively, mated with DY3438.

To monitor BIR/G2 crossover/chromosome loss events, we created two strains identical to DY3515-13 and SW3516-4, except that *HIS3* was located near the telomere linked to HO432. We amplified a 1.4-kbp fragment of intergenic DNA present 8 kbp from the telomere on the left arm of chromosome V (telV) with the following primers: 5′-AAGGATCCCGGCAG GAAGAGTTAAAAAGA-3′ and 5′-GGAATTCACGCCTATC ACCATCACCTC-3′ (terminal *Bam*HI and *Eco*RI sites underlined). This DNA was inserted into *Bam*HI/*Eco*RI sites of pUC19, creating pUCtelV. We converted an *Eag*I site in telV to *Bgl*II, and then inserted a 1.8-kbp *HIS3 Bam*HI fragment into the *Bgl*II site. The resulting *HIS3*:telV fragment was transformed into strains DY3439 and SW3440, creating JC3441 and JC3442, respectively. These strains were mated with DY3427 to create JC3517-13 and JC3518-4.

Recombination frequencies and rates: DSB-induced recombination frequencies were measured using selective and nonselective assays performed in parallel (Cho *et al.* 1998). Two-day-old colonies of parent strains were inoculated into 1.5 ml of YPGly medium and grown for 24 hr. Cultures were divided, cells were harvested by centrifugation, and suspended in 1.5 of YPD (uninduced control) or 1.5 ml of YPGal (HO nuclease-induced), grown for 6 hr, and appropriate dilutions were plated on YPD and uracil omission medium. In selective assays, Ura^+ recombination frequencies were calculated as the number of Ura^+ colonies per cell plated on uracil omission medium. In nonselective assays, colonies on YPD plates were replica plated to uracil omission medium, and Ura^+ frequencies were calculated as the number of Ura^+ colonies per colony replica plated. Parent cells and Ura^- recombinants (mainly conversions to homozygous X764) are both Ura^-, but these can be distinguished in reinduction assays (Weng *et al.* 1996). BIR/G2 crossover/chromosome loss events were expected to yield His^- products, which were identified among nonselected colonies.

Spontaneous recombination rates were measured by using fluctuation analysis. For each rate determination, 11 2-day-old colonies on YPD plates were suspended in water, and appropriate dilutions were seeded to YPD and uracil omission plates. After Ura^+ colonies and total viable cells (from YPD plates) were scored, rates were calculated as described by Reenan and Kolodner (1992).

Recombination products, chromosome loss assay, and statistical analysis: All recombinant products were independent

TABLE 1

Yeast strains

Name	Genotype	Source or reference
YPH250	*MAT***a** *ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1 ura3-52*	Sikorski and Hieter (1989)
DY3017	*MAT***a** *ade2-101 his3-200 lys2-801*::pUC*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1 ura3-52*	Sweetser *et al.* (1994)
DY3024	*MAT*α *ade2-101 his3-200 lys2-801*::pUC*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1 ura3-52*	This study
DY3025	*MAT***a***-inc ade2-101 his3-200 lys2-801*::pUC*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1*	Sweetser *et al.* (1994)
DY3028	*MAT***a***-inc ade2-101 his3-200 lys2-801*::pUC*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1 ura3-*X432	Sweetser *et al.* (1994)
DY3031	*MAT*α *ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1*	This study
DY3051	*MAT*α *ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1 ura3-52*	This study
DY3065	*MAT*α *ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1*	This study
DY3066	*MAT***a***-inc ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1*	This study
DY3424	*MAT***a***-inc ade2-101 his3-200 lys2-801*::pHSS*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1*	This study
DY3427	*MAT*α *ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1*, RscBam-*ura3*-X764-*LEU2*[a]	This study
DY3428	*MAT*α *ade2-101 lys2-801 trp1-*Δ*1 leu2-*Δ*1*	This study
DY3438	*MAT*α *ade2-101 lys2-801 trp1-*Δ*1 leu2-*Δ*1* RscBam-*ura3*-X764-*LEU2*[a]	This study
DY3439	*MAT***a***-inc ade2-101 his3-200 lys2-801*::pHSS*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1* RscRI-*ura3*R-HO432-*LEU2*[a]	This study
SW3440	*MAT***a***-inc ade2-101 his3-200 lys2-801*::pHSS*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1* RscRI-*ura3*-HO432-*LEU2*[a]	This study
JC3441	*MAT***a***-inc ade2-101 his3-200*:*HIS3*:telV[b] *lys2-801*::pHSS*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1*, RscRI-*ura3*R-HO432-*LEU2*[a]	This study
JC3442	*MAT***a***-inc ade2-101 his3-200*:*HIS3*:telV[b] *lys2-801*::pHSS*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1*, RscRI-*ura3*-HO432-*LEU2*[a]	This study
JC3443	*MAT***a***-inc ade2-101 his3-200 lys2-801*::pHSS*GALHO::LYS2 trp1-*Δ*1 leu2-*Δ*1* RscRI-*ura3*-HO432-Bss14-409-*LEU2*[a]	This study
JC3444	*MAT***a***-inc ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1* RscRI-*ura3*R-HO432-*LEU2*[a]	This study
JC3445	*MAT***a***-inc ade2-101 his3-200 lys2-801 trp1-*Δ*1 leu2-*Δ*1*, RscRI-*ura3*-HO432-*LEU2*[a]	This study
JD1000	Diploid product of YPH250 × DY3024	This study
JD1001	Diploid product of DY3051 × DY3025	This study
JD1003	Diploid product of DY3031 × DY3028	This study
DY3515-13	Diploid product of DY3438 × DY3439	This study
SW3516-4	Diploid product of DY3438 × SW3440	This study
JC3517-13	Diploid product of DY3427 × JC3441	This study
JC3518-4	Diploid product of DY3427 × JC3442	This study
JC3519-5	Diploid product of DY3427 × JC3443	This study
JC3520-13	Diploid product of DY3438 × JC3444	This study
JC3521-4	Diploid product of DY3438 × JC3445	This study

[a] RscBam and RscRI replace *URA3* with pUC19-*ura3-LEU2* (Figure 2A), with various *ura3* alleles as indicated.
[b] *HIS3*:telV designates a copy of *HIS3* located 8 kbp from the telomere on the left arm of chromosome V (Figure 2B).

since each was isolated from independent parent cultures. For the densely marked strain (DY3515-13), all markers in both alleles were scored in plasmids rescued using *Bsp*DI (Figure 2B). For events in G2, only half of products are expected to carry the interacting alleles. Typically, >95% of rescued plasmids had expected structures (data not shown); incorrect structures may have resulted, for example, from partial *Bsp*DI digestion or insertion of an extra *Bsp*DI fragment into the released plasmid during ligation. Among Ura^+ products, the two alleles were usually recovered at equal frequencies (distinguished by mapping X764 with *Xba*I), requiring the isolation of two to four plasmids per product. For some Ura^- products, all markers converted, and the two alleles were identical. If six or more plasmids rescued from a single Ura^- recombinant had identical structures (matching the donor: *ura3*-X764), we assumed complete LOH (97% confidence $\approx 2^6 \times 2$); this is a good assumption since we always identified distinct alleles in 45 of 45 Ura^+ products among six or fewer rescued plasmids per product (data not shown). The four markers in SW3516-4 products were mapped in genomic DNA by Southern hybridization with a ^{32}P-labeled *URA3* probe and four digestions. *Nco*I/*Hin*dIII and *Xba*I/*Hin*dIII were used to score HO432 and X764, respectively. The 5′ marker (*Eco*RI or *Bam*HI) was mapped with *Eco*RI; the 3′ marker (*Bam*HI or no site) was mapped by comparing *Bst*EII/*Bam*HI patterns with the *Eco*RI pattern. Chromosome loss was assayed by using dual-probe quantitative Southern hybridization, with signals measured using a Molecular Dynamics (Sunnyvale, CA) phosphorimager. Hybridization was performed with two probes simultaneously, including the telV PCR product, and a second 889-bp chromosome VII PCR product (primers: 5′-AATGGTTGTGG TGGTAATGGCA-3′ and 5′-ATAAGTATTGGCGCCCGACA TT-3′). The ratio of the telV:chromosome VII signals in a control strain with two copies of chromosome V (DY3515-13) were normalized to a value of 1.0, and then compared to normalized ratios from Ura^- His^- products; chromosome loss was indicated when a Ura^- His^- ratio was approximately twofold lower than the DY3515-13 ratio. Chromosome loss was

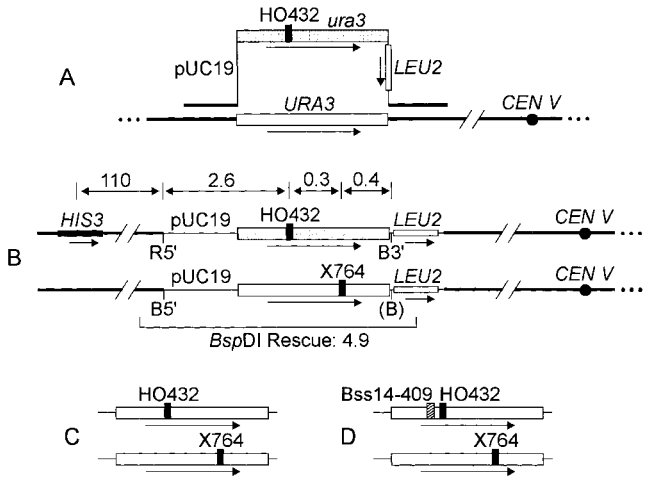

Figure 2.—Recombination substrates. (A) Targeting vectors replace *URA3* with pUC19-*ura3-LEU2*. (B) Map of DY3515-13/JC3517-13 showing relative positions of HO432, X764 and the flanking 5′ and 3′ markers. R5′, *Eco*RI; B5′, *Bam*HI; B3′, *Bam*HI; B3′ is absent in the X764 chromosome. Sizes are given in kilobase pairs. JC3517-13 has *HIS3* linked to *ura3*-HO432 near the telomere (*HIS3*:telV); DY3515-13 lacks *HIS3*:telV. In DY3515-13 and JC3517-13 there are nine additional RFLP markers (shading; see Figure 4). *ura3* alleles linked to pUC19 are excised by digestion with *Bsp*DI during rescue. (C) SW3516-4 and JC3518-4 are identical to DY3515-13 and JC3517-13, respectively, but they lack the nine RFLPs. (D) JC3519-5 is identical to SW3516-4, but has a 14-bp palindromic insertion 23 bp upstream of HO432 that creates a *Bss*HII site (Bss14-409).

not verified by tetrad analysis since HO induces conversion from *MAT*α/*MAT***a**-*inc*/*MAT***a**-*inc* diploids to *MAT***a**-*inc*/*MAT***a**-*inc*, which do not sporulate. Statistical analyses were performed by using Fisher exact tests unless otherwise specified.

RESULTS

Allelic recombination system: Two diploid strains were constructed with allelic recombination substrates that were sparsely or densely marked in a 3.4-kbp interval. In both strains, one copy of *ura3* was inactivated by insertion of a 24-bp HO site (HO432), and the second copy by a +1 frameshift mutation (X764; Sweetser *et al.* 1994). In both strains, flanking RFLP markers defined the 3.4-kbp interval. In the densely marked strain (DY3515-13), nine additional phenotypically silent RFLP mutations were present at ~100-bp intervals in *ura3*; the sparsely marked strain (SW3516-4) lacked these markers. Prior to mating, haploid parents were constructed such that *URA3* on chromosome V was replaced by pUC19-*ura3-LEU2* (Figure 2). This design maintains essentially complete homology along homologous chromosomes V while allowing recombinant alleles to be rescued for RFLP analysis. Allele rescue is superior to PCR and Southern hybridization approaches because rescue permits independent analysis of the two alleles and it simplifies analysis of marker linkage relationships. Each strain carried an integrated copy of *GALHO* to allow delivery of DSBs to HO sites when cells are grown in medium with galactose, which greatly stimulates recombination. This system allows detection of gene conversion and crossovers within the 3.4-kbp interval, providing information about gene conversion tract lengths, directionality, and symmetry relative to a defined DSB. LOH at all markers centromere-distal to HO432 may result from gene conversion, BIR, or G2 crossovers; LOH at all markers may result from these processes as well as from chromosome loss. However, gene conversion was the dominant outcome (see below). Unlike direct repeat substrates, sister chromatid exchange and nonconservative, single-strand annealing events are not detected.

Ura^+ frequencies were determined by directly selecting for Ura^+ products and by using a nonselective replica-plate assay; Ura^- frequencies can only be determined with the nonselective assay. As expected, expression of HO nuclease enhanced recombination by ~100-fold. DSB-induced Ura^+ frequencies for strain SW3516-4 were similar in selective and nonselective assays (Table 2, experiments 1a *vs.* 1b, and 2a *vs.* 2b). In one experiment, Ura^+ frequencies for strain DY3515-13 were significantly higher (1.5-fold) with nonselective assays (3a *vs.* 3b; $P < 0.01$, *t*-test). In a second experiment, this same trend was seen, but the difference was not significant (4a *vs.* 4b; $P = 0.3$). A greater difference between selective and nonselective assays (1.7-fold) was seen with multiply marked *ura3* direct repeats (Cho *et al.* 1998); these differences do not reflect differential plating efficiencies for Ura^+ and Ura^- cells on nonselective medium, additional spontaneous recombination occurring during nonselective colony growth, or differential persistence of HO nuclease in the two assays. Apparently, selective conditions do not permit the timely conclusion of all recombination events.

DSB-induced events initiate at HO432, and this allows us to define three gene conversion parameters: tract lengths, tract directionality, and conversion frequencies for individual markers as a function of distance from the initiating DSB. Gene conversion can yield Ura^+ or Ura^- products. For DSB-induced events, conversion tracts in Ura^+ products generally do not extend past X764 since most conversion tracts are continuous (Petes *et al.* 1991; Nickoloff and Hoekstra 1998). Although Ura^+ products could result from crossing over in the HO432-X764 interval without associated conversion, such products were not detected (see below). Ura^- products were all homozygous at X764. Selection can bias product spectra (Sweetser *et al.* 1994; Weng *et al.* 1996; Cho *et al.* 1998), but combining Ura^+ and Ura^- data yields unbiased spectra.

One percent heterology in a 1.2-kbp region reduces spontaneous, but not DSB-induced, allelic recombination: Spontaneous ectopic recombination is reduced seven- to eightfold by 1% heterology (Datta *et al.* 1997). We measured spontaneous allelic recombination rates

TABLE 2

Spontaneous and DSB-induced recombination frequencies

				Recombination frequency ($\times 10^4$)[a]				
				Ura$^+$		Ura$^-$		Ura$^-$/(Ura$^+$ + Ura$^-$):
Expt.	Strain[b]	Assay[c]	n[d]	Glu	Gal	Glu	Gal	Gal
1a	SW3516-4	Selective	4	2.2 ± 1.8	212 ± 115	NA	NA	NA
1b	SW3516-4	Nonselective	4	1.8 ± 3.5	237 ± 85	21 ± 29	208 ± 71	0.52 ± 0.02
2a	SW3516-4	Selective	4	ND	295 ± 111	NA	NA	NA
2b	SW3516-4	Nonselective	4	ND	321 ± 44	ND	318 ± 42	0.47 ± 0.09
3a	DY3515-13	Selective	4	5.9 ± 5.1	269 ± 90	NA	NA	NA
3b	DY3515-13	Nonselective	4	0[e]	402 ± 122	6 ± 12	843 ± 297	0.67 ± 0.04
4a	DY3515-13	Selective	4	ND	298 ± 160	NA	NA	NA
4b	DY3515-13	Nonselective	4	ND	378 ± 84	ND	753 ± 75	0.67 ± 0.04
5	JC3520-13	Selective	11	0.01 ± 0.01	ND	NA	NA	NA
6	JC3521-4	Selective	11	0.05 ± 0.03	ND	NA	NA	NA

NA, not applicable (since Ura$^-$ products are not recovered in selective assays); ND, not determined; Expt., experiment.

[a] Recombination frequencies (averages ± SD) are given for glucose (Glu) and galactose (Gal) grown cultures (uninduced and DSB-induced, respectively).

[b] DY3515-13, JC3520-13, SW3516-4, and JC3521-4 have *GALHO*; JC3520-13, and JC3521-4 lack *GALHO*.

[c] In selective assays Ura$^+$ recombinants were identified by directly plating on selective medium; in nonselective assays, Ura$^+$ recombinants were identified by replica plating colonies grown initially on YPD medium. In nonselective assays, 1300–11,000 individual colonies were scored per experiment.

[d] Number of independent populations tested.

[e] No Ura$^+$ colonies arose from any of the four populations, totaling 7790 cells.

in sparsely and densely marked intervals (0.2 *vs.* 1% heterology) in strains lacking *GALHO* (JC3520-13 and JC3521-4). The Ura$^+$ recombination rate was fourfold lower in the densely marked strain (2.4×10^{-7} *vs.* 9.9×10^{-7} events/cell/generation). This reduction is also apparent from the significantly different spontaneous recombination frequencies (Table 2, experiments 5 and 6; $P = 0.0002$, *t*-test). Thus, 1% heterology in a limited region reduces the frequency of allelic recombination events. In contrast, total DSB-induced recombination frequencies (Ura$^+$ + Ura$^-$), determined in analogous strains carrying *GALHO*, were not lower in the presence of the additional markers; in fact, induced frequencies were approximately twofold higher in the densely marked strain (DY3515-13) than the sparsely marked strain (SW3516-4), as shown in Table 2, experiments 1–4. These results indicate that extra markers do not inhibit conversion when events are stimulated by a targeted DSB.

Most DSB-induced allelic conversion tracts are long and bidirectional: We analyzed all markers in both alleles in 45 Ura$^+$ and 30 Ura$^-$ products of DY3515-13. A product spectrum was constructed by combining Ura$^+$ and Ura$^-$ tract data in proportion to the frequencies that these product types arose (Ura$^-$ products arose twice as often as Ura$^+$; Table 2). Most products (76%) were simple gene conversions of alleles suffering a DSB; these had continuous conversion tracts, no detectable crossovers in the 3.4-kbp interval, and no alterations of unbroken alleles (Figure 3, class A). Interestingly, 57 simple conversion products were distributed among only 15 of 48 possible continuous tract types for events initiated at HO432 (Figure 4). Absent were most short tracts and the majority of unidirectional tracts. This is in marked contrast to the tract spectrum obtained with

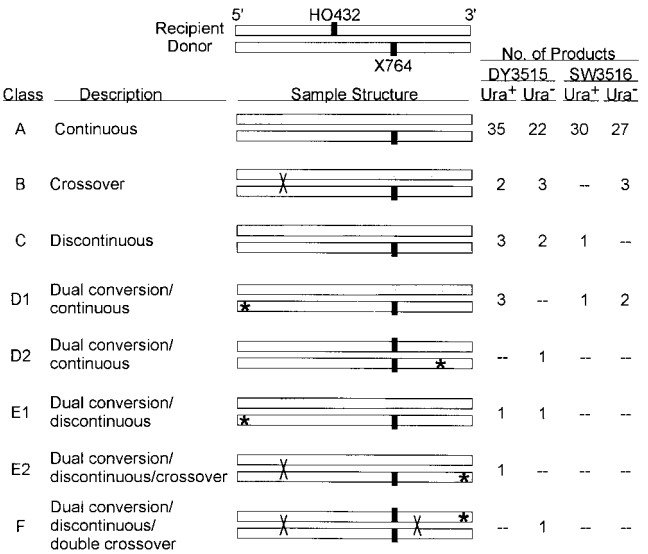

Figure 3.—Representative structures of DSB-induced recombination products. The parent marker configuration is shown above. For Ura$^+$ products, tracts generally do not extend 3′ of X764 (as shown in class A); in Ura$^-$ products, X764 is homozygous (not illustrated except in classes D2 and F). Crossovers are shown by "X" and markers converted in unbroken alleles by an asterisk.

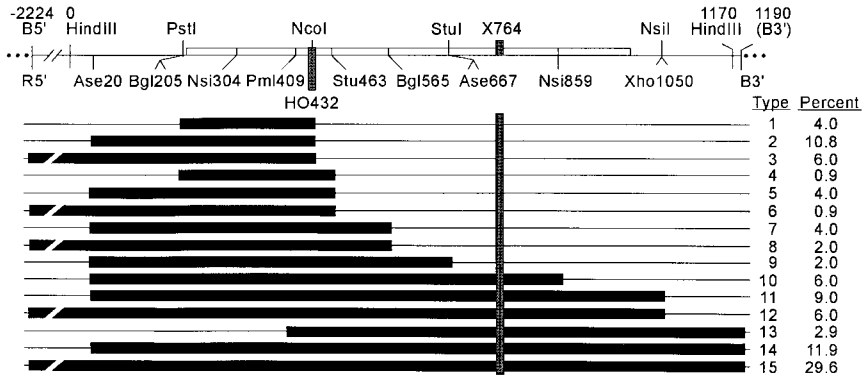

Figure 4.—Tract spectrum for DY3515-13 products with continuous tracts. Markers in the X764 chromosome are shown above, HO432 below. Sites in parentheses are absent. *Hin*dIII sites flank *ura3*; these are not heterozygous. Conversion tracts are shown below for 15 of the 48 possible continuous tracts recovered among 57 DY3515-13 products (black bars). This spectrum was generated by combining Ura^+ and Ura^- products in proportion to their frequencies given in Table 2; each product was isolated from an independent population of parent cells.

ura3 direct repeats (Cho *et al.* 1998), as summarized in Table 3. For example, 47% of direct-repeat tracts were confined to the Bgl205-Ase667 interval, but these short-tract classes were not recovered in the allelic cross. Also, unidirectional tracts were in slight majority in the direct-repeat cross (55%), but were significantly less frequent in the allelic cross (21%; $P < 0.00001$).

Chromosome loss and break-induced replication are rare in wild-type, diploid yeast: All markers in the 3.4-kbp interval were lost in 30% of products. These could have arisen by gene conversion, BIR, G2 crossovers, or chromosome loss. An additional 15% of products lost all markers 5′ (centromere-distal) of HO432 and could have arisen by gene conversion, BIR, or G2 crossovers. To distinguish gene conversion from these other possibilities, we constructed two strains identical to DY3515-13 and SW3516-4 but with *HIS3* linked to *ura3* alleles carrying HO432; *HIS3* was located 100 kbp from *ura3* near the telomere on the left arm of chromosome V (strains JC3517-13 and JC3518-4). Among *ura3* recombinants (either Ura^+ or Ura^-), gene conversion results in retention of *HIS3*, whereas BIR, chromosome loss, and some G2 crossovers result in loss of *HIS3* (Figure 1). *HIS3* loss was not detected among uninduced colonies (data not shown). Upon HO induction, *HIS3* was lost in only 5–7% of *ura3* recombinants (including both His^- and sectored $His^{+/-}$ products) from both the densely and sparsely marked strains (Table 4). Thus, additional markers at *ura3* do not affect *HIS3* loss. About 35% of the His^- or $His^{+/-}$ products were Ura^+; these are unlikely to arise by chromosome loss. Ura^- His^- products (and the Ura^- His^- sectors of $Ura^{-/-}$ $His^{+/-}$ colonies) could have arisen by chromosome loss. We PCR amplified a region carrying the B3′ marker from Ura^- His^- products of JC3517-13 and JC3518-4: 5 of 15 JC3517-13 products and 3 of 12 JC3518-4 products remained heterozygous at the B3′ marker, ruling out chromosome loss for 25–33% of Ura^- His^- products. The remaining 19 Ura^- His^- products were chromosome loss candidates. We determined chromosome V copy number in these candidates by using quantitative Southern hybridization (data not shown). Of the 10 JC3517-13 candidates tested, 3 arose by chromosome loss. In total, we analyzed 479 JC3517-13 products by genetic and physical assays, and only these 3 products (0.6%) reflected chromosome loss. None of the 9 candidates from JC3518-4 lost chromosome V (loss rate $<0.3\%$). Thus, DSBs rarely lead to chromosome loss in diploid yeast.

The assays above do not distinguish between BIR and G2 crossovers for His^- products. However, G2 crossovers can be identified among His^+ products as those that gain a second copy of *HIS3*; neither BIR nor chromosome loss will lead to gain of a second *HIS3*. Since G2 crossovers will lead to gain or loss of *HIS3* at equal frequencies, the measurement of *HIS3* gain provides an estimate of *HIS3* loss via G2 crossovers. In strain JC3517-13, 2 of 20 Ura^+ His^+ products and 1 of 20 Ura^- His^+

TABLE 3

Conversion tract directionality

Strain[a]	Cross[b]	% Bidirectional	% Undirectional		
			HO only	5′	3′
DY3515-13	Allelic	79	0	19	2
JW3082	Direct repeat	45	12	35	8

[a] Both strains carry *ura3*R-HO432 (with nine RFLPs) and *ura3*-X764; the direct repeats in JW3082 are separated by pUC19 and *LEU2* (Cho *et al.* 1998).

[b] Allelic data are for markers in recipient allele only, from 75 products, including simple gene conversions and complex products. Direct repeat data from Cho *et al.* (1998) from 86 products, all of which were simple conversions. In both data sets, Ura^+ and Ura^- products were combined to give unbiased spectra.

products had two copies of *HIS3* (assayed by PCR amplification of the *HIS3*:telV region; data not shown). These values translate to His^+ G2 crossover frequencies of 78×10^{-4} and 53×10^{-4}, respectively, for a net His^+ G2 crossover frequency of 131×10^{-4}, which is similar to the combined His^- and $His^{+/-}$ frequency in JC3517-13 of 140×10^{-4}. We conclude that most His^- products arise by G2 crossovers and that BIR is infrequent, consistent with the results of Malkova *et al.* (1996). Thus, nearly all DSB repair in diploid yeast occurs by gene conversion, with proximal LOH usually resulting from associated G2 crossovers.

Most DSB-induced allelic conversion involves mismatch repair of hDNA: Most or all meiotic gene conversion in yeast involves mismatch repair of hDNA. To determine whether allelic conversion events in mitotic cells arise from hDNA intermediates (and hence reflect mismatch repair), we constructed strain JC3519-5, which is identical to SW3516-4 except for the addition of a 14-bp palindromic frameshift insertion near HO432 (Bss14-409). If included in hDNA, this insertion is expected to produce a poorly repaired stem-loop mismatch (Nag and Petes 1991; Weng and Nickoloff 1998) that will segregate in the next mitosis and yield a sectored ($Ura^{+/-}$) colony; these are detected in the *ade2* background as half pink/half white colonies (Weng and Nickoloff 1998). HO nuclease was induced in JC3519-5 for only 2 hr to minimize segregation prior to plating as this maximizes sensitivity of sector detection. We scored an average of 242 colonies that were either Ura^+ or $Ura^{+/-}$ in each of four determinations, and $87 \pm 2\%$ of colonies were sectored $Ura^{+/-}$, indicating that most DSB-induced allelic gene conversion reflects mismatch repair of hDNA.

DSB-induced allelic gene conversion is asymmetric: Among unidirectional tracts from both direct-repeat and allelic crosses, 5′ (promoter-proximal) tracts were four- to ninefold more frequent than 3′ tracts (Table 3). Another form of asymmetry is apparent from the analysis of individual marker conversion rates. In DY3515-13, four pairs of markers are essentially equidistant from HO432, and for each pair we found that 5′ markers converted at higher rates than 3′ markers (Figure 5). Note that these asymmetries are not simply reflections of each other since individual marker conversion rates were calculated by using all products, 80% of which had bidirectional tracts, whereas the difference in 5′ *vs.* 3′ unidirectional tracts derives from 20% of products that have unidirectional tracts.

Complex events occur at similar rates in densely and sparsely marked intervals: In DY3515-13, 25% of DSB-induced allelic recombinants had complex marker patterns reflecting additional processing beyond conversion of *ura3*R-HO432, including crossovers, discontinuous conversion tracts, and conversions of markers in the unbroken allele; representative examples of seven distinct classes of complex patterns are shown in Figure 3. Conversion in unbroken alleles was restricted to the flanking (5′ and 3′) markers (classes D1, E1, E2, and F) except for one product (class D2). Some conversions of unbroken alleles were continuous with the conversion tract in the broken allele (classes D1 and D2), but just as often the two tracts were discontinuous (classes E1, E2, and F). One product had a very complex structure, reflecting double crossovers flanking the conversion tract in the broken allele, plus a discontinuous conversion of the 3′ marker in the unbroken allele (class F). Crossovers in the 3.4-kbp interval were detected in ~10% of DY3515-13 products (7% associated with simple gene conversions plus 3% among those that had converted the unbroken allele). In SW3516-4, crossovers in this interval were less frequent (~5%), but this difference was not significant ($P = 0.13$). From the *HIS3*:telV data above, we estimate an additional 5% of products had undetected G2 crossovers. Discontinuous tracts were more common in DY3515-13 than SW3516-4 (Figure 3), but the greater number of markers in DY3515-13 provides greater sensitivity for detecting discontinuities. When only those markers shared by DY3515-13 and SW3516-4 are considered, discontinuous tracts arose at equal frequencies in the two strains (data not shown).

A hallmark of DSB-induced gene conversion is the strong preference for conversion of alleles suffering a DSB (McGill *et al.* 1993; Nickoloff and Hoekstra 1998). In strain DY3515-13, 10% of DSB-induced recombinants converted one or more markers in unbroken alleles. With fewer markers in strain SW3516-4 there is less opportunity to detect conversion of the unbroken allele. Despite this limitation, 3 of 64 SW3516-4 products (5%) converted a marker in the unbroken allele (Figure 3, class D1); again, these values are not significantly different ($P = 0.11$). These values are likely underestimates of unbroken allele conversion frequencies since only half of events in G2 would lead to cosegregation of the donor and recipient chromosomes. In any case, these data indicate that multiple markers do not increase the frequency of complex events.

Multiple markers increase DSB-induced gene conversion tract lengths: DY3515-13 and SW3516-4 share four markers, including HO432, X764, and the 5′ and 3′ flanking markers; only the last three are informative since the HO site converts in all DSB-induced events. Nonselective assays give relative measures of Ura^+ and Ura^- recombinants. Since Ura^- recombinants reflect conversion of X764, the ratio of Ura^- recombinants to total recombinants provides a measure of the X764 conversion frequency. In SW3516-4, Ura^- recombinants comprised 50% of DSB-induced recombinants (Table 2, experiments 1b and 2b). In contrast, Ura^- recombinants were more frequent in the densely marked DY3515-13 cross, comprising 66% of DSB-induced recombinants (Table 2, experiments 3b and 4b); these differences in the fractions of Ura^- recombinants in SW3516-4 and DY3515-13 were significant in both sets

TABLE 4

Frequency of *HIS3*:telV loss and retention

		DSB-induced recombination frequencies × 10^4						
		Ura$^+$			Ura$^-$			
Strain[a]	n[b]	His^{+c}	His$^-$	His$^{+/-}$	His^{+c}	His$^-$	His$^{+/-}$	Ura$^{+/-d}$
JC3517-13	2436	768	29	8	1063	41[e]	25	33
JC3518-4	3145	464	3	0	512	10	10	22

[a] JC3517-13 and JC3518-4 both carry *HIS3*:telV on the *ura3*-HO432 chromosome.
[b] Number of colonies analyzed.
[c] ~15% of His$^+$ products gained a second copy of *HIS3*:telV; the rest remained heterozygous.
[d] Includes four classes: Ura$^{+/-}$ His$^{+/+}$, Ura$^{+/-}$ His$^{-/-}$, Ura$^{+/-}$ His$^{+/-}$, and Ura$^{+/-}$ His$^{-/+}$.
[e] About 30% of these nonsectored, Ura$^-$ His$^-$ products from JC3517-13 arose by chromosome loss; no chromosome loss was detected in JC3518-4.

of experiments ($P < 0.007$, *t*-tests). Thus, X764 converts at higher rates in the densely marked interval. DSB-induced conversion frequencies for the 5′ and 3′ flanking markers, determined by physical mapping of recipient alleles from 64 recombinants of SW3516-4 and 75 recombinants of DY3515-13, revealed an even greater difference than that seen at X764, as both flanking markers converted significantly more often (twofold) in the densely marked interval (Figure 6). Average minimum tract lengths, calculated using only the markers shared by DY3515-13 and SW3516-4, were significantly longer in the multiply marked cross (1414 ± 1464 bp *vs.* 714 ± 1194; $P = 0.007$, *t*-test). The DY3515-13 value is comparable to meiotic values measured in multiply marked intervals (Judd and Petes 1988; Borts and Haber 1989). We conclude that multiple markers increase gene conversion tract lengths. Since the 5′ marker in DY3515-13 is 2.6 kbp from the DSB, and separated from the adjacent marker (Ase20) by 2.2 kbp of perfect homology, we further conclude that marker-dependent increases in conversion occur at considerable distances from a DSB, and across considerable distances of perfect homology.

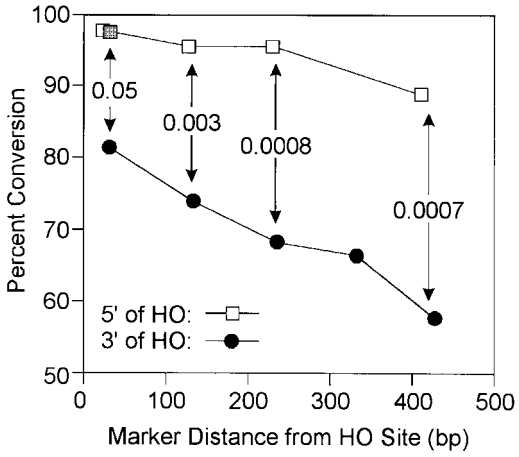

Figure 5.—Asymmetric conversion in DY3515-13. *P* values are given for each pair of equidistant markers 5′ and 3′ of HO432. For the two markers closest to HO432, we compared an estimated value (shaded box) to correct for the 50% difference in the distances from HO432 (23 *vs.* 31 bp). Data are from all 75 DY3515-13 products.

DISCUSSION

Heterology reduces spontaneous, but not DSB-induced, allelic recombination: Sequence divergence has variable effects among different organisms/genetic contexts. For example, very limited sequence divergence effectively eliminates recombination in *Escherichia coli* (Rayssiguier *et al.* 1989) and in mammalian chromosomal, but not extrachromosomal, substrates (Waldman and Liskay 1987; Taghian and Nickoloff 1997); in yeast the effects are generally weaker (Bailis and

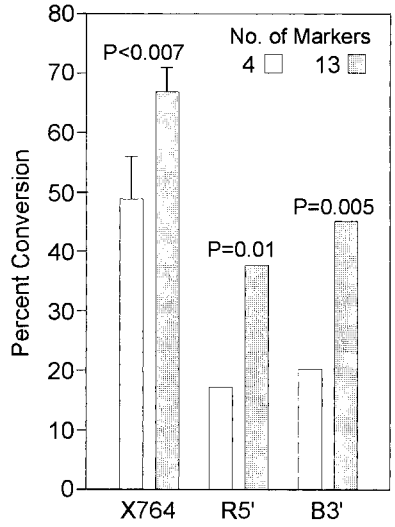

Figure 6.—Marker-dependent increases in conversion. Conversion frequencies in DY3515-13 (shaded bars) compared to SW3516-4 (open bars). Data are given for three markers shared by DY3515-13 and SW3516-4, from 75 and 64 products, respectively.

Rothstein 1990; Harris *et al.* 1993; Chambers *et al.* 1996; Porter *et al.* 1996; Datta *et al.* 1997). We found that 1% divergence in a 1.2-kbp region reduces by fivefold the rate of spontaneous allelic recombination, similar to the reduction with 1% diverged ectopic substrates (Datta *et al.* 1997). Thus, complete homology for more than 100 kbp on either side of a 1.2-kbp diverged region does not overcome the inhibition of spontaneous recombination. It is not known whether spontaneous recombination is initiated by DSBs; the differential effect of 1% sequence divergence on spontaneous and HO-induced events might indicate otherwise, although other differences might be important. For example, HO-induced DSBs have short nonhomologous ends while most spontaneous DSBs have homologous ends, and HO-induced events initiate within the diverged region whereas random DSBs might initiate within or outside this region. Others have found that sequence divergence has little or no inhibitory effect on DSB-induced recombination (Resnick *et al.* 1992; Larionov *et al.* 1994; Mezard and Nicolas 1994). In contrast, gene targeting in yeast, stimulated by DSBs that release linear targeting fragments, is reduced by even a single mismatch (Leung *et al.* 1997; Negritto *et al.* 1997), and mismatches also inhibit DSB-induced single-strand annealing (Sugawara *et al.* 1997). For allelic events, the relaxed control for DSB-induced recombination may reflect the greater need to repair breaks to prevent chromosome loss and cell death relative to the need to maintain sequence differences. Alternatively, the different degrees of hDNA rejection may reflect distinct modes of initiation and/or subsequent processing for spontaneous and DSB-induced events in different chromosomal/topological contexts. The greater sensitivity of hDNA rejection in mammalian cells (Waldman and Liskay 1987; Taghian and Nickoloff 1997) is likely required to maintain stability in genomes with large amounts of repetitive sequence. Interestingly, hDNA rejection for DSB-induced events may also be relaxed in mammalian cells (Taghian and Nickoloff 1997).

Repair of DSBs by recombination *vs.* break-induced replication: Meiotic gene conversion (Petes *et al.* 1991) and DSB-induced mitotic conversion (Ray *et al.* 1991; Weng and Nickoloff 1998) are mediated primarily by mismatch repair of hDNA; here we generalize this finding to DSB-induced mitotic events at allelic loci. An alternative DSB-repair mechanism is BIR, which has been seen in yeast during transformation with linear DNA (Morrow *et al.* 1997), with persistent chromosomal DSBs (Bosco and Haber 1998), and when significant homology existed on only one side of a DSB (Bosco and Haber 1998). BIR was common in *rad51* mutants but rare in wild-type yeast (Malkova *et al.* 1996), as in the present study. Chromosome loss was rare in the present study and in the study by Malkova *et al.* (1996). The dominant mode of DSB repair in wild-type diploid yeast is gene conversion.

Conversion tract directionality and asymmetry: For DSB-induced ectopic events bidirectional tracts are in the minority, ranging from 10 to 20% in plasmid–chromosome crosses to 45% in direct repeats (Sweetser *et al.* 1994; Nelson *et al.* 1996; Cho *et al.* 1998), contrasting with the 80% value for allelic events (Table 3). The analysis of tract directionality is influenced by marker placement relative to the initiating DSB, and in meiosis, additional DSBs may be a confounding factor (Schultes and Szostak 1990). McGill *et al.* (1993) found 40% of allelic tracts were bidirectional, but the defining markers were several hundred base pairs from the DSB. In meiotic yeast bidirectional tracts were common at *ARG4* in one study (Schultes and Szostak 1990), but not another (Gilbertson and Stahl 1996), and they were rare at *HIS4* (Porter *et al.* 1993). Tract directionality likely reflects several factors, including end invasion (one- or two-ended), extent of hDNA (potentially controlled by branch migration of Holliday junctions), and mismatch repair. Linkage of the two ends (*i.e.*, when a plasmid is linearized) does not influence tract directionality, nor does homology (or lack thereof) at termini (Cho *et al.* 1998). In our studies, ectopic events were studied in *MAT***a**-*inc* haploids and the allelic events in *MAT***a**-*inc*/*MAT*α diploids. It is possible that tract directionality is influenced by *MAT* status since this has been shown to influence recombination frequencies (Friis and Roman 1968). However, it seems more likely that these differences reflect effects of substrate topology or chromosome environment, with the high frequency of allelic bidirectional tracts reflecting enhanced pairing on opposite sides of the DSB due to (essentially) unlimited homology flanking the DSB.

In the present study and previous plasmid–chromosome crosses (Sweetser *et al.* 1994; Cho *et al.* 1998), 5′ unidirectional tracts were more common than 3′ tracts, and 5′ markers converted more often than equidistant 3′ markers. In direct repeats, conversion frequencies of equidistant 5′ and 3′ markers were not significantly different, but the same trend of higher 5′ conversion was apparent (Cho *et al.* 1998). A fourfold conversion bias was found for nearly equidistant markers in a meiotic study, but only in the presence of intervening markers (Borts and Haber 1989). It will be interesting to test whether the 5′ bias is also marker dependent for mitotic events.

How can these asymmetries be explained? In our crosses, it is possible that the nonpalindromic HO site biases events toward 5′ markers. However, this is unlikely since parallel crosses with HO sites oriented in opposite directions have never shown detectable differences in tract spectra or other endpoints (Nickoloff *et al.* 1986; Rudin *et al.* 1989; Sweetser *et al.* 1994). An alternative explanation derives from the fact that once a DSB is created, only 5′ sequences remain transcriptionally active (at least until the break is repaired). Since transcription is known to stimulate recombination in a variety

of contexts (Thomas and Rothstein 1989; Voelkel-Meiman and Roeder 1990; Nickoloff 1992), it is possible that the observed 5′ biases reflect transcriptional effects. We recently found that increased transcription in donor alleles increased DSB-induced conversion frequencies of promoter-proximal markers (Y.-S. Weng, D. Xing and J. A. Nickoloff, unpublished results).

Conversions associated with crossovers and source of complex events: In meiosis, 30–70% of gene conversions are associated with crossovers (Petes *et al.* 1991). For mitotic allelic events, we found 10% of conversions associated with crossovers in the 3.4-kbp interval, and we estimate that an additional 10% are associated with crossovers outside this interval; the net 20% association is similar to the reported 25% for allelic events initiated at *MAT* (Malkova *et al.* 1996). Crossovers associated with meiotic conversion typically occur adjacent to the conversion tract (Borts and Haber 1987), and this was true for all crossovers detected in the present study (data not shown). In meiosis, crossovers are required for accurate chromosome segregation (Carpenter 1994). The somewhat higher frequency of associated crossovers in meiosis may be an effect of multiple DSBs in each chromosome and/or the function of meiosis-specific proteins.

Complex recombination events, particularly conversions of unbroken alleles, might result from secondary recombination events (Borts and Haber 1989). Alternatively, they might reflect end-directed mismatch repair of symmetric hDNA produced by branch migration of Holliday junctions (Figure 7). Such conversions might be more likely to occur at markers far from a DSB, since these are more likely to occur in symmetric hDNA and are farther from ends directing repair. This view can also explain why unbroken alleles are converted less frequently during ectopic recombination (Roitgrund *et al.* 1993; Cho *et al.* 1998; Weng and Nickoloff 1998) compared to allelic recombination (Figure 3) since limited homology lengths in ectopic substrates would restrict Holliday junction migration and the formation of symmetric hDNA. Alternatively, symmetric hDNA in ectopic substrates may be too close to the DSB to avoid end-directed mismatch repair. For ectopic events, branch migration may be restricted to only part of the region of shared homology since markers near homology borders are rarely converted (Ahn and Livingston 1986; Sweetser *et al.* 1994; Cho *et al.* 1998). This is unlikely to be due to limitations of DNA base pairing near a border since large heterologies are easily incorporated into hDNA *in vitro* (Bianchi and Radding 1983) and *in vivo* (Lichten and Fox 1984; Holbeck and Smith 1992); instead it may reflect a difficulty in resolving events near a border. Allelic events have no homology borders to restrict branch migration, so there is a greater probability that distant markers would be included in symmetric hDNA. Consistent with this view, we observed nine products with markers converted in unbroken alleles, and in seven of these, only the most distant (5′ or 3′ flanking) markers converted.

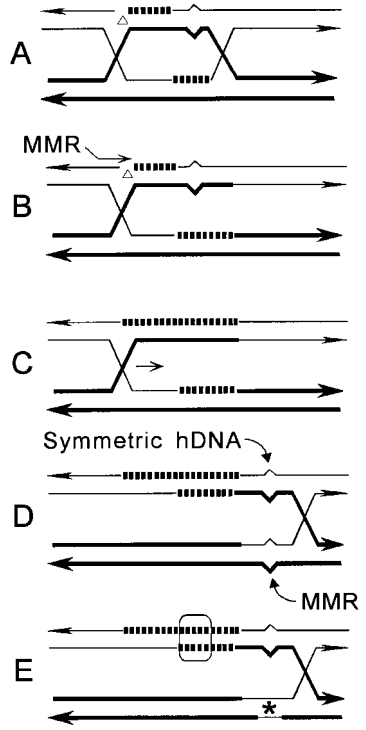

Figure 7.—Mechanism for conversion of unbroken alleles. The allele suffering a DSB is shown by thin lines, an unbroken homolog is shown by thick lines, repair synthesis by dashed lines, and single-strand nicks by triangles. This model is related to those described previously (Gilbertson and Stahl 1996; Weng and Nickoloff 1998). (A) Processing of ends by 5′ to 3′ exonuclease exposes 3′ extensions that invade the unbroken allele and prime repair synthesis, producing the canonical DSB repair intermediate with two Holliday junctions; a mismatch formed upon strand invasion is indicated in the broken allele. (B) End-directed mismatch repair (MMR) initiates at the nick indicated by the open triangle; this type of repair preferentially converts the broken allele; we show one Holliday junction resolving at this stage, but this is not required. (C) Branch migration of the remaining Holliday junction produces symmetric hDNA, with mismatches in both duplexes. (D) Non-nick-directed (or directed from random nicks in either strand) can result in conversion of the unbroken allele, as shown in (E). The complex product has markers converted in the broken allele (boxed region) and the unbroken allele (indicated by an asterisk). The final intermediate may be resolved to give crossover or noncrossover products (not shown).

Multiple markers do not increase complex events: Several studies have shown that multiple markers can alter recombination outcomes. In meiosis, adding seven to nine markers to a 9-kbp *MAT-ura3-MAT* interval decreased crossovers by twofold and increased gene conversion (Borts and Haber 1987). Since *pms1* mutants displayed normal recombination frequencies/spectra in this interval, it was argued that the altered spectrum in wild-type cells resulted from secondary rounds of recombination stimulated by mismatch repair (Borts *et al.* 1990). Support for this model comes from the

observation that complex events (*i.e.*, three-strand double crossovers) are more frequent in multiply marked intervals (Symington and Petes 1988; Borts and Haber 1989). In contrast, additional markers did not increase complex events in the present study, nor in a meiotic study at *ARG4* (Schultes and Szostak 1990). These conflicting results might be explained by marker/DSB spacing or selection bias. For example, recombinants in the *MAT-ura3-MAT* interval were generally selected as those displaying non-Mendelian segregation at *MAT* or *ura3* (Borts and Haber 1987, 1989; Borts *et al.* 1990). We identified recombinants only by the loss of the HO site; there was no selection bias due to X764 since both Ura^+ and Ura^- products were recovered, and all other markers were phenotypically silent. The analysis at *ARG4* was similarly unbiased as both selected (*ARG4*-convertants) and nonselected tetrads were analyzed (Schultes and Szostak 1990).

Multiple markers increase gene conversion tract lengths: Marker-dependent increases in DSB-induced gene conversion tract lengths were observed in the present study and in a meiotic study (Schultes and Szostak 1990). Although we cannot rule out the possibility that this reflects mismatch repair-stimulated secondary events, this is unlikely because the markers failed to induce complex events; Schultes and Szostak (1990) obtained similar results and reached the same conclusion. Although Borts and Haber (1989) found that multiple markers increased conversion frequencies, there was no apparent increase in conversion tract lengths. This might be a reflection of the greater spacing of the markers (averaging 1 kbp apart), interference from multiple DSBs, a greater distance separating the initiating DSB(s) from the markers, or selection bias.

A possible explanation for marker-dependent increases in tract lengths is that a multiply mismatched region is processed by Rad1p/10p endonuclease as if it were part of the nonhomologous tail that includes the HO recognition sequence. However, this seems unlikely since the markers in the present study were present at ~100-bp intervals, and we showed previously that markers present at 3-bp intervals flanking a DSB are processed similarly in *RAD1* and *rad1* cells (Nelson *et al.* 1996), suggesting that even densely spaced markers are processed by the mismatch repair system rather than by Rad1p/10p.

Other alternative explanations for marker-dependent increases in tract lengths derive from two models proposed to explain meiotic polarity gradients. In the first model, polarity gradients are thought to reflect limiting hDNA due to hDNA rejection, with rejection sensitive to very low levels of sequence divergence (Alani *et al.* 1994). However, this model is inconsistent with the lack of (or minimal) hDNA rejection for allelic events induced by DSBs, as discussed above. In the second model, termed "restoration/conversion" (Detloff and Petes 1992; Kirkpatrick *et al.* 1998), hDNA is nonlimiting,

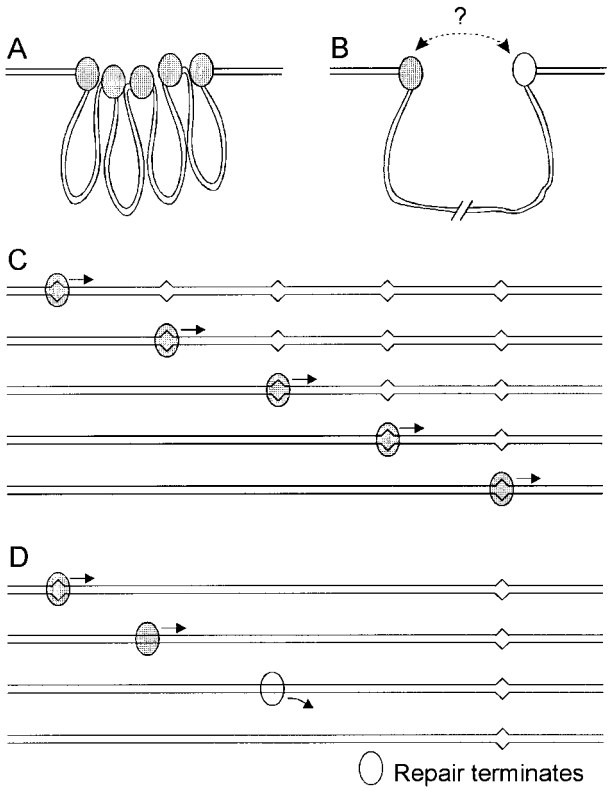

Figure 8.—Models for marker-dependent increase in excision repair processivity. (A) In a densely marked interval, mismatch bound Msh complexes (ovals) interact with each other, forming loops, and this signals the excision repair machinery to repair all mismatches in the same direction. (B) In a sparsely marked interval, complexes bound to distant mismatches do not interact and are free to repair mismatches in opposite directions (shaded *vs.* open complexes). (C, D) RecBCD-like model: Processivity of excision repair complex is increased (shading) each time it encounters a mismatch. In a sparsely marked interval, the complex has reduced processivity (no shading) and does not reach the distant mismatch (repair terminates, as shown), or it could switch from conversion-type to restoration-type repair (not shown).

with polarity gradients reflecting a switch from conversion-type repair at markers near a DSB to restoration-type repair at more distant markers. It is possible that the switch in repair direction reflects limited processivity of end-directed mismatch repair. In this light, marker-dependent increases in gene conversion tract lengths might reflect an alteration of the conversion/restoration switch. Although the elimination of marker effects in mismatch repair-defective *pms1* mutants was taken as evidence in support of the idea that mismatch repair induces secondary events (Borts *et al.* 1990), the restoration/conversion model also predicts a dependence on a functional mismatch repair system. In this way, the restoration/conversion model can accommodate marker-dependent increases in DSB-induced conversion tract lengths, as well as the seemingly contradictory finding by Chen and Jinks-Robertson (1998) that spontaneous tract lengths are shorter in mismatch re-

pair-proficient *vs.* -deficient cells. If we assume that hDNA is nonlimiting, processing in repair-proficient cells will (eventually) switch from conversion-type to restoration-type and this will limit tract lengths, whereas in repair-deficient cells, all markers are free to segregate, and the products will reflect the full length of the hDNA, a possibility raised by Chen and Jinks-Robertson (1998, 1999).

There are at least two ways to envision a role for mismatch repair in marker-dependent increases in tract lengths. One model suggests that Msh2p/6p complexes bound to mismatches along hDNA "communicate" with each other, perhaps forming a multi-looped structure as shown in Figure 8A. This idea is consistent with loops formed by MutS/L/H in *E. coli* (Grilley *et al.* 1990), and with evidence that MutS and Msh2p interact with themselves and with other Mut/Msh/Mlh/Pms proteins (Crouse 1997; Rasmussen *et al.* 1998). If many mismatches are present, communication between bound complexes could sustain the signal to the end-directed, excision-based repair machinery, thus increasing its processivity. With lower mismatch density, we imagine the communication link is broken, repair processivity is reduced, and this increases the probability for independent repair of distant markers (Figure 8B). Alternatively, a single excision repair complex might undergo a conformational change that increases processivity each time a mismatch is encountered (Figure 8C); in this view, excision repair would terminate sooner in sparsely marked intervals (Figure 8D). This model is analogous to activation of RecBCD when it encounters Chi (Myers and Stahl 1994). Each of these mechanisms would promote complete repair of hDNA over long distances, thus reducing the possibility of mismatch segregation with its attendant mutagenic consequences.

We thank Tom Petes and James Haber for helpful comments, and Heather Hough and Kim Spitz for expert technical assistance. This work was supported by grant CA 55302 to J.A.N. from the National Institutes of Health.

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Communicating editor: L. S. Symington