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Effects of Terminal Nonhomology and Homeology on Double-Strand-Break-Induced Gene Conversion Tract Directionality

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Double-strand breaks (DSBs) greatly enhance gene conversion in the yeast *Saccharomyces cerevisiae*. In prior plasmid × chromosome crosses, conversion tracts were often short (<53 bp) and usually extended in only one direction from a DSB in an HO recognition sequence inserted into *ura3*. To allow fine-structure analysis of short and unidirectional tracts, phenotypically silent markers were introduced at 3- and 6-bp intervals flanking the HO site. These markers, which created a 70-bp homeologous region (71% homology), greatly increased the proportion of bidirectional tracts. Among products with short or unidirectional tracts, 85% were highly directional, converting markers on only one side (the nearest marker being 6 bp from the HO site). A DSB in an HO site insertion creates terminal nonhomologies. The high degree of directionality is a likely consequence of the precise cleavage at homology/nonhomology borders in hybrid DNA by Rad1/10 endonuclease. In contrast, terminal homeology alone yielded mostly unidirectional tracts. Thus, nonhomology flanked by homeology yields primarily bidirectional tracts, but terminal homeology or nonhomology alone yields primarily unidirectional tracts. These results are inconsistent with uni- and bidirectional tracts arising from one- and two-ended invasion mechanisms, respectively, as reduced homology would be expected to favor one-ended events. Tract spectra with terminal homeology alone were similar in *RAD1* and *rad1* cells, indicating that the high proportion of bidirectional tracts seen with homeology flanking nonhomology is not a consequence of Rad1/10 cleavage at homology/homeology boundaries. Instead, tract directionality appears to reflect the influence of the degree of broken-end homology on mismatch repair.

DNA double-strand breaks (DSBs) and double-strand gaps greatly stimulate homologous recombination (24, 28, 30, 38, 39, 46, 47, 51, 54). Recombinational repair of DSBs can result in gene conversion, a nonreciprocal transfer of information from one homologous region to another, although reciprocal exchange (crossing over) is frequently associated with conversion (33). DSBs usually stimulate conversion of the broken allele (20, 24, 28–30, 32, 39, 46, 47, 54; reviewed in reference 57), but occasionally unbroken alleles are converted (24, 44, 54). Gene conversion has been explained by models that invoke heteroduplex DNA (hDNA) intermediates formed during strand exchange, with conversion resulting from mismatch repair of hDNA (18, 25, 37). This mechanism is thought to mediate most or all meiotic gene conversion in yeast cells (reviewed in reference 33).

DSB or gap repair (56) is an alternative model for conversion that suggests that DSBs are processed to double-stranded gaps, with both 3′ ends invading an undamaged homologous duplex to produce two cross-strand structures (Holliday junctions [18]). The 3′ ends prime DNA synthesis, using the undamaged duplex as a template to fill the gap. In this model, most conversion occurs in a gap (explaining why conversion occurs in broken alleles), but conversion is also possible by repair of hDNA adjacent to gaps, formed during strand invasion and branch migration of Holliday junctions. Both hDNA and gap repair models suggest that Holliday junction resolution accounts for associated crossovers (18, 25, 56). Although double-strand gaps produced in vitro are repaired in vivo with information donated by an endogenous homologous duplex (32), there is no evidence that DSBs are processed into gaps in vivo. In fact, studies of mitotic and meiotic DSB-induced conversion indicate that DSBs are processed by a unidirectional exonuclease to give long 3′ single-strand extensions (51, 53). Also, there is no requirement for both ends to invade, as repair synthesis primed from one end can produce a region complementary to the opposite 3′ extension (reviewed in reference 2). However, the recently described double Holliday junctions in meiotic recombination intermediates indicate that these events involve two-ended invasions (49).

Recombination between related but not identical (homeologous) sequences occurs at low levels compared with homologous recombination, helping to preserve distinct family members. Spontaneous homeologous recombination occurs at lower frequencies than homologous recombination in bacterial (41), yeast (1, 16, 23, 50), and mammalian (58) cells. Three studies have shown that reduced homology also reduces recombination stimulated by DSBs and gaps (23, 26, 43). Homeologous recombination is thought to be reduced because strand transfer is inhibited (6) and because mismatch repair systems scan hybrid DNA and abort recombination when too many mismatches are detected (41).

To clarify the mechanism(s) of DSB-induced gene conversion, we previously examined conversion tract structures by using *ura3* heteroalleles with phenotypically silent markers spaced at ~50- to 100-bp intervals flanking an HO nuclease recognition site. We found that a significant fraction of tracts from these plasmid × chromosome conversions were very short (<53 bp) and that more than 70% extended unidirectionally from the DSB (at a 23- to 31-bp resolution level [54]). DSBs in HO site insertions produce ends with terminal nonhomologies, which can be removed by Rad1/10 endonuclease cleavage at nonhomology/homology boundaries following strand invasion (11, 35). In addition to Rad1/10 processing, tract spectra likely also reflect the extent of hybrid DNA on one or both sides of the DSB and mismatch repair processing.

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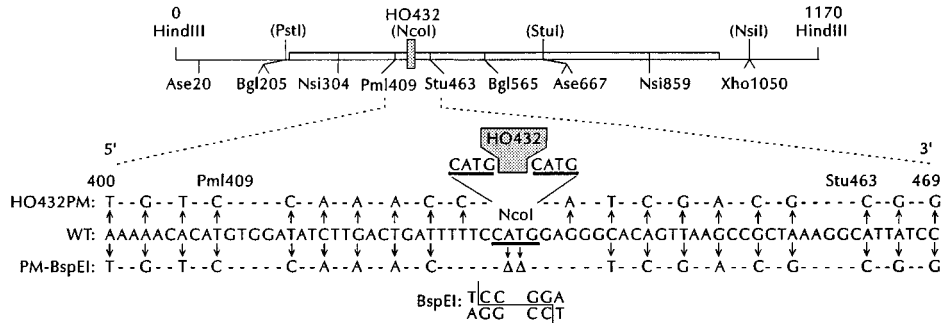

FIG. 1. Structures of recipient *ura3* alleles. At the top is a map of the 1,170-bp *Hin*dIII fragment containing *ura3*-HO432 (54). The promoter is to the left of the coding region, which is shown by the open box. Wild-type (WT) restriction sites are shown above the line and silent RFLP mutations are shown below, with numbers indicating positions within *ura3*. The HO site is located in the *Nco*I site at position 432. Below the map is the 70-bp sequence around *Nco*I, with base changes from wild-type *URA3* in HO432PM and PM-BspEI alleles indicated by arrows. The HO432 allele has only Pml409 and Stu463 mutations in this region. Filling in *Nco*I duplicates the four central bases (underlined) before the HO site is inserted. The PM-BspEI allele has all PM markers except C-430 and A-439; positions of strand breaks in the *Bsp*EI site are shown below. The chromosomal (donor) allele is wild type except for the X764 frameshift mutation (not shown).

One possibility is that nonhomologous ends inhibit strand invasion, favoring one-ended invasions that lead to the observed unidirectional tracts. The present study was designed to examine the effects of terminal nonhomology and homeology on conversion tract spectra. We introduced 20 additional silent markers in the 70-bp region flanking the HO site, revealing highly directional conversion tracts that extended less than 6 bp into homology in one or both directions. Most markers in the homeologous region were separated by 2-bp stretches of homology, but four 5- to 6-bp stretches were present, and nearly all short and unidirectional tracts ended in these regions. We propose that the high degree of directionality results from precise cleavage by Rad1/10 at homology/nonhomology boundaries. The additional mutations created a short region of homeologous DNA, but instead of further increasing unidirectional tracts, reduced homology greatly increased bidirectional tracts, arguing against the idea that unidirectional tracts result from one-ended invasion. We also ruled out the possibility that Rad1/10 cleavage at homeology/homology boundaries produces the bidirectional tracts, showing that bidirectional tracts are common only when both nonhomology and homeology are present at invading ends; most tracts are unidirectional when ends have only homeology or nonhomology. These results are consistent with mismatch repair being influenced by the degree of homology at broken ends.

MATERIALS AND METHODS

Plasmids and yeast strains. Plasmid pUCUraRFLP is a pUC19 derivative carrying a functional *URA3* gene with nine silent mutations creating restriction fragment length polymorphisms (RFLPs) at ~100-bp intervals (54). Additional silent mutations were introduced by two rounds of unique site elimination mutagenesis (7). The first mutagenic primer introduced nine mutations promoter proximal to the *Nco*I site (5′-ATTTGTTTACTTAAGACTCACGTGGACAT ATTAACAGACTTCTCCATGGAGG-3′); the second primer introduced nine more mutations downstream from *Nco*I (5′-CTTCTCCATGGAAGGTACCGT GAAACCCCTGAAGGCCTTGTCGGCCAAGTACAA-3′) (mutant bases are underlined; boldface type indicates the two preexisting RFLPs, Pml409 and Stu463). The second primer overlapped the first and included two mutations introduced by the first primer. The closely spaced polymorphisms (PMs), shown in Fig. 1, were confirmed by DNA sequence analysis (U.S. Biochemicals), and their phenotypic silence was demonstrated as described previously (54). The *Nco*I site at position 432 was made blunt, 10-bp *Eco*RI linkers were inserted, 24-bp HO sites with *Eco*RI-cohesive ends were inserted in two orientations (creating HO432PM and OH432PM alleles), and *TRP1*/*ARS1*/*CEN4* (TAC) derivatives were constructed as described previously (54). A Ura$^+$ product of the HO432PM-TAC derivative in which the HO site was converted but all RFLP and PM sites were retained was isolated (see Results). This product was modified for use in transformation studies as follows. A unique *Bsp*EI site formed during construction of the Bgl205 RFLP (Fig. 1) was destroyed by *Bsp*EI digestion and fill-in/ligation reactions. The unique *Nco*I site at position 432 was converted to a unique *Bsp*EI site by unique site elimination mutagenesis with a primer (5′-TATTAACAGACTTTTCCΔΔGGAGGGTACCGTGAAAC-3′) that deletes the 2 central bp in *Nco*I (ΔΔ) and restores the two PM markers nearest *Nco*I to wild type (underlined). The resulting allele, termed PM-BspEI (Fig. 1), is Ura$^-$ as a result of the frameshift caused by the 2-bp deletion but otherwise has wild-type coding potential, as all remaining RFLP and PM mutations are silent.

Saccharomyces cerevisiae strains were cultured as described previously (54) and transformed by using lithium acetate (19). Strains carrying TAC plasmids were selected and maintained on medium lacking tryptophan. Recombination was induced in vivo in DY3026 (*MAT***a**-*inc lys2*-Δ*1*:*LYS2-GALHO ura3*-X764 *ade2-101 his3-200 leu2*-Δ*1 trp1*-Δ*1* [54]) carrying appropriate plasmids. The *MAT***a**-*inc* mutation prevents mating-type switching and diploidization when the *GAL1* promoter-driven HO nuclease (*GALHO*) is induced by growth in medium with galactose, and the *ura3*-X764 mutation is a +1-bp frameshift mutation in the chromosomal *ura3* gene (54). Strain DY3085 is identical to DY3026 except that the 1.2-kbp *Hin*dIII fragment with *ura3* was completely replaced with *LEU2* and pUC19 in a single-step transplacement (45). Strain DY3437 is a *rad1* derivative of DY3026 created by transplacing *RAD1* with *rad1*::*LEU2* by using plasmid pL962 (kindly provided by R. Keil). Chromosome structures in DY3085 and DY3437 were confirmed by Southern hybridization analysis; DY3437 shows UV hypersensitivity typical of *rad1* mutants (data not shown).

In vivo induction of recombination. Ura$^+$ recombinants were isolated following induction of *GALHO* in 1-cm^2 patches of independent cell populations on solid medium (54). Plasmids with converted alleles were transferred to *Escherichia coli* HB101 by electroporation of total yeast DNA (13) or by a semidirect electrotransfer method in which yeast cells are vortex mixed with glass beads for 5 min, heated to 100°C for 1 min, cooled on ice, mixed with electrocompetent *E. coli*, and electroporated (14). RFLP markers were scored essentially as described previously (54), and PM markers were scored by DNA sequence analysis. All Ura$^+$ recombinants were independent since they were derived from independent Trp$^+$ Ura$^-$ parent colonies, and only a single product was analyzed per induced culture. The system does not allow analysis of conversions to Ura$^+$ associated with single crossovers, as these integrate *CEN4* plasmids into the chromosomal *ura3* gene and produce lethal dicentric chromosomes. Recombination frequencies were measured in four independent induced (galactose-grown) or noninduced (glucose-grown) populations as described previously (30). All statistical tests were done with Fisher's exact test.

In vivo measurements of DSB induction. Transformants of strain DY3085 with TAC derivatives carrying either HO432 or HO432PM were transferred from 3-day-old lawns on medium lacking tryptophan into 100 ml of rich medium with 2% glycerol and grown overnight to derepress the *GAL1* promoter. Cells were harvested by centrifugation and resuspended in 200 ml of rich medium with 5% galactose to induce *GALHO*, and total yeast DNAs were prepared from 40-ml aliquots removed at intervals (13). Four-microgram samples were digested with *Xho*I, electrophoresed on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with a ^{32}P-labeled, 1.2-kbp *Hin*dIII fragment with *ura3*, using standard methods (48). Signals were quantified by scanning densitometry.

Transformation assays. A TAC derivative with the PM-BspEI allele (Fig. 1) was linearized with *Bsp*EI and transformed into DY3026 (*RAD1*) and DY3437 (*rad1*), and Ura$^+$ recombinants were selected. The PM-BspEI allele does not revert under these conditions; no Ura$^+$ colonies arose when DY3085 (lacking a chromosomal copy of *ura3*) was transformed. Transformation frequencies were similar in *RAD1* and *rad1* strains (~5 Ura$^+$ colonies per μg of DNA).

RESULTS

Recombination system. Our previous analysis of DSB-induced gene conversion tracts indicated that most tracts ex-

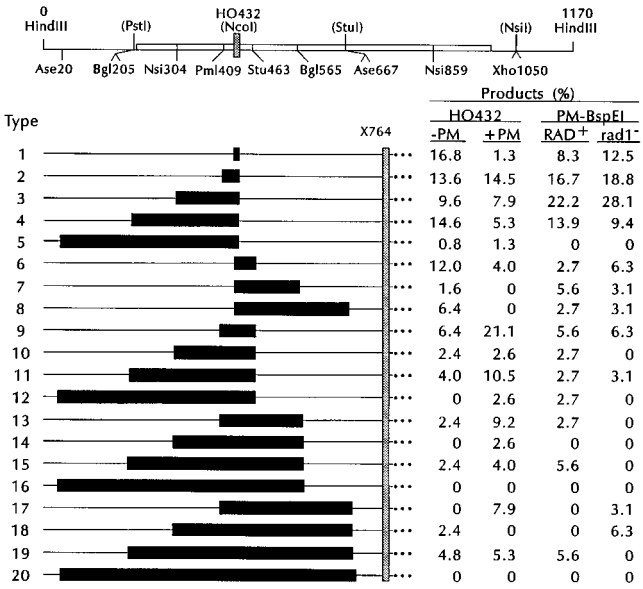

FIG. 2. Conversion tract spectra. At the top is a map of the *ura3* allele as in Fig. 1. Below, 20 types of gene conversion products with continuous tracts are shown; black bars indicate converted markers. The chromosomal X764 frameshift mutation in strain DY3026 is shown by the shaded box. Continuous tracts that include HO sites cannot extend beyond X764. Percentages of each type are given for HO432 conversions induced by HO nuclease without PM markers (125 products) or with PM markers (76 products) and for PM-BspEI cleaved with *Bsp*EI in Rad$^+$ and *rad1* cells (36 and 32 products, respectively).

tended unidirectionally from the DSB and that tract lengths were frequently shorter than 53 bp (54). In that study, the markers closest to the HO site were 23 and 31 bp away. To increase our ability to detect very short and highly directional tracts, we introduced phenotypically silent, single-base mutations (PMs) at 3- to 6-bp intervals surrounding the HO site, creating a *ura3* allele termed HO432PM (Fig. 1). This allele was introduced on a *CEN4* plasmid into strain DY3026, which carries the heteroallelic *ura3*-X764 allele on chromosome V, with a frameshift mutation at position 764. As expected, nearly all induced Ura$^+$ products arose from conversion of the allele suffering the DSB (data not shown). Induction of HO nuclease increased the Ura$^+$ frequency more than 80-fold in strain DY3026:HO432PM, but this value is 10-fold lower than in DY3026:HO432, which lacks the PM mutations. Although this reduction might partly reflect reduced recombination efficiency because of the homeologous DNA flanking the HO site, Southern analysis showed that in a strain lacking a chromosomal *ura3*, the HO432PM allele is cleaved less efficiently than HO432 (data not shown). Previous studies indicated that these 24-bp HO sites are cleaved with various efficiencies at different positions in *ura3* (54, 59), suggesting that cleavage efficiency is affected by flanking sequences, probably because these HO sites are near minimal length (28, 29).

Effect of PM markers on large-scale HO-induced conversion tract structures. Two crosses were performed with PM alleles which differed only by HO site orientation. Although previous studies indicated that HO site orientation had no detectable effects on the types of tracts recovered (28, 47, 54), we thought it possible that the additional markers in the HO432PM allele might reveal an effect of HO site orientation on tract structure spectra. However, no significant differences were found, and the data from the two crosses have been pooled. Large-scale tract structures were determined by RFLP analysis in 78 plasmids isolated from Ura$^+$ recombinants of DY3026:HO432PM and DY3026:OH432PM. Of these, 76 had continuous conversion tracts distributed among 15 of 20 possible types (Fig. 2). This spectrum differs in two respects from that obtained previously in crosses without the PM mutations (54 [reproduced in Fig. 2]). First, PMs reduced by more than 10-fold the percentage of short tracts (<53 bp). Second, PMs significantly reduced other highly directional tracts (types 2 to 8) (58% versus 33%; $P = 0.0002$).

Very long tracts, discontinuous tracts, and tracts extending past the Ase20 border site were rare, and RFLPs promoter proximal to the HO site were converted more frequently than equidistant promoter-distal RFLPs; these results are similar to those obtained previously (54). The reductions in very short and unidirectional tracts by the PMs were balanced primarily by increases in short, bidirectional tracts (types 9, 11, 13, and 17). Thus, the markers defining the homeologous region reduced the chance of a conversion tract terminating in this region.

Conversion tracts extending from HO site insertions are highly directional and rarely terminate in 2-bp stretches of homology. Of the 78 products described above, RFLP mapping defined 26 products with unidirectional tracts or tracts less than 53 bp in length. We consider these short and unidirectional tracts together since these have at least one end that terminates near the HO site. To increase the sample size of short and unidirectional tract types, an additional 96 plasmids from Ura$^+$ products were isolated and mapped with enzymes that define these types (*Pml*I and *Stu*I). From these two sets of products, 60 products with unidirectional or short tracts were identified, and their PM regions were sequenced. As a control, we sequenced two bidirectional products (i.e., both Pml409 and Stu463 converted); as expected, these had converted all PM mutations. The PM mutations allow the identification of a large number of different continuous tract structures, including 20 types that are unidirectional (at a 6-bp level of detection) and 97 types that are <53 bp in length (i.e., retaining both Pml409 and Stu463). Interestingly, only five different types were found among 60 sequenced products (Fig. 3). Of these, 85% were highly directional, extending less than 6 bp in one or both directions (Fig. 3, types 1 to 3 and 5). The remaining 15% had tracts that ended in the 5-bp stretch of homology between positions 457 and 463 (Fig. 1). Of the two products with tracts shorter than 53 bp, one converted only the HO site and the other converted only two PM markers in addition to HO (Fig. 3, types 1 and 2). Thus, 59 of 60 short and unidirectional tracts terminated in the 5- to 6-bp regions of homology. There was no evidence of discontinuous conversion in the PM region among

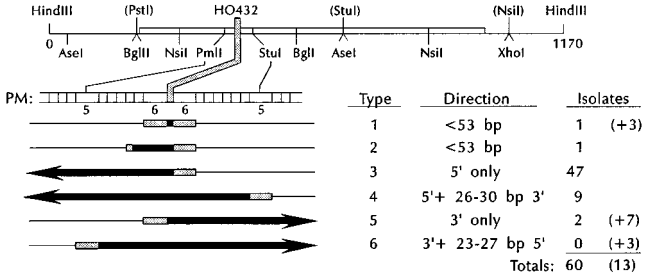

FIG. 3. Spectrum of conversion tract termini within the PM region of the HO432PM allele. At the top is a map of *ura3*-HO432PM, with the PM region represented graphically below (see Fig. 1). All PM markers are separated by 2 bp of homology except where numbered, indicating 5- and 6-bp stretches of homology. Below the map is the spectrum of conversion tract termini within the PM region. Converted markers are indicated by black bars; gray bars indicate regions in which tracts may have ended. A total of 73 Ura$^+$ isolates, including 13 selected as Pml409$^+$ (shown in parentheses), were sequenced.

TABLE 1. Conversion tract spectra for DSB-induced Ura$^+$ recombinants

ura3 allele[a]	Termini	*RAD1* genotype	n^b	Tract distribution (%)[c]	
				<53 bp or unidirectional	Bidirectional
HO432	Nonhomology	+	125	75.2	24.8
HO432PM	Nonhomology and homeology	+	76	34.3	65.8
PM-BspEI	Homeology	+	36	72.2	27.8
PM-BspEI	Homeology	−	32	81.3	18.7

[a] Recipient alleles, shown in Fig. 1.
[b] Number of products analyzed with continuous tracts. Discontinuous tracts, which constituted 0 to 5% of total sample sizes, are not included.
[c] Percentages of each tract class are given for continuous tracts.

the 60 sequenced products. Also, there were no PM discontinuities in two products with gross tract discontinuity identified by RFLP mapping: one of these converted only markers promoter proximal to HO, including all promoter-proximal PMs and Bgl205, but not Nsi304; the other converted all PM markers and all RFLPs except Ase20, Bgl565, Nsi859, and Xho1050.

Because unidirectional tracts extending promoter distal from the HO site and tracts <53 bp were rare (Fig. 2), we devised a selection and screening strategy to generate additional products of these types. By definition, such products would retain Pml409. To select such products, we isolated total DNA from pools of 600 to 1,200 Ura$^+$ recombinants from each of 18 independent populations of DY3026:HO432PM following induction of HO nuclease in 1.5-ml cultures. We made replicas of the original selective plates to reduce the number of parental cells containing unconverted plasmids (that also carry Pml409) before harvesting and preparing total DNA from each pool of recombinants. Each yeast DNA preparation was electroporated into *E. coli*, yielding pools of bacterial transformants that contained 80 to 3,000 members each, and these pools were grown en masse in 10-ml cultures. Plasmid DNA was prepared from each transformant pool and digested with *Pml*I, which releases 3.4-kbp fragments from molecules that retained Pml409. These 3.4-kbp fragments contained the 3′ end of *ura3*, pUC19, and 51 bp of TAC; those that converted Pml409 were cleaved only once and yielded a single unit-length parental fragment (7.0 kbp). The 3.4-kbp fragments were gel purified, self-ligated, and transformed into *E. coli*. Recovered plasmids were mapped with *Nco*I to identify those that converted HO and with *Stu*I to distinguish products that converted or retained Stu463 (and Stu664). We previously determined that Ura$^+$ recombinants sometimes contain both converted and unconverted plasmids (59), and so it was not surprising that 64% of plasmids recovered by this procedure were derived from unconverted (parental) plasmids, as these are also Pml409$^+$. However, by screening 2 to 9 products from each pool, we identified 13 independent Pml409$^+$ products that had converted the HO site and subjected these to sequence analysis. Three products retained Stu463 and therefore had tracts <53 bp (assuming no discontinuities; Fig. 3, type 1); the rest extended promoter-distal from HO (Fig. 3, types 5 and 6). All tracts from this set of selected products (shown in parentheses in Fig. 3) terminated in the 5- and 6-bp stretches of homology at Pml409 and immediately adjacent to the HO site. Thus, of 73 products in which one or both tract ends terminated in the PM region, only 1 terminated in a 2-bp stretch of homology (type 2, Fig. 3).

Terminal nonhomology or homeology favors unidirectional tracts. Unidirectional tracts are favored when nonhomologous termini are flanked by homology (54), but bidirectional tracts are favored when nonhomologous termini are flanked by homeology (Table 1). These bidirectional tracts might result from efficient mismatch repair driven by the highly mismatched region formed by pairing in homeology (Fig. 4). Since Rad1/10 cleaves at homology/nonhomology boundaries (11, 35), a second possibility is that bidirectional tracts result from Rad1/10 cleavage at homology/homeology boundaries (Fig. 4). Despite the presence of *CEN4*, TAC plasmids can exist in multiple copies per yeast cell (59). Therefore, segregation analysis is not informative with these substrates, and this precludes their use to directly test the first possibility in mismatch repair mutants. However, we were able to test the second possibility by transforming a *ura3*-PM-BspEI plasmid linearized with *Bsp*EI (which produces DSBs flanked by homeology [Fig. 1]) into *RAD1* and *rad1* cells carrying a chromosomal *ura3*-X764 allele. If Rad1/10 cleaves at homeology/homology boundaries, *RAD1* cells should yield mostly bidirectional tracts and *rad1* mutants should yield mostly unidirectional tracts. This prediction was not met: both *RAD1* and *rad1* mutant strains showed predominantly unidirectional tracts (Table 1). These gross tract spectra with terminal homeology were very similar to that seen with HO432, in which terminal nonhomology is flanked by homology (Fig. 2). The PM region was sequenced in 15 (*RAD1*) and 19 (*rad1*) products with short or unidirectional tracts, and all were highly directional (analogous to types 1, 3, and 5 in Fig. 3). We draw three conclusions from these experiments. First, nonhomologous or homeologous termini alone are not sufficient to bias toward bidirectional tracts. Second, bidirectional tracts do not result from Rad1/10 processing at homology/homeology boundaries. Third, the predominant, unidirectional or short tracts are highly directional, indicating that tracts efficiently

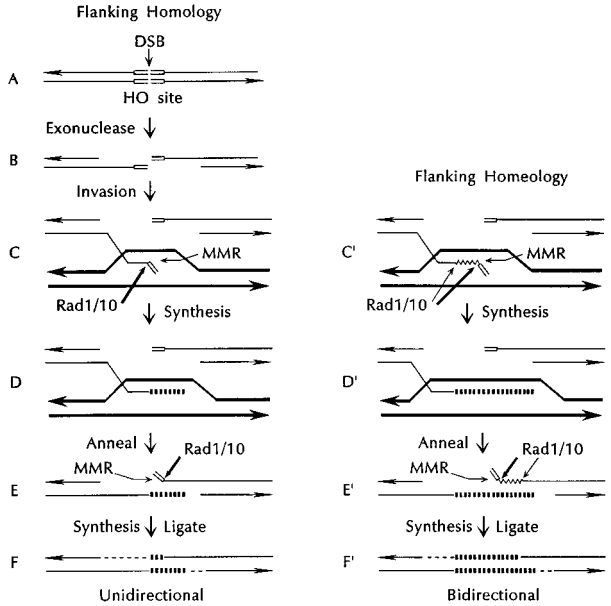

FIG. 4. One-ended invasion producing uni- and bidirectional conversion tracts. A DSB is shown in the nonhomologous HO site insertion (open boxes); 3′ ends are marked by arrowheads. After 3′ ends are exposed (B), one end invades an unbroken homolog (thick lines) (C). Rad1/10 endonuclease removes the nonhomologous region, DNA polymerase effects repair synthesis (dashed lines), and DNA ligase seals nicks in both strands. hDNA may form and be repaired by mismatch repair (MMR) in steps C and E. Flanking homeology (shown by zigzag lines) produces highly mismatched hDNA (C′ and E′). See text for additional details.

terminate in the 4-bp homologies left at 3′ ends when the PM-BspEI allele is cleaved by *Bsp*EI (Fig. 1).

DISCUSSION

Our previous finding (54) that most HO nuclease-induced conversion tracts extend less than 23 or 31 bp into homology in one or both directions led us to examine more closely the structures of these unidirectional and short conversion tracts. We report here that the unidirectional character of such tracts holds at a 6-bp level of resolution. To perform these measurements, we created a short homeologous region (71% homologous over 70 bp) flanking an HO site insertion. In this case, most products had bidirectional tracts and short tracts were extremely rare, contrasting sharply with spectra from analogous crosses with HO sites flanked by homology or with only terminal homeology (Table 1).

Length of homology required for tract termination. In designing our allele for measuring short and unidirectional tracts, we required an intact *Nco*I site to allow insertion of the HO site. We also wanted to retain the two RFLP markers closest to the HO site (Pml409 and Stu463) so that we could rapidly identify products with short or unidirectional tracts. This design created a homeologous region having 5- and 6-bp stretches of homology within regions with 2-bp stretches of homology. Only 1 of 73 products with tracts terminating in the homeologous region had a tract terminate in a 2-bp stretch of homology. These results parallel those of Priebe et al. (36), who studied homeologous conversion between *his4* alleles from *S. cerevisiae* and *S. carlsbergensis* stimulated by a double-strand gap. In that study, homologies flanking the gap ranged from 1 to 50 bp, and none of the 23 tracts examined terminated in regions with less than 5 bp of homology. It is possible that successful conversion requires that the invading strand be anchored to the donor allele through pairing in homologous stretches more than 2 bp in length. Here we show that tracts terminate relatively frequently in 5 and 6 bp of homology (with terminal nonhomologies) and in 4 bp of homology (with terminal homeologies).

DSB-induced conversion by gap or hDNA repair? Although unidirectional tracts were found about twofold less frequently when HO sites were flanked with homeology than when they were flanked with homology, they were still fairly common (34% [Table 1]). The prevalence of unidirectional tracts indicates that DSB-induced conversion is unlikely to involve double-strand gap formation (56) since gaps are unlikely to be formed at only one end, a conclusion also reached by Priebe et al. (36). Considerable evidence supports an alternative model for DSB-induced conversion involving single-stranded degradation at DSBs, leaving 3′ extensions at both ends (31, 40, 51–53, 61). This model suggests that conversion results from mismatch repair of hDNA following 3′-end invasion and is consistent with meiotic conversion in *S. cerevisiae* (33), which is thought to be initiated by DSBs (31, 52, 53). DSB-induced gene conversion normally involves preferential conversion of broken alleles (e.g., this study and references 24, 30, and 54). Although preferential conversion is easily explained by conversion occurring in a gap (56), it is less easily explained by hDNA repair models in view of early studies showing that most mismatches are repaired efficiently and with parity (or at most a slight, twofold disparity [3, 4, 21]). However, these studies used artificial mismatches, and a more recent study of mismatch repair in meiosis showed marked disparity in favor of conversion-type repair over restoration-type repair, suggesting that donor or recipient strands are tagged (9). For DSB-induced events, preferential conversion of broken alleles may reflect differential strand tagging such that invading strands are targeted for repair. A likely tag is the broken end itself directing the repair to that invading strand, analogous to nick-directed mismatch repair in *E. coli* (22) and mammalian cells (8, 15).

Conversion tract directionality: invasion by one end or two? It is possible that uni- and bidirectional tracts result from one-ended invasions (2) and two-ended invasions, respectively. It is reasonable to suppose that one-ended events would be favored if invasion is rate limiting. Studies in vivo and in vitro indicated that invasion by two ends is temporally distinct (5, 55), and integration-based recombination systems that required both ends of a DSB to interact with a homolog showed no evidence for cooperativity between the two invading ends (17), a result consistent with the idea that invasion is limiting. Although no studies have yet identified factors that might influence a choice between one- and two-ended invasion pathways, the degree of terminal homology is likely to be important. Unidirectional tracts may predominate for HO-induced events because DSBs in an inserted HO site produce nonhomologous ends (Fig. 4C); such ends may inhibit strand invasion, promoting one-ended events. The homeologous conversions examined by Priebe et al. (36) were stimulated by double-strand gaps created in vitro, and therefore termini were homeologous, yet nearly every tract characterized in that study was highly directional. We found similar results with terminal homeologies. It is possible that terminal homeology and nonhomology may act similarly to reduce the probability of double-ended invasion. Priebe et al. (36) found most tracts extended only one direction from the gap. This bias may reflect the asymmetric distribution of homologous stretches flanking the gap. Most tracts characterized in that study extended from a 14-bp stretch of homology only 8 bp from the gap on one side. They did not observe tracts that terminated in other stretches of homology of similar lengths (12 and 20 bp) that were farther from the gap (26 and 38 bp). Since our data show that 5 to 6 bp of homology is sufficient to allow fairly efficient tract termination (Fig. 3), 14 bp is likely long enough to allow efficient tract termination and produce the bias observed by Priebe et al. (36). In our HO-induced homeologous crosses, stretches of homology were symmetrical around the DSB, but we observed more frequent conversion of markers promoter proximal to the DSB. This bias was also seen with homologous crosses (reference 54 and Fig. 2), and recent work has indicated that it reflects a selection bias against Ura$^-$ products that arise when the promoter-distal chromosomal X764 frameshift mutation is transferred to the plasmid allele (59). While homeology had no effect on the distribution of unidirectional tracts extending promoter proximal to HO, the unidirectional promoter-distal tract types 6 and 8 were significantly reduced from three- to sixfold ($P = 0.03$ and 0.02, respectively). It is unclear how the symmetric PM mutations might cause such differences on only one side; these may reflect statistical fluctuations.

The idea that unidirectional tracts arise from one-ended invasions and that one-ended invasions are favored when ends have reduced or no homology is consistent with some but not all meiotic conversion data. For example, most meiotic conversion products at *ARG4* have bidirectional tracts (53), and these events are stimulated by DSBs (52) that occur in regions of total homology. In contrast, unidirectional tracts were found for meiotic conversions at *HIS4*, although it has been questioned whether these events are stimulated by nicks (10, 34) or by DSBs that are processed asymmetrically (34). It is possible that unidirectional tracts result from differential mismatch repair on either side of the DSB in two-ended invasion intermediates. If repair on both sides were random, half of tracts would

be unidirectional, but we found that more than half were unidirectional with nonhomology flanked by homology and that less than half were unidirectional with nonhomology flanked by homeology. Also, if only 3′ ends invade, it is unlikely that strands with identical polarity on either side would frequently be repaired in opposite directions. Another possibility is that one-ended invasions are favored in these plasmid × chromosome crosses because the two ends of the DSB are linked through the plasmid backbone. A single-end invasion followed by repair synthesis can produce a complementary strand to which the noninvading end can anneal (Fig. 4E). Since the noninvading end is linked to the invading end in these crosses, such an intramolecular reaction might be favored and produce predominantly unidirectional tracts. Since ends are not linked when DSBs occur in chromosomes, two-ended invasions might be expected to be more frequent for chromosomal events. We recently obtained evidence supporting this view: bidirectional tracts arose about twice as often (42% versus 22%; $P = 0.01$) in an intrachromosomal cross with *ura3*-X764 and *ura3*-HO432 alleles (60) than in a plasmid × chromosome cross using identical alleles (54). Double Holliday junctions in meiosis (49) also are consistent with two-ended invasions.

Conversion tract directionality: effect of reduced terminal homology and possible role of mismatch repair. Although the one- and two-ended invasion hypothesis is simple and intuitively attractive, it cannot completely explain the spectra reported here. If nonhomology (or homeology) at ends reduces strand invasion, and uni- or bidirectional tracts result from one- and two-ended invasions, respectively, then reduced homology flanking the HO site should further reduce bidirectional tracts, but they were sharply increased (Table 1). To understand these results, we draw on current models of DSB-induced gene conversion (2, 12, 27, 53) as diagrammed in Fig. 4. This is a one-ended invasion model (2) similar to that proposed by Resnick (42; reviewed in reference 57) except that the donor never suffers a strand break. The model includes the production of 3′ extensions (12, 51, 53, 61), Rad1/10 endonuclease activity to remove 3′ nonhomologous regions (11, 35), and end-directed mismatch repair (8, 15, 22). With an HO site flanked by homology, 5′-to-3′ exonuclease activity from both ends of a DSB exposes 3′ ends, one of which invades the unbroken homolog (Fig. 4A to C). The short nonhomology at the 3′ end is removed by Rad1/10 endonuclease, exposing a 3′ homologous end that primes repair synthesis (Fig. 4C and D). Precise cleavage by Rad1/10 produces the observed highly directional tracts. The newly synthesized strand then anneals to the exposed 3′ end on the other side of the DSB, producing another substrate for Rad1/10 endonuclease, and the single-strand gaps are filled by DNA polymerase and ligase (Fig. 4E and F). Depending on the extents of exonucleolytic degradation, pairing upon invasion, and repair synthesis, hDNA may be formed in hybrid DNA and would be subject to mismatch repair. If mismatch repair occurs prior to ligation, it might be end directed, involving exonucleolytic removal of one strand (and leading to preferential conversion of the broken allele [MMR in Fig. 4C and E]). Note that this model does not require two-ended invasions to create bidirectional tracts. This allows us to retain the attractive ideas that invasion is rate limiting and inhibited by nonhomology or homeology at DNA ends.

In this model, final tract structures are determined by mismatch repair and/or Rad1/10 activities on hDNA, and hDNA formation depends on two factors. First, increasing hybrid DNA formation during strand invasion increases the chance that hDNA will form on the invasion side of the DSB. Second, increasing the extent of repair synthesis increases the chance that hDNA will form on the opposite side of the DSB. If either of these factors is normally limiting, most tracts would be unidirectional. Studies in mismatch repair mutant strains with chromosomal substrates amenable to segregation analysis are in progress to determine how hDNA formation depends on strand invasion and repair synthesis.

How are homeologous tract spectra shifted from primarily unidirectional to primarily bidirectional tracts by the addition of terminal nonhomology? One explanation is that bidirectional tracts might result from Rad1/10 cleavage at homeology/homology borders (Fig. 4C′ and E′, thin arrows) rather than nonhomology/homeology borders (thick arrows). Rad1/10 cleavage at mismatches was first suggested by Bailis and Rothstein (1). A second possibility is that pairing must occur in homology, as this would produce intermediates with hybrid DNA that always included both homeologous regions. If the highly mismatched regions present a strong signal to the mismatch repair system, they would be repaired efficiently and most tracts would be bidirectional. However, tract spectra with terminal homeology alone, showing Rad1-independent, predominantly unidirectional tracts (Table 1), are inconsistent with these ideas. A more likely explanation is that terminal nonhomology acts as a supplementary signal to the mismatch repair system. An intriguing alternative is that one or more mismatch repair proteins may interact with Rad1/10 endonuclease, as these enzymes are each involved in processing recombinational repair intermediates.

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