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Effect of Limited Homology on Gene Conversion in a *Saccharomyces cerevisiae* Plasmid Recombination System

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Plasmids containing heteroallelic copies of the *Saccharomyces cerevisiae HIS3* gene undergo intramolecular gene conversion in mitotically dividing *S. cerevisiae* cells. We have used this plasmid system to determine the minimum amount of homology required for gene conversion, to examine how conversion tract lengths are affected by limited homology, and to analyze the role of flanking DNA sequences on the pattern of exchange. Plasmids with homologous sequences greater than 2 kilobases have mitotic exchange rates as high as 2×10^{-3} events per cell per generation. As the homology is reduced, the exchange rate decreases dramatically. A plasmid with 26 base pairs (bp) of homology undergoes gene conversion at a rate of approximately 1×10^{-10} events per cell per generation. These studies have also shown that an 8-bp insertion mutation 13 bp from a border between homologous and nonhomologous sequences undergoes conversion, but that a similar 8-bp insertion 5 bp from a border does not. Examination of independent conversion events which occurred in plasmids with heteroallelic copies of the *HIS3* gene shows that markers within 280 bp of a border between homologous and nonhomologous sequences undergo conversion less frequently than the same markers within a more extensive homologous sequence. Thus, proximity to a border between homologous and nonhomologous sequences shortens the conversion tract length.

Numerous examples of genetic exchange between repeated sequences along individual chromosomes or between such elements present on different chromosomes have been observed in eucaryotic organisms. Many of the observed exchanges have been gene conversions in which a sequence of the donor replaces the homologous sequence of the recipient without concomitant change in the donor (12). In *Saccharomyces cerevisiae*, for which detailed studies have been possible, some of the events include crossing over concomitant with the conversion event (8, 9, 14), whereas others are mostly limited to conversion alone (5, 6, 11, 24). These exchanges are effected by the homology which exists between the repeated sequences. Although studies of homology requirements for exchange have been made with *Escherichia coli* (21, 23, 29) and mammalian cells (3, 15, 20), no systematic study has been made with *S. cerevisiae* to determine the requirements for homology in effecting exchange, particularly conversion, between elements of limited homology.

Because exchange of this nature is thought to mimic the normal chromosomal exchange which occurs between DNA molecules of lengthy homology, exchange between elements of limited homology offers an opportunity to examine fundamental questions which relate to the basic mechanism of exchange. Current models of exchange postulate that homology is required to synapse two homologous sequences by the formation of heteroduplex DNA in which a double helix is formed from complementary strands of the participating homologs (7, 17, 27, 30). Examination of exchange between sequences of limited homology should define the absolute length of DNA needed to effect gene conversion and to find whether homology must exist on both sides of a marker for it to be included in the conversion event.

Previously (1, 4) we have constructed plasmids which undergo intramolecular gene conversion and crossing over in *S. cerevisiae* between heteroallelic copies of the *HIS3* gene which are held as an inverted repetition in the circular contour of the plasmid. The plasmids have a number of properties which are well suited for studies of the role of homology in promoting exchange. The plasmids have limited homology between the exchanging allelic segments, which can be varied over a 300-fold range. The mitotic exchange rate of plasmids with the largest homologous sequences can exceed 10^{-3} events per cell per generation, whereas rates as low as 10^{-10} events per cell per generation can be measured when the homology is substantially decreased. The mutations in the plasmid sequences are 8- or 10-base-pair (bp) restriction site linkers which have been inserted into blunt-end restriction sites within the wild-type sequence. Because the linker insertions do not revert, exchange rates which are lower than the reversion rate of many base substitution mutations are readily measured. Furthermore, because the linker insertions are restriction sites within restriction sites, the fidelity of conversion events which include the site can be checked by appropriate digestions.

MATERIALS AND METHODS

The methodology used in constructing plasmids, the *S. cerevisiae* strain, SSL204, which contains the *his3Δ200* deletion, the measurement of mitotic exchange rates by fluctuation analyses, and the means by which independent exchange events are analyzed have been described previously (1).

Sequences flanking the *HIS3* gene were integrated at a *Pvu*II site 588 bp upstream of the transcription start site of the *HIS4* gene by using plasmid pL191 in *S. cerevisiae* K144 and K150 as described by Keil and Roeder (10).

RESULTS

Minimal homology requirement for conversion. To determine the minimal length of homology needed to effect gene conversion, we constructed plasmids with one complete copy of the *HIS3* gene, mutated with a restriction site linker

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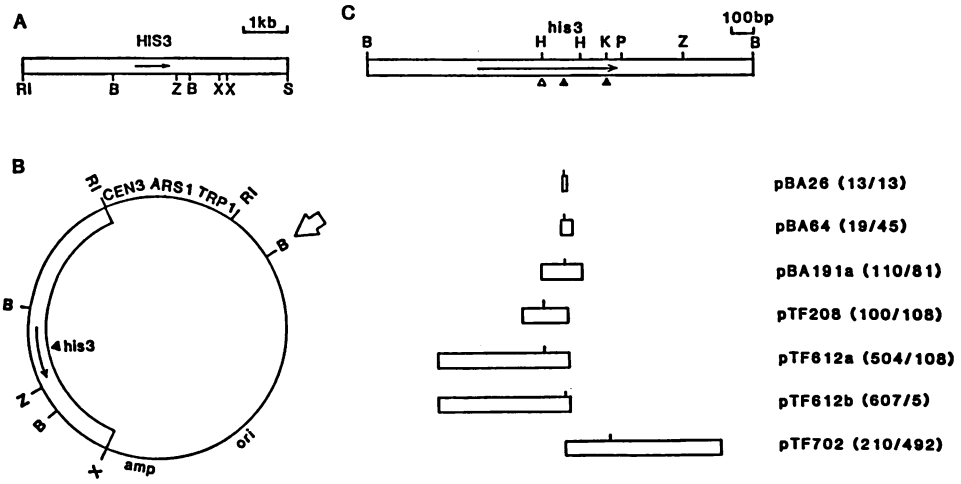

FIG. 1. Map of the yeast *HIS3* region and plasmids used to study the effect of homology on gene conversion. (A) Restriction map of the 6.1-kb *HIS3* region from chromosome XV of *S. cerevisiae* (26). The arrow shows the direction and the extent of the *HIS3* coding region. Abbreviations: RI, *Eco*RI; B, *Bam*HI; Z, *Xho*I; X, *Xba*I; S, *Sal*I. (B) Diagram of one of three plasmid vectors. Symbols: ▭, 4.4-kb *Eco*RI-*Xba*I *HIS3*-containing sequence from chromosome XV; —, bacterial and yeast sequences needed for the selection and propagation of the plasmids in the two organisms; ▲, *Sac*I linker inserted inside the *HIS3* coding region (it is one of the three insertion mutations used in these plasmids); , *Bam*HI site where various subgenic fragments of the wild-type *HIS3* sequence are inserted. Each plasmid contains one subgenic fragment opposite a complete copy with a single linker insertion. All subgenic fragments are oriented as inverted repetitions as previously described (1). (C) Top: Expanded map of the 1.78-kb *Bam*HI fragment containing *HIS3*. Abbreviations: H, *Hin*dIII; K, *Kpn*I; P, *Pst*I. Symbols: △, *Cla*I linkers; ▲, *Sac*I linkers. All three insertions are shown, even though each plasmid contains only one. The position of the linker insertions and their distance from the leftmost *Bam*HI site are an *Alu*I site (790 bp), a *Rsa*I site (900 bp), and a *Rsa*I site (1,107 bp), respectively (25). Bottom: Subgenic fragments of *HIS3* used in each plasmid. The fragments are all small restriction fragments of the wild-type sequence. The position within each wild-type subgenic sequence corresponding to the site of the linker insertions which they cover is indicated as a short line protruding above the bar. The amount of flanking sequence (in base pairs) on each side of the marker is indicated in parentheses after the name of each plasmid.

in the coding region, and a second, subgenic portion of the wild-type sequence, which covered the linker insertion (Fig. 1). The plasmids were transformed into yeast cells containing a deletion of the chromosomal *HIS3* gene, and the cells were grown mitotically in the presence of histidine to permit plasmid-limited exchange events which generate a wild-type *HIS3* allele. We used fluctuation analysis (16) to measure the rate of exchange, by monitoring the number of histidine prototrophs which arose in the cultures. For the plasmids in which the subgenic fragment was completely contained within the reading frame of the gene, only conversion events, which did not include an associated crossover, or double-reciprocal recombinants would yield a complete, wild-type *HIS3* allele. A graph of the relationship between the rate and the homology (Fig. 2) shows that the rate decreases precipitously with the length of homology, such that a 10-fold decrease in homology decreases the rate by approximately 1,000-fold. A plasmid with 26 bp of homology yielded one recombinant in each of two fluctuation cultures out of a set of 198 cultures, each containing 5×10^7 cells. These values yield a rate of 10^{-10} events per cell per generation.

To ensure that the recombinants which arose in plasmids with homologies of 64 and 26 bp resulted from conversion of the linker insertion and not from forms of reversion such as frameshift mutations in adjacent regions or from other forms of exchange such as double-reciprocal recombination, we isolated plasmid DNAs from independent recombinants and subjected them to restriction digestions. In both plasmids the insertion mutation was a *Sac*I linker placed in a *Rsa*I site of the wild-type sequence. Digestion of plasmids showed that all tested (10 of 10 for the 64-bp homology and 2 of 2 for the 26-bp homology) had lost the *Sac*I site and regained the *Rsa*I

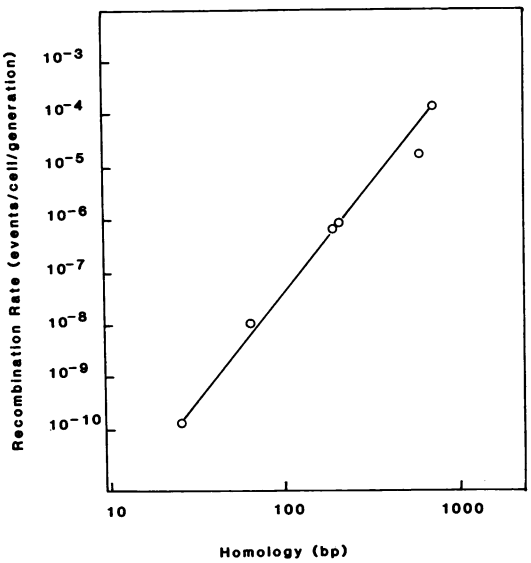

FIG. 2. Rate of mitotic recombination as a function of homology. Fluctuation analyses were used to measure exchange rates (16). For most of the plasmids, 7 to 20 cultures were started, and the rate was calculated by the method of the median frequency (13). For plasmid pBA26 the rate was calculated on the basis of the result that only 2 of 198 fluctuation cultures yielded recombinants. The rate and homology are each plotted on a logarithmic scale.

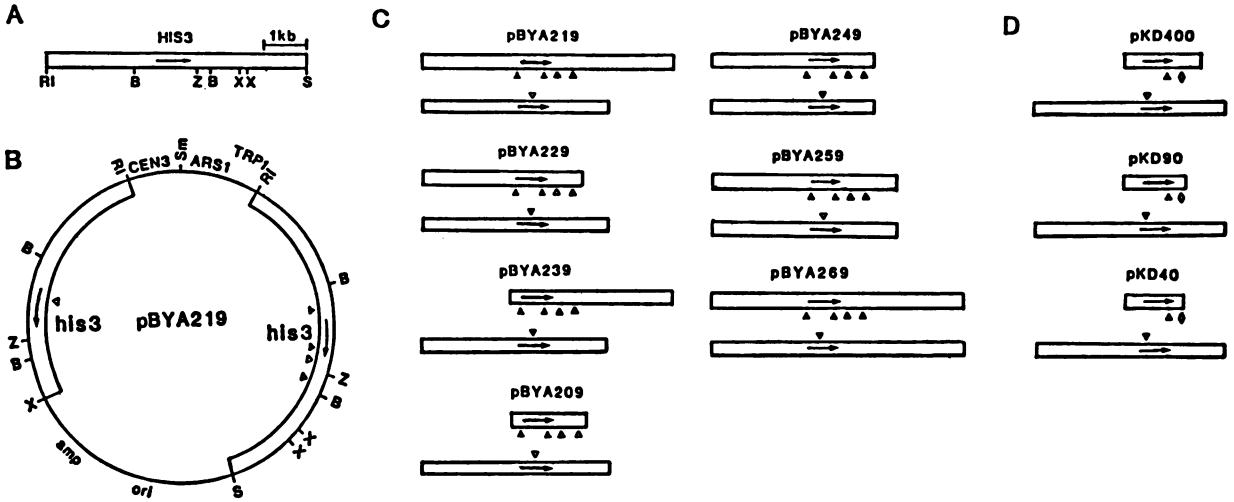

FIG. 3. Map of the yeast *HIS3* region and heteroallelic plasmids used to examine the effect of flanking sequences on conversion. (A) Map of the *HIS3* gene as described in Fig. 1. (B) Complete diagram of one of the heteroallelic plasmids. The linker mutations are the same as those described in Fig. 1. (C) Diagrams of the marker placement and homologous sequences within one set of plasmids. Only the two *HIS3*-containing segments from each plasmid are shown. The top bar of each segment represents the right-hand *HIS3* copy within the plasmid as diagrammed, and the bottom bar represents the left-hand copy within the plasmid. In all plasmids except pBYA249 and pBYA269, the 4.4-kb *Eco*RI-*Xba*I fragment occupies the left side. The right side contains the following fragments, all oriented as indirect repeats with respect to the left-hand copy: 6.1-kb *Eco*RI-*Sal*I (pBYA219); 3.8-kb *Eco*RI-*Bam*HI (pBYA229); 4.0-kb *Bam*HI-*Sal*I (pBYA239); 1.8-kb *Bam*HI-*Bam*HI (pBYA209); 4.4-kb *Eco*RI-*Xba*I (pBYA259). Plasmid pBYA249 contains the 3.8-kb *Eco*RI-*Bam*HI fragment in both cloning sites, and plasmid pBYA269 has the 6.1-kb *Eco*RI-*Sal*I fragment in both sides. The positions of the five linker insertions, reading from left to right, are 280, 625, 900, 1,165, and 1,600 bp, respectively, from the leftmost *Bam*HI site. (D) The second set of plasmids contain the 4.4-kb *Eco*RI-*Xba*I fragment in their left side. Plasmid pKD400 contains a full-size 1.78-kb *Bam*HI-*Bam*HI fragment in its right side. The three markers shown are at 280, 1,106, and 1,347 bp, respectively, from the leftmost *Bam*HI site. Symbols: ◇, *Sma*I site generated by inserting 10-bp *Sma*I-*Sal*I adapters into the *Xho*I site in the wild-type sequence; △, *Cla*I linkers; ▲, *Sac*I linkers. In plasmids pKD90 and pKD40, either *Bal* 31 or a combination of exonuclease III and S1 nuclease has been used to shorten the homology flanking the 3′ side of the *Sma*I marker. Nuclease action brought the *Sma*I site to 90 and 40 bp of the homology border in pKD90 and pKD40, respectively.

site. Thus, the events which give rise to the wild-type allele in plasmids with very limited homology result from the same form of exchange, namely, gene conversion, as that which we (1) have found to occur in plasmids with extensive homology.

To determine whether deletion of the linker insertion could occur in the absence of a homologous sequence, a plasmid with a single copy of the *HIS3* gene containing a linker insertion, but with no subgenic fragment, was tested for its ability to mutate to a form capable of supporting prototrophic growth. To carry out this test, 1 liter of transformed cells was grown in the presence of histidine to a density of 5×10^7 cells per ml and then transferred to medium lacking histidine to permit the growth of rare prototrophs. After 3 days a sample was plated on agar lacking histidine. A few colonies grew, and plasmid DNA was purified from 10 of these. Molecular analysis revealed that the plasmids were all the same and contained a *Sac*I linker insertion at the same site as that in the starting plasmid. Further analysis has revealed that the plasmids contain a small insertion (7 to 10 bp) of unknown origin to the 3′ side of the *Sac*I insertion (B.-Y. Ahn and D. M. Livingston, unpublished observation). Thus, although nonrecombinational mechanisms may exist to yield pseudorevertants, the complete reversion which occurs in plasmids with small lengths of homology does so by conversion.

Minimal homology requirement to one side of a converted marker. In addition to the requirement for the total amount of homology which must surround a marker, we attempted to determine the minimal amount of homology which must flank one side of a converted marker. Thus, a marker within a region of extensive homology may fail to convert if it is close to the border between homologous and nonhomologous DNA. From the successful conversion events undergone by the plasmid with 26 bp of homology, we knew that 13 bp must be sufficient, because the linker insertion was exactly in the middle of the homologous sequence. To generate a marker within a region of substantial homology that lay very near the homology border, we constructed plasmid pTF612b (Fig. 1), in which the 612-bp homologous segment of the wild-type sequence covered a linker insertion mutation 5 bp from the homology border within the complete gene copy. When the exchange rate of this plasmid was measured, it failed to yield recombinants. In this case, a single recombinant would have meant a rate equal to 1.5×10^{-10} events per cell per generation. This value is nearly 5 orders of magnitude lower than the rate measured for the same length of homology when the marker was farther from the border (Fig. 2, plasmid pTF612a). Thus, in our plasmid system, in which the mutations are 8-bp linker insertions, 13 bp of flanking homology is sufficient, whereas 5 bp is not.

Effect of flanking sequences on exchange within the *HIS3* gene. Having examined exchange within the *HIS3* gene with limiting amounts of homology, we proceeded to add flanking homologous sequences to observe whether the exchange rate would continue to rise logarithmically or whether it would reach a plateau. Two sets of plasmids were constructed (Fig. 3C and D), both of which contained two complete copies of the *HIS3* gene appropriately marked with heteroallelic mutations. Thus, in these plasmids, exchange could take place in either direction between the allelic segments to yield a wild-type *HIS3* allele and events involv-

TABLE 1. Analysis of plasmid recombination events which give rise to a wild-type *HIS3* allele

Plasmid	Homology (kb)	Rate (10^{-4} events/ generation ± SD)	No. of 5′ allele convertants[a]		No. of 3′ allele convertants[a]			No. of complex conversion events[b]	No. of reciprocal recombinants[c]	Total[d]
			Single site	Coconvertants	Single site	Coconvertants: First marker	Coconvertants: First and second marker			
Set I										
pBYA219	4.4	12.0 ± 2.8	27 (2)	25 (10)	8 (1)	9 (0)	22 (3)	32 (9)	1	124 (26)
pBYA229	3.8	18.0 ± 3.9	13 (3)	8 (2)	3 (2)	11 (0)	5 (5)	6 (2)	2	48 (16)
pBYA239	2.3	0.33 ± 0.06	19 (0)	5 (0)	3 (0)	5 (0)	11 (3)	6 (2)	1	50 (6)
pBYA209	1.5	1.3 ± 0.22	20 (1)	5 (0)	3 (1)	10 (3)	6 (1)	6 (0)	0	50 (6)
pBYA249	3.8	23.0 ± 5.1	ND[e]	ND	ND	ND	ND	ND	ND	
pBYA259	4.4	22.0 ± 4.8	ND	ND	ND	ND	ND	ND	ND	
pBYA269	6.1	4.6 ± 1.1	ND	ND	ND	ND	ND	ND	ND	
Set II										
pKD400	1.78	4.8 ± 1.2	26 (4)		6 (1)	11 (5)		3 (2)	3	49 (15)
pKD90	1.31	2.3 ± 0.61	27 (5)		8 (2)	3 (2)		6 (3)	4	48 (16)
pKD40	1.26	1.6 ± 0.41	36 (23)		13 (10)	1 (1)		3 (1)	5	58 (35)

[a] In these events, conversion of either the 5′ or 3′ *HIS3* heteroallele occurs to generate a wild-type allele. If coconversion of flanking markers also occurs, the pattern is continuous. Some events are associated with reciprocal exchange, which alters the linkage of flanking markers (numbers in parentheses).

[b] In these events, conversion of one of the heteroalleles occurs, but coconversion of noncontiguous markers on either the same or homologous allelic segment has also occurred. These events have been described as asymmetric discontinuous and symmetric in our previous work (1). The events associated with reciprocal recombination of flanking markers are indicated in parentheses.

[c] In these events, no conversion occurs in the *HIS3* coding region, but a crossover between the heteroalleles generate a wild-type allele.

[d] The numbers in parentheses are the total number of events, which include reciprocal recombination.

[e] ND, Not determined.

ing reciprocal recombination could be productive. Additional markers were placed within the flanking sequences, with the two sets differing in the number and placement of markers. Within each set the plasmids differed in the amount of flanking sequences. As in our previous work (1, 4), the allelic segments were held as an inverted repetition so that any reciprocal exchange which might accompany a conversion event or be the cause of a *HIS3*$^+$ allele would result in the reorientation of vector sequences.

The exchange rates for these plasmids are listed in Table 1. In contrast to the exchange rates for plasmids with limited homology, the rates for these plasmids were not a simple function of the amount of homology. For example, plasmid pBYA249, which contained 3.8 kb of homologous sequences, had the highest rate of exchange, whereas pBYA 269, with a larger amount of homology (6.1 kb), had a lower rate. Similarly, plasmid pBYA239, which contained 2.3 kb of homology, had the lowest exchange rate, whereas pBYA209, with less homology (1.8 kb), had a fourfold-higher rate. Indeed, pBYA239 had a lower rate of exchange than pTF702 (Fig. 2), even though it had three times the amount of homology and exchange could take place in either direction between the heteroalleles. Because all the other plasmids had rates higher than that of pTF702, we conclude that additional homology does increase the exchange rate within the *HIS3* coding region. The rate increase is not directly proportional to the amount of homology, nor does it follow the logarithmic progression seen with the plasmids with subgenic fragments (Fig. 2). Consequently, we are unsure whether a plateau has been reached whereby further increases in flanking homology will have no further effect on the exchange rate.

Truncation of conversion tracts. Plasmids with small amounts of homology might limit exchange not only by reducing the rate at which events initiate, but also by restricting the growth of conversion tracts once an event has been initiated. The plasmids with more extensive homology provided the opportunity to examine whether tract growth might be truncated as the result of limited homology. These plasmids, which included one or more markers in the flanking homologous sequences, varied in the extent of the flanking homology, but not in the relative placement of markers. Thus, the distance between the homology borders and the markers differed among these plasmids.

Using these plasmids, we measured the length of the conversion tracts which occurred during exchange by analyzing independent exchange events and determining the frequency of coconversion of the multiple markers. Data on the frequency of coconversion events are given in Table 1. The appropriate data were then plotted (Fig. 4) to show the relative efficiency of coconversion of the flanking markers as a function of their distance from the homology border. The results indicate that the coconversion frequencies of markers which are farther than 400 bp from the homology border are no different from the coconversion frequencies of the same markers in plasmids with more extensive flanking homology. The coconversion frequency was reduced when the markers were located within 280 bp of the homology border. Thus, conversion tract lengths are truncated by a homology border.

The truncation of conversion tracts close to a border between homologous and nonhomologous DNA could result either from a decrease in the number of sites at which exchange may initiate or from a failure to elongate events once they are initiated. For plasmids pBYA209, pBYA219, pBYA229, and pBYA239, two measurements suggest that initiation was not impaired. First, if initiation were affected by the alterations in the position of the homology border, then the exchange rate should have diminished as homology was decreased. Although the exchange rates of the plasmids differed from one another, the rates did not correlate with the examples of shortened tract length. For example, pBYA229 had one of the highest exchange rates, yet it exhibited truncated tract lengths to the 3′ side. The fact that

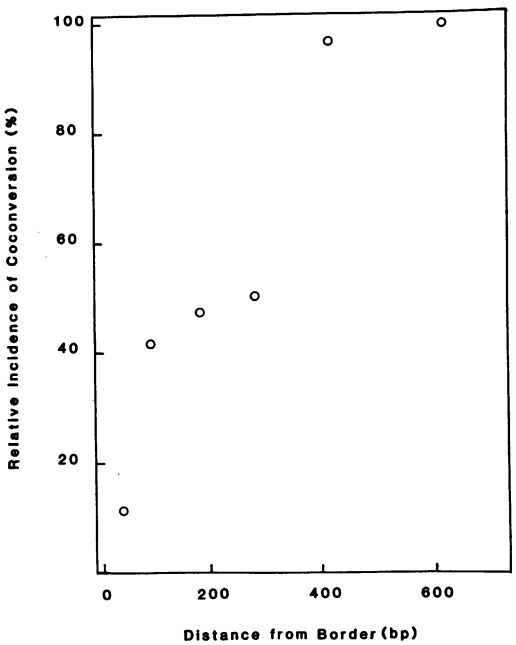

FIG. 4. Relative incidence of coconversion as a function of distance from the end of homology to a coconverting marker. The relative frequency of coconversion is the percentage of coconversion in a plasmid with shortened homology divided by the percentage of coconversion of the same marker in a plasmid with more extensive homology. The distance is that of the marker from the homology border in the plasmid with the limited homology. From left to right, the ratios are for the following sets of markers: the *SmaI* marker in pKD40 versus that in pBYA819 and pBYA918; the *SmaI* marker in pKD90 versus that in pBYA819 and pBYA918; the second *ClaI* marker to the 3′ side in pBYA229 and pBYA209 versus that in pBYA219 and pBYA239; the *SacI* marker to the 5′ side in pBYA239 and pBYA209 versus that in pBYA219 and pBYA229; the *SmaI* marker in pKD400 versus that in pBYA819 and pBYA918; and the first *ClaI* marker to the 3′ side in pBYA229 and pBYA209 versus that in pBYA819 and pBYA918. The data for plasmids pBYA819 and pBYA918 are from our previous report (1).

the rates, which are measures of exchange within the *HIS3* gene, were not clearly diminished by the changes in the position of the homology border suggests that the same number of events were initiated in cases in which borders were closer to flanking markers as in cases with more extended homology. Second, examination of independent events showed that the frequency with which each of the two *HIS3* heteroalleles was converted to the wild type remained nearly constant in all the constructions. (As previously reported [1], a slight preference for conversion of the allele on the side of the plasmid close to the ampicillin marker exists no matter whether the heteroallele is the 5′ or 3′ member of the pair.) Limitations in homology which would diminish initiation would be expected to decrease the frequency with which the allele nearest the homology border is converted. Again, the constancy of the data on allele preference argues that initiation is not affected.

For plasmids pKD40, pKD90, and pKD400, these justifications do not hold, since both the exchange rate and the allele preference are affected by the truncations. Nevertheless, we note that the frequency of coconversion of the marker nearest the homology border decreases nearly 10-fold between plasmids pKD400 and pKD40, whereas the difference between exchange rates is only 3-fold and that for allelic preference is only 2-fold. Thus, in these plasmids, both initiation and elongation may be affected.

DISCUSSION

Requirement for minimal amount of homology. We examined how limited homology affects the exchange rate of plasmids which must undergo gene conversion to produce productive exchange events. The exchange rate dropped logarithmically from 10^{-3} events per cell per generation for a plasmid with 702 bp of homology to 10^{-10} events per cell per generation for a plasmid with 26 bp of homology. Analyses of independent examples of recombined plasmids revealed that the molecular outcome was the same unidirectional exchange characteristic of gene conversion, regardless of the length of homology. In addition, the exchange rate of plasmids with very limited homology fit the same logarithmic relationship as did that of plasmids with longer homology. These two results suggest that events in all plasmids occur by a common pathway of gene conversion and that this process is strongly dependent on the length of homology. The drop of nearly 3 orders of magnitude with each logarithmic decrease in homology was greater than expected for the pairing of complementary single strands, which may occur at the outset of exchange. Possibly, steps in the pathway which occur after the initial recognition of homology are also very sensitive to homology.

We also attempted to define the minimum length of homology required for successful conversion, but were limited by the technical problem of measuring rates below 10^{-11} events per cell per generation. Nevertheless, we could detect exchange in a plasmid with 26 bp of homology.

Our finding that 26 bp of homology is sufficient to effect exchange by gene conversion in a yeast plasmid system is very similar to the minimal homology requirements measured for exchange events in other organisms. Measurements of the minimal homology requirements for bacteriophage T4 recombination in *E. coli* (23), host-dependent exchange between plasmid and phage DNAs in *E. coli* (21, 29), and intermolecular plasmid exchange in mammalian cells (3, 20) have shown that approximately 20 bp of homology is sufficient in all cases. The constancy of the value is interesting in that some of the systems selected for reciprocal recombination events (20, 21, 23, 29), some selected for conversion events (our own), and some selected for either form of exchange (3). Thus, the value is constant among organisms and between forms of exchange. What distinguishes the various systems, especially our own, is the functional dependence of the exchange rates on homology. The steep exponential dependence of rate within a homology range which spans 2 orders of magnitude is distinct to our system.

Homology requirement to one side of the conversion event. Although we found conversion of a marker 13 bp from the border between homologous and nonhomologous sequences in the plasmid with 26 bp of homology, we did not detect any when a marker was 5 bp from the border in a plasmid with 612 bp of homology. We note a number of examples in which conversion has occurred close to homology borders. Amstutz et al. (2) found conversion within 35 bp of a homology border in events between *Schizosaccharomyces pombe* tRNA genes, Netter et al. (18) found that an event in *S. cerevisiae* mitochondrial DNA proceeded within 11 bp of a homology border, and Reynaud et al. (19) showed a conversion between a immunoglobulin V-chain pseudogene and a rearranged V-J joint which had only 5 bp of homology to one

side of the event. These results and ours demonstrate that conversion tracts can come within 5 to 10 bp of a homology border. However, conversion is severely restricted in situations in which a marker within an extensive homology lies close to a border. Molecular models of exchange postulate that one or both ends of a conversion tract exist as a Holliday junction in which single strands from one or both participating duplexes cross each other to form a heteroduplex with the opposing partner (7, 17, 27, 30). Model building of Holliday junctions (22) shows that they should be capable of proceeding to the very border of homology by heteroduplex formation without unpaired bases in and around the point of the crossed strands. If the supposition of Holliday junctions flanking conversion events is correct, then in our system the heteroduplex supporting the Holliday junction must be interrupted by an 8-bp insertion. We presume that to accommodate this loop within the heteroduplex close to a border, a small amount of perfect homology must exist between the marker and the homology border. Our results establish a threshold value for perfect homology in yeast recombination of approximately 10 bp.

Truncation of conversion tracts. In addition to a requirement for a small amount of perfect homology between a homology border and a marker, branch migration may play a role in reducing conversion of a marker near such a border. If Holliday junctions are present, they should be able to branch migrate up to a homology border and then migrate away. If branch migration up to and away from the border occurs in a random pattern, markers close to a homology border may seldom be found within heteroduplex and consequently infrequently convert. This explains not only why markers near borders seldom convert, but also why conversion tracts are truncated as they proceed close to such borders. That markers as far as 280 bp from a homology border are affected may reflect the confines of the one-dimensional random walk taken by a Holliday junction.

APPENDIX

Effect of 5'- and 3'-flanking sequences. In reviewing the data, we noticed that the addition of 5'- and 3'-flanking sequences had opposite effects on the rate of plasmid exchange (Table 1). Plasmids with the 5'-flanking 2.1-kilobase (kb) *Eco*RI-*Bam*HI sequence in homologous copies had 15-fold-higher exchange rates than did plasmids which lack this homology (compare pBYA229 with pBYA209). This rate increase most probably results from the increase in homology. To support this conclusion, we note that additional plasmids, analogous to pBYA229 but with this fragment inserted either in the opposite orientation or to the 3' side of the gene or with the fragment replaced by a 12-kb fragment of bacteriophage lambda DNA, all had recombination rates near that of pBYA209 (data not shown). Furthermore, we tested whether this fragment could act as a recombinational hot spot by integrating it to the 5' side of the chromosomal *HIS4* gene in two haploid strains with heteroallelic markers of this gene, mating the strains, and examining the effect of the chromosomal insertions on mitotic recombination at the *HIS4* locus (10, 28). When integrated in front of both chromosomal *HIS4* copies of the diploid, the 5' *HIS3* sequence stimulated exchange by approximately 20% over that found in a diploid in which the sequence was placed in front of only one of the two *HIS4* copies. Thus, the sequence may have a very mild stimulatory effect, but most of the effect observed in the plasmid constructs results from the addition of homology.

In contrast, the 3'-flanking *Xba*I-*Sal*I fragment had the opposite effect. Addition of this fragment decreased the plasmid exchange rate approximately fourfold (compare pBYA239 with pBYA209 and pBYA269 with pBYA249). (That this inhibition is not caused by the 3'-flanking DNA between the 3' *Bam*HI and *Xba*I sites can be substantiated by comparing pBYA269 with pBYA259.) To examine whether the inhibitory property of the *Xba*I-*Sal*I fragment was independent of its plasmid context, we also integrated this fragment to the 5' side of the *HIS4* gene and measured its effect on chromosomal mitotic recombination. The fragment had no effect on chromosomal recombination, and, thus, its properties in the plasmid may be limited to this system.

Relationship of reciprocal recombinations. As we reported previously (1, 4), some of the plasmid conversion events are accompanied by reciprocal recombination. In addition, a few of the exchange events occur by crossovers between the heteroallelic markers. The number of such events is given in Table 1. Because we had previously found that the percentage of conversion events associated with reciprocal recombination was correlated with the length of the conversion tracts (1), we expected that the percentage of conversion events accompanied by crossovers would be correlated with the length of homology. Examination of the data for plasmids pBYA219, pBYA229, pBYA239, and pBYA209 suggests some correlation, in that the two plasmids with more extensive homology (pBYA219 and pBYA229) have higher frequencies than the other two. The correlation does not hold, though, when the data for plasmids pKD400, pKD90, and pKD40 are included, since these plasmids have small homologies, yet have high frequencies of associated reciprocal recombination. Although little correlation between homology and associated crossing over exists, two correlations which we previously noted can be found in the current data. First, when compared with single-site conversions, a higher percentage of coconversion events is associated with crossovers for plasmids pBYA219, pBYA229, pBYA239, and pBYA209 (27 of 122 versus 10 of 96). Second, for the plasmids with extensive homology (pBYA219 and pBYA229), the more complex conversion events, which include discontinuities in conversion tracts and symmetric events (1), also have a more frequent association with reciprocal recombination than do the less complex events for the plasmids with more extensive homology (pBYA219 and pBYA229). Thus, the current data support the conclusion that length and complexity of an event are two of the factors which contribute to whether a conversion event is associated with a crossover.

Having found little correlation between the length of homology and the frequency of events which include reciprocal recombination, we examined the data to determine whether shortening the homology to one side of the *HIS3* gene might affect the association. For convertants of the 5' marker for plasmids pBYA219, pBYA229, pBYA239, and pBYA209, the number of associated crossovers is decreased in the plasmids with shortened homology to this end of the gene (1 of 49 versus 17 of 73). For convertants of the 3' marker and shortened homology to this end, the correlation does not hold. Instead, associated crossovers are slightly more frequent when the homology is shortened (12 of 38 versus 7 of 58). (This effect at the 3' end is especially noticeable with plasmids pKD40, pKD90, and pKD400). Thus, like the effect of each of the flanking sequences on the overall rate of recombination, each flanking sequence has its own effect on the crossovers which are associated with conversion tracts. Indeed, the data support the correlation that for plasmids pBYA209, pBYA219, pBYA229, and pBYA239, the flanking sequence which increases the overall exchange rate also increases the overall frequency of associated crossovers, whereas the flanking sequence which decreases the rate also decreases the percentage of associated reciprocal recombination.

Conclusion. The effects of the sequences flanking the *HIS3* gene on exchange within the gene are divergent. As might be expected from the relationship between homology length and exchange rate, the 5'-flanking sequence enhances the exchange rate when it is present on both of the allelic segments of the plasmid. Examination of the sequence of possible chromosomal recombination enhancement revealed that it has a mild stimulatory effect. The sequence also increases the amount of reciprocal recombination associated with conversion. Unexpectedly, the 3'-flanking sequence has a negative effect on the rate of exchange and also appears to decrease the incidence of associated crossovers. The negative effect is present even when the sequence is present as a single copy without a homologous pair. The effect was limited to plasmid exchange, since chromosomal insertion showed no effect on chromosomal mitotic exchange. The causes of these effects are not apparent, and a molecular explanation is obscured by a number of factors.

Foremost of these is that because we selected for histidine prototrophs, we recovered only a subset of all recombination events. Furthermore, we do not know how vector sequences within the plasmids influence exchange. That flanking sequences may have distinct effects on exchange is not surprising, and further study will undoubtedly reveal how specific sequences can alter the course of conversion and crossovers.

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