MOLECULAR AND CELLULAR BIOLOGY, Aug. 1985, p. 2029–2038
0270-7306/85/082029-10$02.00/0

Vol. 5, No. 8

Recombination Hot Spot in the Human β-Globin Gene Cluster: Meiotic Recombination of Human DNA Fragments in *Saccharomyces cerevisiae*

DOUGLAS TRECO, BARBARA THOMAS, AND NORMAN ARNHEIM*

Biochemistry Department and Molecular Biology Graduate Program, State University of New York at Stony Brook, Stony Brook, New York 11794

Received 6 February 1985/Accepted 7 May 1985

We describe a novel system for the analysis of sequence-specific meiotic recombination in *Saccharomyces cerevisiae*. A comparison of three adjacent restriction fragments from the human β-globin locus revealed that one of them, previously hypothesized to contain a relative hot spot for genetic recombination, engages in reciprocal exchange during yeast meiosis significantly more frequently than either of the other two fragments. Removal of the longest of four potential Z-DNA-forming regions from this fragment does not affect the high frequency of genetic recombination.

Molecular and genetic studies have suggested that recombination events in eucaryotic chromosomes do not occur with equal frequencies in all regions of the genome. Examples include the suppression of recombination between *H-2* haplotypes and t-complex alleles in mice (15, 16), within heterochromatin in *Drosophila* (6, 44), and within the tandemly arranged rRNA genes in the yeast *Saccharomyces cerevisiae* (35). In addition, extensive genetic studies of *S. cerevisiae* indicate that the frequency of gene conversion varies considerably from one heterozygous site to another (12).

Recombination within gene clusters has also been shown to be nonrandom with respect to positions within the clusters. Using restriction site differences between mouse *H-2* haplotypes, Hood and co-workers have demonstrated that genetic exchanges leading to recombinant congenic mice in the I region of the mouse *H-2* locus are restricted to a 2-kilobase (kb) region between the I-A and I-E subregion (24). Likewise, Kazazian, Orkin, and co-workers have undertaken an extensive molecular genetic analysis of the β-globin gene cluster in humans (31, 32). Based on population studies of the linkage relationships of 12 restriction enzyme polymorphisms spread over a 65-kb region encompassing the human β-globin locus, they concluded that an 11-kb region immediately 5′ of the β-globin gene contains a hot spot for genetic recombination.

The 65-kb β-globin locus includes a 32-kb 5′ region and a 20-kb 3′ region, and each region exhibits nonrandomness with respect to the combinations of restriction site polymorphisms found within it. The 5′ and 3′ regions are represented by a very limited number of frameworks which are defined by the particular pattern of restriction site polymorphisms. Between the 5′ and 3′ frameworks is an 11-kb segment of DNA which includes the δ-globin gene and within which enough recombination occurs to allow all combinations of 5′ and 3′ frameworks to exist within the β-globin-carryng chromosomes in a population (2–4, 22, 31, 32). Figure 1 is a detail of the 11-kb sequence between the 5′ and 3′ frameworks. A polymorphic *Hin*fI site is in likage equilibrium with restriction sites in both the 5′ and 3′ frameworks and with a single-site polymorphism lying 700 base pairs (bp) 3′ to it (29), and Kazazian et al. (22) have suggested that the *Hin*fI site lies in a region of relative sequence randomization.

Also shown in Fig. 1 is the presence of the highly repetitive evolutionarily conserved sequence $d(TG \cdot AC)_n$ first described by Miesfeld et al. (26), where $n = 17$ at this genomic position. The presence of a stretch of $d(TG \cdot AC)_n$ of a similar length at the breakpoint of a gene conversion event between the duplicated $^{G}\gamma$ and $^{A}\gamma$ fetal globin genes first led Slightom et al. (48) to suggest that the sequence may have initiated the intergenic exchange.

In this report we take advantage of the yeast transformation technique (18, 20) and recombination analysis by tetrad dissection (12, 46) to introduce several β-globin locus fragments into identical positions within the *S. cerevisiae* genome and to study their potential to engage in homologous meiotic recombination. We have chosen *S. cerevisiae* for our molecular recombination studies because we can recover all of the DNA molecules involved in a meiotic recombination event. Our results indicate that a restriction fragment within the 11-kb region of sequence randomization is two to four times as active in reciprocal meiotic exchange in *S. cerevisiae* as compared to the ability of the two adjacent β-globin locus fragments to engage in homologous exchanges. The recombinogenic nature of this fragment is not due only to the sequence $d(TG \cdot AC)_{17}$, since deletion of this sequence from the fragment does not significantly decrease the high frequency of exchange.

MATERIALS AND METHODS

Protocol for insertion of homologous β-globin DNA at the *S. cerevisiae HIS3* locus. Plasmids pHLΔV and pHUΔV are described below. *Eco*RI linkers (Collaborative Research) were attached to the 1.8-kb *Bam*HI *HIS3* fragment from pSZ62 (33), kindly provided by J. Szostak. The yeast *LEU2* and *URA3* genes used in the constructions of pHLΔV and pHUΔV were subcloned from pCV03 (8) and pMM2D (21) and were kindly provided by M. McLeod and J. Broach. Each of the three *Bam*HI fragments from the human β-globin locus was inserted into the unique *Bam*HI site of both pHLΔV and pHUΔV to generate pairs of plasmids for introduction into *S. cerevisiae*. The β-globin derivatives of pHLΔV and pHUΔV are designated pHLMG-1 and pHUMG-1, pHLI800 and

* Corresponding author.

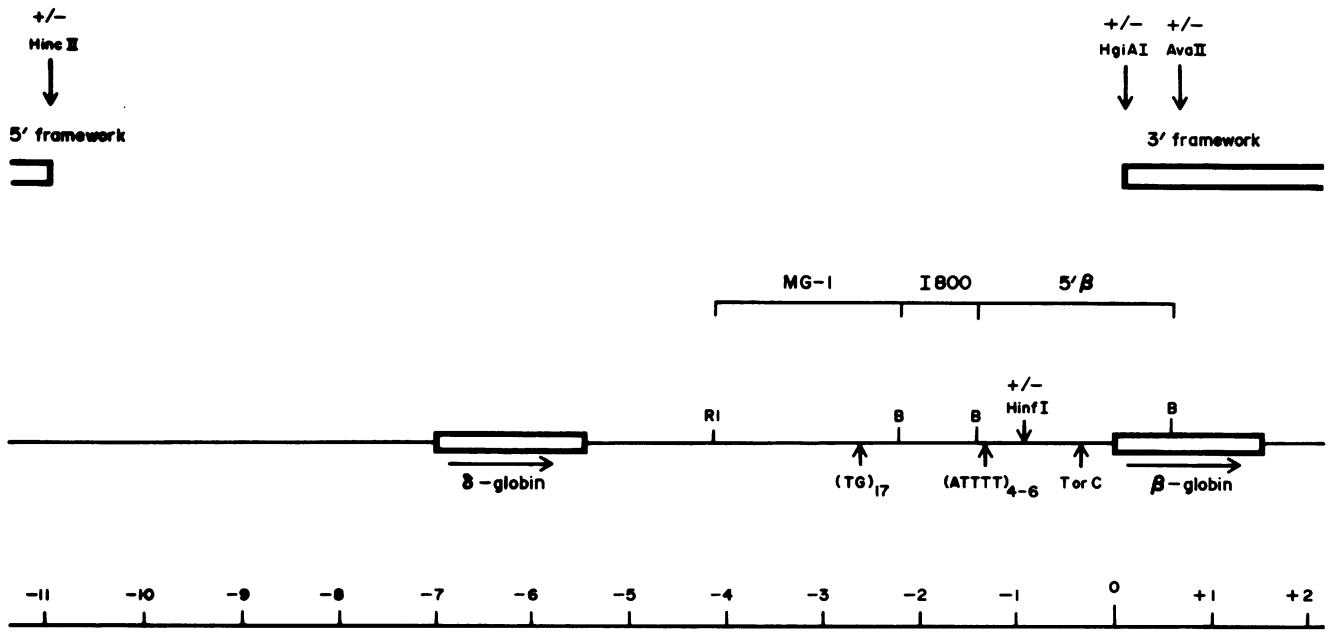

FIG. 1. Intergenic region between the δ- and β-globin genes. The horizontal arrows below the δ- and β-globin genes indicate the direction of mRNA transcription. $(TG)_{17}$ is a 34-bp stretch of the alternating copolymeric sequence $d(TG \cdot AC)_n$ (26). T or C is a sequence polymorphism detected by direct DNA sequencing (29). The bracketed regions denote the positions of the β-globin fragments used in this study (see text). The numbered line represents kilobase subdivisions of the region. The *Hin*cII, *Hin*fI, *Hgi*AI, and *Ava*II sites are 4 of the 12 polymorphisms described in reference 31. B, *Bam*HI; RI, *Eco*RI.

pHUI800, and pHL5'β and pHU5'β. The orientation of the *Bam*HI β-globin fragment in each pair was judged to be the same by restriction enzyme mapping studies (data not shown).

Covalently closed circular plasmids were transformed into strains of *S. cerevisiae* by a modification of the lithium acetate transformation technique (10, 20). Stable transformants were selected by plating transformation mixtures onto minimal medium plates containing amino acids and adenine but lacking either leucine or uracil (46). Transformation frequencies ranged from 0.1 to 0.5 stable transformant per μg of DNA.

pHUΔV and its derivatives carrying β-globin fragments were transformed into *S. cerevisiae* 16d with the genotype *MATα leu2-3,112 ura3-52 his3-Δ1 TRP1 CAN1 ade2. TRP1* and *CAN1* are unlinked to *HIS3*, and the *ADE2* locus is 38 centimorgans (cM) centromere-proximal to *HIS3* (27). pHLΔV and its derivatives were transformed into strain 1b with the genotype *MATa leu2-3,112 ura3-52 his3-Δ1 trp1-289 can1-101 ADE2*. Strains 16d and 1b were generated using standard genetic techniques by first crossing DBY747 (*MATa leu2-3,112 ura3-52 his3-Δ1 trp1-289 gal2*, kindly provided by T. Petes) to DBY869 (*MATα ade2 can1-101*, kindly provided by D. Shortle). Spores of the genotype *MATα leu2 ura3 his3 trp1 can1 ade2* were isolated by tetrad analysis and crossed to DBY946 (*MATa ura3-52 suc2*$^-$, S288C background provided by D. Shortle). The back-cross of these markers to DBY946 was repeated six times to generate strains 1b and 16d, which have good sporulation efficiency (ca. 50%) and excellent spore viability (greater than 99%). Aside from the two marker genes (*LEU2* or *URA3*) inserted at *HIS3*, four additional markers (*CAN1*, *TRP1*, *ADE2*, and *MAT*) were heterozygous in our experimental crosses.

Transformants were selected as Leu$^+$ or Ura$^+$ prototrophs after transformation by pHLΔV (and derivatives) or pHUΔV (and derivatives), respectively. Yeast genomic DNA was prepared from stable transformants (48) and screened by restriction enzyme (*Bam*HI and *Eco*RI) and Southern blot hybridization (49) analysis for single-copy plasmid insertions at the *HIS3* locus. Plasmid insertions at the *HIS3* locus on chromosome XV were subsequently confirmed as such by tight genetic linkage to the *HIS3* locus (data not shown). For all transformants, however, restriction enzyme and Southern blot analysis using pHLΔV as a hybridization probe could unambiguously identify insertions at *HIS3*.

The *his3*$^-$ allele used, *his3-Δ1*, has a 200-bp deletion in the *HIS3* coding region (45). All integrations at *HIS3* result in the duplication of *HIS3* sequences which end up flanking the inserted plasmid. However, due to the nature of the integration event, which often involves gene conversion (33), some transformants are flanked by two-wild type genes (++), some are flanked by two *his3-Δ1* genes (ΔΔ), and some have *HIS3*$^+$ and *his3-Δ1* sequences on opposite sides of the insertion (Δ+ or +Δ). The distribution of the two *HIS3* sequences in each transformant can be determined by restriction enzyme and Southern blot analysis. The absolute distribution of the *HIS3* alleles in each of the two homologous chromosomes undergoing meiotic recombination was not identical. Thus, recombination in the region between the duplicated *HIS3* sequence could usually be analyzed independently of events occurring in the *LEU2-URA3* interval.

Southern transfer techniques. Yeast DNA was prepared as described by Sherman et al. (46). Restriction enzymes were purchased from Bethesda Research Laboratories, and digestions were carried out under the recommended conditions. Digested DNA (1 to 2 μg) was electrophoresed on 1.3% vertical agarose gels, transferred to nitrocellulose (Millipore), and hybridized to ^{32}P-labeled pHLΔV (41) essentially as described (50).

RESULTS

Development of a novel system to study sequence-specific meiotic recombination. To study the ability of various β-globin locus sequences to participate in meiotic recombination in *S. cerevisiae*, we needed to introduce identical human β-globin locus DNA fragments into homologous positions within the genomes of *S. cerevisiae* strains of opposite mating type. We also needed to associate the human DNA fragments in each of the mating strains with flanking marker genes to allow the identification of recombinant spores. The construction of these defined genetic loci is illustrated in Fig. 2.

Two integrating vectors were constructed, pHLΔV and pHUΔV, both of which are derivatives of pBR322 (7). Each vector has a 1.7-kb DNA fragment containing the wild-type yeast *HIS3* gene (54) inserted in the same orientation into the *Eco*RI site of pBR322 (56). pHLΔV has a 2.2-kb *Sal*I-*Xho*I fragment containing the wild-type yeast *LEU2* gene (1, 19, 39) inserted into the pBR322 *Sal*I site. This plasmid can transform *leu2*$^-$ *his3*$^-$ yeast strains to leucine and histidine prototrophy when integrated into the yeast genome. In addition to the yeast *HIS3* gene, plasmid pHUΔV has a 1.2-kb *Hin*dIII fragment containing the wild-type yeast *URA3* gene (5) inserted into the pBR322 *Hin*dIII site. This plasmid can transform *ura3*$^-$ *his3*$^-$ yeast strains to uracil and histidine prototrophy when integrated into the yeast genome. Neither vector carries a sequence that allows the plasmid to replicate autonomously in *S. cerevisiae*. Therefore, the transformation to prototrophy demands that the plasmid integrate by homologous recombination into the yeast genome (18). In both vectors the *Bam*HI site of pBR322 is unoccupied. Any *Bam*HI fragment can be cloned into this site in pHLΔV and pHUΔV. By the protocol outlined in Fig. 2 we have the ability to place exogenous *Bam*HI fragments at the yeast *HIS3* locus on chromosome XV by homologous recombination between plasmid and chromosomal *HIS3* sequences. Figure 2 also shows that, upon integration, pHLΔV positions *LEU2* to the left of the *Bam*HI cloning site, whereas pHUΔV positions *URA3* on the right. Exchanges between sequences inserted into the *Bam*HI site will be recombinant for *LEU2* and *URA3* as well as the flanking *HIS3* sequences, while exchanges that are limited to *HIS3* and pBR322 sequences will only yield spores recombinant for the *HIS3* sequences.

The transformation of yeast strains of opposite mating types with pHLΔV or pHUΔV permits us to generate diploids heterozygous for *URA3* and *LEU2* at the *HIS3* locus. If such a diploid is stimulated to enter meiosis, homologous exchange in the interval between these two markers will generate a chromosome with *LEU2* and *URA3* now physically linked on the same chromosome at the *HIS3* locus, as well as a chromosome with neither *LEU2* nor *URA3* at *HIS3*. Both chromosomes will still have pBR322 sequences, as well as any sequence inserted in the *Bam*HI site. Gene conversion events involving *LEU2* or *URA3* sequences will also create novel recombinant chromosomes. Tetrad analysis and restriction enzyme and Southern blotting (49) of DNA from recombinant spores allows us to identify recombinant tetrads and the DNA sequences that were involved in the genetic exchanges.

Three DNA fragments from the human β-globin locus were examined for their ability to promote exchanges in the vicinity of the *LEU2* and *URA3* genes inserted at the *HIS3* locus. Figure 1 shows the three β-globin locus sequences that were used in this study. The 5′-most fragment, MG-1 (26), is 1.9 kb in length and contains several members of the alternating copolymer d(TG · AC)$_n$ family that is repeated approximately 50,000 times in the human genome (14, 26). A *Bam*HI linker has been added to the *Eco*RI site on the left of MG-1, and the *Eco*RI site is retained. I800 is approximately 800 bp in length and is normally flanked on both sides by *Bam*HI sites. 5′β is a 1.9-kb *Bam*HI fragment which contains about 500 bp from the 5′ end of the β-globin gene as well as 1.4 kb of DNA 5′ to the β-globin coding region. Combined, the three fragments span 4.6 kb. The entire nucleotide sequence has been published (36). All three fragments were subcloned from λ phage carrying human β-globin DNA isolated by Fritsch et al. (13). Each DNA fragment was inserted in the same orientation into the unique *Bam*HI site in both pHUΔV and pHLΔV.

Differential recombination of human β-globin locus fragments in *S. cerevisiae*. Figure 3 illustrates the physical organization of the *HIS3* locus when homologous exchange between the 5′β-fragments from the human β-globin locus was studied. Genetic exchanges in the interval between the *LEU2* and *URA3* sequences were monitored by scoring 354 tetrads for their *LEU2*, *URA3*, and *HIS3* segregation patterns. Nonrecombinant tetrads should always segregate 2 Leu$^+$ Ura$^-$ His$^+$:2 Leu$^-$ Ura$^+$ His$^+$ spores. Of 354 tetrads, 13 contained one or more spores with nonparental arrangements of the *LEU2*, *URA3*, or *HIS3* genes (Table 1). By genetic and Southern blot analysis, five of these nonparental segregations were shown to be due to a reciprocal exchange in the 2.5-kb interval between *LEU2* and *URA3* (1.9-kb 5′β fragment + 0.6 kb of pBR322 DNA between the *Sal*I and *Hin*dIII sites). An autoradiogram of a Southern blot of the parental DNA samples cut with *Bam*HI, along with DNA samples from the four spores from representative recombinant (tetratype ascus) and nonrecombinant (parental ditype ascus) tetrads, is shown in Fig. 4. The recombinant nature of the tetrad can be visualized in the *Bam*HI-digested spore DNA samples by the association of the 3.4-kb fragment containing the *URA3* gene with the 8-kb fragment that carries the *LEU2* gene (spore d, Fig. 4, lane f). This same tetrad has a spore (spore b, lane d) with the 5.6-kb and 2.0-kb fragments that represent plasmid insert sequences lacking *LEU2* and *URA3* sequences completely. An examination of the physical maps of the recombining parental inserts (Fig. 3) reveals that the 8-kb and the 5.6-kb *Bam*HI fragments are effectively allelic, while the same is true of the 3.4-kb and 2.0-kb fragments. *Eco*RI digests (Fig. 4) of the spore DNA samples reveal fragments that are not seen in the parental DNA samples (lanes k and l) and are diagnostic for the recombinant configuration (5.0-kb *Eco*RI fragment in spore d [lane p] and 6.4-kb *Eco*RI fragment in spore b ([lane n]). The rearrangement of the *LEU2* and *URA3* genes results in the tetratype segregation of restriction fragments homologous to pBR322 sequences. All 55 of the reciprocal exchanges reported in Table 1 (all crosses combined) have tetratype segregation patterns similar to that of the recombinant tetrad shown in Fig. 4. In Fig. 5 we illustrate the physical maps of the *HIS3* regions from the four spores of the recombinant tetrad as deduced from the genetic and restriction enzyme analyses. The genetic distance between *LEU2* and *URA3*, based on the number of reciprocal recombinants, is calculated to be 0.7 cM (Table 1).

Of the 13 nonparental tetrads, 3 were 3:1 segregations for *LEU2* or *URA3* sequences and represent gene conversion events. One of these three events resolved by reciprocal exchange in pBR322 or *HIS3* sequences in addition to converting the pBR322 region of one of the 5′β-*URA3*

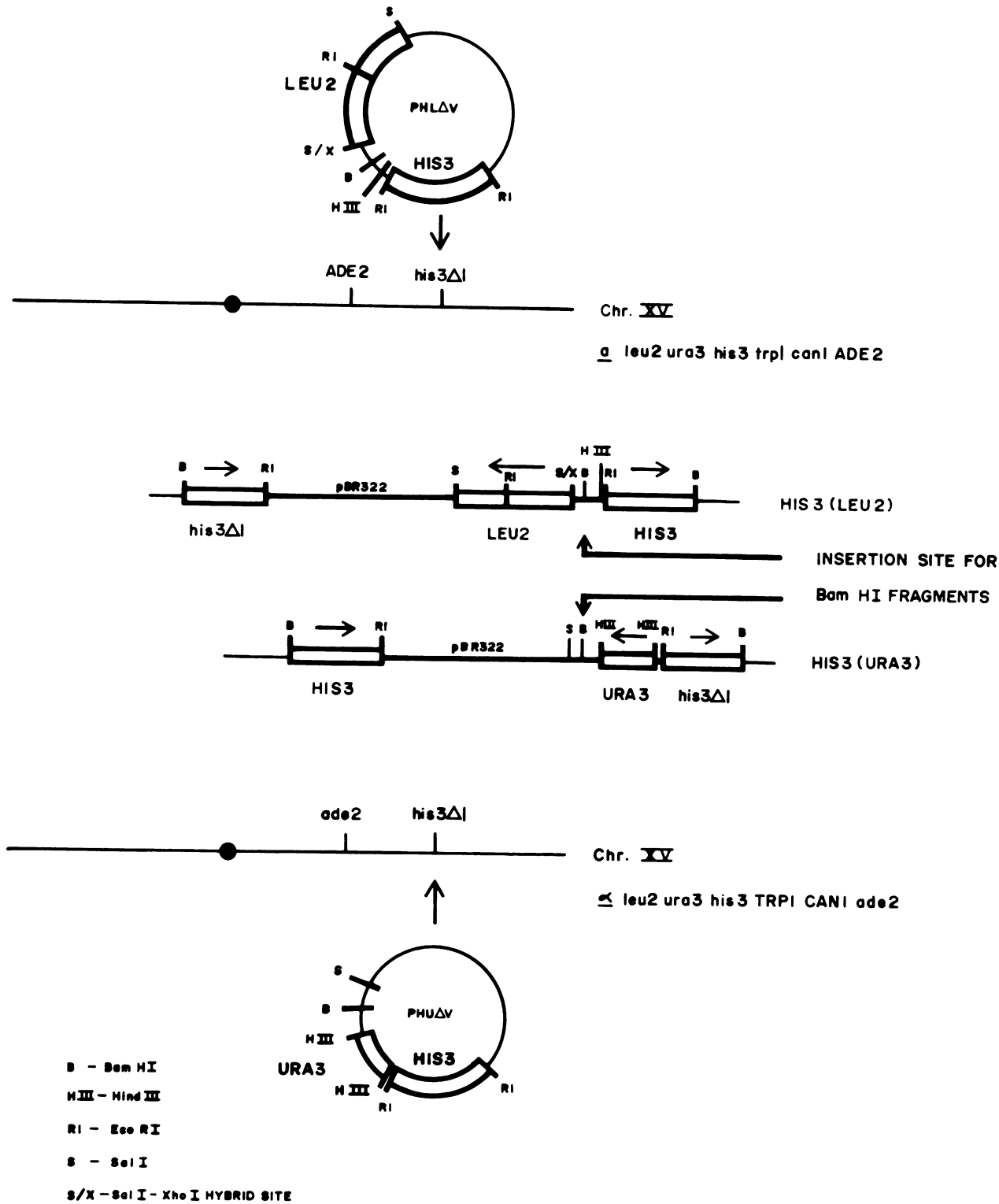

FIG. 2. Plasmids pHLΔV and pHUΔV are described in the text. The central portion of the figure illustrates the physical organization of *HIS3* loci in a diploid cell where no β-globin DNA has been inserted into the transforming plasmids. The arrows above the *LEU2*, *URA3*, and *HIS3* marker genes indicate the direction and extent of transcription of these genes (1, 43, 55). B, *Bam*HI; HIII, *Hin*dIII; RI, *Eco*RI; S, *Sal*I; S/X, *Sal*I-*Xho*I hybrid site. The black circle represents the chromosome centromere. In the absence of reciprocal exchange in the interval between *LEU2* and *URA3* or gene conversion events involving these markers, tetrads will contain two Leu^+ Ura^- spores and two Leu^- Ura^+ spores (parental segregation). The 2:2 segregation pattern for four additional markers (*CAN1*, *TRP1*, *ADE2*, and *MAT*) was always used to confirm that the tetrads with nonparental segregations for *LEU2*, *URA3*, or *HIS3* were true tetrads. Unsporulated diploids that were accidentally dissected along with ascospores were identified by microscopic examination for their ability to sporulate and their inability to mate with *MAT***a** and *MAT*α tester strains. Fewer than 1% of the tetrads did not segregate 2:2 for all unlinked markers, and these were discarded, as were the 3 to 5% of the dissected tetrads that contained diploids.

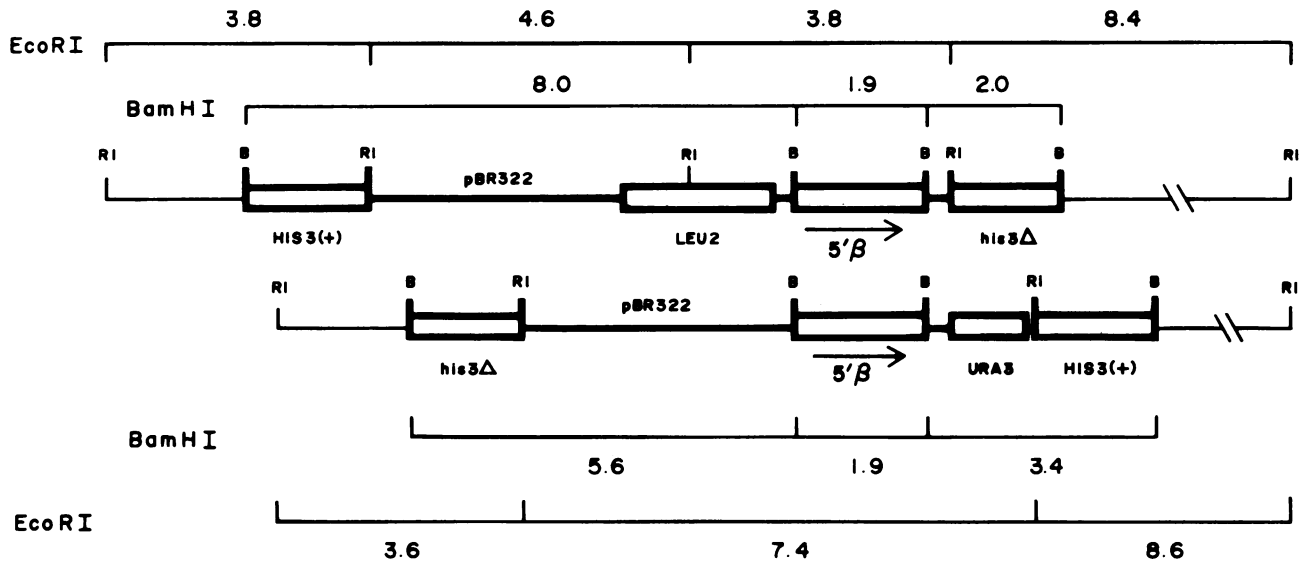

FIG. 3. Physical organization of homologous *HIS3* loci in a diploid homozygous for the 5'β fragment. The haploid strains used in this experiment were generated and identified by the protocol outlined in the text. The diploid is heterozygous for the *HIS3* alleles on both sides of the inserted DNA. The numbers above and below the bracketed lines represent restriction fragment sizes in kilobase pairs.

chromatids to *LEU2*$^+$. Physical maps of the *HIS3* region in the four spores from this recombinant tetrad, based on restriction enzyme and Southern blot analysis (data not shown), are illustrated in Fig. 6. Notice the 2:2 segregation for the wild-type (+) and deletion (Δ) *HIS3* markers on both the left and right sides, while the *LEU2* gene segregates $3^+:1^-$. This confirms, with molecular evidence, the notion that gene conversion and reciprocal exchange can be intimately linked processes (12). By restriction enzyme and Southern blot analysis we could show that 4 of the 26 gene conversion events reported in Table 1 (all crosses combined) were associated with reciprocal recombination of the flanking *HIS3* markers (data not shown). In 10 of the remaining 22 events, one endpoint of the converted segment of DNA lies outside the integrated plasmid sequences, and the conversion could not be unambiguously identified as having been associated with crossing over. In these cases a flanking *HIS3* sequence was converted along with the *LEU2* or *URA3* marker gene that exhibited nonparental segregation. These events, where two markers were converted together (coconversion; 12), dictate conversion tracts 3 to 5 kb in length. Since the *LEU2* and *URA3* marker genes are opposite nonhomologous DNA, all of the conversion events must involve the conversion of a large deletion or insertion (2.2 kb for *LEU2* and 1.2 kb for *URA3*). Molecular models for how such events can occur have been described (38, 51, 57). In six of the conversion tetrads reported in Table 1 (all crosses combined), the *LEU2* and *URA3* markers were converted together. In three of these tetrads one of the flanking *HIS3* markers was also converted. Such events require conversion tracts of 5 to 9 kb and span three large heterologous DNA segments (the *LEU2* and *URA3* markers and the 200-bp *HIS3* deletion).

As shown in Table 1, 10/363 tetrads with nonparental segregations for either *LEU2*, *URA3*, or *HIS3* were identified when homologous exchange between I800 fragments was examined. By genetic and restriction enzyme and Southern blot analysis, five of these nonparental segregations were shown to be due to a reciprocal exchange in the 1.4 kb of DNA between *LEU2* and *URA3* (data not shown). The genetic distance between *LEU2* and *URA3* in the cross is 0.7 cM. One of the remaining five nonparental segregations resulted from a gene conversion event in which *LEU2* segregated $3^+:1^-$ while *URA3* segregated $2^+:2^-$. The other four events were due to rearrangements of the flanking *HIS3* sequences and did not involve *LEU2* or *URA3* (Table 1).

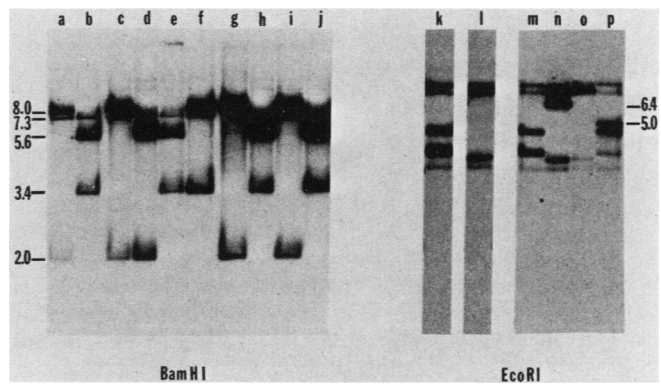

FIG. 4. Restriction enzyme and Southern blot analysis of the four spores from recombinant (lanes c through f) and nonrecombinant (lanes g through j) tetrads. *Bam*HI digests of DNA isolated from the parental strains (lanes a and b) and from the four spores of the tetrads (lanes c through j) were run on a 1.2% vertical agarose gel and transferred to nitrocellulose. Hybridization was carried out with nick-translated pHLΔV as a probe. The 7.3-kb fragment present in all lanes digested with *Bam*HI derives from the mutant *LEU2* locus on chromosome III. Spores a and c (lanes c and e, respectively) in the recombinant tetrad are parental in genotype and restriction map at the *HIS3* locus. Spores b and d (lanes d and f, respectively) are the reciprocal products of a cross-over event in the vicinity of the 5'β fragment and have nonparental restriction maps (see text). In the nonrecombinant tetrad (lanes g through j), only the two parental patterns are seen. Lanes k through p, *Eco*RI digests. Lanes k and l, *LEU2* and *URA3* haploid parents, respectively. *Eco*RI digests of the DNA from the same recombinant tetrad (lanes m through p) reveal fragments at 6.4 kb (spore b, lane n) and 5.0 kb (spore d, lane p) that are unique to the recombinant configurations.

TABLE 1. Compilation of data from the tetrad analysis

Fragment studied	No. of tetrads analyzed	No. of nonparental segregations (*LEU2*, *URA3*, *HIS3*)	Reciprocal exchanges in *LEU2-URA3* interval	Gene conversions[a] (*LEU2* or *URA3*)	*LEU2-URA3*		Specific recombination efficiency[d] (cM/kb)	Nonparental segregations (*HIS3* only)	Total reciprocal exchanges between *HIS3* sequences[e]	*HIS3-HIS3*	
					Genetic distance[b] (cM)	Physical distance[c] (kb)				Genetic distance (cM)	Physical distance[f] (kb)
5′β	354	13	5	3	0.7	2.5	0.28	5	11	1.6	7.8
I800	363	10	5	1	0.7	1.4	0.46	4	9	1.2	6.7
MG-1	443	33	23	9	2.6	2.5	1.0	1	25	2.8	7.8
MG-1 opp	133	11	6	4	2.3	2.5	0.9	1	8	3.0	7.8
MG-1 deleted for d(TG.AC)$_{17}$	295	21	15	4	2.5	2.26	1.1	2	15	2.5	7.56
No insert	420	15	1	5	0.12	0.62	0.19	9	11	1.3	5.9

[a] Defined as 3 *LEU2*$^+$: 1 *leu2*$^-$, 1 *LEU2*$^+$: 3 *leu2*$^-$, 3 *URA3*$^+$: 1 *ura3*$^-$, or 1 *URA3*$^+$: 3 *ura3*$^-$.
[b] cM = [100 (T + 6NPD)]/[2(PD + NPD + T)], where T, PD, and NPD are tetratype, parental ditype, and nonparental ditype, respectively (28).
[c] Defined as the total length of homologous DNA between the insertion sites of the *LEU2* and *URA3* genes (includes both pBR322 and exogenous DNA sequences).
[d] *LEU2-URA3* genetic distance/*LEU2-URA3* physical distance.
[e] Cross-overs in *LEU2-URA3* interval plus all events in pBR322 and *HIS3* sequences that result in the reciprocal exchange of flanking *HIS3* markers.
[f] Defined as the total length of homologous DNA between the sites of the deletions in the *HIS3* sequences on the left and right of the integrated DNA (excludes *LEU2* and *URA3* sequences since they share no homology with the non-sister chromatid).

We next examined homologous exchange between chromosomes carrying the MG-1 fragment. Of 443 asci dissected and examined for segregation of the *LEU2*, *URA3*, and *HIS3* markers, 33 tetrads with nonparental segregation patterns were identified (Table 1). By genetic and Southern blot analysis, 23 of these were shown to be due to a reciprocal exchange within the 2.5 kb of DNA between *LEU2* and *URA3*. All 23 of the nonparental segregations listed in Table 1 that were identified as reciprocal exchanges by their marker segregation patterns showed identical tetratype patterns of restriction fragment association by Southern blot analysis. Nine of the 33 nonparental segregations involved gene conversion of either *LEU2* or *URA3* sequences.

The genetic distance between *LEU2* and *URA3*, when MG-1 is inserted between them, is 2.6 cM (Table 1). This is the largest distance observed when the three β-globin fragments are compared. As compared to the 5′β fragment, which is virtually identical in length to MG-1 (both are 1.9 kb), reciprocal exchanges in the vicinity of the MG-1 insertion occur 3.7 times more frequently. Chi-square analysis indicates that the frequency of reciprocal exchange in the MG-1 experiment is significantly greater than the frequencies observed for either the 5′β ($P < 0.005$) or I800 ($P < 0.005$) fragments. To determine whether the high frequency of exchange associated with the MG-1 insertion was dependent on a particular orientation, we inserted this fragment into both pHLΔV and pHUΔV and subsequently into homologous yeast chromosomes at the *HIS3* locus in the opposite orientation (MG-1 opp, Table 1). In 133 tetrads examined, six reciprocal exchanges in the *LEU2-URA3* interval were identified. Although fewer tetrads were examined, the calculated map distance is very close to the distance obtained from the frequency of exchanges when MG-1 is in the original orientation (MG-1, Table 1). This frequency of exchange is also significantly greater than when the 5′β fragment ($P < 0.005$) or I800 fragment ($P < 0.005$) was studied. Based on restriction enzyme mapping of the transforming plasmids (data not shown), it can be shown that the 5′β and I800 fragments were inserted into pHLΔV and pHUΔV in the same orientation relative to each other as they are found in the human β-globin locus (36). Of the two orientations of the MG-1 fragment studied, MG-1 opp (Table 1) is the cross in which the MG-1 fragment lies in the same orientation relative to 5′β and I800 as it does in the human genome (36).

Long tracts of the alternating purine-pyrimidine sequence d(TG · AC)$_n$ have been postulated to be initiation sites for genetic exchange (see below). We sought to determine whether the sequence d(TG · AC)$_{17}$ that is found within MG-1 contributes to the high frequency of exchange associated with this fragment. A deletion derivative of the MG-1 fragment was created in vitro by removing a 240-bp *Hin*dIII-*Acc*I restriction fragment that contains the d(TG · AC)$_{17}$ sequence. This derivative does not hybridize to a nick-translated probe of pure d(TG · AC)$_n$ (data not shown). However, it does contain the other two short runs of d(TG · AC)$_n$, where $n = 5$ and 7. These are located at positions −430 and −930, respectively, relative to the d(TG · AC)$_{17}$ stretch (36). An additional sequence of 11 bp of alternating purine-pyrimidine residues is found at position −530 (36). The loss of the longest d(TG · AC)$_n$ sequence has no effect on the recombination efficiency of MG-1. Fifteen reciprocal exchanges (tetratype asci) were observed in 295 segregations examined when the MG-1 deletion derivative was inserted at the *HIS3* locus (Table 1). This can be translated into a genetic distance for the *LEU2-URA3* inter-

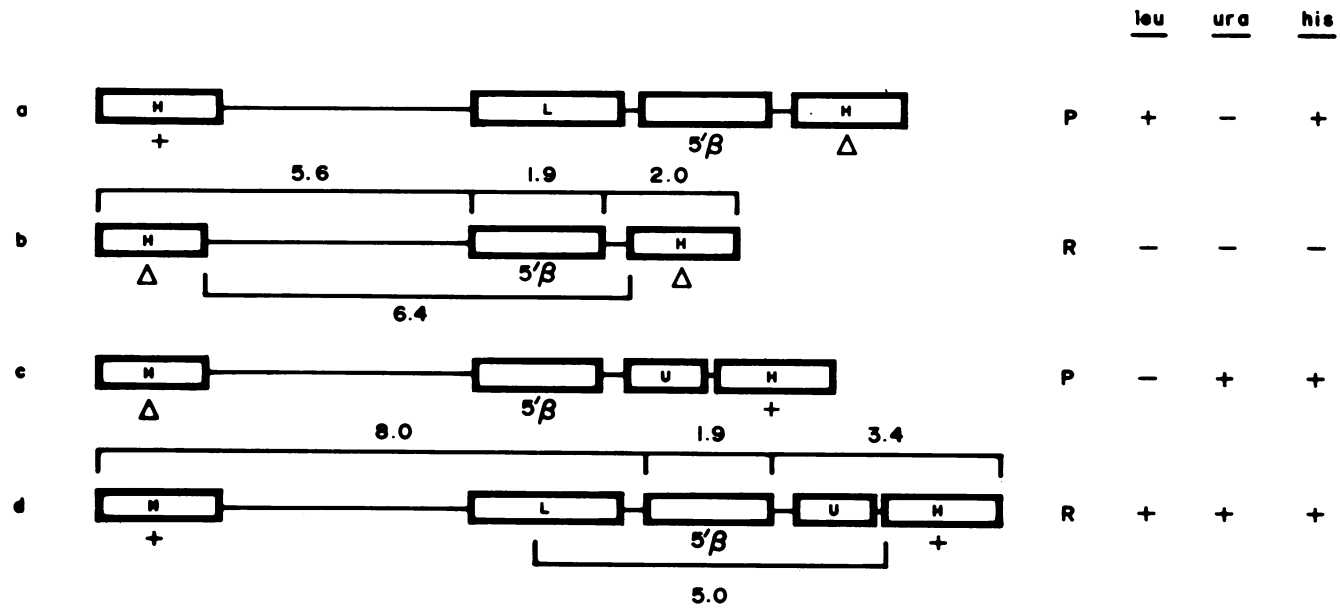

FIG. 5. Physical maps of the *HIS3* loci in all four spores of the recombinant tetrad. For the two recombinant chromosomes (b and d) the sizes of restriction fragments in kilobases are shown above (*Bam*HI fragments) and below (*Eco*RI fragments) the maps. P, Parental genotype; R, recombinant genotype.

val of 2.5 cM, roughly the same as when the intact, 1.9-kb MG-1 fragment was studied ($P > 0.9$) and significantly higher than when either the 5'β or I800 fragments were examined (both P values <0.005).

Also listed in Table 1 are data from 420 tetrads dissected and analyzed for *LEU2*, *URA3*, and *HIS3* marker segregation when no exogenous DNA was inserted into the *Bam*HI site between *LEU2* and *URA3*. Fifteen aberrant segregations of the marker genes were identified. Six of these events involved *LEU2* or *URA3* sequences. Only one of these was due to a reciprocal exchange in the 0.62 kb of pBR322 between the *LEU2* and *URA3* marker sequences (data not shown). In this cross, the most common events involving *LEU2* and *URA3* were nonreciprocal gene conversion events which exhibited $3^+:1^-$ or $1^+:3^-$ segregations for these markers.

Considering all events involving *LEU2* or *URA3* sequences (combining data in Table 1 for reciprocal exchanges in the *LEU2-URA3* interval with all gene conversions for *LEU2* or *URA3*), the frequencies of such events in crosses involving MG-1 are significantly greater than the frequencies observed for the I800, 5'β, and "no-insert" crosses (all probability [P] values <0.005). Tetratype asci resulting from reciprocal recombination in the *LEU2-URA3* interval were observed in all of our crosses, and since this frequency allows one to calculate the genetic distance between *LEU2* and *URA3* (28), it is a very useful parameter to use in comparing the β-globin fragments for their ability to participate in meiotic recombination.

DISCUSSION

We have analyzed three contiguous DNA fragments from the human β-globin locus for their ability to engage in homologous meiotic recombination at identical positions in a yeast chromosome. Our data indicate that the 1.9-kb MG-1 fragment expands the genetic map between very closely linked marker genes by a factor of 3.7 as compared to the 1.9-kb 5'β fragment (2.6 cM versus 0.7 cM). As compared to the smaller, 0.8-kb I800 fragment, MG-1 expands the genetic map by a factor of 3.7 (2.6 cM versus 0.7 cM). In this paper and other studies presented elsewhere (D. Treco and N. Arnheim, manuscript in preparation), we demonstrate that

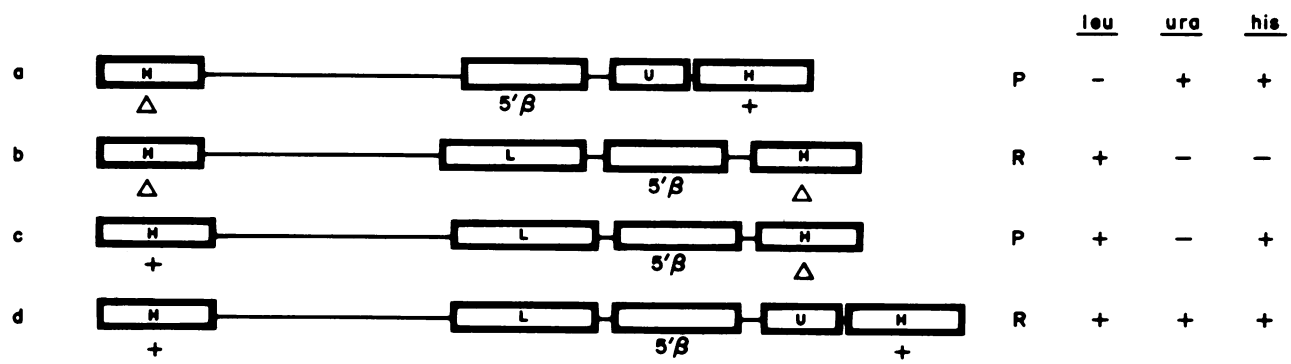

FIG. 6. Physical maps of the four spores from a tetrad where gene conversion of the *LEU2* gene was associated with a reciprocal exchange in pBR322 or *HIS3* sequences (see the text).

the physical distance between *LEU2* and *URA3* is a poor indicator of the genetic distance between them. The MG-1 and 5′β fragments are identical in size; however, they differ significantly in their ability to engage in reciprocal exchanges ($P < 0.005$). Likewise, 5′β and I800 differ in size but show no significant difference in their ability to recombine ($P > 0.9$). By comparing the genetic distance between *HIS3* (left) and *HIS3* (right) with the genetic distance between *LEU2* and *URA3* (Table 1), we can calculate that the 5.3 kb of *HIS3* and pBR322 DNA outside the *LEU2-URA3* interval contributes 1 cM or less to the total map distance between *HIS3* (left) and *HIS3* (right). In other experiments (Treco and Arnheim, in preparation) we show that the addition of sequences consisting entirely of the repeating dinucleotide $d(TG \cdot AC)_n$, which are on the order of 0.1 kb in length, to the 0.62 kb of pBR322 DNA between the *LEU2* and *URA3* genes results in approximately a sevenfold increase in map distance. It is therefore difficult to directly compare I800 with MG-1 and 5′β. However, we can compare each fragment in terms of the map distance it contributes per kilobase of DNA between the two marker genes. By calculating such a specific recombination efficiency parameter for each segment of DNA (Table 1), we see that MG-1 sequences contribute 1.0 cM/kb to the *LEU2-URA3* interval, whereas I800 sequences contribute only 0.46 cM/kb. The specific recombination efficiency of the 5′β fragment is not much greater than that of the pBR322 sequences present in the no-insert experiment (0.28 cM/kb compared to 0.19 cM/kb). As compared with an average value relating map distance to physical distance in *S. cerevisiae* (0.4 cM/kb; 42, 52), MG-1 is much better than average, I800 is approximately average, and 5′β is below average in the ability to engage in reciprocal exchanges.

It is important to note that the *LEU2* and *URA3* sequences positioned as marker genes can be thought of as large insertions of DNA that are opposite nonhomologous pBR322 sequences. In other organisms, regions of nonhomology are known to influence the frequencies and patterns of heteroduplex formation and gene conversion in adjacent homologous intervals (12, 17, 25). Nonhomology could also influence the exchange pattern and the overall frequency of recombination in the *LEU2-URA3* intergenic region where we have positioned the β-globin fragments. The frequency of exchange is not particularly low, however, since none of the values reported here for centimorgans per kilobase are less than one half of the published average of 0.4 cM/kb. The potential effects that heterology may have on the mode of resolution of gene conversion events that initiate in the *LEU2-URA3* interval have not been clearly established. However, since each cross is subject to the same effects, a direct comparison of recombination frequencies between crosses should be meaningful. We therefore feel that the differences in specific recombination efficiencies between DNA sequences in the same chromosomal environment during meiosis reflect inherent differences in the ability of different DNA sequences to engage in homologous recombination. We would like to point out that the large heterologies do not have a major effect on recombination in the adjacent chromosomal regions. In all of the crosses, the *ADE2* locus is heterozygous, which permits us to calculate the genetic distance between *ADE2* and *HIS3*. In every case, regardless of whether plasmid inserts were present at the *HIS3* locus, the genetic distance was approximately 39 cM, the same as in the absence of the heterologies (data not shown).

Borts et al. (7a) have made a rather striking observation using a similar experimental strategy. When *URA3* was positioned within pBR322 sequences flanked by a nontandem, direct duplication of the *MAT* locus on chromosome III, reciprocal meiotic exchange within the inserted DNA leading to recombined flanking *MAT* alleles occurred in up to 20% of all tetrads examined. When neither of the chromosome III homologs carried a *URA3* sequence, reciprocal exchange was only observed in 5.6% of all tetrads, but a partial stimulatory effect was observed when the *URA3* sequence was heterozygous. In experiments in which we studied recombination near the *URA3* gene when it was positioned between a nontandem, direct duplication of the *HIS3* locus on chromosome XV, reciprocal exchange between the duplicated sequences was observed in only about 2.5% of all tetrads examined (Table 1, no-insert experiment). The most obvious difference between the two intervals being monitored, aside from their distinct chromosomal locations, is the fact that in addition to the *URA3* heterozygosity, all of our crosses also have *LEU2* as a tightly linked heterozygous marker. The 2.2-kb *LEU2* heterology near the *URA3* gene may obscure the ability of the *URA3* gene to stimulate recombination. Alternatively, recombination in the vicinity of the *HIS3* locus may normally be very rare, and *URA3* is inefficient in promoting exchange when located there. The MG-1 fragment, however, is capable of raising the frequency of exchange when inserted at the *HIS3* locus.

We initially chose the 5′β fragment for our studies because previous work demonstrated that the polymorphic *Hin*fI site within this fragment is not in disequilibrium with either the 5′ or 3′ framework, or to a polymorphic marker lying 700 bp 3′, suggesting that it lies in a region of relative sequence randomization (22). This fragment was the least recombinogenic of the three β-globin fragments tested. Thus, although this fragment may be near a hot spot for recombination (22), the hot spot could lie at some distance 5′ or 3′ of this region and still generate sequence randomization in the vicinity of the *Hin*fI site.

The ability of the MG-1 fragment to participate in frequent genetic exchanges due to the presence of runs of alternating purines and pyrimidines capable of forming Z DNA (30, 34, 40, 47) was suggested by other studies implicating $d(TG \cdot AC)_n$ in promoting recombination events in mammalian cells (11, 23, 37, 48, 53). However, our data show that a deletion derivative of MG-1 lacking the $d(TG \cdot AC)_{17}$ sequence is still able to recombine efficiently in *S. cerevisiae*, suggesting that additional sequence(s) within the MG-1 fragment must play a dominant role in its genetic behavior in this organism. Such sequences may include the three additional short stretches of alternating purines and pyrimidines (11, 12, and 15 bp) that also exist in this fragment. In fact, in experiments to be reported elsewhere (Treco and Arnheim, in preparation), a region of DNA that consists only of a 150-bp-long stretch of $d(TG \cdot AC)_n$ was found to be frequently involved in reciprocal recombination during yeast meiosis, independent of unique β-globin sequences; in addition, it generates unusual classes of recombinant tetrads when heterozygous.

Although our data on the MG-1 fragment in *S. cerevisiae* are consistent with the presence of a hyperrecombinogenic region in this fragment in the human genome, we have no specific evidence that yeast cells and human cells recognize the same recombination signals. It is important to note, however, that analysis of the population genetics data on the restriction fragment polymorphisms in the β-globin cluster suggests that even as little as a threefold-higher frequency of recombination in a hot spot relative to surrounding segments

could account for the linkage equilibrium between the 5′ and 3′ frameworks (9). In this regard it will be interesting to analyze other suggested recombination hot spots in our system. In any case, our approach has great promise in dissecting the recombinogenic nature of sequences such as MG-1 which recombine very efficiently in yeast meiosis. Our hope is that this novel system for the study of genetic recombination can be used to elucidate the molecular basis for the differences we observed in the ability of different DNA sequences to engage in homologous recombination.

ACKNOWLEDGMENTS

We thank Steve DiNardo and David Shortle for helpful suggestions and Jim Broach for the use of his laboratory for tetrad dissections. We also thank Dee Sedivec for help in the preparation of the manuscript and Cathy Ahern for the artwork.

This work was supported by Public Health Service grants GM-25365 and GM-28632 from the National Institutes of Health. D.T. was supported by Public Health Service predoctoral training grant GM-08065 from the National Institutes of Health.

LITERATURE CITED

1. **Andreadis, A., Y.-P. Hsu, G. B. Kohlhaw, and P. Schimmel.** 1982. Nucleotide sequence of yeast *LEU2* gene shows 5′-noncoding region has sequences cognate to leucine. Cell **31**:319–325.
2. **Antonarakis, S. E., C. D. Boehm, P. J. V. Giardin, and H. H. Kazazian, Jr.** 1982. Nonrandom association of polymorphic restriction sites in the β-globin gene cluster. Proc. Natl. Acad. Sci. U.S.A. **79**:137–141.
3. **Antonarakis, S. E., C.D. Boehm, G. R. Serjeant, C. E. Theisen, G. J. Dover, and H. H. Kazazian, Jr.** 1984. Origin of the β^S-globin gene in Blacks: the contribution of recurrent mutation or gene conversion or both. Proc. Natl. Acad. Sci. U.S.A. **81**:853–856.
4. **Antonarakis, S. E., S. H. Orkin, H. H. Kazazian, Jr., S. C. Goff, C. D. Boehm, P. G. Waber, J. P. Sexton, H. Ostrer, V. F. Fairbands, and A. Chakravarti.** 1982. Evidence for multiple origins of the β^E-globin gene in southeast Asia. Proc. Natl. Acad. Sci. U.S.A. **79**:6608–6611.
5. **Bach, M.-L., F. Lacroute, and D. Botstein.** 1979. Evidence for transcriptional regulation of orotidine-5′-phosphate decarboxylase in yeast by hybridization of mRNA to the yeast structural gene cloned in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:386–390.
6. **Baker, W. K.** 1958. Crossing-over in heterochromatin. Am. Nat. **92**:59–60.
7. **Bolivar, R., R. L. Rodriquez, P. J. Greene, M. C. Betlachk, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.

7a. **Borts, R. H., M. Lichten, M. Hearn, L. S. Davidow, and J. E. Haber.** 1984. Physical monitoring of meiotic recombination in *Saccharomyces cerevisiae*. Cold Spring Harbor Symp. Quant. Biol. **49**:67–76.

8. **Broach, J. R., and J. B. Hicks.** 1980. Replication and recombination functions associated with the yeast plasmid 2μ circle. Cell **21**:501–508.
9. **Chakravarti, A., K. H. Buetow, S. E. Antonarakis, P. G. Waber, C. D. Boehm, and H. H. Kazazian.** 1984. Nonuniform recombination within the human β-globin gene cluster. Am. J. Human Genet. **36**:1239–1258.
10. **Dunn, B., P. Szauter, M. L. Pardue, and J. W. Szostak.** 1984. Transfer of yeast telomeres to linear plasmids by recombination. Cell **39**:191–201.
11. **Flanagan, J. G., M.-P. Lefranc, and T. H. Rabbitts.** 1984. Mechanisms of divergence and convergence of the human immunoglobulin γ1 and γ 2 constant region gene sequences. Cell **36**:681–688.
12. **Fogel, S., R. Mortimer, K. Lusnak, and F. Tavares.** 1979. Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43**:1325–1341.
13. **Fritsch, E. F., R. M. Lawn, and T. Maniatis.** 1980. Molecular cloning and characterization of the human β-like globin gene cluster. Cell **19**:959–972.
14. **Hamada, H., M. G. Petrino, and T. Kakunaga.** 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc. Natl. Acad. Sci. U.S.A. **79**:6465–6469.
15. **Hammerberg, C., and J. Klein.** 1975. Linkage relationships of markers on chromosome 17 of the house mouse. Genet. Res. **26**:203–211.
16. **Hammerberg, C., and J. Klein.** 1975. Linkage disequilibrium between H-2 and t complexes in chromosome 17 of the mouse. Nature (London) **258**:296–299.
17. **Hamza, H., V. Haedens, A. Mekki-Berrada, and J.-L. Rossignol.** 1981. Hybrid DNA formation during meiotic recombination. Proc. Natl. Acad. Sci. U.S.A. **78**:7648–7651.
18. **Hicks, J. B., A. Hinnen, and G. R. Fink.** 1978. Properties of yeast transformation. Cold Spring Harbor Symp. Quant. Biol. **43**:1305–1313.
19. **Hsu, Y.-P., and G. B. Kohlhow.** 1982. Overproduction and control of the *LEU2* gene product, β-isopropylmalate dehydrogenase, in transformed yeast strains. J. Biol. Chem. **257**:39–41.
20. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
21. **Jayaram, M., Y.-Y. Li, M. McLeod, and J. R. Broach.** 1983. Analysis of site-specific recombination associated with the yeast plasmid 2 micron circle, p. 685–694. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), Mechanisms of DNA replication and recombination. Alan R. Liss, Inc., New York.
22. **Kazazian, H. H., Jr., S. H. Orkin, S. E. Antonarakis, J. P. Sexton, C. D. Boehm, S. C. Goff, and P. G. Waber.** 1984. Molecular characterization of seven β-thalassemia mutations in Asian Indians. EMBO J. **3**:593–599.
23. **Kilpatrick, M. W., J. Klysik, C. K. Singleton, D. A. Zarling, T. M. Jovin, L. H. Harau, B. F. Erlanger, and R. D. Wells.** 1984. Intervening sequences of human fetal globin genes adopt left-handed Z helices. J. Biol. Chem. **259**:7268–7274.
24. **Kobori, J. A., A. Winoto, J. McNicholas, and L. Hood.** 1984. Molecular characterization of the recombination region of six murine major histocompatibility complex (MHC) I-region recombinants. J. Mol. Cell. Immunol. **1**:125–131.
25. **Lichten, M., and M. S. Fox.** 1983. Effects of nonhomology on bacteriophage lambda recombination. Genetics **103**:5–22.
26. **Miesfeld, R., M. Krystal, and N. Arnheim.** 1981. A member of a new repeated sequence family which is conserved throughout eucaryotic evolution is found between the human δ and β globin genes. Nucleic Acids Res. **9**:5931–5947.
27. **Mortimer, R. K., and D. C. Hawthorne.** 1966. Genetic mapping in *Saccharomyces*. Genetics **53**:165–173.
28. **Mortimer, R. K., and D. Schild.** 1981. Genetic mapping in *Saccharomyces cerevisiae*, p. 11–25. *In* J. N. Strathern et al. (ed.), Molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Moschonas, N., E. de Boer, and R. A. Flavell.** 1982. The DNA sequence of the 5′ flanking region of the human β-globin gene: evolutionary conservation and polymorphic differences. Nucleic Acids Res. **10**:2109–2120.
30. **Nordheim, A., and A. Rich.** 1983. The sequence $(dC\text{-}dA)_n$ $(dG\text{-}dT)_n$ forms left-handed Z-DNA in negatively supercoiled plasmids. Proc. Natl. Acad. Sci. U.S.A. **80**:1821–1825.
31. **Orkin, S. H., S. E. Antonarakis, and H. H. Kazazian, Jr.** 1983. Polymorphism and molecular pathology of the human beta-globin gene. Prog. Hematol. **13**:49–73.
32. **Orkin, S. H., H. H. Kazazian, Jr., S. E. Antonarakis, S. C. Goff, C. D. Boehm, J. P. Sexton, P. G. Waber, and P. J. V. Giardina.** 1982. Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. Nature (London) **296**:627–631.
33. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981.

Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. U.S.A. **78**:6354–6358.
34. **Peck, L. J., and J. C. Wang.** 1983. Energetics of B-to-Z transition in DNA. Proc. Natl. Acad. Sci. U.S.A. **80**:6206–6210.
35. **Petes, T. D., and D. Botstein.** 1977. Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc. Natl. Acad. Sci. U.S.A. **74**:5091–5095.
36. **Poncz, M., E. Schwartz, M. Ballantine, and S. Surrey.** 1983. Nucleotide sequence analysis of the β-globin gene region in humans. J. Biol. Chem. **258**:11599–11609.
37. **Proudfoot, N. J., and T. Maniatis.** 1980. The structure of a human α-globin pseudogene and its relationship to α-globin gene duplication. Cell **21**:537–544.
38. **Radding, C. M.** 1979. The mechanism of conversion of deletions and insertions. Cold Spring Harbor Symp. Quant. Biol. **43**:1315–1316.
39. **Ratzkin, B., and J. Carbon.** 1977. Functional expression of cloned yeast DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:487–491.
40. **Rich, A., A. Nordheim, and A. H.-H. Wang.** 1984. The chemistry and biology of left-handed DNA. Annu. Rev. Biochem. **53**:791–846.
41. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling DNA to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
42. **Roeder, G. S.** 1983. Unequal crossing-over between yeast transposable elements. Mol. Gen. Genet. **190**:117–121.
43. **Rose, M., M. J. Casadaban, and D. Botstein.** 1981. Yeast genes fused to β-galactosidase in *Escherichia coli* can be expressed normally in yeast. Proc. Natl. Acad. Sci. U.S.A. **78**:2460–2464.
44. **Schalet, A.** 1969. Exchanges at the bobbed locus of *Drosophila melanogaster*. Genetics **63**:133–153.
45. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosomal segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **76**:4951–4955.
46. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
47. **Singleton, C. K., M. W. Kilpatrick, and R. D. Wells,** 1984. S_1 nuclease recognizes DNA conformational junctions between left-handed helical $(dT\text{-}dG)_n$ $(dC\text{-}dA)_n$ and contiguous right-handed sequences. J. Biol. Chem. **259**:1963–1967.
48. **Slightom, J. L., A. E. Blechl, and O. Smithies.** 1980. Human fetal $^{G}\gamma$- and $^{A}\gamma$-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell **21**:627–638.
49. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
50. **Southern, E. M.** 1979. Gel electrophoresis of restriction fragments. Methods Enzymol. **68**:152–176.
51. **Stahl, F. W.** 1979. Summary. Cold Spring Harbor Symp. Quant. Biol. **43**:1353–1356.
52. **Strathern, J. N., C. S. Newlon, I. Herskowitz, and J. B. Hicks.** 1979. Isolation of a circular derivative of yeast chromosome III: implications for the mechanism of mating type interconversion. Cell **18**:309–319.
53. **Stringer, J. R.** 1982. DNA sequence homology and chromosomal deletion at a site of SV40 DNA integration. Nature (London) **296**:363–366.
54. **Struhl, K., and R. W. Davis.** 1977. Production of a functional eukaryotic enzyme in *Escherichia coli*: cloning and expression of the yeast structural gene for imidazoleglycerolphosphate dehydratase (*HIS3*). Proc. Natl. Acad. Sci. U.S.A. **74**: 5255–5259.
55. **Sugawara, N., and J. W. Szostak.** 1983. Recombination between sequences in nonhomologous positions. Proc. Natl. Acad. Sci. U.S.A. **80**:5675–5679.
56. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
57. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33**:25–35.