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The Evolutionarily Conserved Repetitive Sequence d(TG · AC)$_n$ Promotes Reciprocal Exchange and Generates Unusual Recombinant Tetrads During Yeast Meiosis

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We have studied the genetic behavior of the alternating copolymer d(TG · AC)$_n$ inserted into a defined position in the genome of the yeast *Saccharomyces cerevisiae*. When d(TG · AC)$_n$ sequences were present at the *HIS3* locus on homologous chromosomes, diploid cells undergoing meiosis generated an excess of tetrads containing reciprocally recombined products with crossover points close to the repetitive DNA insert. Most of these tetrads exhibited gene conversion of a d(TG · AC)$_n$ insert. However, the insertion of d(TG · AC)$_n$ sequences had no effect on the frequency of gene conversion of closely linked marker genes. Surprisingly, when d(TG · AC)$_n$ sequences were present on only one homolog at the *HIS3* locus, one-half of the tetrads exhibiting nonparental segregation for marker genes that flanked the repetitive DNA insert were very unusual and appeared to have arisen by multiple recombination events in the vicinity of the d(TG · AC)$_n$ insert. Similar multiply recombinant tetrads were seen in crosses in which d(TG · AC)$_n$ sequences were present on both homologs. Combined, the data strongly suggest that d(TG · AC)$_n$ sequences significantly enhance reciprocal meiotic recombination and may be important in causing multiple recombination events to occur within a relatively small region of the yeast chromosome. Molecular evidence is presented that clearly documents the postmetiotic segregation of an 80-base stretch of d(TG · AC)$_n$.

We have previously described an in vivo system for analyzing the ability of specific DNA sequences to engage in homologous meiotic recombination in the yeast *Saccharomyces cerevisiae* (58). This system was used to demonstrate that different DNA sequences from the human β-globin locus participate in genetic exchanges at different frequencies during yeast meiosis. One fragment from the human β-globin locus, MG-1 (24), was associated with reciprocal exchange during meiosis significantly more frequently than either of two other fragments normally found near MG-1 in the β-globin cluster. All three sequences lie within an 11-kilobase (kb) region between the human δ- and β-globin genes that has previously been hypothesized to be a relative hot-spot for genetic exchange within the β-globin gene cluster (1, 18, 29).

One element within MG-1 that is of particular interest is a highly repetitive, evolutionarily conserved sequence (EC-1) originally described by Miesfeld et al. (24). Its nucleotide sequence reveals a substantial stretch of the simple repeating dinucleotide d(TG · AC)$_n$. Similar sequences of d(TG · AC)$_n$ (n = 17 in the MG-1 fragment) are found repeated approximately 50,000 times in the human genome and appear to be highly repetitive in all eucaryotic cell genomes examined by Southern blot analysis (13, 24). Long tracts of the sequence d(TG · AC)$_n$ have been observed by direct DNA sequencing in or near many eucaryotic genes (26, 27, 38, 43, 49), and it has been suggested that such sequences may be involved in the initiation of genetic exchanges (28, 39, 43). In addition, the d(TG · AC)$_n$ element has been shown to be repeated approximately 100 times in the yeast genome. Many of the restriction fragments hybridizing to a labeled d(TG · AC)$_n$ probe in yeast are not due to strictly alternating T and G (or A and C) residues, but consist of tandem irregular repeats of the general form $C_{1-3}A$ and have been localized to the telomeres of yeast chromosomes (37, 60, 61). The $C_{1-3}A$ tracts present at yeast telomeres that hybridize with d(TG · AC)$_n$ probes are on the order of one to several hundred base pairs in length (37, 60). However, it is predicted that there are 15 to 20 d(TG · AC)$_n$-hybridizing sequences that are not telomeric or telomere associated (60).

An analysis of the MG-1 fragment in yeast showed that it was very active in meiotic recombination even in the absence of the d(TG · AC)$_{17}$ sequence (58). However, in this experiment two small d(TG · AC)$_n$ runs of 10 and 14 base pairs (bp) (n = 5 and 7, respectively) remained in the tested fragment. We were therefore interested in determining whether d(TG · AC)$_n$ sequences are frequently associated with meiotic exchanges when separated from other, unique β-globin sequences. Using our in vivo assay for meiotic recombination, we show here that the addition of cloned fragments of d(TG · AC)$_{40}$, d(TG · AC)$_{60}$, and d(TG · AC)$_{75}$ (80, 120, and 150 bp, respectively) to a 620-bp interval between two yeast genes enhances reciprocal recombination and increases the genetic distance between the two genes by a factor of seven. In addition, the presence of d(TG · AC)$_n$, when homozygous or heterozygous, generated unusual tetrad classes at higher than expected frequencies.

MATERIALS AND METHODS

To study the ability of d(TG · AC)$_n$ sequences to participate in meiotic recombination in yeast, we needed to introduce these sequences into homologous positions within the

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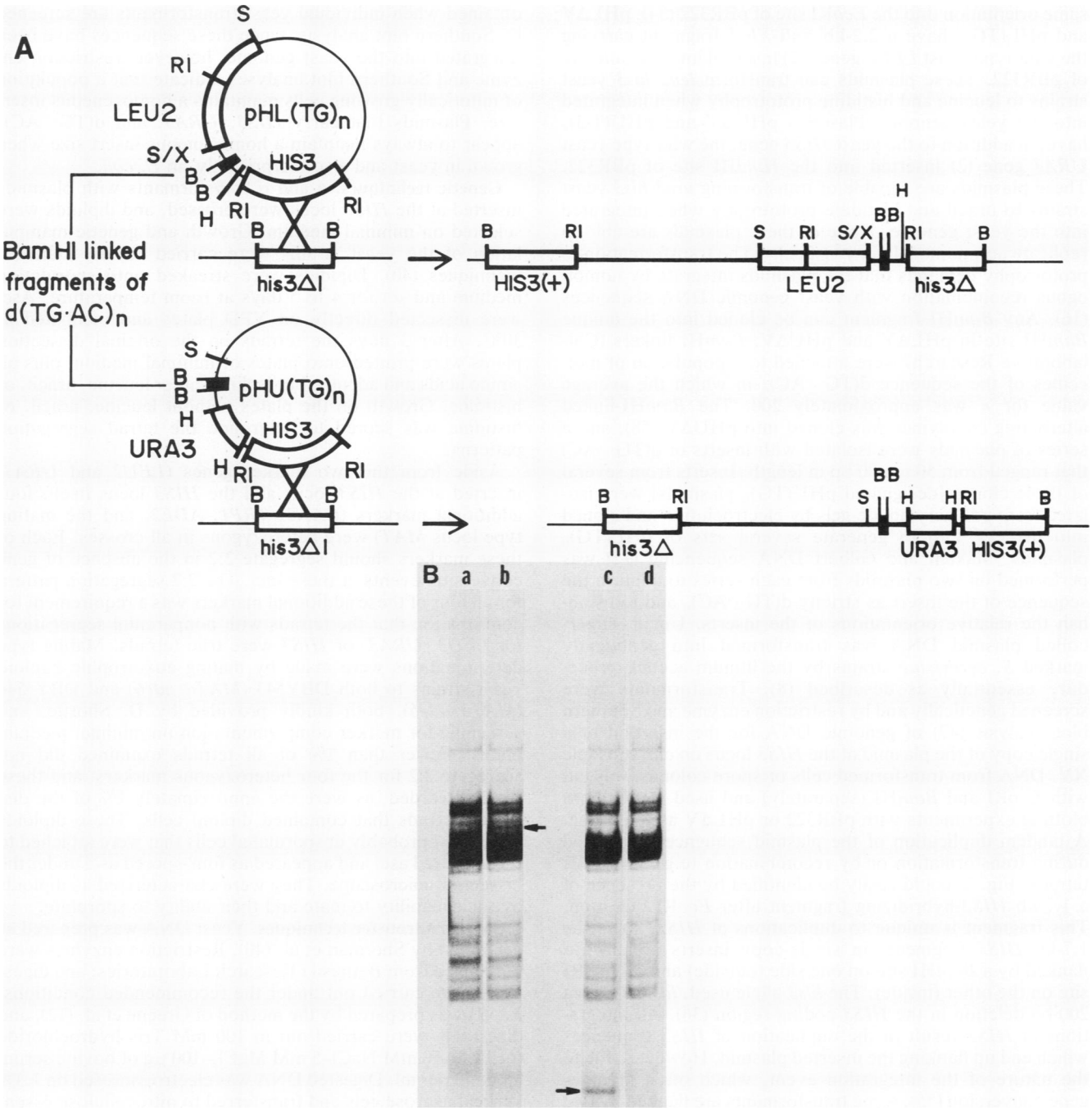

FIG. 1. (A) Construction and characterization of plasmids pHL(TG)$_n$ and pHU(TG)$_n$ is described in the Materials and Methods section. The right side of this figure illustrates the physical organization of homologous *HIS3* loci in a diploid generated after integration of d(TG · AC)$_n$ sequences and marker genes into haploid yeast strains. B, *Bam*HI; RI, *Eco*RI; S, *Sal*I; H, *Hin*dIII; S/X, *Sal*I-*Xho*I hybrid site. (B) Autoradiogram of a Southern blot to identify newly inserted d(TG · AC)$_n$ sequences in the yeast genome. Hybridization was with a ^{32}P-labeled d(TG · AC)$_n$ probe. All DNA samples were cut with *Hin*cII. Lane a, Untransformed strain 1b; lane b, strain 1b transformed with pHL(TG)$_n$ clone carrying 80 bp of d(TG · AC)$_n$; lane c, strain 16d transformed with pHU(TG)$_n$ clone carrying 150 bp of d(TG · AC)$_n$; lane d, untransformed strain 16d. The arrows point to bands that were absent in the untransformed strains and present in the transformed strains and of the size predicted from restriction maps of the transforming plasmids (data not shown).

genomes of yeast strains of opposite mating types. We also needed to associate the sequences to be monitored with flanking marker genes to allow the identification of recombinant spores. The construction of these defined genetic loci is illustrated in Fig. 1A.

Two pairs of integrating vectors were constructed, pHLΔV and pHUΔV, and their d(TG · AC)$_n$-carrying derivatives pHL(TG)$_n$ and pHU(TG)$_n$. All plasmids are derivatives of pBR322 (4). Each vector has a 1.7-kb DNA fragment containing the wild-type yeast *HIS3* gene (53) inserted in the

same orientation into the *Eco*RI site of pBR322 (54). pHLΔV and pHL(TG)$_n$ have a 2.2-kb *Sal*I-*Xho*I fragment carrying the wild-type yeast *LEU2* gene (32) inserted into the *Sal*I site of pBR322. These plasmids can transform *leu2 his3* yeast strains to leucine and histidine prototrophy when integrated into the yeast genome. Plasmids pHUΔV and pHU(TG)$_n$ have, in addition to the yeast *HIS3* gene, the wild-type yeast *URA3* gene (2) inserted into the *Hin*dIII site of pBR322. These plasmids are capable of transforming *ura3 his3* yeast strains to uracil and histidine prototrophy when integrated into the yeast genome. None of these plasmids are able to replicate autonomously in yeast cells. The transformation to prototrophy demands that the plasmids integrate by homologous recombination with yeast genomic DNA sequences (16). Any *Bam*HI fragment can be cloned into the unique *Bam*HI site in pHLΔV and pHUΔV. *Bam*HI linkers (Collaborative Research) were attached to a population of molecules of the sequence d(TG · AC)$_n$ in which the average value for *n* was approximately 200. The *Bam*HI-linked alternating copolymer was cloned into pHUΔV (58), and a series of plasmids were isolated with inserts of d(TG · AC) that ranged from 50 to 400 bp in length. Inserts from several of these clones [designated pHU(TG)$_n$ plasmids] were isolated from polyacrylamide gels by electroelution and cloned into pHLΔV (58) to generate several sets of pHL(TG)$_n$ plasmids. Maxam and Gilbert DNA sequencing (22) was performed on two plasmids from each series to confirm the sequence of the insert as strictly d(TG · AC)$_n$ and to establish the relative orientations of the inserts. Uncut, supercoiled plasmid DNA was transformed into genetically marked *S. cerevisiae* strains by the lithium acetate procedure essentially as described (8). Transformants were screened genetically and by restriction enzyme and Southern blot analysis (47) of genomic DNA for the insertion of a single copy of the plasmid at the *HIS3* locus on chromosome XV. DNA from transformed cells or spore colonies was cut with *Eco*RI and *Bam*HI (separately) and used in Southern blotting experiments with pBR322 or pHLΔV as the probe. A tandem duplication of the plasmid sequences generated during transformation or by recombination (e.g., class VII tetrads, Fig. 2) could easily be identified by the presence of a 1.7-kb *HIS3*-hybridizing fragment after *Eco*RI digestion. This fragment is unique to duplications at *HIS3*, since the 1.7-kb *HIS3* fragments in single-copy inserts are always flanked by a *Bam*HI site on one side (outside) and an *Eco*RI site on the other (inside). The *his3* allele used, *his3Δ1*, has a 200-bp deletion in the *HIS3* coding region (36). 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 (55), some transformants are flanked by two wild-type genes (++), some are flanked by two *his3Δ1* genes (ΔΔ), and some have *HIS3* and *his3Δ1* sequences on opposite sides of the insertion (Δ+ or +Δ). The distribution of the two *HIS3* sequences in each transformant or spore DNA sample can be determined by restriction enzyme (*Bam*HI) and Southern blot analysis.

Plasmids pHL(TG)$_n$ and pHLΔV were transformed into a strain of the genotype *MAT***a** *leu2-3,112 ura3-52 his3Δ1 trp1-289 can1-101* (strain 1b) (58). Plasmids pHU(TG)$_n$ and pHUΔV were transformed into a strain of the genotype *MATα leu2-3,112 ura3-52 his3Δ1 ade2* (strain 16d) (58). When grown in *Escherichia coli* a population of plasmids carrying *HIS3*, *LEU2*, and d(TG · AC)$_n$ harbors a significant fraction (10 to 50%) of molecules with deletions in the *Bam*HI insert, and a range of d(TG · AC)$_n$ insert sizes are obtained when individual yeast transformants are screened by Southern blot analysis. Once these sequences have been integrated into the yeast genome, however, restriction enzyme and Southern blot analyses indicate that a population of mitotically growing cells maintains a homogeneous insert size. Plasmids that carry *HIS3*, *URA3*, and d(TG · AC)$_n$ appear to always maintain a homogeneous insert size when grown in yeast and also, surprisingly, in *E. coli*.

Genetic techniques. **a** and α transformants with plasmids inserted at the *HIS3* locus were crossed, and diploids were selected on minimal medium. Growth and genetic manipulation of the yeast strains were carried out by standard techniques (40). Diploids were streaked onto sporulation medium and set for 4 to 5 days at room temperature. Asci were dissected directly on YPD plates and incubated at 30°C. After 3 days the tetrads on the original dissection plates were printed onto plates of minimal medium plus all amino acids and adenine but lacking either leucine, uracil, or histidine. Growth on the plates without leucine, uracil, or histidine was scored to determine the tetrad segregation patterns.

Aside from the two marker genes (*LEU2* and *URA3*) inserted at the *HIS3* locus and the *HIS3* locus itself, four additional markers (*CAN1*, *TRP1*, *ADE2*, and the mating type locus *MAT*) were heterozygous in all crosses. Each of these markers should segregate 2:2 in the absence of gene conversion events at these loci. The 2:2 segregation pattern for all four of these additional markers was a requirement for confirmation that the tetrads with nonparental segregations for *LEU2*, *URA3*, or *HIS3* were true tetrads. Mating type determinations were made by mating auxotrophic haploid yeast strains to both DBY543 (*MATα ade6*) and DBY 544 (*MAT***a** *ade6*), both kindly provided by D. Shortle, and screening for marker complementation on minimal medium plates. Fewer than 1% of all tetrads examined did not segregate 2:2 for the four heterozygous markers, and these were discarded, as were the approximately 1% of the dissected tetrads that contained diploid cells. These diploids were most probably unsporulated cells that were attached to three-spored asci and appeared as four-spored asci under the dissection microscope. They were characterized as diploids by their inability to mate and their ability to sporulate.

Southern transfer techniques. Yeast DNA was prepared as described by Sherman et al. (40). Restriction enzymes were purchased from Bethesda Research Laboratories, and digestions were carried out under the recommended conditions. *Eco*RI was prepared by the method of Greene et al. (12), and digestions were carried out in 100 mM Tris hydrochloride (pH 7.5)–50 mM NaCl–5 mM $MgCl_2$–100 μg of bovine serum albumin per ml. Digested DNA was electrophoresed on 1.3% vertical agarose gels and transferred to nitrocellulose essentially as described (48).

The gel in the autoradiograph shown in Fig. 4B (lanes l through p) was transferred to Genatran 45 filter paper (D&L Filter Corp.). To avoid nicking the DNA the gel was not stained with ethidium bromide or photographed. The filter was hybridized with a d(TG · AC)$_n$ probe labeled by nick translation (34) with [α-^{32}P]dCTP. Hybridization was done in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–3× Denhardt solution (7)–0.5% sodium dodecyl sulfate (SDS) at 65°C for 12 to 16 h. The filter was washed in 3× SSC–0.5% SDS for 2 h at 65°C. All of the other gels were transferred to nitrocellulose (Millipore Corp.) and hybridized with labeled pHLΔV in 3× SSC–3× Denhardt solution–0.1% SDS. These filters were washed in 0.2× SSC–0.1% SDS for 2 h at 65°C.

TABLE 1. Recombinant tetrads in each class in three experimental crosses

Class[a]	% of tetrads in class (actual no. observed)		
	HL(TG)$_n$ × HU(TG)$_n$ (n = 1,660)	HLΔV × HUΔV (n = 1,526)	HL(TG)$_n$ × HUΔV and HLΔV × HU(TG)$_n$ (n = 981)
I	1 (17)	0.13 (2)	0.20 (2)
II-A	0.24 (4)	0.07 (1)	0.10 (1)
II-B	0.24 (4)	0.33 (5)	0 (0)
III-A	0.30 (5)	0.46 (7)	0.31 (3)
III-B	0.36 (6)	0.39 (6)	0.10 (1)
IV	0.36 (6)	0.26 (4)	0.10 (1)
V	0 (0)	0.07 (1)	0 (0)
VI	2.7 (45)	2.2 (34)	3.0 (29)
VII	0.18 (3)	0.26 (4)	0.20 (2)
VIII	0.36 (6)	0 (0)	0.71 (7)

[a] The recombination events involved in each class are diagrammed in Fig. 2.

RESULTS

By the protocol outlined in Fig. 1A, we have the ability to place defined d(TG · AC)$_n$ sequences at the yeast *HIS3* locus on chromosome XV. Figure 1B shows an autoradiogram of a Southern blot probed with ^{32}P-labeled d(TG · AC)$_n$ to identify the newly inserted d(TG · AC)$_n$ sequences in the yeast genome. The intensity of hybridization of the newly inserted d(TG · AC)$_n$ sequences relative to endogenous hybridizing fragments suggests that even a 150-bp stretch of d(TG · AC)$_n$ is not extraordinarily long, although no yeast sequence of strictly d(TG · AC)$_n$ longer than 34 bp has been documented by DNA sequencing (60). This 34-bp tract is one of perhaps 15 to 20 d(TG · AC)$_n$-hybridizing sequences that are not telomeric or telomere associated (60).

The transformation of a yeast strain of the **a** mating type with pHLΔV or pHL(TG)$_n$ and a strain of the α mating type with pHUΔV or pHU(TG)$_n$ permitted us to generate diploids heterozygous for *URA3* and *LEU2* at the *HIS3* locus. If such a diploid is stimulated to enter meiosis and allowed to sporulate, the segregation of the *URA3* and *LEU2* markers can be studied by tetrad analysis (11, 40). Homologous exchange in the interval between these two markers will generate a chromosome with *LEU2* and *URA3* now physically linked on the same chromosome as well as a chromosome with neither marker gene. Both chromosomes will still carry pBR322 sequences flanked by direct duplications of *HIS3* sequences and, in experiments involving pHL(TG)$_n$ and pHU(TG)$_n$, the d(TG · AC)$_n$ sequences inserted in the *Bam*HI site. Gene conversion events involving *LEU2* or *URA3* sequences will also create novel recombinant chromosomes. Exchanges that are limited to *HIS3* sequences on either side of the integration unit or pBR322 sequences to the left of *LEU2* will yield chromosomes recombinant for only the *HIS3* sequences.

Meiotic recombination in diploids lacking d(TG · AC)$_n$ sequences at the *HIS3* locus. In the first experiment, no d(TG · AC)$_n$ sequences were included in the plasmids used in the yeast transformation (transformation with plasmids pHLΔV and pHUΔV). The physical distance between the *LEU2* and *URA3* genes on homologous chromosomes where recombination can take place is 0.62 kb and consists entirely of the sequence of pBR322 between the *Hin*dIII and *Sal*I sites (54). The organization of the homologous *HIS3* loci undergoing recombination in this cross (cross HLΔV × HUΔV, Table 1) is identical to that illustrated in Fig. 1A except for the absence of inserts in the *Bam*HI site. Recombination events giving rise to tetrads with nonparental segregation patterns for *LEU2*, *URA3*, or *HIS3* sequences are diagrammed in Fig. 2. Class I events are reciprocal exchanges in the interval between *LEU2* and *URA3*. Class II and class III events are gene conversions for only *LEU2* or *URA3* sequences and show 3+:1− or 1+:3− segregation for these markers. The *LEU2* and *URA3* marker genes are opposite nonhomologous DNA and can be thought of as allelic with pBR322 sequences. Gene conversion events involving these sequences must involve the conversion of a large deletion or insertion (2.2 kb for *LEU2* and 1.1 kb for *URA3*). Class IV and V events are converted for large segments of DNA extending from pBR322 sequences on the left of the *LEU2* gene through to sequences on the right-hand side of the *URA3* gene. These "coconversion" events (11), in which two markers are included in the same tract of converted DNA, here involve two large heterologies and cover a minimum of 4 kb. Some events extended further to the right or left and also showed nonparental segregation for a flanking *HIS3* sequence. Since the *his3* mutation *his3Δ1* is a 200-bp deletion (36), these events involve conversion tracts that span three substantial heterologies over 5 to 9 kb of DNA.

A total of 1,526 asci were dissected and analyzed for recombination events that involved *LEU2*, *URA3*, or the flanking *HIS3* sequences. Sixty-four nonparental segregations involving these genes were identified (Table 1). Twenty-four of the events were gene conversions (3+:1− or 1+:3− segregations) for either *LEU2* or *URA3* (classes II, III, IV, and V, Fig. 2). In a diploid the *HIS3* sequences on both the left and right sides of the inserted DNA were heterozygous. In 19 of these 24 *LEU2* or *URA3* gene conversion events, the flanking *HIS3* markers were nonrecombinant, suggesting that in these cases the gene conversion resolved between the *HIS3* sequences without reciprocal exchange (data not shown). Two of the remaining five conversion events segregated 3+:1− for the *HIS3* marker on right side of the insert (data not shown). Since in these two events one endpoint of the converted segment lay outside the inserted DNA, these events could not be unambiguously identified as having been associated with crossing-over. Only 3 of the 24 gene conversion events involving *LEU2* or *URA3* sequences were associated with reciprocal exchange between the duplicated *HIS3* sequences.

Only 2 tetrads of the 64 nonparental segregations from this cross were tetratype for the *LEU2* and *URA3* markers, indicative of a reciprocal exchange in the 0.62-kb interval between these two genes (class I events). This was confirmed by restriction enzyme and Southern blot analysis (data not shown). Thus, only two reciprocal exchanges in the *LEU2-URA3* interval were observed in 1,526 dissected tetrads. Using these two events to calculate the genetic distance (25) between the *LEU2* and *URA3* genes, we obtained a value of 0.07 centimorgans (cM) (Table 2). Most of the remaining nonparental segregations involved only *HIS3* sequences. Not all of these were necessarily the result of crossover events, since gene conversion of a wild-type *HIS3* marker to the *his3Δ1* deletion mutation on either side could yield tetrads that are phenotypically 3 His$^+$:1 His$^-$. Likewise, events that convert a deletion to wild type on either side would generate tetrads that appear to segregate normally (4 His$^+$:0 His$^-$) even though one chromatid has two wild-type *HIS3* genes flanking the inserted DNA. However,

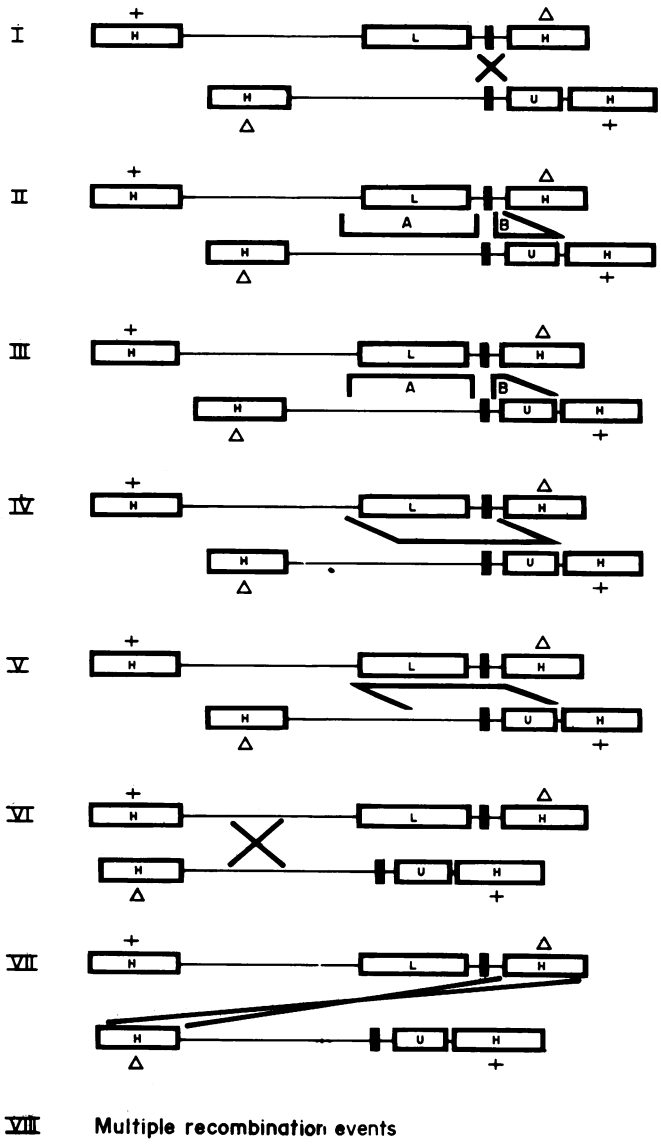

FIG. 2. Recombination events involving markers inserted at the *HIS3* locus; generation of eight classes of recombinant tetrads. The percentage of recombinant tetrads observed in each class in each of three experimental crosses is listed in Table 1. Classes I, VI, and VII are reciprocal exchange events. For class I events the crossover can occur within, to the right, or to the left of the *Bam*HI cloning site or it can involve a gene conversion event covering $d(TG \cdot AC)_n$ sequences inserted in the *Bam*HI site (see Results). Class II and III events are gene conversions detected as 3+:1− or 1+:3− segregations for *LEU2* or *URA3*. In class II events the top chromatid is the donor in gene conversion. Class II-A events are phenotypically 3 Leu^+:1 Leu^-, while class II-B events are 1 Ura^+:3 Ura^-. In class III events the bottom chromatid is the donor in gene conversion. Class III-A events are phenotypically 1 Leu^+:3 Leu^-, while class III-B events are 3 Ura^+:1 Ura^-. Class IV and V events are gene conversions that span both the *LEU2* and *URA3* markers (coconversions) and are detected as 3 Leu^+ Ura^-:1 Leu^- Ura^+ (class IV, top chromatid is donor) and 3 Leu^- Ura^+:1 Leu^+ Ura^- (class V, bottom chromatid is donor). The open side of the brackets denotes the sequences used as the donors in gene conversion events. The closed side of the brackets runs along the DNA segments that are the recipients in the information transfers. The endpoints of the converted segments are not specifically as shown. The converted region in some recombinants can extend through a *HIS3* marker on the left or right. In addition, some of the gene conversion events in each experimental cross resolved by crossing-over between the flanking *HIS3* genes. This was true for six, three, and three gene conversion events (class II, III, IV or V) in the experimental crosses $HL(TG)_n \times HU(TG)_n$ $HL\Delta V \times HU\Delta V$, and $HL(TG)_n \times HU\Delta V/HL\Delta V \times HU(TG)_n$, respectively (see Table 1). These events were added to the class I and class VII events to calculate the total number of reciprocal exchanges in the *HIS3-HIS3* duplication interval and the genetic distance between the duplicated *HIS3* genes listed in Table 2. Only 12 of 64 gene conversion events for *LEU2* or *URA3* markers resolved by reciprocal exchange in the *HIS3-HIS3* duplication interval. Class VIII tetrads are described in the text (see Results). H, *HIS3*; L, *LEU2*; U, *URA3*; +, wild-type *HIS3* gene; Δ, *his3Δ1* mutation; ■, *Bam*HI cloning site.

in a cross in which the organization of flanking *HIS3* genes allowed an unambiguous distinction between reciprocal recombinations and gene conversions, we found that 17 of 17 nonparental segregations for the *HIS3* phenotype were reciprocally recombined for the flanking *HIS3* markers (data not shown). The organization of markers in this cross was *HIS3*-pBR322-*LEU2-his3Δ1* × *his3Δ1*-pBR322-*URA3-his3Δ1*, and reciprocal exchanges could be identified phenotypically. An additional 11 tetrads with nonparental segregation for *HIS3* were analyzed by Southern blotting and were shown to be the result of reciprocal exchange. Thus, a large fraction of the 108 class VI tetrads must be the result of reciprocal exchange. This is consistent with the observation of Borts et al., in a similar experimental design, that 95% of all nonparental segregations of duplicated *MAT* genes flanking pBR322 sequences were reciprocal recombinants (5). Likewise, Jackson and Fink have shown that the major type of nonparental segregation for *HIS4* sequences in crosses in which duplicated, mutant *HIS4* genes flank pBR313 or pBR322 sequences is a reciprocal exchange between equally paired homologs (17). We can therefore take the nonparental segregations for *HIS3* as reflecting the maximum frequency of crossing-over in *HIS3* or pBR322 sequences and calculate a map distance between the flanking *HIS3* sequences of 1.28 cM (Table 1).

Meiotic recombination in diploids carrying $d(TG \cdot AC)_n$ insertions at the *HIS3* locus. We then performed an experiment identical to that just described except that *Bam*HI fragments of DNA composed entirely of the sequence $d(TG \cdot AC)_n$ were added to the pBR322 sequences in the *LEU2-URA3* interval. In some of the diploids constructed, the *LEU2* chromatid had 120 bp of this sequence, while in others it had 80 bp inserted. The *URA3* chromatid had 150 bp of $d(TG \cdot AC)_n$ inserted. The orientation of the simple repetitive sequence in each of the transforming plasmids was determined to be the same by direct DNA sequencing (22) before integration into the yeast genome.

Figure 1A illustrates the physical organization of homologous *HIS3* loci where $d(TG \cdot AC)_n$ sequences were inserted into homologous positions in the *LEU2-URA3* interval. We identified 96 nonparental segregations for the *LEU2*, *URA3*, and *HIS3* marker genes in 1,660 dissected tetrads (Table 1). Twenty-five of these were gene conversions for *LEU2* or *URA3* sequences (3+:1− and 1+:3−), and 45 were nonparental segregations for *HIS3* sequences only (3 His^+:1 His^-). Seventeen of the remaining nonparental segregations were tetratype for *LEU2* and *URA3* and were the result of reciprocal exchanges that occurred in the *LEU2-URA3* interval, as judged by restriction enzyme and Southern blot analysis (data not shown). Compared with the results of the experiment reported above, there was a sevenfold increase in the frequency with which crossover events occurred in the

TABLE 2. Tetrad analysis

Cross[a]	No. of tetrads analyzed	No. with reciprocal exchanges in *LEU2-URA3*	*LEU2-URA3* distance		Specific recombination efficiency (cM/kb)[d]	Total no. of reciprocal exchanges between duplicated *HIS3* sequences[e]	*HIS3-HIS3* distance		% of tetrads with gene conversion events involving *LEU2* or *URA3* (no.)[g]
			Genetic (cM)[b]	Physical (kb)[c]			Genetic (cM)	Physical (kb)[f]	
LEU2-URA3 interval homozygous									
HL(TG)$_n$ × HU(TG)$_n$[h]	1,660	17	0.51	0.75	0.68	68	2.05	6.0	1.5 (25)
HLΔV × HUΔV	1,526	2	0.07	0.62	0.11	39	1.28	5.9	1.6 (24)
LEU2-URA3 interval heterozygous[i]	981	2	0.1	0.6	0.16	34	1.7	5.9	0.6 (6)

[a] Homozygous or heterozygous for sequences in the *LEU2-URA3* interval.

[b] Distance in centimorgans = [100(T + 6NPD)]/[2(PD + NPD + T)], where T, PD, and NPD are tetratype, parental ditype, and nonparental ditype, respectively (25).

[c] Defined as the total length of homologous DNA between the insertion sites of the *LEU2* and *URA3* genes.

[d] Calculated as *LEU2-URA3* genetic distance/*LEU2-URA3* physical distance.

[e] Crossovers in *LEU2-URA3* interval plus all events in pBR322 and *HIS3* sequences that result in the reciprocal exchange of the 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 nonsister chromatid).

[g] Class II, III, IV, and V tetrads combined (see Fig. 2A). The numbers in parentheses are the actual number of events observed.

[h] Two different diploids were analyzed. A total of 195 tetrads were dissected in which the *LEU2* chromatid had 80 bp of the d(TG · AC)$_n$ sequence and the *URA3* chromatid carried 150 bp of this sequence. Three reciprocal exchanges in the *LEU2-URA3* interval were isolated from this cross. A total of 1,465 tetrads were dissected in which the *LEU2* chromatid had 120 bp of the d(TG · AC)$_n$ sequence and the *URA3* chromatid had 150 bp. Fourteen reciprocal exchanges in the *LEU2-URA3* interval were isolated from this cross.

[i] Four different diploids were analyzed. A total of 177 tetrads were dissected in which 80 bp of d(TG · AC)$_n$ was on the *URA3* chromatid; 383 tetrads had 150 bp of d(TG · AC)$_n$ on the *URA3* chromatid (in both crosses the plasmid integrated on the *LEU2* chromatid was pHLΔV); 179 tetrads were dissected with 80 bp of d(TG · AC)$_n$ on the *LEU2* chromatid and pHUΔV integrated on the *URA3* chromatid; 242 tetrads were dissected in which the *LEU2* chromatid had 120 bp of d(TG · AC)$_n$ and the *URA3* chromatid had pHUΔV integrated.

LEU2-URA3 interval. This increase was statistically highly significant (G test of independence [45] yielded $P < 0.005$). The frequency of gene conversion events involving the *LEU2* or *URA3* marker genes was virtually identical in the two experiments [1.5% for the experiment with d(TG · AC)$_n$ sequences versus 1.6% for the experiment with no insert; Table 2].

Six events did not fall into the recombination classes I through VII that were seen in the first experiment in which diploids lacked d(TG · AC)$_n$ sequences at *HIS3*. These events (class VIII tetrads, Fig. 2) were characterized by more than one recombination event occurring at the *HIS3* locus. The four spores from these tetrads were analyzed in detail by restriction enzyme and Southern blot analysis and will be discussed below.

Meiotic recombination in diploids heterozygous for d(TG · AC)$_n$ at *HIS3*. We next decided to examine the recombination properties of d(TG · AC)$_n$ sequences present on only one chromosome at *HIS3* (Table 1). A total of 981 tetrads were examined for segregation of the *LEU2*, *URA3*, and *HIS3* markers. Forty-six nonparental segregations for *LEU2*, *URA3*, or *HIS3* sequences were identified (Fig. 2, Table 1). Aside from class VI and VII events, there were 15 events that involved *LEU2* or *URA3* sequences. Two of these 15 nonparental segregations for *LEU2* or *URA3* were reciprocal exchanges in the *LEU2-URA3* interval. Since only two reciprocal exchanges in the *LEU2-URA3* interval were detected, we conclude that reciprocal exchange is enhanced maximally when d(TG · AC)$_n$ sequences are homozygous. A G test of independence (45) indicated that the frequency of reciprocal exchange in this experiment was not significantly different from the frequency in crosses with no d(TG · AC)$_n$ inserts on either chromosome ($P > 0.5$). However, genetic and restriction enzyme and Southern blot analysis of the 13 remaining nonparental segregations for *LEU2* or *URA3* revealed unusual segregation patterns.

Tetrads i and ii in Fig. 3A were isolated from a cross with 150 bp of d(TG · AC)$_n$ on the *URA3* chromatid and no insert on the *LEU2* chromatid. Both had a Leu$^-$ Ura$^-$ spore that was apparently the result of a deletion of the entire inserted plasmid by *HIS3-his3Δ1* intrachromatid recombination. Alternatively, these chromatids could result from gene conversion between sister or nonsister chromatids in which the event paired a contiguous *HIS3* gene from one chromatid with the two halves of *HIS3* genes from opposite sides of the insert on another molecule. In this case there would be a large heterology consisting of the entire plasmid insert. Tetrad i had a Leu$^+$ Ura$^+$ spore (spore a) that was probably the recipient of genetic information from one of the *LEU2* chromatids that ended up in tetrad i spores c and d. The chromatids in these latter spores, however, were recombinant for *HIS3* sequences. In all, this tetrad had four recombinant spores. We can conclude that at least three recombination events were required to generate the chromosomes found in this tetrad, although the event that gave rise to the recombinant *HIS3* arrangements in spores c and d may have been mitotic in origin. We have not analyzed the effect of d(TG · AC)$_n$ sequences on mitotic recombination.

The molecular basis for the nonparental segregation in tetrad ii was the deletion of the entire plasmid insert from *HIS3* in spore d. Southern blot analysis for the segregation of the d(TG · AC)$_n$ sequence revealed that spore b, which originally had d(TG · AC)$_n$ linked to *URA3*, now had no simple sequence DNA inserted at the *Bam*HI site (data not shown). In this tetrad the loss of d(TG · AC)$_n$ and the event giving rise to the Leu$^-$ Ura$^-$ spore were coincident but probably independent events (see Discussion). It thus appears that two rearrangements have occurred in the meiosis that generated this tetrad; however, we cannot rule out the possibility that the d(TG · AC)$_n$ insert was lost by a mitotic event.

Tetrads iii and iv in Fig. 3B were isolated from a cross in

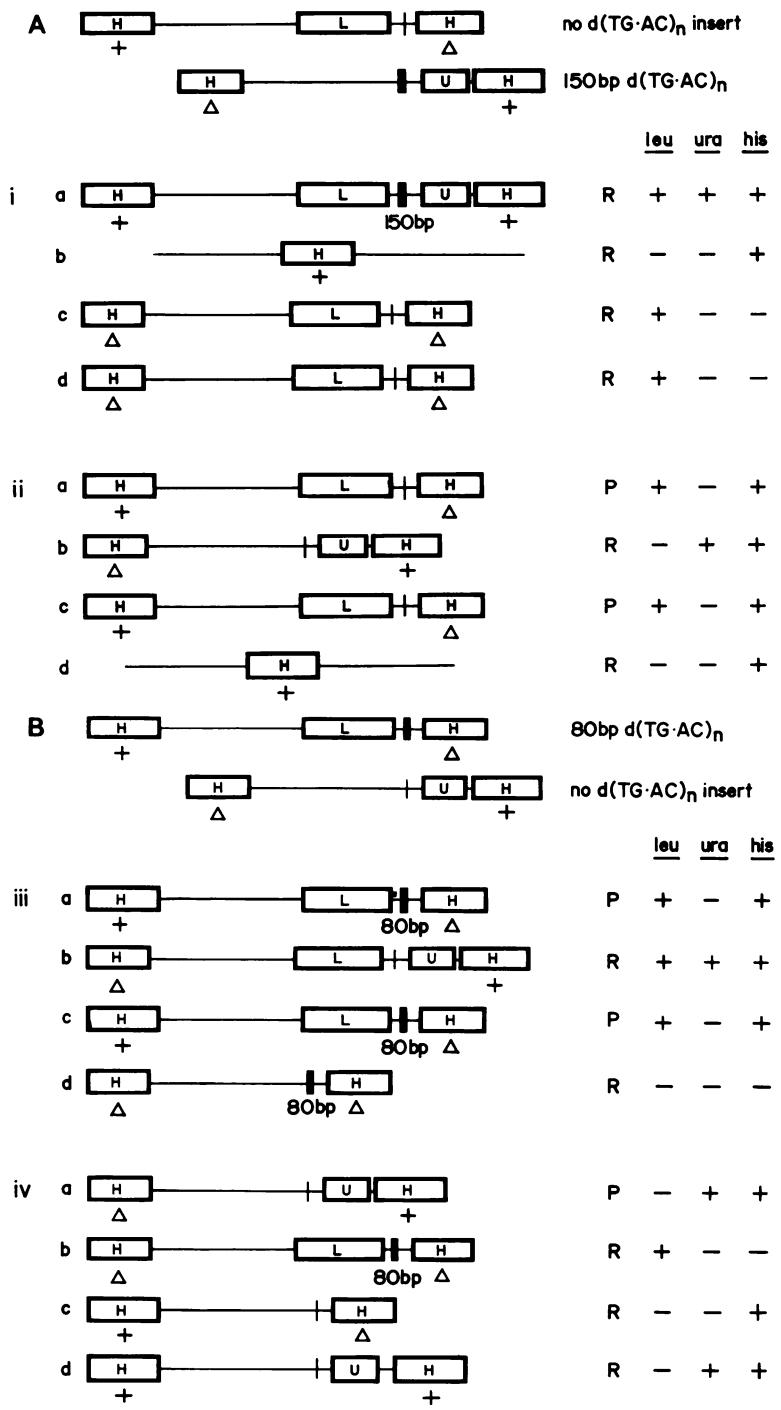

FIG. 3. Recombinant tetrads from crosses in which d(TG · AC)$_n$ is found on only one chromosome in the *LEU2-URA3* interval. (A) At the top is illustrated the physical organization of homologous *HIS3* loci where 150 bp of d(TG · AC)$_n$ is on the *URA3* chromatid. pHLΔV is the plasmid insert on the *LEU2* chromatid. Below are physical maps and phenotypes of the four *HIS3* loci from the four spores of two different unusual tetrads that are described in the text. (B) At the top is illustrated the organization of homologous *HIS3* loci when 80 bp of d(TG · AC)$_n$ is on the *LEU2* chromatid. pHUΔV is the plasmid insert on the *URA3* chromatid. Below are physical maps and phenotypes of the four *HIS3* loci from the four spores of two different unusual segregations that are described in the text. P, Parental configurations; R, recombinant configuration.

which an 80-bp insert of d(TG · AC)$_n$ was on the *LEU2* chromatid with no insert on the *URA3* chromatid. Tetrad iii exhibited 3+:1− segregation for *LEU2* and 1+:3− for *URA3*. Restriction enzyme and Southern blot analysis allowed us to construct the physical maps shown in Fig. 3B. The *URA3* and *LEU2* segregation pattern as well as the *HIS3* arrangements were not consistent with a single conversion-associated exchange event. A likely explanation is that

spores b and d are the products of two separate gene conversion events, both of which use a *LEU2* chromatid as the donor for the genetic information and have one endpoint of the converted segment in the *LEU2-URA3* interval. Tetrad iv in Fig. 3B had a total of three recombinant spores. Spores b and d appeared to be related by a reciprocal exchange in pBR322 or *HIS3* sequences to generate a recombinant *HIS3* arrangement with no alterations in the linkages of *LEU2* and *URA3* to their *HIS3* markers on the right. Spore c appeared to have been the recipient for a gene conversion event that replaced *LEU2* sequences with pBR322 to generate the *Leu*$^-$ chromatid. Two recombination events can account for the three recombinant chromosomes in this tetrad.

Tetrad v in Fig. 4A was also quite unusual. It was isolated as a gene conversion for the *LEU2* gene (3+:1−) from a cross with 80 bp of d(TG · AC)$_n$ on the *URA3* chromosome and no insert on the *LEU2* chromosome. Spores b and c appeared to be related by a crossover that generated the reciprocally recombined *HIS3* marker configuration. By restriction enzyme and Southern blot analysis (Fig. 4B, lane d) we noticed that DNA from the colony derived from spore d had a lower than expected intensity of the 2.0-kb *Eco*RI fragment that is diagnostic for the parental *LEU2* chromosome, and a new *Eco*RI fragment of about 2.1 kb was observed. The restriction map of the *HIS3* region in this DNA sample suggested that it came from a mixed population of cells that were either fully parental or parental except for a small insert in the *Bam*HI site (Fig. 4A). The original spore colony was sectored into quadrants, and three single colonies from each quadrant were examined by *Eco*RI digestion and Southern hybridization. Two of the four sectors were homogeneous for the 2.0-kb *Eco*RI fragment, and the remaining two sectors were homogeneous for the 2.1-kb *Eco*RI fragment. *Bam*HI digests of the DNA from all four sectors revealed that the two sectors with the larger *Eco*RI fragment had picked up the 80-bp stretch of d(TG · AC)$_n$ that is characteristic of the *URA3* chromatid. That exactly one-half of the cells contained the insert and that the two types of chromosome were found in cells organized in a defined spatial orientation strongly suggest that two parental strands in a heteroduplex intermediate segregated in the first mitotic division after meiosis. This phenomenon has been referred to as postmeiotic segregation (PMS) (11). We hypothesize that the *LEU2* chromosome in spore d entered its ascospore as a heteroduplex between pBR322 from the *LEU2* parent and the homologous pBR322 sequences with 80 bases of the repetitive DNA insert from the *URA3* parent. The heteroduplex would have an 80-base-long region of single-stranded DNA in which there is no homology to the parental DNA from the *LEU2* chromosome. Extensive genetic evidence suggests that if such heteroduplexes ever form in yeast, they do not show PMS (9, 11). However, the *ADE8* mutation *ade8-18* exhibits PMS at a high frequency and has recently been characterized as a 38-bp deletion in the *ADE8* structural gene (63). Thus, certain deletions and insertions may be capable of exhibiting PMS. We have demonstrated PMS for a d(TG · AC)$_n$ sequence by using strictly molecular data. It remains to be determined whether this observation is a reflection of the unusual genetic phenomena involving chromosomes carrying d(TG · AC)$_n$ sequences.

An alternative hypothesis is that the *LEU2* chromatid in spore d carried d(TG · AC)$_n$ sequences on both strands and that an unequal sister chromatid gene conversion event occurred between misaligned d(TG · AC)$_n$ tracts prior to the first mitosis after germination. Such an event would have to retain some d(TG · AC)$_n$ sequences on both chromatids. While we did not detect any inserted DNA in the *LEU2-URA3* interval in one-half of the spore colony from spore d, we cannot detect less than 10 bp inserted. However, if any remaining d(TG · AC)$_n$ sequences could be identified by cloning and squencing DNA from this region, it would eliminate our PMS hypothesis.

Tetrad vi in Fig. 5 was isolated from a cross in which the *LEU2* chromatid had 120 bp of d(TG · AC)$_n$ inserted at the *Bam*HI site and the *URA3* chromatid had 150 bp of this sequence (identical to the organization shown in Fig. 1A). Tetrad vii was isolated from a cross in which the *URA3* chromatid had no insert in the *Bam*HI site while the *LEU2* chromatid had 120 bp of d(TG · AC)$_n$ inserted there [identical to the organization shown in Fig. 3B except for the length of the d(TG · AC)$_n$ insert]. The marker segregation patterns in these two tetrads were remarkably similar. Both tetrads had a pair of chromatids that were related by a reciprocal exchange in the *LEU2-URA3* interval (spores b and c in tetrad vi and spores a and c in tetrad vii). Both reciprocal exchanges were associated with a gene conversion event in the *LEU2-URA3* interval that covered the *Bam*HI site. The most striking fact was that both tetrads carried a third recombinant chromosome that was the product of a gene conversion event. Spore a in tetrad vi had a chromosome that lacked *LEU2* or *URA3* and was the recipient in a recombination event in which pBR322 sequences replaced *LEU2* DNA. Spore d in tetrad vii had a chromosome that carried both *LEU2* and *URA3* and was the recipient in an event in which a *URA3* gene replaced pBR322 DNA between the *LEU2* gene and the right-hand *HIS3* marker.

Combined, the seven unusual segregations described above had a total of 20 recombinant chromosomes which required a total of at least 15 recombination events to account for their existence. Three tetrads isolated from a cross in which d(TG · AC)$_n$ sequences were present on both homologs at *HIS3* arose by an intrachromatid deletion or gene conversion event resulting in the loss of the entire integrated plasmid on one chromatid coupled to a second gene conversion event involving *LEU2* or *URA3* sequences (data not shown). These are remarkably similar to the tetrads illustrated in Fig. 3A, which were isolated from a cross in which d(TG · AC)$_n$ sequences were present on only one homolog at *HIS3*. Three additional tetrads (data not shown) that arose by multiple recombination events in the vicinity of the *HIS3* locus were the result of a reciprocal exchange between the duplicated *HIS3* genes coupled to a second recombination event, either a reciprocal exchange in the *LEU2-URA3* interval [one tetrad, d(TG · AC)$_n$ homozygous] or a gene conversion event involving *LEU2* or *URA3* [two tetrads, one from a cross with d(TG · AC)$_n$ homozygous and one from a cross with heterozygous d(TG · AC)$_n$ sequences]. In all, 13 tetrads in which multiple recombination events occurred in a single meiosis were observed among 2,641 tetrads from crosses with d(TG · AC)$_n$ sequences inserted at the *HIS3* locus (0.5%). In contrast, no tetrads reflecting the occurrence of multiple exchanges in a single meiosis were observed among 1,526 tetrads analyzed in crosses in which d(TG · AC)$_n$ sequences were absent from the plasmids integrated at *HIS3* (cross HLΔV × HUΔV, Table 1).

DISCUSSION

Three important conclusions can be drawn from the data presented here.

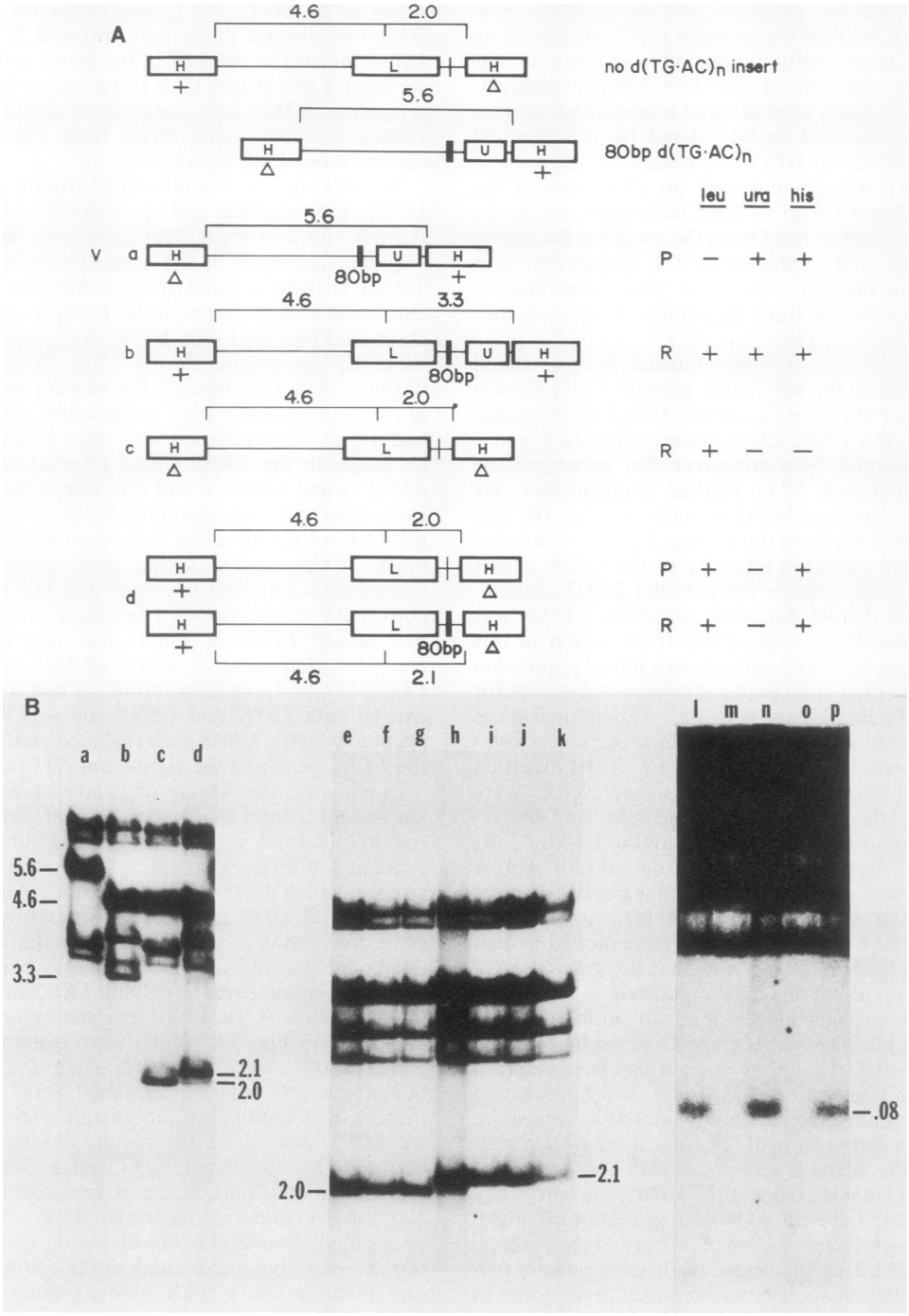

FIG. 4. (A) At the top is illustrated the physical organization of homologous *HIS3* loci where 80 bp of d(TG · AC)$_n$ is on the *URA3* chromatid. pHLΔV is the plasmid insert on the *LEU2* chromatid. Below are physical maps and phenotypes of the *HIS3* locus in the four spores of tetrad v. The maps of the two halves of the spore d colony are identical except for the 80-bp *Bam*HI insert of d(TG · AC)$_n$ in one-half of the colony (see text). The origin of the *Eco*RI restriction fragments labeled in panel B are shown by the brackets. P, Parental configuration; R, recombinant configuration. (B) Restriction enzyme and Southern blot analysis of DNA from recombinant tetrad v. Hybridization was with ^{32}P-labeled pHLΔV (58). Lanes a through d, *Eco*RI digests of DNA from spores a through d, respectively, of tetrad IV. Two bands are evident around 2.0 kb in lane d. Lanes e through g, *Eco*RI digests of DNA from three single colonies from the east sector of the spore d colony. Lane h, original spore d sample digested with *Eco*RI. Lanes i through k, *Eco*RI digests of DNA from three single colonies from the west sector of the spore d colony. Three single colonies from both the north and south sectors appeared identical to those from the east and west sectors, respectively. Lanes l through p, *Bam*HI digests of the parents and four sectors. The agarose gel was processed for transfer to Genatran 45 paper as described in the Materials and Methods section. Hybridization was with a ^{32}P-labeled d(TG · AC)$_n$ probe. Lane l, *URA3* haploid parent with 80 bp of d(TG · AC)$_n$ in the *Bam*HI site; lanes m through p, DNA from the north, south, east, and west sectors, respectively. Only two of the four sectors have the 80-bp repetitive DNA insert. Sizes of some of the hybridizing restriction fragments are indicated (in kilobase pairs).

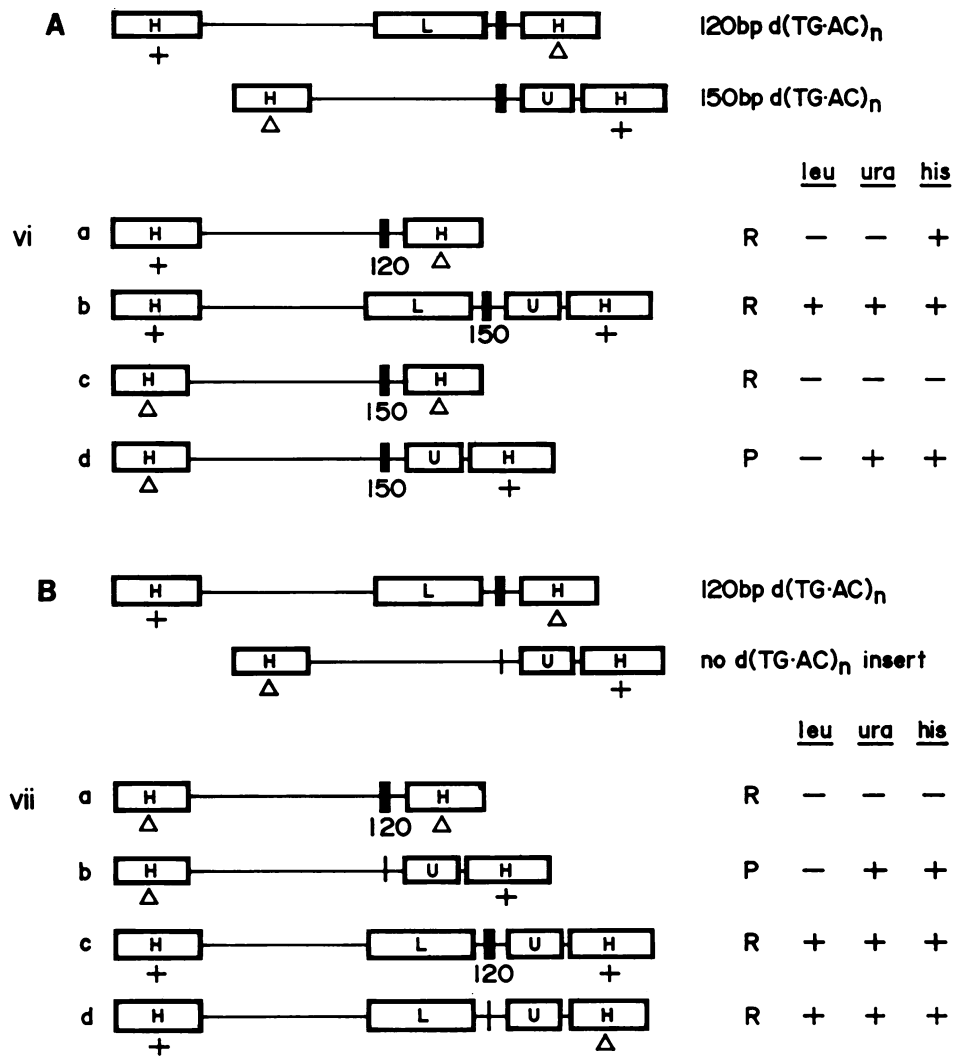

FIG. 5. Two class VIII tetrads. (A) At the top is illustrated the physical organization of homologous *HIS3* loci used in a cross in which 150 bp of d(TG · AC)$_n$ is on the *URA3* chromatid and 120 bp of d(TG · AC)$_n$ is on the *LEU2* chromatid. Below are restriction maps and phenotypes of the *HIS3* loci in the four spores from a class VIII tetrad isolated from this cross. (B) At the top is illustrated the physical organization of homologous *HIS3* loci used in a cross in which 120 bp of d(TG · AC)$_n$ is on the *LEU2* chromatid. pHUΔV is the plasmid insert on the *URA3* chromatid. Below are restriction maps and phenotypes of the *HIS3* loci in the four spores of a class VIII tetrad isolated from this cross. P, Parental configuration; R, recombinant configuration.

(i) When sequences of the alternating copolymer d(TG · AC)$_n$ were introduced into homologous positions at the *HIS3* locus of *S. cerevisiae*, meiotic crossing-over was enhanced sevenfold. With nothing inserted at the *Bam*HI site there were 0.62 kb of pBR322 DNA between the *LEU2* and *URA3* genes on homologous chromosomes. We inserted stretches of d(TG · AC)$_n$ into this interval that were on the order of 0.1 kb in length, increasing the total homology in the *LEU2-URA3* interval by about 15 to 20%. In doing this the genetic distance between *LEU2* and *URA3* was increased by a factor of seven. The addition of approximately 0.1 kb of d(TG · AC)$_n$ resulted in an increase of 0.44 cM to the interval between *LEU2* and *URA3* (Table 2). This corresponds to a specific increase of 4.4 cM per kilobase inserted, approximately 11 times the average of 0.4 cM/kb for regions of the yeast genome studied by others (35, 50). When normalized for the increase in the length of the *LEU2-URA3* interval, we observed a sixfold increase in the specific recombination efficiency of the DNA sequences between the *LEU2* and *URA3* genes [0.68 cM/kb in crosses with homozygous d(TG · AC)$_n$ sequences versus 0.11 cM/kb in crosses with no d(TG · AC)$_n$ inserts, Table 2]. Since the *LEU2-URA3* interval is approximately 20% longer in the crosses with d(TG · AC)$_n$ sequences inserted on both chromosomes, one might expect more reciprocal exchanges to occur simply because of more sequence homology. To correct for this we could reduce the number of reciprocal exchanges observed in this cross by 20% to account for the length difference. This calculation predicted that only 13.7 reciprocal exchanges would occur in the *LEU2-URA3* interval if it were the same length as the control interval. After statistical analysis of this corrected value we found that the difference in recombination frequency between crosses with d(TG · AC)$_n$ sequences homozygous and crosses with no insert in the *LEU2-URA3* interval was still highly significant (G test of independence yielded $P < 0.005$). We therefore consider it unlikely that the striking enhancement in reciprocal exchange was due to the relatively small increase in homologous DNA between

LEU2 and *URA3*, and therefore propose that the effect is due to the specific sequence introduced. This proposal is supported by our previous observation (58) that β-globin sequences of identical lengths are quite different in their ability to engage in meiotic crossing-over in yeast and that the length of homologous DNA is a poor indicator of how often the sequence is involved in reciprocal exchanges. Gene conversion events involving *LEU2* and *URA3* were equally frequent in the experiments with and without d(TG · AC)$_n$ in the *LEU2-URA3* interval (1.6 and 1.5%, respectively). However, since the d(TG · AC)$_n$ inserts on a pair of homologs were different in length and could be followed through meiosis, we had the opportunity to study gene conversion in the *LEU2-URA3* interval.

The 17 reciprocal exchanges that took place within the *LEU2-URA3* interval were examined for the segregation of d(TG · AC)$_n$ sequences (unpublished observations.) Ten of seventeen events segregated 3:1 for length [gene conversion spanning a d(TG · AC)$_n$ tract associated with crossing-over in the *LEU2-URA3* interval]. Three of the crossover events were associated with the generation of new length variants, although variation of less than 10 bp would be difficult to detect. These new length variants were associated with only one of the two recombinant chromatids, suggesting that they were the result of gene conversion between misaligned homologs. Thus, the majority of the reciprocal exchange events were associated with gene conversion. This strongly suggests that meiotic recombination events stimulated by d(TG · AC)$_n$ sequences are of a nature that is typical of classical meiotic exchange in yeast, since extensive genetic studies suggest that conversion-associated exchange is the hallmark of normal meiotic recombination in yeast (11). The stimulation of conversion-associated exchange is also characteristic of the only hot-spot for meiotic recombination in yeast that has been studied in detail, which was found near the *ARG4* gene (11).

(ii) The presence of the sequence d(TG · AC)$_n$ generated tetrads in which multiple events occurred in the vicinity of the repetitive sequence. When d(TG · AC)$_n$ was present on both chromatids in the *LEU2-URA3* interval, 3 of 51 nonparental segregations for *LEU2* or *URA3* had a chromatid with the entire plasmid insert deleted in addition to a gene conversion event between two homologous chromosomes (data not shown). A tetrad in which a similar event had occurred was isolated when d(TG · AC)$_n$ was present on only one homolog (tetrad i, Fig. 3A). Another tetrad with a plasmid insert entirely deleted (tetrad ii, Fig. 3A) lost the d(TG · AC)$_n$ insert on the sister of the deleted chromatid. Thus, 5 of the 68 nonparental segregations for *LEU2* or *URA3* reported in Fig. 2 and Table 1 [all crosses, with d(TG · AC)$_n$ sequences heterozygous or homozygous] contained intrachromatid deletions that were at least spatially linked to other recombination events. Tetrads iii and iv in Fig. 3B were isolated from a cross with d(TG · AC)$_n$ on only one chromosome. Both of these tetrads required at least two distinct meiotic recombination events within the inserted plasmid sequences to generate the rearranged structures. Likewise, tetrads vi and vii in Fig. 5 each required a reciprocal exchange and a separate gene conversion event to account for the three recombinant chromosomes in each tetrad. Finally, one tetrad, v in Fig. 4, exhibited PMS for an 80-base insertion of d(TG · AC)$_n$ in a spore clone that was apparently not involved in the conversion-associated exchange by which the tetrad was first recognized. Based on the physical organization of the DNA inserted at the *HIS3* locus in the spores of these and additional unusual segregations, it appears that the presence of d(TG · AC)$_n$ sequences in the vicinity of a region where recombination is occurring or has occurred makes it likely that a second event will occur. The presence of heterologous DNA in the vicinity of the *HIS3* locus did not affect the frequency of recombination in adjacent chromosomal intervals. The genetic distance between *ADE2* and *HIS3* remained constant at about 40 cM regardless of the heterozygosities introduced by *LEU2*, *URA3*, or d(TG · AC)$_n$ sequences (data not shown).

In summary, among 68 tetrads with nonparental segregations for *LEU2* or *URA3*, 13 particularly unusual segregations were observed. Excluding class VII unequal crossovers, about one-half (7 of 15) of the nonparental segregations for *LEU2* or *URA3* isolated from crosses with d(TG · AC)$_n$ sequences on only one chromatid at the *HIS3* locus were multiply recombined in this region. In contrast, among a total of 75 tetrads with nonparental segregations for *LEU2* or *URA3* that were studied in the analysis of the recombination properties of human β-globin gene fragments in yeast (58) and 30 additional tetrads analyzed in this paper in which no d(TG · AC)$_n$ sequences were present on either chromosome, no examples of multiple exchanges within inserts at the *HIS3* locus were identified.

Tetrads carrying three and four chromosomes recombinant for a single interval have been described by others (11, 17) and have been explained by two independent events that involve all four chromatids within the genetic interval monitored. The fact that they were observed could be attributed to the high frequency of single events occurring in the regions studied. In fact, both groups observed these multiple exchange tetrads at approximately 10% of the expected frequencies, suggesting some interference in pairing of all four chromatids in one genetic interval during meiosis.

In the absence of interference, the frequency at which we observe the intrachromatid deletion coupled with a gene conversion event might be expected to be equal to the product of the frequencies at which we see each event occurring individually. We saw events resulting in the loss of the entire plasmid insert in crosses with d(TG · AC)$_n$ sequences on one or both chromosomes at a frequency of 0.003 (data not shown). Gene conversions occurred at a frequency of 0.01 (Table 2), so the two events should co-occur at a frequency of $0.003 \times 0.01 = 3 \times 10^{-5}$. Among 2,641 tetrads, we would expect to find 0.08 tetrads of this type. In fact, we found five, or 60 times the expected number, a significant excess (G test for goodness of fit [45] yielded $P < 0.005$). Following the same arguments for the tetrads shown in Fig. 5, in which a reciprocal exchange in the *LEU2-URA3* interval was associated with a gene conversion event, we found a significant (ninefold) excess over the number expected ($P < 0.005$). The existence of most of the multiple exchange tetrads in these experiments cannot be explained simply by the random occurrence of two independent events.

It should be emphasized that in our experiments with d(TG · AC)$_n$ sequences inserted on both homologs there was always a small heterology present in the *LEU2-URA3* interval since the two tracts of d(TG · AC)$_n$ were not identical in length (80 by 150 bp or 120 by 150 bp). We cannot rule out the possibility that such heterologies are responsible for the unusual genetic properties that we attribute to the d(TG · AC)$_n$ inserts. However, such effects would presumably be specific to heterologies involving d(TG · AC)$_n$ tracts since neither the 2.2-kb *LEU2* heterology or the 1.1-kb *URA3* heterology showed any unusual properties in the crosses with no d(TG · AC)$_n$ sequences on either homolog.

In addition, the 200-bp *his3Δ1* deletion was heterozygous on both sides of the plasmid insert and did not appear to generate a high frequency of tetrads with multiple recombination events in the absence of d(TG · AC)$_n$ tracts. Any effect that these heterologies may have on recombination frequency would be controlled for in the cross in which no d(TG · AC)$_n$ sequence was present on either homolog.

(iii) The enhancement of reciprocal exchange was maximal when d(TG · AC)$_n$ sequences were homozygous. The slight increase in reciprocal exchange frequency in the *LEU2-URA3* interval observed when d(TG · AC)$_n$ sequences were heterozygous compared with that in crosses that lacked these sequences was not statistically significant ($P > 0.5$). However, the data presented suggest that particular unusual tetrad classes are shared between the crosses in which d(TG · AC)$_n$ sequences were homozygous and heterozygous at the *HIS3* locus and were not seen when d(TG · AC)$_n$ fragments were absent at *HIS3*. If d(TG · AC)$_n$ sequences initiate genetic exchange by enzymatic recognition and nicking of these sequences, then a chromatid with d(TG · AC)$_n$ sequences might be expected to be the preferred donor or recipient of information in gene conversion events as predicted by current models for recombination (23, 55). The marker segregation patterns of 15 gene conversion events isolated from crosses with d(TG · AC)$_n$ sequences on only one homolog at the *HIS3* locus can be analyzed to determine which chromatids were the donors or recipients in the genetic exchange. Six of the conversion tetrads were class II, III, or IV events, with each tetrad representing a single event (Fig. 2). Nine of the conversion events were found in the seven tetrads in which multiple exchanges had occurred (class VIII, tetrads v [Fig. 4] and iii [Fig. 3B], have two converted chromosomes). In these 15 events, 8 used the d(TG · AC)$_n$ chromatid as the recipient of information in the genetic exchange and seven used the d(TG · AC)$_n$ chromatid as the donor of information (data not shown). There was no bias in the use of d(TG · AC)$_n$ chromatids as donors or recipients in genetic exchange.

Using a different approach to the analysis of the ability of d(TG · AC)$_n$ sequences to promote recombination, Stringer measured the frequency of intramolecular deletions between nontandem duplications of d(TG · AC)$_n$ inserted into simian virus 40 (SV40) genomes (52). He reported that on a per-nucleotide basis, recombination between duplicated d(TG · AC)$_n$ elements was at the most eight times more frequent than exchanges in nontandem duplications of more complex sequences, a result consistent with the data reported here on the enhancement of meiotic crossing-over in yeast chromosomes.

The d(TG · AC)$_n$ repetitive sequence has recently attracted a great deal of attention since it has been shown to adopt a Z-DNA conformation in supercoiled plasmids (14, 28). In general, DNA polymers of alternating purine and pyrimidine residues, with the exception of poly(dAT) · poly(dTA), have been shown to be capable of forming Z-DNA when chemically modified, under high-salt conditions or when present in supercoiled plasmids (3, 14, 28, 41, 42, 59, 62, 64). The possibility of a unique genetic role for d(TG · AC)$_n$ and other sequences capable of adopting the left-handed helical conformation has been raised in several reports suggesting eucaryotic gene conversion and recombination (6, 10, 21, 31, 43, 51).

Even more recently it has been shown that the transition region where B-DNA becomes Z-DNA is partially unwound and is sensitive to S1 nuclease (30, 41). These reports have prompted speculation that the single-stranded nature of the Z-DNA conformation may be a substrate for recombination enzymes that generate the free ends to invade homologous duplex DNA (15, 28, 33). We have previously shown that the removal of the longest potential Z-DNA-forming region from a human β-globin locus restriction fragment which recombines at a high frequency in yeast had no effect on the frequency of reciprocal exchange (58). Only two of the eight potential B-Z boundaries were removed from this fragment, and we may not have been able to detect a small drop in recombination frequency. Thus, the total length of the repetitive element may not be as important as the number of repetitive elements, and thus the number of B-Z junctions, within a segment of DNA.

Our observations suggest two additional possible mechanisms by which this simple repetitive sequence influences crossing-over in our system. First, it is possible that any simple repetitive sequence can promote exchange simply by increasing the likelihood that a pairing partner will encounter homologous sequences. Crossing-over between misaligned DNA is expected to generate length variation in the recombinant products (44, 46, 56, 57). In the experiments of Stringer discussed earlier, most or all of the recombinant SV40 genomes that were generated by recombination between arrays of d(TG · AC)$_n$ sequences displayed length heterogeneity. Only 3 of the 17 reciprocal exchanges reported here were associated with the generation of new length variants (see Results), although variation of less than 10 bp would have been difficult to detect. If the mechanism by which d(TG · AC)$_n$ sequences promote recombination requires crossing-over within the repetitive DNA, then misalignment is usually not associated with these events in yeast meiosis. However, recombination in contiguous regions may be enhanced by the ability of these and other simple sequences to misalign. One would obviously like to study the recombination properties of other alternating copolymeric sequences that differ in their ability to form Z-DNA.

The second possibility is that d(TG · AC)$_n$ promotes crossing-over by presenting the meiotic recombination apparatus with a unique and recognizable DNA sequence or conformation. This structure could be a signal to initiate genetic exchange nearby, or it could be a place where homologous DNA segments are drawn together at synapsis as the initial step for the meiotic recombination apparatus to recognize homologous chromosomes. If initiation of genetic exchange is directed to occur near d(TG · AC)$_n$ inserts, then we might predict that within a small region of DNA more than one of the four chromatids that are present when meiotic recombination takes place may be initiating an event at the same time. Such a model could account for the 13 multiply recombinant tetrads that we have described.

The idea that d(TG · AC)$_n$ sequences promote synapsis is supported by the recent observations of Holloman and colleagues (19, 20) which demonstrate that the Rec1 protein from *Ustilago maydis* promotes homologous pairing of DNA molecules and binds much more strongly to Z-DNA than to B-DNA. In addition, paranemic joints, the initial synaptic structures formed between homologous DNA duplexes that are topologically prevented from interwinding, were shown to contain Z-DNA, as judged by anti-Z-DNA antibody binding. Thus, there is strong biochemical evidence for the involvement of Z-DNA and a Z-DNA-binding protein in genetic recombination in lower eucaryotes. The absence of a bias in d(TG · AC)$_n$ chromatids as donors or recipients in genetic exchange and the lack of an enhancement of crossing-over when d(TG · AC)$_n$ sequences are heterozygous support a model in which these sequences are not substrates

for an endonuclease that nicks or cuts DNA to promote a recombination event, but instead promote recombination by promoting an interaction between two chromosomes carrying $d(TG \cdot AC)_n$ in homologous positions.

The most important question raised here is that of the role of this highly repetitive element in the evolution of eucaryotic genomes. If $d(TG \cdot AC)_n$ can promote homologous meiotic recombination in yeast, as our data suggest, then do the 50 to 100 copies of this repetitive sequence that are normally found in the yeast genome serve similar functions? Alternatively, recombination may be an activity that $d(TG \cdot AC)_n$ sequences participate in by virtue of their unique DNA structure, but this activity may be unrelated to some other function that the sequence performs within eucaryotic cells.

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