Position Effects in Ectopic and Allelic Mitotic Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

We have examined the role that genomic location plays in mitotic intragenic recombination. Mutant alleles of the *LEU2* gene were inserted at five locations in the yeast genome. Diploid and haploid strains containing various combinations of these inserts were used to examine both allelic recombination (between sequences at the same position on parental homologs) and ectopic recombination (between sequences at nonallelic locations). Chromosomal location had little effect on mitotic allelic recombination. The rate of recombination to *LEU2* at five different loci varied less than threefold. This finding contrasts with previous observations of strong position effects in meiosis; frequencies of meiotic recombination at the same five loci differ by about a factor of forty. Mitotic recombination between dispersed copies of *leu2* displayed strong position effects. Copies of *leu2* located approximately 20 kb apart on the same chromosome recombined at rates 6–13-fold higher than those observed for allelic copies of *leu2*. *leu2* sequences located on nonhomologous chromosomes or at distant loci on the same chromosome recombined at rates similar to those observed for allelic copies. We suggest that, during mitosis, parental homologs interact with each other no more frequently than do nonhomologous chromosomes.

THERE is now considerable evidence that genetic exchange occurs between repeated sequences that are present at different locations in the eucaryotic genome. Various important evolutionary roles have been ascribed to such recombination events, including the maintenance of sequence homogeneity in multigene families and the formation of duplications, deletions, and other chromosome rearrangements (EDELMAN and GALLY 1970; TARTOF 1973; SMITH 1973; HOOD, CAMPBELL and ELGIN 1975; WALSH 1987). Examples of both kinds of events in the genomes of metazoan organisms are numerous (KOURILSKY 1986). However, the absence of quantitative information on the rates at which such events occur in these organisms confounds assessment of the importance of recombination between repeated sequences in evolutionary terms. Recombination between dispersed homologous sequences (hereafter referred to as ectopic recombination) has been most extensively characterized in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and it is from these two organisms that most quantitative measurements derive (for reviews, see KLEIN 1988; PETES and HILL 1988).

Studies using gene duplications created by integrative transformation have revealed that ectopic recombination can occur frequently during meiosis in *S. cerevisiae*, often at levels approaching that of recombination between sequences at the same location on parental homologs (hereafter referred to as allelic recombination) (KLEIN and PETES 1981; BORTS *et al.* 1984; JINKS-ROBERTSON and PETES 1985; JINKS-ROBERTSON and PETES 1986; LICHTEN, BORTS and HABER 1987). These same studies indicated that the rate at which a sequence recombines during meiosis is profoundly affected by its genomic location. Frequencies of allelic meiotic recombination between heteroallelic *leu2* sequences at five different genomic locations varied almost 40-fold (LICHTEN, BORTS and HABER 1987). Similar position effects were observed in frequencies of ectopic meiotic recombination.

The observation of strong position effects in meiotic recombination raises the question of whether similar effects are active in mitosis. Numerous studies have shown that ectopic mitotic recombination between both naturally occurring and artificially created sets of repeated sequences occurs at rates similar to those observed for allelic recombination (SCHERER and DAVIS 1980; JACKSON and FINK 1981; MIKUS and PETES 1982; SUGAWARA and SZOSTAK 1983; JINKS-ROBERTSON and PETES 1986; ROTHSTEIN, HELMS and ROSENBERG 1987; KUPIEC and PETES 1988). However, most of these studies used haploid strains, thus precluding a direct examination of the relationship between allelic and ectopic recombination. In particular,

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TABLE 1

Yeast strains

Haploid strains

H326 *MAT***a** *leu2-R ura3 ade1 his6 met13-4 lys2-d*
H330 *MATα leu2-K ura3 ade1 trp5 met13-2 lys2-c can1 cyh2*
H378 *MAT***a** *leu2-R ura3::URA3-leu2-K ade1 his6 met13-4 lys2-d*
H379 *MMTαleu2-K ura3::URA3-leu2-R ade1 trp5 met13-2 lys2-c can1 cyh2*
H386 *MATα leu2-K his4::URA3-leu2-R ura3 ade1 trp5 met13-2 lys2-c can1 cyh2*
H395 *MAT***a** *leu2-R his4::URA3-leu2-K ura3 ade1 met13-4 lys2-d cyh2*
H495[a] *MATα leu2-K HML::URA3-leu2-R ura3 trp5 met13-2 lys2-c cyh2 can1*
H497[a] *MAT***a** *leu2-R HML::URA3-leu2-K ura3 his6 met13-4 lys2*
H587 *MAT***a***::URA3-leu2-K leu2-R ura3 ade1 his6 met13-4 lys2-d*
H589 *MATα::URA3-leu2-R leu2-K ura3 ade1 trp5 met13-2 lys2-c can1 cyh2*

Diploid strains[b]

RHB422 *leu2-K MATα ura3 can1 met13-2 lys2-c HIS6 trp5 cyh2* / *leu2-R MAT***a** *ura3 CAN1 met13-4 lys2-d his6 TRP5 CYH2*

MJL161[a] *leu2-K,R MATα::URA3-leu2-K ura3 can1 met13-2 lys2 HIS6 cyh2* / *leu2-K,R MAT***a***::URA3-leu2-R ura3 CAN1 met13-4 lys2 his6 CYH2*

MJL182 *leu2-K,R MATα ura3::URA3-leu2-K CAN1 met13-2 lys2 trp5 cyh2* / *leu2-K,R MAT***a** *ura3::URA3-leu2-R can1 met13-4 lys2 TRP5 CYH2*

MJL189 *his4::URA3-leu2-R leu2-K,R MATα ura3 can1 met13-2 lys2 trp5 cyh2* / *his4::URA3-leu2-K leu2-K,R MAT***a** *ura3 CAN1 met13-4 lys2 TRP5 cyh2*

MJL227 *HML::URA3-leu2-R leu2-K,R MATα ura3 CAN1 met13-2 lys2 ADE1 trp5 cyh2* / *HML::URA3-leu2-K leu2-K,R MAT***a** *ura3 can1 met13-4 lys2 ade1 TRP5 CYH2*

MJL225 *leu2-K MATα ura3::URA3-leu2-R can1 met13-2 lys2-c trp5* / *leu2-K MAT***a** *ura3 CAN1 met13-4 lys2-c TRP5*

MJL224 *leu2-R MATα ura3 met13-2 lys2-d HIS6 trp5* / *leu2-R MAT***a** *ura3::URA3-leu2-K met13-4 lys2-d his6 TRP5*

MJL178 *his4::URA3-leu2-R leu2-K MATα ura3 can1 met13-2 lys2-c trp5 cyh2* / *HIS4 leu2-K MAT***a** *ura3 CAN1 met13-4 lys2-c TRP5 CYH2*

MJL176 *HIS4 leu2-R MATα ura3 can1 met13-2 lys2 trp5 cyh2* / *his4::URA3-leu2-K leu2-R MAT***a** *ura3 CAN1 met13-4 lys2-d TRP5 cyh2*

MJL330, MJL331 *HIS4 leu2-R MATα ura3 can1 met13-2 lys2-d trp5 cyh2* / *his4::URA3-leu2-K leu2-K,R MAT***a** *ura3 CAN1 met13-4 lys2-c TRP5 cyh2*

MJL192 *his4::URA3-leu2-R leu2-K,R MATα ura3 met13-2 lys2-c trp5 cyh2* / *HIS4 leu2-K MAT***a** *ura3 met13-4 lys2-c TRP5 CYH2*

MJL195 *HIS4 leu2-K,R MATα ura3 met13-2 lys2 trp5 cyh2* / *his4::URA3-leu2-K leu2-R MAT***a** *ura3 met13-4 lys2-d TRP5 cyh2*

MJL196 *his4::URA3-leu2-R leu2-K MATα ura3 can1 met13-2 lys2-c trp5 cyh2* / *HIS4 leu2-K,R MAT***a** *ura3 can1 met13-4 lys2 TRP5 CYH2*

MJL237[a] *HML::URA3-leu2-R leu2-K MATα ura3 can1 met13-2 lys2-c trp5 ADE1 cyh2* / *HML leu2-K MAT***a** *ura3 CAN1 met13-4 lys2-c TRP5 ade1 CYH2*

MJL243[a] *HML leu2-R MATα ura3 met13-2 lys2-d HIS6 trp5 ade1* / *HML::URA3-leu2-K leu2-R MAT***a** *ura3 met13-4 lys2 his6 TRP5 ADE1*

MJL322, MJL323 *leu2-K MATα::URA3-leu2-R ura3 CAN1 met13-2 lys2-c trp5 CYH2* / *leu2-K MAT***a** *ura3 can1 met13-4 lys2-c TRP5 cyh2*

MJL324, MJL325 *leu2-R MATα ura3 met13-2 lys2-d HIS6 trp5* / *leu2-R MAT***a***::URA3-leu2-K ura3 met13-4 lys2-d his6 TRP5*

[a] Strains which are not entirely derived from H326 and/or H330.
[b] Unless otherwise indicated, all diploid yeast strains are also homozygous for the *ade1* marker. All strains were either *lys2-c* or *lys2-d*; loci where the particular allele is unknown are denoted as *lys2*.

little is known about the effect that genomic location might have on the ability of sequences to participate in mitotic recombination.

In this paper, we demonstrate that the marked position effects observed for meiotic allelic recombination are absent in mitosis, and report evidence consistent with the suggestion that a major determinant of the rate of mitotic ectopic recombination between dispersed sequences is the physical distance separating them.

MATERIALS AND METHODS

Strains: Genotypes of the yeast strains used in this work are presented in Table 1. Strains which contain *leu2-K* or *leu2-R* inserted at loci other than the normal *LEU2* locus (designated as X::*leu2*) contain a pBR322-derived plasmid flanked by a duplication of the indicated locus. This plasmid contains a 1.2-kb *URA3* fragment at the *Hin*dIII site and a 2.2-kb *LEU2* fragment (bearing either the *leu2-K* or *leu2-R* mutations) at the *Sal*I site of pBR322. The *leu2-K* and *leu2-R* mutations destroy a *Kpn*I and *Eco*RI site, respectively. The genomic positions of the four insert loci (*HIS4*, *HML*, *MAT*, and *URA3*) and the structure of these inserts are illustrated in Figure 1. Further details of plasmid structure, plasmid construction, and strain construction are given in LICHTEN, BORTS and HABER (1987).

The yeast strains used in this work are closely related, although not isogenic. All are derived from strains which have been backcrossed extensively to the Y55 background. In addition, with a few exceptions, all are derived from transformants of the haploid parents of RHB422 (H326 and H330), from meiotic segregants of RHB422, or from backcrosses of H326, H330, and meiotic segregants of RHB422 with Y55.

Determination of mitotic recombination rates: Colonies (10–20) of equal size (1.5–2 mm in diameter), freshly grown on YPD plates, were individually suspended in 0.5 ml of water and vortexed vigorously. Samples were diluted in water and plated on YPD (to measure total cell number) and on synthetic complete plates lacking either leucine or methionine (to measure *LEU2* or *MET13* recombinants). Rates of recombination were estimated using the median method of LEA and COULSON (1948). Pilot experiments indicated that the use of freshly grown single colonies yielded rates of mitotic recombination similar to those obtained using log phase liquid cultures (data not shown).

The rate of recombination to *MET13* [r(*MET13*)] was measured as a way of estimating the effects that nonuniformity in genetic background might have on measurements of *LEU2* recombination. The mean r(*MET13*) for all diploids presented in this report was $2.6 \times 10^{-7} \pm 8.7 \times 10^{-8}$ recombinants/generation. Duplicate measurements of r(*MET13*) were made in eight different strains. The average deviation from the mean for these duplicate measurements was 6.4×10^{-8} recombinants/generation. It is therefore unlikely that differences in strain background made a major contribution to the differences in recombination rates observed.

Analysis of mitotic recombinants: *LEU2* recombinants from several diploids were checked for heterozygosity at *MAT*, *MET13* and *CAN1*, to ensure that the recombinants recovered were not the products of meiotic recombination. Of 240 recombinants analyzed, 236 were heterozygous at all three loci.

Recombinants between *leu2* and *his4*::*leu2*: Freshly grown single colonies were replica-plated to synthetic complete plates lacking leucine. Leu$^+$ papillae were picked, patched on YPD, and analyzed. To ensure independence and purity, papillae were picked only from colony replicas which contained a single *LEU2* papilla. Structures of *LEU2* recombinants isolated from haploid strains (H386 and H395) were determined by a combination of genetic and Southern analyses as previously described (LICHTEN, BORTS and HABER 1987).

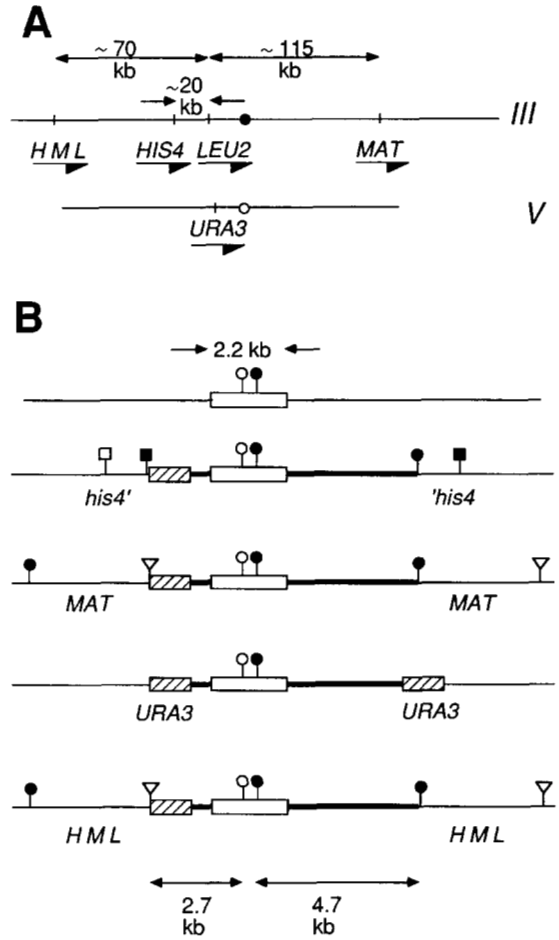

FIGURE 1.— A, Loci used in this study. Physical distances on chromosome *III* are from NEWLON *et al.* (1985). Since distances along chromosome *III* vary significantly among different strain backgrounds, these distances should be taken as approximations only. Arrows indicate the direction of transcription of *LEU2* at each location. B, Structure of the *leu2* plasmid inserts. Open rectangles indicate the 2.2-kb *LEU2 Xho*I-*Sal*I fragment; cross-hatched rectangles, the 1.2-kb *URA3 Hin*dIII fragment; thick lines, pBR32I sequences. Relevant restriction sites are as follows: (○) *Kpn*I; (●) *Eco*RI; (□) *Cla*I; (■) *Pvu*II; and (▽) *Hin*dIII. The four *leu2* inserts share 2.2 kb of homology with the normal *LEU2* locus. In addition, these four insert loci share 2.7 kb of homology to the left of the 0.4-kb *leu2-K*–*leu2-R* interval, and 4.7 kb of homology to the right. Integration at *HIS4* produces two terminally deleted copies of the *HIS4* gene. These are referred to as *his4'* and *'his4* in this figure and the text.

Media: Complex and synthetic media were prepared as described by SHERMAN, FINK and HICKS (1986).

RESULTS

Genomic location does not markedly affect allelic recombination in mitosis: A series of diploid strains

TABLE 2

Allelic mitotic recombination

Diploid name	Location of mutant alleles	r(*LEU2*)[a] × 10^7	Normalized[b] r(*LEU2*)
RHB422	*LEU2*	1.2	1.0
MJL189	*HIS4*	1.0	0.8
MJL227	*HML*	1.9	1.6
MJL161	*MAT*	0.71	0.6
MJL182	*URA3*	1.4	1.2

[a] Rate of recombination (recombinants per generation).
[b] Recombination rates were normalized to that observed for RHB422.

were constructed which contained two *leu2* mutations, *leu2-K* and *leu2-R*, inserted at identical positions on the two parental homologs. Five diploid strains were used, each with *leu2* at a different location: at *LEU2*, at *HIS4*, at *HML*, at *MAT* and at *URA3*. Strains which were marked with *leu2-K* and *leu2-R* at an insert locus (*HIS4*, *HML*, *MAT*, and *URA3*) contained these *leu2* alleles in a 7.8-kb plasmid insert (see Figure 1); in these strains, the normal *LEU2* locus contained the double mutation *leu2-K,R*.

Rates of allelic recombination to *LEU2* are presented in Table 2. Rates of allelic recombination varied less than three-fold, with a mean of 1.2 x 10^{-7} recombinants/generation. This value is identical to that observed for the diploid strain RHB422, which contained *leu2-K* and *leu2-R* at the normal *LEU2* locus. It is likely, in fact, that none of the values in Table 2 reflect significant differences in the actual rate of recombination to *LEU2* at the five different loci, as the estimated standard deviation for these values (4.5 × 10^{-8} recombinants/generation, or 38%) is similar to that observed for mitotic recombination to *MET13* (34%; see MATERIALS AND METHODS). This result stands in marked contrast to the strong position-dependence observed for meiotic allelic recombination. Frequencies of allelic meiotic recombination measured using *leu2* inserts at the same five loci varied almost 40-fold (LICHTEN, BORTS and HABER 1987).

Ectopic recombination is as frequent as allelic recombination: Ectopic recombination was examined using diploid and haploid yeast strains in which *leu2-K* and *leu2-R* were at different locations. In all cases, the normal *LEU2* locus was marked with either *leu2-K* or *leu2-R*; the other *leu2* heteroallele was inserted elsewhere. The insert loci used were the same as those used to examine allelic recombination: *HIS4*, about 20 kb centromere-distal to *LEU2* on the left arm of chromosome *III*; *HML*, about 70 kb centromere-distal to *LEU2* on the left arm of chromosome *III*; *MAT*, about 100 kb from *LEU2* on the opposite arm of chromosome *III*; and *URA3*, on chromosome *V*. Rates of recombination to *LEU2* in these strains are presented in Table 3. *LEU2* recombinants were recovered from diploids at rates ranging from 5.6 × 10^{-8} to 1.6 × 10^{-6} recombinants/generation (Table 3A). The highest rates were observed in the case of recombination between *leu2* at its normal locus and *his4::leu2*, where the two interacting sequences were separated by about 20 kb; the lowest rates were obtained in diploids where the two copies of *leu2* were on opposite arms of the same chromosome (*LEU2* and *MAT::leu2*) or on nonhomologous chromosomes (*LEU2* and *URA3::leu2*). These latter rates (1.5 × 10^{-7} and 5.6 × 10^{-8} recombinants/generation for *leu2-R* × *URA3::leu2-K* and *leu2-K* × *URA3::leu2-R* respectively) are indistinguishable from those observed in allelic diploids, where *leu2-K* and *leu2-R* were at the same location on the two parental homologs. Slightly lower rates of recombination were observed in haploid strains (Table 3B), but the overall pattern was preserved.

This observation, that ectopic recombination between nonhomologous chromosomes occurs as frequently as does allelic recombination, indicates that sequences at allelic positions on homologous chromosomes interact with one another during mitosis no more frequently that do sequences on nonhomologous chromosomes.

Intra- versus interchromosomal recombination: A notable feature of the data presented in Table 3 is an apparent proximity effect. *leu2* sequences at loci separated by about 20 kb (*his4::leu2* and the *leu2* at its normal locus) recombined about ten times more frequently than did *leu2* sequences on separate chromosomes (between *leu2* sequences at allelic positions or between *URA3::leu2* and *leu2* at its normal locus). *leu2* sequences at loci separated by ca. 70 kb on the same arm of chromosome *III* (*HML::leu2* and the normal *LEU2* locus) recombined at a lower rate, but one which was still substantially greater than that observed for *leu2* sequences on different chromosomes. These results can be explained by suggesting that sequences which are located at nearby loci on the same homolog recombine more frequently that do well separated sequences (*i.e.*, sequences on different homologs, on nohomologus chromosomes, or at distant loci on the same chromosome).

Support for this suggestion was provided using the diploid strains illustrated in Table 4. One set of diploids (MJL191, MJL330 and MJL331) contained, on one copy of chromosome *III*, *leu2-K* or *leu2-R* inserted at *HIS4* and the *leu2-K,R* double mutation at its normal locus; the normal *LEU2* locus on the other copy of chromosome *III* was marked with the opposite heteroallele (either *leu2-R* or *leu2-K*). Since exchange involving *leu2-K,R* and either of the two single mutants would not produce *LEU2* recombinants, the leucine prototrophs produced by this diploid must be the products of interchromosomal recombination. *LEU2* recombinants were recovered at a low rate from these diploids (2–3 × 10^{-8} recombinants/generation).

TABLE 3

Ectopic mitotic recombination

Structure	Strain name	r(*LEU2*)[a] × 10^7	Normalized[b] r(*LEU2*)
A. Diploids			
-his4-URA3-leu2-K-his4———— *leu2-R-* *---------HIS4*———— *leu2-R-*	MJL176	16	13
-his4-URA3-leu2-R-his4———— *leu2-K-* *---------HIS4*———— *leu2-K-*	MJL178	7.5	6.3
-HMLα-URA3-leu2-K-HMLα----------leu2-R- *---------HMLα----------leu2-R-*	MJL243	5.4	4.5
-HMLα-URA3-leu2-R-HMLα----------leu2-K- *---------HMLα----------leu2-K-*	MJL237	2.8	2.3
*-leu2-R--------MAT***a***-URA3-leu2-K-MAT***a***-* *-leu2-R----------MATα---------*	MJL324,325[c]	1.1	0.9
-leu2-K--------MATα-URA3-leu2-R-MATα- *-leu2-K----------MAT***a***---------*	MJL322,323[c]	0.75	0.6
---leu2-R--- --URA3-leu2-K-URA3-- *---leu2-R--- --------ura3-------*	MJL224	1.5	1.3
---leu2-K--- --URA3-leu2-R-URA3-- *---leu2-K--- --------ura3-------*	MJL225	0.56	0.5
B. Haploids			
-his4-URA3-leu2-K-his4-------leu2-R-	H395	11	9.2
-his4-URA3-leu2-R-his4-------leu2-K-	H386	5.1	4.3
-HMLα-URA3-leu2-K-HMLα--------leu2-R-	H497	4.2	3.5
-HMLα-URA3-leu2-R-HMLα--------leu2-K-	H495	1.9	1.6
*-leu2-R--------MAT***a***-URA3-leu2-K-MAT***a***-*	H587	1.7	1.4
-leu2-K--------MATα-URA3-leu2-R-MATα-	H589	0.38	0.3
---leu2-R------URA3-leu2-K-URA3--	H378	0.46	0.4
---leu2-K------URA3-leu2-R-URA3--	H379	0.4	0.3

[a] Rate of recombination (recombinants per generation).
[b] Recombination rates were normalized to that observed for RHB422 (Table 2).
[c] Results obtained with this pair of isogenic diploids were pooled to calculate recombination rates.

TABLE 4

Chromosome specificity

Structure	Strain name	r(*LEU2*)[a] × 10^7	Normalized[b] r(*LEU2*)
A. Heteroalleles in *trans*			
-his4-URA3-leu2-K-his4-----leu2-K,R- *---------HIS4----------leu2-R---*	MJL330,331[c]	0.2	0.2
-his4-URA3-leu2-R-his4-----leu2-K,R- *---------HIS4----------leu2-K---*	MJL192	0.3	0.2
B. Heteroalleles in *cis*			
-his4-URA3-leu2-K-his4-----leu2-R--- *---------HIS4----------leu2-K,R-*	MJL195	12	10
-his4-URA3-leu2-R-his4-----leu2-K--- *---------HIS4----------leu-2K,R-*	MJL196	4.8	4.0

[a] Rate of recombination (recombinants per generation).
[b] Recombination rates were normalized to that observed for RHB422.
[c] Results obtained with these two isogenic diploids were pooled to calculate recombination rates.

The other set of diploids (MJL195 and MJL196) contained both *leu2-K* and *leu2-R* on the same copy of chromosome *III*, one copy at its normal locus and the other inserted at *HIS4*. The opposite chromosome *III* homolog was marked with the *leu2-K,R* double mutation. *LEU2* recombinants from these diploids will be the products of intrachromosomal exchange; these were recovered at the expected high level (5–12 ×

TABLE 5

Crossovers associated with mitotic ectopic recombination

Parent structure	Structure of *LEU2* segregants		
		Crossovers	
	Parental	Duplication	Deletion
-his4-URA3-leu2-K-his4----leu2-R-o-	101	10	1
-his4-URA3-leu2-R-his4----leu2-K-o-	80	4	17

Chromosome *III* structures were determined as described (LICHTEN, BORTS and HABER 1987). Segregants marked "deletion" contain a deletion of sequences between *LEU2* and *his4::leu2* and have the structure *-his-URA3-LEU2-o-*. Segregants marked "duplication" contain a duplication of these sequences, and have the structure *-his4-URA3-leu2-his4----LEU2-his4----leu2-o-*. In addition, two *LEU2* recombinants were obtained (one from each haploid) with structures that defied interpretation.

10^{-7} recombinants/generation). Therefore, the apparent proximity-associated elevation in the rate of mitotic recombination between two sequences depends on the sequences being present on the same physical chromosome.

Ectopic mitotic recombination is accompanied by crossing over: Previous studies of both allelic and ectopic mitotic recombination have shown that recombination between closely linked mutant alleles is occasionally accompanied by exchange of flanking sequences; these values range from 10–20% for allelic recombination (ESPOSITO 1978; HABER and HEARN 1985; JINKS-ROBERTSON and PETES 1986) and 6–12% in ectopic recombination (JACKSON and FINK 1981; MIKUS and PETES 1982; SUGAWARA and SZOSTAK 1983; JINKS-ROBERTSON and PETES 1986). To determine the extent that recombination between dispersed copies of *leu2* is accompanied by exchange of flanking sequences, we analyzed *LEU2* recombinants from strains that contained *leu2* heteroalleles at *LEU2* and *HIS4*. Crossovers involving these duplicated copies of *leu2* delete or duplicate the *ca.* 20 kb of DNA between *LEU2* and *HIS4*. Since this interval contains no essential sequences (ROEDER 1983), the majority of crossover products can be recovered in viable cells. The results of this analysis are presented in Table 5.

Exchange of flanking sequences was detected in 10% of the *LEU2* products isolated from H395 (*his4::leu2-K* × *leu2-R*) and in 20% of the *LEU2* products isolated from H386 (*his4::leu2-R* × *leu2-K*). Both values are within the ranges cited above. A marked asymmetry in the recovery of deletions and duplications of the *HIS4-LEU2* region was observed. In H395 (*his4::leu2-K* × *leu2-R*), the majority of crossover products were duplications. When the positions of the two *leu2* alleles were reversed (H386), most crossover products were deletions. This asymmetry in the direction of crossing over is similar to that observed in previous studies of both mitotic (JACKSON and FINK 1981; HABER and HEARN 1985) and meiotic recombination (FOGEL and Hurst 1967; LICHTEN, BORTS and HABER 1987). It is consistent with the hypothesis that intragenic recombinants (at least those associated with crossovers) are mostly the products of events in which exchange terminates in the interval bounded by the two markers.

It has been suggested that most mitotic recombination occurs in G_1, prior to DNA replication (ESPOSITO 1978). Although the deletion crossover recombinants we observed could have been produced at any time during the cell cycle, the duplication crossovers observed are the products of sister-strand exchange, and therefore must have been produced after DNA replication. Our recovery of duplication crossovers and deletion crossovers in similar yields is consistent with the suggestion that a substantial fraction of mitotic recombination events occur during the S or G_1 phases of the cell cycle. Similar conclusions have been reached by JACKSON and FINK (1981).

DISCUSSION

Allelic mitotic recombination is relatively position-insensitive: We have examined the effect of genomic location on mitotic recombination between a pair of *leu2* mutants at five different positions. Both recombination between copies of *leu2* at the same location on parental homologs (allelic recombination) and recombination between copies of *leu2* at dispersed loci (ectopic recombination) were examined. No effect of chromosomal location could be discerned for allelic mitotic recombination. Rates of recombination to *LEU2* at the five loci examined varied less than threefold, and displayed no more variation than did rates of recombination at a control locus (*MET13*). In contrast, levels of meiotic allelic recombination at the same five loci differ significantly, consistent with the suggestion that sequences located more that 2 kb from a locus can exert profound effects on meiotic recombination at that locus (LICHTEN, BORTS and HABER 1987). The observed uniformity in rates of allelic mitotic recombination may indicate that flanking sequences have little, if any, effect on mitotic recombination.

It is important to recognize the limitations imposed on this conclusion by the design of these experiments. In four of the five cases examined, the *leu2* gene was present in the context of a plasmid insert. In these four cases, the *leu2-K–leu2-R* interval was flanked by 2.7 kb of identical sequence to the left and and 4.7 kb of identical sequence to the right (see Figure 1). Thus, only effects which were transmitted across at least 2.7 kb would have been detected. Sequences which regulate transcription and which exert their effects over distances of over 1000 nucleotides have been described (BRAND *et al.* 1985; NAYSMYTH 1985). Since high levels of transcription can markedly in-

crease the level of mitotic recombination in an interval (VOELKEL-MEIMAN, KEIL and ROEDER 1987; THOMAS and ROTHSTEIN 1989), sequences which act at a distance to modulate transcription might also modulate mitotic recombination. The experiments reported here do not exclude the possibility that sequences exist in the yeast genome which can affect recombination in intervals several kilobases away. However, they do allow the conclusion that such sequences were not present at the loci examined.

Is mitotic recombination between homologous and heterologous chromosomes equivalent? Dispersed copies of *leu2* were found to recombine during mitosis at rates which were similar to, if not greater than, those observed for allelic recombination. For example, the rate of recombination in MJL224, where *leu2-K* and *leu2-R* were located on nonhomologous chromosomes, was 1.5×10^{-7} recombinants/generation, a rate similar to the rate displayed by "allelic" diploids (1.2×10^{-7}). In the former diploids, the two copies of *LEU2* shared only 2.2 kb of homology; in the latter, they were flanked by continuous homology. These results indicate that 2.2 kb of homology are sufficient to allow frequent mitotic recombination. Studies of mitotic recombination between plasmids have indicated that considerably shorter stretches of homology are sufficient to allow mitotic recombination (KUNES *et al.* 1987; AHN *et al.* 1988). These studies did not establish a lower bound for the extent of homology above which dispersed sequences recombine as efficiently as do allelic sequences. The finding that dispersed sequences sharing 2.2 kb of homology recombined at levels comparable to that of allelic sequences allows at least a rough assignment of this limit.

These observations also allow the question of whether the distinction between allelic and ectopic recombination is a useful one, at least regarding its use in describing mitotic recombination. The concept of allelic positions implies that sequences present at the same location on maternal and paternal homologs share a relationship which is not shared by homologous sequences which are at dispersed (or "nonallelic") loci. Such a relationship may exist during meiosis, where parental homologs become paired (ZICKLER and OLSON 1975; BYERS and GOETSCH 1975; DRESSER and GIROUX 1988; GIROUX 1988), although the relevance of such pairing to meiotic recombination has been questioned (JINKS-ROBERTSON and PETES 1986; LICHTEN, BORTS and HABER 1987). There is no compelling evidence that such chromosomal pairing or association occurs during mitosis in yeast. In fact, the observation that mitotic recombination between dispersed sequences on nonhomologous chromosomes occurs at frequencies comparable to frequencies of allelic recombination can be taken as evidence against such an association.

Vicinity effects in ectopic mitotic recombination: One feature which has emerged from these studies is an apparent distance-dependence of the rate of mitotic ectopic recombination. Dispersed copies of *leu2* which were on the same arm of chromosome *III* recombined at a rate higher than that observed for allelic copies or for more widely separated copies of *leu2*. In the most striking example, *leu2* loci separated by about 20 kb (*his4*::*leu2* and *leu2*) recombined about ten times more frequently than did *leu2* sequences on separate chromosomes (*URA3*::*leu2* and *leu2*). This elevated level of recombination was dependent on both copies of *leu2* being present on the same homolog. Dispersed copies of *leu2* separated by *ca.* 70 kb recombined at an intermediate level. Similar results have been reported for mitotic recombination between Ty elements (KUPIEC and PETES 1988; ROEDER, SMITH and LAMBIE 1984). In particular, a Ty element at *HIS4* was observed to recombine most often with other Ty elements near *HIS4*, at lower levels with other Ty elements on chromosome *III*, and least often with Ty elements on nonhomologous chromosomes (ROEDER, SMITH and LAMBIE 1984). Similar results have also been reported in studies of the repair of double-strand breaks generated by the *HO* endonuclease (RUDIN and HABER 1988). Interchromosomal interactions to repair HO-cleaved *MAT* sequences inserted on chromosome *V* were much less efficient than was intrachromosomal recombination between flanking regions of homology.

All these results are consistent with the suggestion that the rate at which two sequences recombine during mitosis is determined primarly by the average physical distance separating the two sequences. Homologous sequences which are physically linked (present in *cis* on a homolog) and separated by a relatively short length of chromosome would encounter one another more frequently than well-separated or physically unlinked sequences; thus, a sequence which has suffered a recombinogenic lesion will be more likely to recombine with a close neighbor than with an unlinked homologous sequence. If this is the case, then ectopic recombination may prove to be a useful tool to probe the structure of the mitotic nucleus.

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