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Expansions and Contractions of the Genetic Map Relative to the Physical Map of Yeast Chromosome III

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To examine the relationship between genetic and physical chromosome maps, we constructed a diploid strain of the yeast *Saccharomyces cerevisiae* heterozygous for 12 restriction site mutations within a 23-kilobase (5-centimorgan) interval of chromosome III. Crossovers were not uniformly distributed along the chromosome, one interval containing significantly more and one interval significantly fewer crossovers than expected. One-third of these crossovers occurred within 6 kilobases of the centromere. Approximately half of the exchanges were associated with gene conversion events. The minimum length of gene conversion tracts varied from 4 base pairs to more than 12 kilobases, and these tracts were nonuniformly distributed along the chromosome. We conclude that the chromosomal sequence or structure has a dramatic effect on meiotic recombination.

In general, one expects to find that the frequency of recombination between two genes should be proportional to the physical distance between the two markers. Deviations from this expectation have been observed, however, and are usually attributed to one of two factors: the proximity of chromosomal structural elements (such as the centromere) to the genetic interval, or the presence (or absence) of sequence-specific "hotspots" for recombination within the genetic interval.

The clearest example of an effect of chromosome structure on recombination of adjacent sequences is the effect of the centromere on meiotic exchange in *Drosophila melanogaster*. The genetic map is contracted relative to the physical map at the centromere (6). Several experiments have indicated the possibility of a similar centromeric repression of meiotic exchange in fungi. First, in *Aspergillus nidulans* and *Saccharomyces cerevisiae*, there is relatively more mitotic than meiotic recombination near the centromere (23, 31). The second type of experiment concerned the physical analysis of cloned centromeric sequences of *S. cerevisiae* (3, 8, 13). Two short conserved sequences (8 and 25 base pairs [bp]) flanking an A+T-rich spacer of 70 to 100 bp are sufficient for centromere function. In some cases, the physical distance between a cloned centromere and a selectable gene was known, and therefore a correlation between genetic and physical distance near the centromere could be established. Although the ratio between centimorgans (cM) and kilobases (kb) for some of these centromeric regions was lower than the average for the genome (37), for others the ratio was about the same. A third type of experiment involved moving the centromere of yeast chromosome III to a new position within the same chromosome. In such yeast strains, Lambie and Roeder (20) found that meiotic recombination increased in an interval near the original position of the centromere, whereas recombination in the interval into which the centromere had been introduced decreased.

In addition to effects of structural elements on recombination, there are sites that appear to stimulate homologous recombination locally (reviewed by Whitehouse [41]). For example, the 8-bp Chi site (36) enhances homologous recombination in *Escherichia coli* by acting as a recognition sequence for the RecBC enzyme (32). In fungi, a number of sites (*cog* in *Neurospora*, M26 in *Schizosaccharomyces pombe*, and YS17 in *Sordaria brevicolis*) have been identified that stimulate meiotic recombination (1, 12, 22). In addition, there appear to be regions of the yeast chromosome that are unusually hot (4) or cold (21) for meiotic crossovers. The mechanism of action of these sites has not yet been determined.

Recombination events in eucaryotes may be either reciprocal or nonreciprocal (gene conversions). In certain organisms, meiotic gene conversion events can be detected at a single heterozygous locus (*A* representing one allele and *a* representing the other) as a departure from 2*A*:2*a* segregation, resulting in either 3*A*:1*a* or 1*A*:3*a* tetrads. Gene conversion events therefore represent the nonreciprocal transfer of information between homologous genes. Since conversion events are frequently associated with reciprocal exchange of flanking markers (16), most current models of recombination invoke the conversion event as an intermediate in the process of forming a reciprocal exchange (15, 25, 39).

The meiotic conversion frequencies for different genes and different alleles vary considerably in a single organism (9, 10, 41). Some of this variation may be the result of the location of the allele in relation to recombination-stimulating sites, such as those described above. Within a single gene, the frequency of gene conversion often varies as a function of the distance from one end of the gene. This polarity is usually explained by postulating the presence of an initiating site for gene conversion near one end of the gene. Recent studies in *S. cerevisiae* with the rRNA gene (19) suggest an association between the initiation of transcription and mitotic recombination.

Estimates of the amount of DNA transferred during a meiotic gene conversion event in *S. cerevisiae* are largely based on coconversion frequencies. Both Fogel et al. (9, 10) and S. R. Judd (personal communication) concluded that conversion tracts were usually several hundred base pairs in length. In addition, Fogel et al. found that meiotic conversion tracts were usually continuous. Although the results of most other studies in fungal systems are consistent with the conclusion that meiotic conversion tracts are generally less

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than 1 kb in length (summarized by Orr-Weaver and Szostak [29]), DiCaprio and Hastings (5) and Fogel et al. (11) reported occasional coconversion of markers in different genes.

In most previous studies on meiotic recombination, the number of heterozygous markers within one chromosomal region is small and the markers represent mutations in expressed genes. In addition, the amount of DNA sequence heterogeneity between the interacting chromosomes other than the heterozygous genetic markers themselves is unknown. As described below, we used recombinant DNA procedures to construct a diploid strain that was heterozygous for 12 restriction site mutations within a 23-kb, 5-cM region of chromosome III. These mutations were constructed in vitro in cloned fragments of DNA, which were then reinserted into a diploid yeast strain. Thus, the physical location and nature of these markers were precisely known. We identified tetrads with a crossover in this genetic interval and used Southern analysis to characterize the position of the exchange; a similar approach has been used by Borts and Haber (personal communication) to investigate meiotic exchanges within pBR322 sequences integrated in the yeast chromosome between duplicated mating type loci. We found that the frequency of both crossovers and gene conversion events was different in different regions of the chromosome.

MATERIALS AND METHODS

Media and growth conditions. Media for yeast growth were prepared as described by Sherman et al. (34). YPD medium (1% yeast extract, 2% dextrose, 2% Bacto-peptone [Difco Laboratories], and 3% agar for plates) was used for nonselective growth. Nutritional markers were scored on synthetic medium lacking one amino acid (2% dextrose, 1.7% yeast nitrogen base [Difco], 0.5% ammonium sulfate, 3% agar). Medium containing 5-fluoro-orotate (5-FOA) was used to select *ura3* mutant strains and was made by supplementing synthetic complete medium to 0.5 mM uracil and 0.88% 5-FOA (2). For sporulation, diploid cells were grown to log phase in YPA (1% yeast extract, 2% Bacto-peptone, 1% potassium acetate) and then transferred to SM (2% potassium acetate plus any required amino acids). Yeast strains were grown vegetatively at 32°C and sporulated at room temperature.

E. coli strains were grown at 37°C in LB medium (1% Bacto-tryptone [Difco], 0.5% yeast extract, 0.5% NaCl plus 1.5% agar for plates). For growth of plasmid-containing strains, the medium was supplemented with 50 μg of ampicillin per ml.

Strains. A complete list of yeast strains is given in Table 1. Most of the strains were derived from XJ24-24a (37); this strain was also used as a source of DNA for construction of the plasmids used in our study (28). The strain LS1 was a spontaneous *ura3* mutant strain isolated from XJ24-24a by growth on 5-FOA plates. The haploid strain LS1 was made into a diploid (LS2) by using a plasmid (YpC50-HO, provided by I. Herskowitz) that contained a cloned gene that catalyzed mating type switching. Since the LS2 strain sporulated poorly, we obtained a spore from this cross (LS2-1a) and crossed it to a haploid strain (SJR14) derived from a different genetic background. The resulting diploid (LS3) sporulated well. We isolated a spore (LS3-17c) from a tetrad derived from this diploid in which no crossing-over had occurred between *LEU2* and the centromere; such tetrads could be identified because they showed first-division segregation relative to the heterozygous centromere-linked *TRP1* gene (26). Thus, strain LS3-17c should contain the same sequences between *LEU2* and the centromere as XJ24-24a.

Most of the other strains in the study were constructed by transformation. The haploid LS18 was derived from LS2-1a by transformation with a *Bam*HI restriction fragment derived from plasmid pLS48 (Table 2). This plasmid contains 10 different filled-in restriction sites in two fused *Bam*HI fragments (C2G and D8B, Fig. 1) derived from chromosome III; in addition, the fragment contains an insertion of the gene *URA3*, which was used to select transformants. To ensure that the sequences on the transforming fragment replaced the wild-type sequences (33), we analyzed the transformed strain by Southern analysis. The haploid LS20 was derived by transformation of LS2-1a with a *Bam*HI

TABLE 1. Yeast strains

Strain	Relevant genotype or description (reference)
XJ24-24a	**a** *trp1-1 arg4-17 tyr7-1 ade6 MAL2* (37)
LS1	**a** *trp1-1 arg4-17 tyr7-1 ade6 MAL2 ura3*
LS2	**a** *trp1-1 arg4-17 tyr7-1 ade6 MAL2 ura3* α *trp1-1 arg4-17 tyr7-1 ade6 MAL2 ura3*
LS2-1a	α *trp1-1 arg4-17 tyr7-1 ade6 MAL2 ura3*
SJR14	**a** *leu2-3,112 his3Δ1 ura3-52 can1*
LS3	Diploid formed by crossing SJR14 with LS2-1a
LS3-17c	**a** *ura3 can1*
LS18	LS2-1a transformed to Ura$^+$ with *Bam*HI fragment of pLS48[a]; contains restriction site changes in C2G and D8B fragments
LS20	LS2-1a transformed to Ura$^+$ with *Bam*HI fragment of pLS37[a]; contains restriction site changes in G4B fragment
LS24	Diploid formed by crossing LS3-17c with LS20
LS25	Ura$^-$ derivative of LS24
LS25-70d	**a** *leu2 ade6 ura3 can1*; contains G4B site changes
LS36	Diploid formed by crossing LS18 with LS25-70d; heterozygous for restriction site changes in fragments C2G, D8B and G4B
LS42	Ura$^-$ derivative of LS36
LS45	Derivative of LS42 that lacks mutant *ura3* insertion in Ty1[b]
LS46	Ura$^+$ diploid derived from LS45 by transformation with plasmid pLS55[a]; contains fill-in of *Cla*I site opposite of centromere from other changes
LS47	Ura$^-$ derivative of LS46; contains changes in CG2, D8B, and G4B fragments in addition to *Cla*I change described for LS46

[a] Plasmids are described in Table 2.
[b] Other details concerning the construction of this strain are given in the text.

TABLE 2. Recombinant plasmids

Plasmid	Description[a] (reference)
G4B	7.2-kb *Bam*HI fragment containing the *LEU2* gene from chromosome III inserted into the *Bam*HI site of YIp5 (28)
D8B	10.5-kb *Bam*HI fragment (located centromere-proximal to G4B) inserted into *Bam*HI site of YIp5 (28)
C2G	8.6-kb *Bam*HI fragment containing centromere of chromosome III inserted into *Bam*HI site of YIp5 (28)
A2C	3.5-kb *Bam*HI fragment contiguous with C2G inserted into *Bam*HI site of YIp5 (28)
pLS31	*Xho*I$^-$ (*Pvu*I$^+$) derivative of G4B
pLS32	*Bst*EII$^-$ (*Mae*II$^+$) derivative of pLS31
pLS33	*Bcl*I$^-$ (*Cla*I$^+$) derivative of pLS32
pLS34	*Sal*I$^-$ (*Pvu*I$^+$) derivative of pLS33
pLS35	1.1-kb *Hin*dIII fragment containing *URA3* gene inserted in *Hin*dIII site of pLS33 within the Ty element
pLS36	1.1-kb *Bam*HI fragment containing the *URA3* gene (17) inserted into *Bcl*I site of pLS32
pLS37	1.1-kb *Hin*dIII fragment containing the *URA3* gene inserted into the *Hin*dIII site of pLS32 within the Ty element
pLS38	*Asp*718$^-$ (*Sna*BI$^+$) derivative of pLS37
pLS41	*Xho*I$^-$ (*Pvu*I$^+$) derivative of D8B
pLS42	*Spe*I$^-$ (*Alu*I$^+$) derivative of pLS41
pLS43	*Bgl*II1$^-$ (*Cla*I$^+$) derivative of pLS42
pLS44	*Bgl*II2$^-$ (*Cla*I$^+$) derivative of pLS43
pLS45	*Bgl*II3$^-$ (*Cla*I$^+$) derivative of pLS44
pLS46	Insertion of 8.6-kb C2G *Bam*HI fragment from pLS54 into one of *Bam*HI sites of pLS45 so that the orientation of the fragments is the same as in chromosome III
pLS47	*Bam*HI$^-$ derivative of pLS46; the site deleted was between the C2G and D8B fragments
pLS48	1.1-kb *Hin*dIII fragment containing *URA3* gene inserted into *Hin*dIII site within yeast DNA insert of pLS47
pLS51	*Mlu*I$^-$ (*Bss*HII$^+$) derivative of C2G
pLS52	*Bgl*II4$^-$ (*Cla*I$^+$) derivative of pLS51
pLS53	*Bcl*I$^-$ (*Cla*I$^+$) derivative of PLS52
pLS54	*Xba*I$^-$ (*Alu*I$^+$) derivative of pLS53
pLS55	*Cla*I$^-$ (*Nru*I$^+$) derivative of A2C

[a] Restriction that were deleted by filling in the single-strand gap are indicated with a − superscript; the new sites formed by the fill-in are indicated by a + superscript.

fragment derived from plasmid pLS37 (Table 2). This fragment contains three different filled-in restriction sites derived from the *Bam*HI fragment G4B and a wild-type *URA3* gene (Fig. 1). The diploid LS24 was constructed by crossing LS3-17c with LS20. A Ura$^-$ derivative of LS24 (LS25) was isolated on 5-FOA plates; this derivative retained a mutant insertion of *ura3* within the G4B *Bam*HI fragment. A spore derived from LS25 (LS25-70d) that contained the filled-in restriction sites in the G4B fragment was crossed to LS18 to create a diploid strain (LS36) that was heterozygous for all 12 restriction site polymorphisms. A Ura$^-$ derivative of LS36 was isolated on 5-FOA plates; this derivative (LS42) was shown to lack the *URA3*$^+$ insertion derived from the LS18 parental strain (presumably as the result of a mitotic gene conversion event involving the homologous chromosome), although the mutant *ura3* insertion derived from LS25-70d was retained. To remove this mutant insertion, we transformed LS42 with a wild-type *URA3* gene (derived from the plasmid MB1068 [17]), and then a Ura$^-$ derivative (LS45) of the strain was selected. To insert an additional restriction site polymorphism in LS45 on the other side of the centromere, we used the two-step transplacement technique (42), first transforming LS45 with plasmid pLS55. This plasmid contains a filled-in restriction site in a *Bam*HI fragment from the other side of the centromere as well as a wild-type *URA3* gene. The resulting transformed diploid (LS46) was plated on 5-FOA medium to isolate a diploid strain (LS47) that retained all restriction site polymorphisms but had lost the *URA3* insertion. The arrangement of the restriction site polymorphisms for the diploid strains LS42, LS45, and LS47 is shown in Fig. 2.

When we attempted to fill in the *Sal*I, *Bcl*I, and *Asp*718 sites within the G4B *Bam*HI fragment, we found that haploid strains containing these changes could not be obtained; this region presumably contains an essential gene. Haploid strains containing a filled-in *Xba*I site in the C2G *Bam*HI fragment grew extremely slowly and were not used in our study.

E. coli RK1448 (38) was used as a host in most of the cloning experiments. Since some of the mutations generated restriction sites that could only be cleaved when unmethylated, *E. coli* MM161 (from M. Marinus) was used as a host for some cloning experiments.

DNA isolation and manipulations. DNA was isolated from 5-ml stationary-phase cultures of *S. cerevisiae* (34). For both small-scale and large-scale plasmid DNA isolations, the alkaline lysis procedure was used (24); large-scale isolations were further purified by CsCl-ethidium bromide density gradients. A complete list of plasmids used for this study is given in Table 2. Standard cloning procedures were performed as described in Maniatis et al. (24). Restriction enzymes were purchased from New England Biolabs, International Biotechnologies Inc., and Boerhinger-Mannheim. *E. coli* DNA polymerase, the Klenow fragment of DNA polymerase, and T4 DNA ligase were purchased from New England Biolabs. ^{32}P-labeled dATP was bought from Amer-

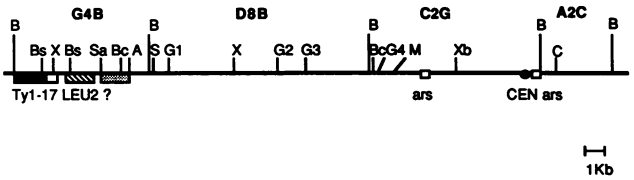


FIG. 1. Restriction map of the *LEU2* to *CEN* region of chromosome III. Abbreviations: A, *Asp*718; B, *Bam*HI; Bc, *Bcl*I; Bs, *Bst*EII; C, *Cla*I; G, *Bgl*II; M, *Mlu*I; S, *Spe*I; Sa, *Sal*I; X, *Xho*I; Xb, *Xba*I. The *Bgl*II sites are designated G1 to G4 from *LEU2* to *CEN3*. Symbols: ?, an essential function mapped within the G4B region; ●, position of *CEN3* sequences; □, approximate locations of the two *ars* elements within the C2G region (30). Several of the restriction sites shown in this figure were determined by Newlon et al. (28).

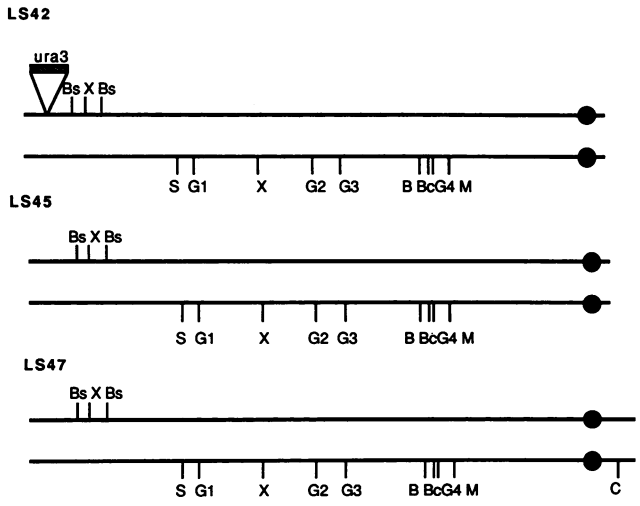


FIG. 2. Locations of the wild-type restriction sites within strains LS42, LS45, and LS47. Abbreviations and symbols are as for Fig. 1.

sham Corp. Southern filters were prepared by using Genescreen (New England Nuclear Corp.) or Hybond nylon membranes (Amersham Corp.) as specified by the manufacturers. Hybridizations were done by methods recommended for these membranes with probes of specific activity of about 5×10^7 cpm/μg in the presence of 5% dextran sulfate.

Yeast transformation. Transformation was performed by the spheroplast technique (14). For transformations involving linear DNA fragments (transplacements [33, 42]), we added 10 to 30 μg of DNA to 200 μl of spheroplasts (10^6 spheroplasts per μl). Following nutritional selection, transformants were screened by Southern analysis (35) to identify those yeast strains with the restriction pattern diagnostic of the desired substitution.

Tetrad analysis. Tetrad dissection was performed as described by Sherman et al. (34). Those tetrads in which a crossover had occurred between *LEU2* and the centromere were identified by looking for second-division segregation between *LEU2* and the centromere-linked heterozygous markers *TRP1* and *ARG4* (26). In DNA samples derived from spores of the diploid LS42 (27 tetrads), all 12 polymorphic sites were analyzed. In samples derived from LS45 (41 tetrads), in most cases all sites except the *Xho*I site centromere-distal to the *LEU2* gene were examined. In samples derived from LS47 (24 tetrads), all sites centromere-proximal to the *LEU2* mutation were analyzed. Restriction digests of DNA with *Bst*EII, *Xho*I, and *Spe*I (after transfer to Hybond) were hybridized to a probe containing all of the G4B region except Ty1-17 (Fig. 1); filters containing DNA treated with *Bgl*II, *Bcl*I, *Mlu*I, and *Xho*I-*Bam*HI were hybridized to the plasmid probe pLS47, which contained DNA derived from the D8B and C2G sequences (Fig. 1).

RESULTS

Construction of plasmids containing restriction site mutations. A gene bank constructed from a circular derivative of chromosome III was used as a source of cloned DNA fragments for this study (28); the bank was prepared by inserting *Bam*HI fragments of yeast DNA into the *Bam*HI site of the yeast vector Ylp5. Three plasmids (C2G, D8B, and G4B) were identified as containing contiguous *Bam*HI fragments from chromosome III (28). The order of the fragments (centromere to telomere) was C2G-D8B-G4B (Fig. 1). The C2G fragment contains the centromere of chromosome III and two *ars* elements (putative origins of replication); the G4B fragment contains a gene involved in leucine biosynthesis (*LEU2*), a $tRNA_3^{Leu}$ gene, and a portion of a Ty element (28, 30, 40).

We constructed restriction site mutations within the cloned plasmids by cleaving them with a restriction enzyme that left recessed 3′ ends. These ends were filled in with DNA polymerase (Klenow fragment), and the cleaved plasmid was religated. The ligated plasmids contain a small (4 to 5 bp) insert at the recognition site of the enzyme used in the initial digestion. For this study, we used restriction enzymes that had sites which, when filled in, produced a site recognized by a different restriction enzyme. For example, when a *Bgl*II site (AGATCT) is filled in, the new site (AGATC GATCT), although no longer recognized by *Bgl*II, is recognized by the *Cla*I enzyme (ATCGAT recognition sequence). By using this procedure, we could be sure that the appropriate sequence changes had been made without sequencing each mutation. Successive restriction site alterations were made within each of the three plasmids until at least four mutations per plasmid were obtained (Table 2).

Construction of yeast strains. Three closely related diploid yeast strains, LS42, LS45, and LS47, were used in these studies. The construction of these strains is described in detail above and outlined in Table 1. In brief, we transformed yeast strains with plasmids (Table 2) derived from chromosome III that contained changes in certain restriction sites. The DNA fragments containing these changes replaced the wild-type sequences on chromosome III. By various genetic crosses, we constructed diploid strains that were heterozygous for these changes. One of the changes was in the gene *LEU2*. The differences among the three strains are summarized in Fig. 2. The results obtained with all strains were very similar and have been pooled.

Analysis of meiotic recombination between *LEU2* and the centromere. The basic plan of these experiments was simple. The diploid strains described above were induced to undergo meiosis. We identified tetrads that had a crossover between *LEU2* and the centromere and mapped the position of the crossover by doing Southern analysis of DNA isolated from cultures of each of the four spores.

Tetrads that had a crossover between *LEU2* and the centromere were identified by comparing the segregation patterns of the heterozygous *LEU2* and *TRP1* mutations (Fig. 3). The *TRP1* gene is very tightly linked to the centromere of chromosome IV, and therefore the wild-type *TRP1* allele will almost always segregate away from the mutant *trp1* allele at the first meiotic division (26). The *LEU2* gene is less tightly linked to the centromere of chromosome III. If there is no crossing-over between either gene and their respective centromeres, then one should obtain only two classes of tetrads (both representing first-division segregation of *LEU2*), those that segregate 2 Trp^+ Leu^+ to 2 Trp^- Leu^- spores and those that segregate 2 Trp^+ Leu^- to 2 Trp^- Leu^+ spores. If there is a single crossover between *LEU2* and the centromere (or between *TRP1* and its centromere), the genotypes for all four spores should be different (tetratype segregation): 1 Trp^+ Leu^+:1 Trp^+ Leu^-:1 Trp^- Leu^+:1 Trp^- Leu^-; this type of tetrad represents a second-division segregation. Since the *TRP1* gene is 0.5 cM from the centromere and the *LEU2* gene is about 5 cM from the centromere, we expected most of the tetratypes would represent crossovers between *LEU2* and the centromere. This expectation was supported by the physical analysis of

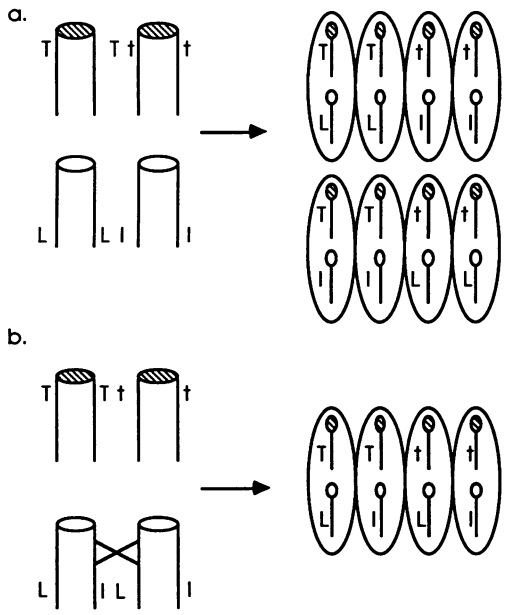


FIG. 3. Genetic screen for crossing-over between *LEU2* and *CEN3*. The *TRP1* gene is very tightly linked to *CEN4* and rarely exhibits crossing over. *LEU2* is less tightly linked to *CEN3*. (a) When no crossovers occur between *LEU2* and *CEN3*, the Leu^+ and Leu^- chromosomes segregate from each other at the first meiotic division, resulting in one of two classes of tetrads, either 2 Trp^+ Leu^+:2 Trp^- Leu^- or 2 Trp^+ Leu^-:2 Trp^- Leu^+. (b) When a crossover occurs between *LEU2* and the centromere, the wild-type and mutant alleles segregate at the second meiotic division, resulting in the formation of a tetratype tetrad (four spores of different genotype, Trp^+ Leu^+:Trp^+ Leu^-:Trp^- Leu^+:Trp^- Leu^-).

the recombinant spores described below. As described below, we also analyzed segregation of *LEU2* relative to *ARG4*, a second centromere-linked marker in the strain.

Of the tetrads derived from LS42, LS45, and LS47, 12.7% showed tetratype segregation between *LEU2* and *TRP1* (26 of 200 for LS42, 41 of 320 for LS45, and 24 of 197 for LS47). Since this frequency of tetratype asci is very similar to that observed for the diploid strain LS25 (12 of 98, 12.2%), which has no heterozygous restriction sites between *LEU2* and the centromere, we conclude that the presence of these sites does not greatly perturb the frequency of meiotic recombination on chromosome III.

In this study, we examined DNA from 91 tetratype tetrads derived from the diploids LS42, LS45, and LS47. As discussed below, 82 of these tetrads represent crossovers between *LEU2* and the centromere; 9 of the tetrads are either crossovers between *LEU2* and the centromere or *TRP1* and the centromere. In recombinant tetrads, DNA samples from all four spores were examined by Southern analysis. We found that the tetrads could be grouped into three classes. In class 1 tetrads (44 tetrads), all restriction site markers segregated 2:2. In addition, in most class 1 tetrads, two of the four spores contained restriction sites in the parental configurations and the other two spores contained the restriction patterns expected as the result of a single reciprocal crossover. In class 2 tetrads (30 tetrads), one or more of the heterozygous restriction sites showed conversion (3:1 or 1:3 patterns of segregation), and the pattern of restriction sites observed in the four spores was consistent with a single crossover adjacent to the conversion tract. Class 3 tetrads (17 tetrads) appeared to be the result of multiple recombination events. This class included tetrads in which the conversion tract was separated from the position of the crossover and those in which multiple crossovers occurred. In addition to examining tetrads that had crossovers between *LEU2* and the centromere, we examined 11 tetrads in which the *LEU2* gene had shown conversion and 19 control tetrads that did not show either conversion of *LEU2* or tetratype segregation between *LEU2* and *TRP1*. Both the experimental and control tetrads are discussed in detail below.

Class 1 tetrads: single crossovers. Of 91 tetrads, 44 were single crossovers, unassociated with gene conversion for any of the heterozygous restriction sites. Three types of class 1 tetrads were observed. The most common type (22 tetrads) had two spores that contained the parental configuration of restriction sites (one resembling each parent) and two spores with the complementary recombinant pattern of restriction sites (Fig. 4).

The second type of class 1 tetrad (13 representatives) had all 12 heterozygous restriction sites segregating 2:2, and all spores had the sites in the parental configurations. In addition, in these tetrads, the *TRP1* and *ARG4* genes showed first-division segregation (ditype tetrads). Since the *LEU2* gene showed second-division segregation with respect to both *TRP1* and *ARG4* (which is also centromere linked), the simplest explanation of these tetrads is that recombination has occurred between the most centromere-proximal restriction site (*Mlu*I) and the centromere. This conclusion is supported by a second argument: all tetrads of this type derived from LS47 (three of three) had a crossover between the *Mlu*I site and the restriction site marker on the other side of the centromere from *LEU2*. Since this restriction site marker (which was about 1 kb from the centromere) showed first-division segregation with *TRP1*, we conclude that these tetrads have a crossover between the *Mlu*I site and the centromere.

The third type of class 1 tetrad (nine tetrads) had the parental configuration of markers for all 12 heterozygous restriction sites between *LEU2* and the centromere. In addition, these tetrads were tetratype for *TRP1* and *ARG4*. This type of tetrad may represent a crossover between *TRP1* and its centromere. Since the *ARG4* gene shows approximately 25% second-division segregation (27), we cannot rule out the alternative possibility that these tetrads represent two events, a crossover between *LEU2* and its centromere proximal to the *Mlu*I site (as described above) and a second crossover between *ARG4* and its centromere. Since we cannot resolve these possibilities, we cannot include these data as representing crossovers between *LEU2* and the centromere.

Thus, of the 82 tetrads containing a crossover between *LEU2* and the centromere, 35 (43%) represent single reciprocal crossovers. The positions of these exchanges are shown in Fig. 5a.

Class 2 tetrads: single crossovers associated with gene conversion of adjacent markers. Since gene conversion events are associated with reciprocal recombination of flanking markers and are assumed to be an intermediate in the generation of a reciprocal exchange (10, 29, 41), it is not surprising that many of the tetrads that had a crossover between *LEU2* and the centromere were associated with conversion events of adjacent markers. These conversion events were detected as restriction site markers that segregated either 3 wild-type restriction sites:1 filled-in site or 1 wild-type site:3 filled-in sites (Fig. 4). We included in this class only those tetrads in which the conversion tract was

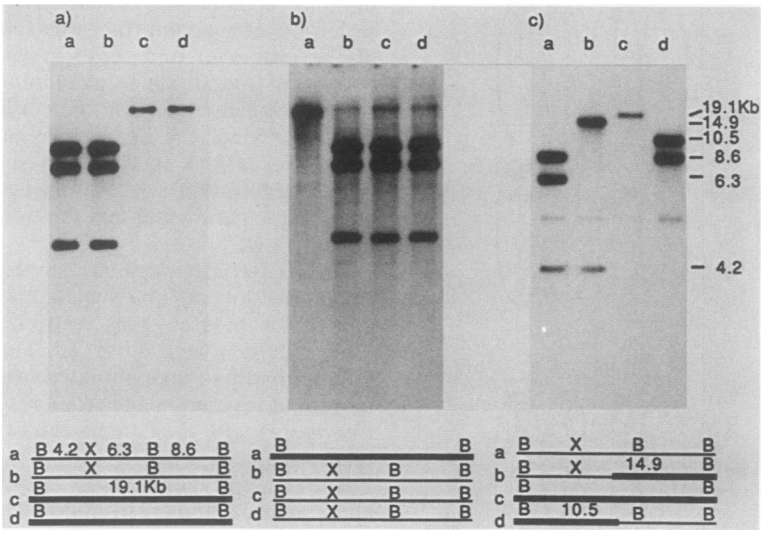
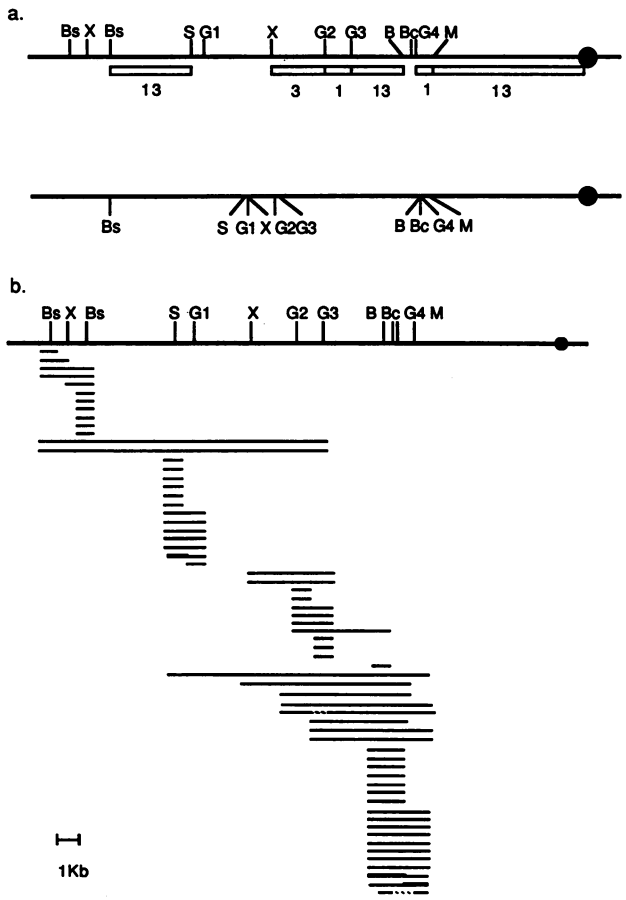


FIG. 4. Physical analysis of DNAs from dissected LS45 tetrads. DNAs were digested with *Bam*HI (B) and *Xho*I (X) and analyzed on a 0.8% agarose gel. Fragments were transferred to Hybond-N and hybridized with a probe containing the 19.1-kb *Bam*HI fragment. Below the Southern blots are shown schematic representations of the strands present in each tetrad. The thin lines represent the cDNA strands from one parent, and the thick lines represent the cDNA strands from the other parent. The parental strains yield fragments of either 19.1 or 8.6, 6.3, and 4.2 kb. (a) Control tetrad in which both heterozygous sites segregate 2:2 and are present in the parental configurations. (b) Conversion tetrad in which all fragments segregate 3:1, indicating coconversion of the heterozygous *Bam*HI and *Xho*I sites. (c) Reciprocal exchange tetrad in which two spores have parental configurations of markers and two spores have novel restriction fragments of 14.9 and 10.5 kb, diagnostic of a reciprocal exchange between the *Xho*I and *Bam*HI sites. The probe used with this filter contained the *URA3* gene, and therefore the 5.5-kb chromosomal copy of *URA3* can also be seen.



immediately adjacent to the position of the crossover. It should be pointed out that in class 2 tetrads, we cannot determine whether the crossover occurred within the conversion tract or at the borders of the tract, and therefore we have not included these crossovers in Fig. 5a. Most recombination models would predict that resolution of the structure responsible for the gene conversion event results in crossing over at one end or the other of the tract (15, 25, 39).

The minimal extent and position of gene conversion tracts are shown in Fig. 5b. Several points concerning these tracts are worth emphasizing. First, we found that the conversion events displayed parity (33 tracts converted in the wild-type direction and 20 tracts converted in the mutant direction). Second, the minimal estimated length of gene conversion

FIG. 5. (a) Distribution of reciprocal recombination events between *LEU2* and *CEN3*. For those tetrads in which an exchange occurred between two chromatids unassociated with conversion of a flanking site, the position of the crossover has been mapped. The crossover locations of class 1 and some class 3 tetrads are shown. The upper line represents the physical map of this region and indicates the number of crossovers that occurred within each interval. Based on these data, the predicted genetic map is shown on the lower line. The total number of crossovers between the *Bst*EII site in the *LEU2* gene and the centromere was 44. The genetic distances were calculated by dividing the number of exchanges in each interval by 44. Abbreviations are as for Fig. 1. (b) Minimal lengths of conversion tracts. It is assumed that when two adjacent sites were converted in the same direction, they were involved in a single recombination event. Therefore, coconversion of sites provides an estimate of the minimum amount of DNA transferred during the gene conversion event. This figure shows the conversion tracts observed in both class 2 and class 3 tetrads. Sites that exhibited a 2:2 segregation within a 3:1 (or 1:3) conversion tract are indicated by a broken line. Sites that exhibited a 4:0 segregation are shown by a thick line. Abbreviations are as for Fig. 1.

tracts varied considerably, from 4 bp (the size of each mutation) to as long as 12 kb. Third, although a few gene conversion tracts were discontinuous (2:2 segregation of a heterozygous site flanked by other sites segregating 3:1 or 1:3), most gene conversion events were continuous. Fourth, different regions of the chromosome had different frequencies of conversion. For example, there were 26 conversion events involving the heterozygous *Bam*HI site and only 6 events involving the *Xho*I site. There were frequent coconversion events involving the contiguous *Bam*HI, *Bcl*I, *Bgl*II (4), and *Mlu*I sites. These conversion tracts sometimes extended as far as the *Spe*I site, suggesting that there may be an initiation site for conversion near the *Mlu*I site from which events extend for variable distances towards the *LEU2* gene. Such polarity gradients for conversion have been observed within some genes (reviewed by Orr-Weaver and Szostak [29]). Fifth, the endpoints of conversion tracts coincided with the hotspots for simple crossovers that were not associated with conversion events, suggesting that conversion-associated crossovers might occur at the same positions.

Class 3 tetrads: multiple recombination events. Of the 82 tetrads that had a crossover between *LEU2* and the centromere, 17 were complex. In these tetrads, there were either more than one crossover or more than one gene conversion event. We also included in this class those tetrads in which the crossover was separated from the conversion tract by a site exhibiting 2:2 segregation. The class 3 events are shown in Fig. 6, as are the number and type of recombination events required to explain the recombinant patterns. The assumptions used in determining the number of required recombination events were that both gene conversion and crossing-over occur after DNA synthesis and that gene conversion is the result of mismatch repair of asymmetric heteroduplexes (25). In addition, we allowed for formation of "patchy" heteroduplexes but only if a region of 2:2 segregation was flanked on both sides by regions of gene conversion. As one example of this type of analysis, we can obtain the pattern of recombination tracts found in tetrad LS42-6 by allowing one crossover and two nonadjacent gene conversion events (Fig. 6). One of the unusual features of tetrad LS45-225 (found also in one of the tetrads that had a gene conversion event at *LEU2* [described below], one of the control tetrads, and one of the class 2 tetrads) is that one of the restriction site markers segregated 4:0. Although 4:0 segregations are usually explained as the result of mitotic recombination events (10), our results suggest that some of these events may be the result of multiple meiotic recombination events, since the 4:0 tracts were often associated with 3:1 or 1:3 conversion tracts. It should also be pointed out

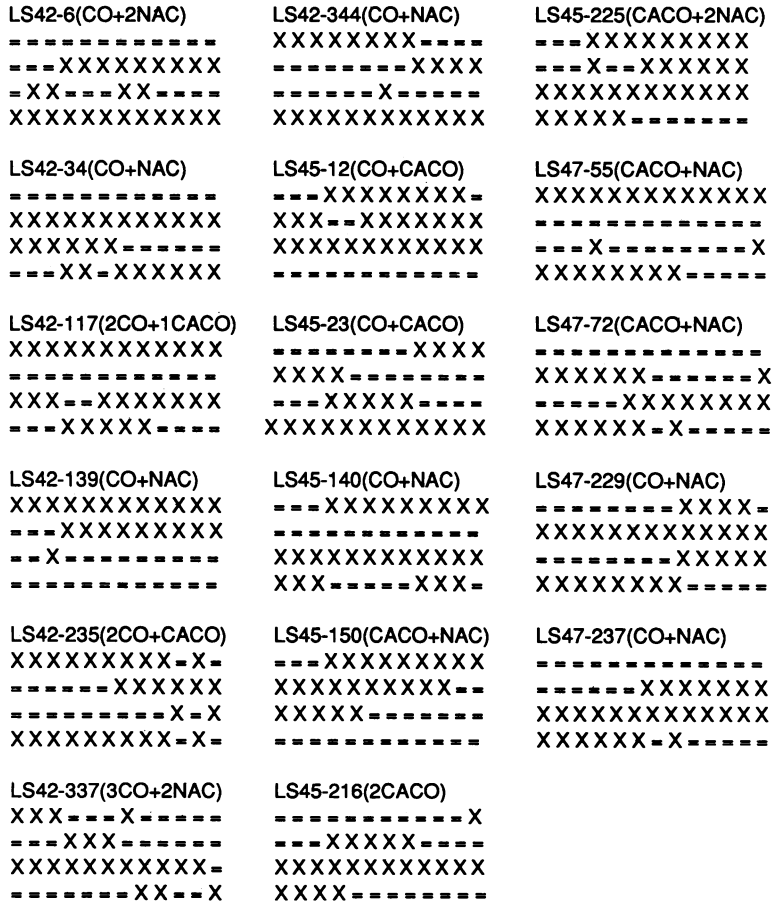


FIG. 6. Recombination patterns in tetrads with multiple events. Each line of X's or ='s represents the pattern for the 12 heterozygous restriction sites in one spore; a parental (nonrecombinant) pattern would be two lines with X's only and two lines with ='s only. The leftmost site is centromere-distal. Combinations of crossovers and gene conversion events required to give the recombinant tetrads are indicated in parentheses (CO, simple crossover; CACO, conversion-associated crossover; NAC, nonadjacent conversion; 2CO and 3CO, double or triple crossover, respectively.

that the patterns found in LS45-225 and similar tetrads can be explained in a number of different ways. In particular, assumptions about the mechanism and timing of gene conversion affect the conclusions. Whatever recombination events generate class 3 tetrads, it is clear that meiotic recombination patterns are often unexpectedly complex.

***LEU2* conversion tetrads.** In the LS42, LS45, and LS47 strains, the *LEU2* gene showed conversion in 3.4% of tetrads (Fig. 5b). We analyzed 11 such tetrads in order to examine the frequency of coconversion of flanking sites. As expected, in all such tetrads, the *Bst*EII site that marked the *leu2* mutation had converted. Two tetrads had converted the two distal markers as well as five proximal markers, resulting in conversion tracts of at least 11.5 kb. Of the 11 conversion tetrads examined, 3 were complex, involving either more than one conversion tract or a nonadjacent crossover. Thus, the frequency of complex events among *LEU2* conversion tetrads was about the same as in crossover tetrads.

Control tetrads. In addition to studying tetrads in which a crossover occurred between *LEU2* and the centromere, we also examined tetrads in which no apparent crossover had occurred (*LEU2* and *TRP1* showing first-division segregation). Of 19 tetrads, 15 had the parental restriction pattern for all spores. Three had gene conversion events, two tetrads with single converted sites and a third with a long conversion tract (4:0 segregation for three of the four centromere-proximal restriction sites, 3:1 segregation for the *Mlu*I site). One tetrad had a two- or four-strand double crossover. Thus, approximately 16% of tetrads that did not have a second-division segregation for *LEU2* had a detectable gene conversion event. Since about 13% (91 of 717) of unselected tetrads had a crossover between *LEU2* and the centromere and since about half of meiotic gene conversion events are not associated with reciprocal recombination, this result was not unexpected; the difference between the expected value of 13% of unselected tetrads with a conversion event without a crossover and the observed value of 16% was not statistically significant (contingency chi-squared value of 0.001).

DISCUSSION

Our results indicate that the frequency and type of meiotic recombination events are a complex function of the structure and/or sequence of the chromosome. Our data can be summarized as follows: the density of crossover events is not uniform along the chromosome, crossover events are frequently associated with conversion events, meiotic conversion tracts in *S. cerevisiae* can be long (at least 12 kb) and are usually continuous, and complex recombination events are fairly common. These conclusions will be discussed in detail below.

The simple crossovers are concentrated in three regions in the *LEU2* to centromere III interval (Fig. 5a). One can calculate the expected number of crossovers in each physical interval (assuming a uniform distribution of exchanges) by multiplying 44 (the total number of crossovers) by the fraction of the total distance (between *LEU2* and the centromere) represented by each interval. We found that the region between the heterozygous *Bam*HI and *Bgl*II (3) restriction sites had significantly more (chi-squared value of 9.3) and the region between the *Xho*I and *Spe*I sites had significantly fewer (chi-squared value of 8.5) crossovers than expected from a uniform distribution. It should be emphasized that we do not know whether the nonuniform distribution of recombination events is the result of the structure of the chromo-

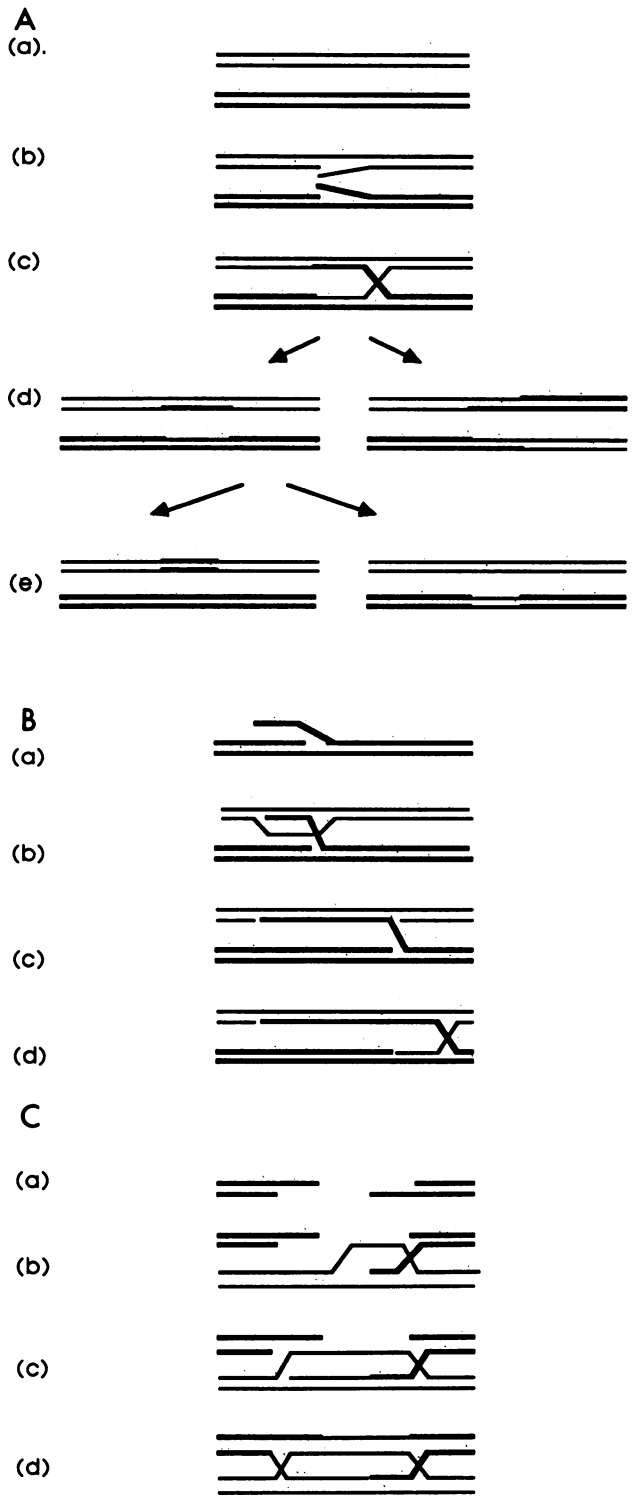


FIG. 7. Models for recombination. (A) Holliday model for recombination. (a) Strands of the same polarity are nicked at homologous positions. (b) The broken strands unwind and exchange to produce a symmetrical heteroduplex. The crossed-strand structure is known as the Holliday junction or Holliday intermediate. (c) Nicks are repaired, and the junction may branch-migrate to enlarge the region of hybrid DNA on both duplexes.(d) The intermediate is resolved by cleavage of the crossed strands. Depending on which strands are cleaved, exchange of flanking markers may result. (e) Mismatch correction of the heteroduplex generates conversion to

some, the sequence of the chromosome, or both factors. For example, it is possible that the certain regions have more recombination events than other regions because the meiotic chromosome is folded in a way that affects the susceptibility of the chromosome to the recombination machinery. Alternatively, the distribution of the recombination events may be primarily the result of differences in DNA sequence. By this interpretation, the hotspots for meiotic recombination may be analogous to Chi sites in *E. coli* (36).

One-third of the simple crossovers occurred within 6 kb of the centromere. This frequency of exchange is approximately the average frequency for the *LEU2* to *CEN3* interval, indicating that there is not a strong repression of meiotic recombination by the centromere. We observed in related studies that a marker 1 kb from *CEN3* also recombined at rates comparable to the average genomic value of 0.37 cM/kb (unpublished data). Lambie and Roeder (20) suggested that the yeast centromere represses recombination, since they found that moving the chromosome III centromere to a new position on the same chromosome increased recombination in the region from which the centromere was removed and decreased recombination at the new position. Our results indicate either that this repression is relatively weak or that moving the centromere has a global effect on recombination within the chromosome that is not directly related to centromeric repression on exchange.

As expected from previous studies (for example, reference 16), crossovers between *LEU2* and the centromere were frequently associated with gene conversion events at adjacent restriction sites. The mechanism by which gene conversion occurs and the association with reciprocal recombination are explained differently by different models of recombination. In the Holliday model (15), gene conversion results from the repair of mismatches in symmetric heteroduplexes (Fig. 7A). In the Meselson-Radding model (25), gene conversion is the result of mismatch repair of a single (asymmetric) heteroduplex (Fig. 7B). In the model of Szostak et al. (39), most conversion events result from gap repair of a double-strand break of the DNA (Fig. 7C). In all models, the structure formed during conversion is resolved into two chromosomes (either recombined or parental) by cleavage of the intermediates at the end of the conversion tract.

The distribution of gene conversion events, like the distribution of crossovers, is not uniform along the chromosome. The length of the conversion tracts was longer than expected on the basis of most previous genetic studies involving coconversion of mutant alleles within single genes (10), although there are two reports of conversion tracts that extended over large genetic distances (5, 11). Although almost all of the observed conversion tracts were continuous (as in previous studies [10]), we found a few tracts in which a site that segregated 2:2 was flanked by sites that were segregating 3:1 or 1:3 (Fig. 5b). Such discontinuous tracts can be explained either as two independent gene conversion events within one region or as a patchy repair of a heteroduplex involving multiple sites (18).

About 22% of the tetrads that had a crossover between *LEU2* and the centromere appeared to have more than one recombination event (class 3 tetrads). Since 20% of the control tetrads (those that did not have a crossover between *LEU2* and the centromere) had either gene conversion or multiple crossover events, it is likely that many of the class 3 tetrads can be explained as representing two independent recombination events. Some of the tetrads, however, appeared to be too complex to represent independent recombination events. For example, tetrad LS42-337 (Fig. 6) required at least three recombination events other than the primary single crossover. Such tetrads can be explained in two ways. First, recombination events within a chromosomal region, although representing different regions of heteroduplex formation and resolution, may be concerted; such concerted recombination events have been suggested previously (summarized by Whitehouse [41] and Fink and Petes [7]). Second, some recombination events may be the result of a different mechanism than repair and resolution of asymmetric heteroduplexes formed in G2 (25). For example, patchy repair of symmetric heteroduplexes formed in G1 could explain any pattern of class 3 tetrads. Whatever mechanisms are involved in formation of the class 3 patterns, it is clear that recombination events that appeared to be simple crossovers between *LEU2* and the centromere by genetic criteria (second-division segregation) are often very complex. In studies of yeast meiotic recombination in plasmid sequences inserted between duplicated *MAT* loci, Borts and Haber (personal communication) detected similar frequencies of complex events.

In summary, genetic distance in *S. cerevisiae* is not simply proportional to physical distance. The frequency of recombination events, both crossovers and gene conversions, varies along the length of the chromosome.

either wild-type or mutant information. (B) Meselson-Radding model. (a) Recombination is initiated by formation of a single-strand nick; the resulting free DNA end serves as a primer for DNA repair synthesis, displacing a single strand. (b) The displaced strand invades a homologous duplex to form a D-loop. (c) The D-loop is degraded, and the asymmetric heteroduplex enlarges by DNA synthesis on the donor chromatid coupled with degradation on the recipient duplex. (d) Ligation of the nicks produces a Holliday junction that may branch-migrate to generate symmetrical heteroduplex adjacent to the region of asymmetrical heteroduplex. Resolution of the Holliday junction yields either crossover or noncrossover products as described for (A) above. Mismatch correction of the asymmetrical heteroduplex yields either gene conversion (3:1) or restoration (2:2) events. (C) Double-strand-break repair model (39). (a) A double-strand break is made in one duplex, which is then enlarged to a gap with 3′ overhangs by the action of nucleases. (b) One 3′ end invades a homologous duplex, displacing a D-loop. (c) The D-loop enlarges by repair synthesis until the other 3′ end can pair with complementary sequences on the displaced strand. (d) Repair synthesis from the other 3′ end repairs the gap. Ligation of the nicks results in the formation of two Holliday junctions that may branch-migrate. Resolution of both junctions in the same sense (both inner or both outer strands) leads to noncrossover products; whereas resolution in the opposite sense generates flanking marker exchange.

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