# MEIOTIC RECOMBINATION BETWEEN DUPLICATED GENETIC ELEMENTS IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

We have studied the meiotic recombination behavior of strains carrying two types of duplications of an 18.6-kilobase *HIS4 Bam*HI fragment. The first type is a direct duplication of the *HIS4 Bam*HI fragment in which the repeated sequences are separated by *Escherichia coli* plasmid sequences. The second type, a tandem duplication, has no sequences intervening between the repeated yeast DNA. The *HIS4* genes in each region were marked genetically so that recombination events between the duplicated segments could be identified. Meiotic progeny of the strains carrying the duplication were analyzed genetically and biochemically to determine the types of recombination events that had occurred. Analysis of the direct *vs.* tandem duplication suggests that the *E. coli* plasmid sequences are recombinogenic in yeast when homozygous. In both types of duplications recombination between the duplicated *HIS4* regions occurs at high frequency and involves predominantly interchromosomal reciprocal exchanges (equal and unequal crossovers). The striking observation is that intrachromosomal reciprocal recombination is very rare in comparison with interchromosomal reciprocal recombination. However, intrachromosomal gene conversion occurs at about the same frequency as interchromosomal gene conversion. Reciprocal recombination events between regions on the same chromatid are the most infrequent exchanges. These data suggest that intrachromosomal reciprocal exchanges are suppressed.

RECOMBINATION between repeated sequences is not restricted to reciprocal and nonreciprocal (gene conversion) exchange between equally paired homologues. For example, STURTEVANT (1925) observed crossing over between unequally aligned chromosomes at the Bar locus in Drosophila. Unequal sister-chromatid exchange has also been observed in Drosophila (TARTOFF 1973) and has been proposed as the mechanism for the maintenance of sequence homogeneity within rDNA repeats (SMITH 1973; TARTOFF 1973). In higher organisms recombination between reiterated genetic elements has been inferred from a comparison of the structure of DNA sequence of genetic variants in the population. The $\alpha$-globin genes, normally duplicated, can also be found in one and three copies in humans and other primates (GOSSENS *et*

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*al.* 1980; ZIMMER *et al.* 1980). These variants are thought to result from unequal reciprocal recombination events. The sequence homogeneity between repeated genes such as the immunoglobulin (BOTHWELL *et al.* 1981; SCHRIER *et al.* 1981) and globin genes (SLIGHTOM, BLECHI and SMITHIES 1980; LIEBHABER, GOOSENS and KAN 1981) is thought to result from gene conversion.

Reciprocal recombination and gene conversion between equally and unequally paired repeated sequences can be demonstrated directly in the yeast, *Saccharomyces cerevisiae*. Unequal sister-chromatid exchange occurs in the rDNA repeats during both mitosis and meiosis (SZOSTAK and WU 1980; PETES 1980). In mitosis, reciprocal exchange between directly repeated sequences has been observed as the excision of intervening sequences (ROEDER and FINK 1982; MIKUS and PETES 1982). These studies led us to inquire about the types of recombination events that occur between duplicated genetic elements. In a previous study (JACKSON and FINK 1981) we analyzed the mitotic recombination behavior of duplications of *HIS4* similar to those studied here. We showed that the major event producing $His^+$ prototrophs is gene conversion unassociated with reciprocal exchange. There is little information concerning the nature of meiotic events, although a study by KLEIN and PETES (1981) found intrachromosomal gene conversion to be the sole meiotic interaction between duplicated *LEU2* genes.

In this study we examine the behavior of duplicated genetic elements in meiosis using two types of duplications. One type is a direct duplication of the *HIS4* fragment separated by *E. coli* plasmid sequences; the other is a tandem duplication with no sequences between the duplicated *HIS4* regions. Unselected tetrads from crosses of duplication-carrying strains were analyzed genetically and biochemically to determine the types of events generated at meiosis. Recombination between the duplicated *HIS4* regions occurs at a high frequency (64% of all meioses show at least one event) and involves predominantly reciprocal exchanges (equal and unequal crossovers) between homologues. Inter- and intrachromosomal gene conversion occurred at approximately equal frequency. In contrast, intrachromosomal reciprocal exchange (both sister-chromatid and intrachromatid) is far less frequent than interchromosomal crossing over. Intrachromatid reciprocal exchanges, in particular, are extremely rare (1/686 tetrads). These results suggest that most intrachromosomal reciprocal exchange is suppressed.

## MATERIALS AND METHODS

*Yeast strains*: The genotypes of the strains used in this study are shown in Table 1. These strains are derived from wild-type strain S288C or strains closely related to it. As detailed in the section on strain construction, $His^+$ duplication strains were created by transformation and strains carrying different mutations in the two *HIS4* genes were constructed by a genetic cross. In this study, *his4*$^-$ mutations are referred to as *his4A*$^-$, *his4B*$^-$ or *his4C*$^-$ to indicate their position in the *HIS4* DNA sequence rather than their complementation behavior (Figure 1). For example, *his4-260* and *his4-39* are polar mutations in the *his4A* region, which do not complement *his4A*$^-$, *his4B*$^-$ or *his4C*$^-$ mutations, and have been previously designated *his4ABC*$^-$ (FINK and STYLES 1974). Throughout this paper *his4-260* and *his4-39* are referred to as *his4A*$^-$ mutations.

The genotype of strains carrying *HIS4* duplication will be symbolized as follows: (1) *HIS4 []*

TABLE 1

*List of strains*

| Strain | Genotype[a] |
|---|---|
| S288C | α *gal2* |
| 5965-20C | **a** *his4-1176, his4-864, leu2-3* |
| 5799-2A | α *his4-260, his4-39* |
| pH4-20C-3 | **a** *his4-1176, his4-864, pBR313 HIS4⁺ leu2-3* |
| pH4-46C-6 | **a** *HIS4⁺ pBR313 his4-1176, his4-864 leu2-3* |
| 7217-30D | α *his4-260, his4-39, pBR313 his4-1176, his4-864* |
| 7168-44C | **a** *his4-1176, his4-864, pBR313 his4-260, his4-39 leu2-3* |
| 118-84A | **a** *his4-260, his4-39 pBR313 his4-260, his4-39 leu2-3* |
| 118-84D | α *his4-1176, his4-864 pBR313 his4-1176, his4-864* |
| T322-5 | α *his4-260, his4-39 pBR322 HIS4⁺* |
| T322-12 | α *HIS4⁺ pBR322 his4-260, his4-39* |
| 150-32C | α *his4-260, his4-39 pBR322 his4-1176, his4-864 leu2-3* |
| 152-9B | **a** *his4-1176, his4-864, pBR322 his4-260, his4-39* |
| Typ5-9 | α *HIS4⁺ YIp5 his4-260, his4-39 ura3-52* |
| Typ5-10 | α *his4-260, his4-39 YIp5 HIS4⁺ ura3-52* |
| 137-47B | α *his4-1176, his4-864 YIp5 his4-260, his4-39 ura3-52* |
| 138-25D | **a** *his4-260, his4-39 YIp5 his4-1176, his4-864 leu2-3 ura3-52* |
| 142-47B | α *his4-260, his4-39 YIp5 his4-260, his4-39 ura3-52* |
| 142-47D | **a** *his4-1176, his4-864 YIp5 his4-1176, his4-864 leu2-3 ura3-52* |
| 205-1A | α *his4-260, his4-39 YIp5 his4-147 ura3-52* |
| 193-3D | **a** *his4-1176, his4-864 YIp5 his4-280 ura3-52* |
| T2 | **a** *his4-1176, his4-864 · HIS4⁺ leu2-3* |
| T12 | **a** *HIS4⁺ · his4-1176, his4-864 leu2-3* |
| 330-3B | α *his4-1176, his4-864 · his4-260, his4-39* |
| 347-27A | **a** *his4-260, his4-39 · his4-1176, his4-864 leu2-3* |

[a] Genotype of duplication-carrying strains includes order of *HIS4* alleles on chromosome *III*. Order of alleles was determined as described in MATERIALS AND METHODS. The order of alleles is: centromere-distal *HIS4* alleles; intervening plasmid sequences; centromere-proximal *HIS4* alleles. "·" indicates that there are no intervening plasmid sequences between the duplicated *HIS4* regions.

*HIS4* is the general designation for all duplications including direct duplications with intervening plasmid sequences and tandem duplications with no plasmid sequences, (2) *HIS · HIS4* is used to designate tandem duplications with no intervening plasmid sequences and (3) direct duplications will often be designated precisely, *e.g.*, *HIS4 pBR322 HIS4*.

*Plasmids*: The yeast plasmids used in this study were YIp300 (HINNEN *et al.* 1979), YIp301 and YIp302 which contain an 18.6-kilobase (kb) *Bam*HI fragment of yeast DNA inserted into the *Bam*HI site of vectors pBR313, pBR322 and YIp5, respectively. The *HIS4⁺* gene is located near the middle of this *Bam*HI fragment. The *E. coli* vector pBR322 is a derivative of plasmid pBR313 (BOLIVAR *et al.* 1977b). The YIp5 plasmid is pBR322 with the yeast *URA3⁺* gene inserted into the *Ava*I site (BOTSTEIN *et al.* 1979).

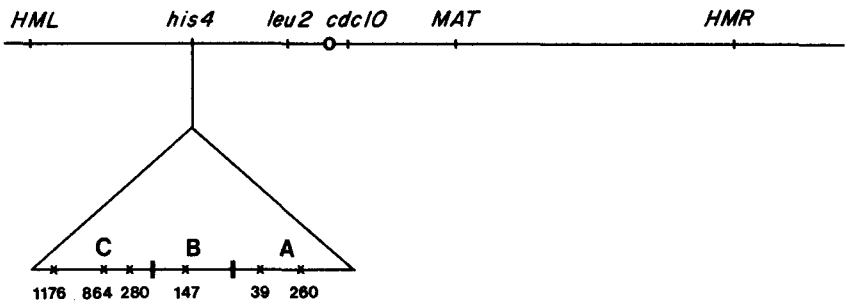


FIGURE 1.—Map of chromosome *III*. The *HIS4* region is shown subdivided into A, B and C. The relevant *his4* mutations are shown.

*Media*: Complete (YEPD), minimal, synthetic complete, presporulation and sporulation media are described in the Cold Spring Harbor Yeast Manual (SHERMAN, FINK and LAWRENCE 1979).

*Genetic procedures*: Procedures for sporulation, tetrad dissection and scoring of genetic markers are described in the Cold Spring Harbor Yeast Manual (SHERMAN, FINK and LAWRENCE 1979). Stable haploid *his4*$^-$ strains carrying one or more copies of the same *his4*$^-$ allele were identified by complementation and recombination tests described by FINK and STYLES (1974). Haploid *his4*$^-$ strains with two or more copies of the *HIS4* region, heteroallelic at the *HIS4* genes, were identified by their ability to give rise to His$^+$ segregants on synthetic complete medium minus histidine plates by mitotic recombination.

*DNA isolation*: The procedures for the preparation of total yeast DNA and *E. coli* plasmid DNA were as described previously (ROEDER and FINK 1980; WINSTON, CHUMLEY and FINK 1982).

*Yeast transformation*: Transformation of yeast strains was carried out as described by HINNEN, HICKS and FINK (1978). His$^+$ transformants were selected by growth in the absence of histidine.

*Colony hybridization*: Meiotic ascospores were micromanipulated onto a YEPD plate which was incubated at 30° for 2 days. The ascosporal clones were then replica-plated to a nitrocellulose filter on the surface of a YEPD plate and allowed to grow at 30° overnight. This filter was then processed according to the procedure for colony hybridization.

Yeast colony hybridization was performed as described in the Cold Spring Harbor Yeast Manual (SHERMAN, FINK and LAWRENCE 1979) using $^{32}$P-labeled pBR322 or pBR313 as the probe. The plasmid was labeled by nick translation using DNA polymerase I (Miles Laboratories) according to the method of MANIATIS, JEFFREY and KLEID (1975).

*Southern hybridization analysis*: Southern analysis was performed on total yeast DNA cut with *Sma*I or *Hin*dIII endonuclease (New England Biolabs). *Sma*I restriction fragments were fractionated by electrophoresis on a horizontal 0.5% agarose slab gel run at 100 V for 18 hr. *Hin*dIII restriction fragments were separated by electrophoresis on a 0.6% agarose slab gel run at 25 V for 18 hr. In both cases electrophoresis was carried out in ½ × E buffer (0.01 M Na acetate, 0.009 M NaCl, 0.001 M disodium ethylene diaminetetraacetate and 0.025 M Tris acetate, pH 8.2).

After electrophoresis, the gel was soaked in 0.06 M HCl for 10 min and then washed in distilled water for 15 min. The fractionated DNA was transferred to nitrocellulose filters by the SOUTHERN (1975) blotting procedure. Filters were hybridized with pBR322 or YIp300, which had been labeled with $^{32}$P by nick translation (MANIATIS, JEFFREY and KLEID 1975). Hybridization conditions were 4 × SSC (0.6 M NaCl, 0.06 M Na citrate) and 0.1% sodium dodecyl sulfate (SDS) at 65° for 16 hr. Filters were washed exhaustively in 0.1 × SSC and 0.1% SDS at 65°, dried and exposed to Kodak XR-5 X-ray film at −70° using Dupont Cronex intensifying screens. Autoradiograms of *Hin*dIII-cut DNA hybridized to $^{32}$P-labeled YIp300 DNA were scanned with a Quick Scan R + D densitometer (Helena Laboratories).

*Construction of strains carrying duplications*: All of the duplications were created by transformation. Hybrid plasmids containing the yeast 18.6-kb *HIS4 Bam*HI fragment in an *E. coli* vector were used as the donor DNAs in the constructions of the direct duplications separated by plasmid sequence. In all constructions the recipient was a haploid strain containing two *his4C*$^-$ mutations (*his4-864* and *his4-1176*) and *leu2-3* (Figure 1). As shown in Figure 2, the orientation of the *HIS4*$^+$,

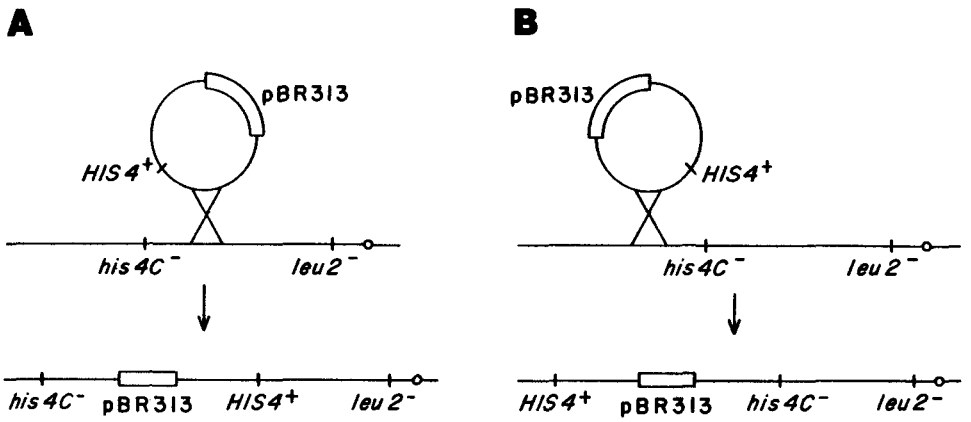


FIGURE 2.—Formation of duplication strains by transformation. The plasmid YIp300, which consists of a *Bam*HI fragment carrying the *HIS4*$^+$ gene inserted into pBR313, integrates into the yeast chromosome *III* by a homologous recombination event as shown here. The recombination event can occur centromere-proximal or centromere-distal with respect to the *his4C*$^-$ mutation on chromosome *III*. A, The orientation of the two *HIS4* genes if the exchange event occurs proximal to the centromere. B, The orientation of the two *HIS4* genes if the exchange event occurs distal to the centromere. The lines represent yeast DNA and the boxes represent pBR313 sequences.

pBR313 and *his4*$^-$ sequences with respect to *LEU2* depends upon the site of integration relative to the *his4C*$^-$ mutations. Strains carrying direct duplications where the *HIS4* regions were separated by vector sequences were identified as His$^+$ transformants that contain vector sequences and segregate His$^-$ derivatives (at a frequency of $10^{-3}$) as a result of mitotic recombination between elements of the duplication.

Yeast strains carrying a tandem duplication without intervening vector sequences were constructed by transforming cells with circularized 18.6-kb *HIS4 Bam*HI fragment made by first cutting YIp300 with *Bam*HI, followed by dilution and ligation. The resulting His$^+$ pBR322$^-$ transformants were identified by colony hybridization using pBR322 DNA as probe. The structure of the *HIS4* region in 29 such transformants was determined by Southern analysis. Two transformants, T2 and T12, were shown to be tandemly duplicated for the *HIS4* region because of the appearance of a novel *Hin*dIII junction fragment of the correct size and by comparing the intensity of hybridization of *Hin*dIII restriction fragments when the *HIS4 Bam*HI fragment was used as the probe (Figure 3).

*Construction of duplication strains heteroallelic for HIS4*

*Heteroallelic direct duplications*: The use of strains carrying duplications in which each copy of the *HIS4* gene carries a different mutation permits identification of each *HIS4* region and of recombinants between the *HIS4* regions. Strains carrying the appropriate alleles in each element of the duplication were constructed by crossing individual *HIS4*$^+$ *leu2*$^-$ transformants with a *LEU2*$^+$ strain carrying two mutations in the *his4A* region, *his4-260* and *his4-39*.

A His$^-$ duplication strain formally represented as *his4A*$^-$ *[] his4C*$^-$ (*his4-260, his4-39 [] his4-1176, his4-864*) can arise from this cross by a meiotic recombination event (Figure 4). Crosses of the two types (Figure 2) of transformants with a *his4A*$^-$ *LEU2*$^+$ strain give rise to recombinant His$^-$ strains with a duplication in either of two orientations (Figure 4). Strains containing the duplication in either orientation I (*his4A*$^-$ *[] his4C*$^-$ *leu2*$^-$) or orientation II (*his4C*$^-$ *[] his4A*$^-$ *LEU2*$^+$) frequently give rise to *HIS4*$^+$ segregants by mitotic recombination events.

The structure and orientation of alleles within a duplication were established by genetic and biochemical tests. These tests were formally identical whether the vector sequences were pBR313, pBR322 or YIp5. The tests used to characterize YIp300 transformants will be described to demonstrate our procedures.

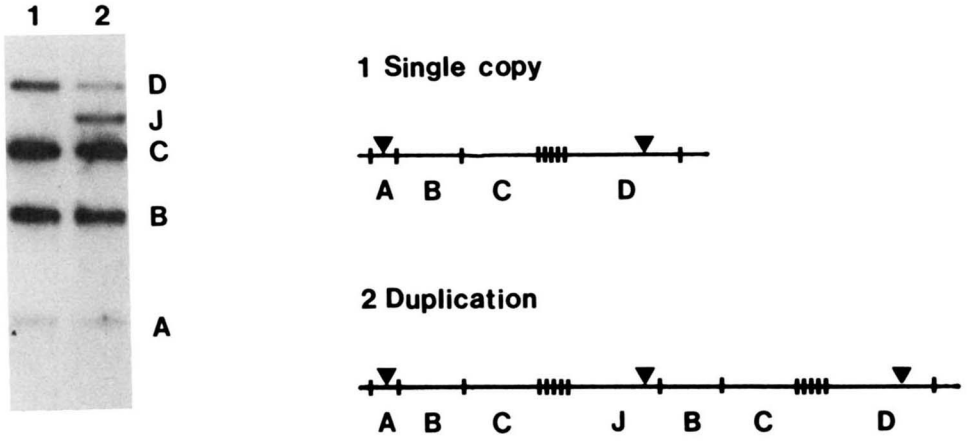


FIGURE 3.—Southern analysis of a tandem duplication of the *HIS4* region. The diagram shows the restriction map of the *HIS4 Bam*HI fragment region of a strain carrying (1) a single copy of the *HIS4 Bam*HI fragment and (2) a tandem duplication of the *HIS4 Bam*HI fragment. The horizontal line represents yeast DNA, the inverted triangles represent *Bam*HI endonuclease sites and the vertical lines represent *Hin*dIII endonuclease sites. The letters A, B, C, D represent the *Hin*dIII restriction fragments that are found in strains carrying both a single copy and a tandem duplication of the *Bam*HI fragment. The letter J represents the novel *Hin*dIII fragment formed by the joining of the two *Bam*HI fragments in the tandem duplication. The autoradiogram shows the hybridization profile of *Hin*dIII digested DNA from (1) a single copy and (2) a tandem duplication. The probe was $^{32}$P-labeled YIp300 which contains the *HIS4 Bam*HI fragment in the vector pBR313.

Transformation with YIp300 yields direct duplications of the *HIS4* alleles separated by pBR313 sequences in either of two orientations (Figure 2). Individual His$^+$ transformants in each orientation were crossed by a *Leu2*$^+$ strain carrying two mutations in the *his4A* region (*his4-260*, *his4-39*). Four types of His$^-$ progeny result from this cross. Three types of His$^-$ progeny are stable and carry a single *his4*$^-$ region. Colony hybridization using plasmid DNA as the probe shows that they have no plasmid sequences. These stable His$^-$ progeny are (1) a nonrecombinant that contains the two *his4A*$^-$ mutations (*his4-260*, *his4-39*), (2) a recombinant that contains the two *his4C*$^-$ mutations (*his4-1176*, *his4-864*) and (3) a recombinant carrying all four mutations (*his4-260*, *hist4-39*, *his4-1176* and *his4-864*). The fourth type is a recombinant containing a duplication of the *Bam*HI fragment. The formation of this recombinant is diagrammed in Figure 4. One element of the duplication contains the *his4A*$^-$ mutations and the other contains the *his4C*$^-$ mutations. The orientation of these mutations in the duplication depends on the site of integration of the YIp300 plasmid into the chromosome (see Figure 2). The Leu phenotype of these recombinants is indicative of the orientation of the alleles within the duplication. The linkage of the *leu2*$^-$ allele to the duplicated segment closest to the *LEU2* locus permits discrimination between orientation I and orientation II. In orientation I most of the His$^-$ recombinants should be Leu$^-$, whereas in orientation II they should be Leu$^+$ (see Figure 4). The His$^-$ recombinants carrying a duplication can be easily detected because they readily give rise to His$^+$ segregants by mitotic recombination between the two *HIS4* regions; whereas the His$^-$ progeny without the duplication are stable. Colony hybridization using plasmid DNA as a probe shows that the unstable His$^-$ progeny contain the pBR313 sequences. The structure of these duplications, inferred by these tests to carry two differently marked *HIS4* regions, was verified by crosses to wild type (*HIS4*$^+$). The identification of recombinant His$^-$ progeny containing each component of the duplication showed that both regions are present.

*Heteroallelic direct duplication strains for intrachromosomal gene conversion study*: Analysis of meiotic gene conversion required two duplication strains in which each of the four *HIS4* regions

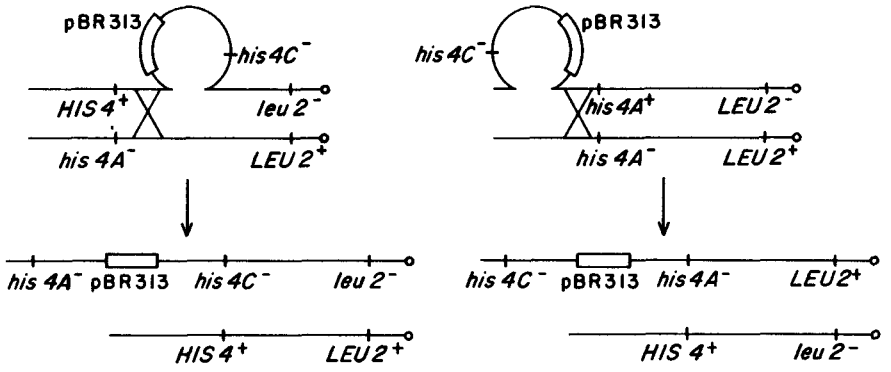


FIGURE 4.—Formation of *his4*⁻ duplication strains in orientation I and orientation II. Transformation with YIp300 yields duplications in two orientations (Figure 2). Individual His⁺ transformants of both orientation were crossed by a *LEU2*⁺ strain carrying two mutations in the *his4A* region (*his4-260* and *his4-39*). Four types of His⁻ progeny results from this cross. The diagram shows the formation of a His⁻ recombinant carrying a duplication of the *Bam*HI fragment. One element of the duplication carries the *his4A*⁻ mutations and the other carries the *his4C*⁻ mutations. The orientation of these mutations within the duplication depends on the site of integration of the YIp300 plasmid into the chromosome (see Figure 2). The relative frequency of Leu⁺ and Leu⁻ among the recombinants is indicative of the orientation of the *HIS4* alleles within the duplication. A, The recombination event giving rise to a *his4*⁻ duplication strain of orientation I. B, shows The recombination event giving rise to a *his4*⁻ duplication strain of orientation II. The lines represent yeast DNA and the boxes represent pBR313 sequences.

carries a different *his4*⁻ mutation. These strains are (1) 193-3D of genotype *his4-1176, his4-864 YIp5 his4-280*, and (2) 205-1A of genotype *his4-260, his4-39 YIp5 his4-147*.

Strain 193-3D was constructed by crossing a His⁺ transformant of genotype *his4-1176, his4-864 YIp5 HIS4*⁺ with a strain carrying the *his4-280* mutation. Strain 193-3D (*his4-1176, his4-864 YIp5 his4-280*) arose from this cross by a meiotic recombination event. Colony hybridization using plasmid DNA as the probe showed that this strain had retained the YIp5 sequences. The duplicated structure of strain 193-3D was confirmed by a cross with a wild-type (*HIS4*⁻) strain. Recombinant His⁻ progeny carrying one or the other component of the duplication were recovered.

Strain 205-1A was constructed by crossing a His⁻ strain carrying the duplication *his4-260, his4-39 YIp5 his4-1176 his4-864* (*his4A*⁻ *YIp5 his4C*⁻), with a strain carrying the polar *his4-147* (*his4B*⁻) mutation. His⁻ recombinants containing a duplication where *his4-147* replaced the *his4-1176, his4-864* segment were identified by tetrad analysis. The genotype of each spore was determined by complementation tests described by FINK and STYLES (1974).

The tetrad that gives rise to the duplication strain of the desired genotype (*his4A*⁻ *YIp5 his4B*⁻) has two nonrecombinant spores: (1) *his4-147* (*his4B*⁻) which complements *his4A*⁻ mutants and (2) *his4-260, his4-39 YIp5 his4-1176, his4-864* (*his4A*⁻ *YIp5 his4C*⁻) which is unstable (giving rise to His⁺ papillae at high frequency) and complements *his4A*⁻ and *his4B*⁻ mutants. This tetrad will also have two recombinant spores: (3) *his4-1176, his4-864* (*his4C*⁻) which is stable and complements *his4A*⁻ and *his4B*⁻ mutants and (4) *his4-260, his4-39 YIp5 his4-147* (*his4A*⁻ *YIp5 his4B*⁻) which is unstable and complements *his4A*⁻ mutants. From these tests the *his4A*⁻ *YIp5 his4B*⁻ strain can be identified unambiguously. Strain 205-1A has the phenotype of spore 4. The genotype and structure of this strain was confirmed by colony hybridization using plasmid DNA as the probe and by tetrad analysis of a cross to a *HIS4*⁺ strain.

*Heteroallelic tandem duplications*: The formation of *his4*⁻ duplication strains of orientation I and

TABLE 2

*Determination of* HIS4 *copy number in tandem duplication strains*

| Strain | No. of *HIS4* regions | *n* | Ratio B/D |
|---|---|---|---|
| 5965-20C | 1 | 5 | 1.9 ± 0.3 |
| 330-33B | 2 | 4 | 3.8 ± 0.4 |
| 347-27A | 2 | 5 | 3.5 ± 0.4 |

The number of copies of the *HIS4* region was determined by comparing the intensity of hybridization of *Hin*dIII restriction fragments B and D of Figure 3. Autoradiograms of the hybridization profile of *Hin*dIII digested DNA, probed with plasmid YIp300, were scanned with a densitometer. Ratio B/D represents the average of the ratio of the intensity of *Hin*dIII restriction fragments B and D from separate scans. *n* represents the number of DNA samples isolated and analyzed for each strain.

orientation II from transformants carrying a tandem duplication of the *HIS4* region without intervening plasmid sequences was substantially the same as for the direct duplications. However, the absence of plasmid sequences in the direct duplications made analysis more difficult. Individual *HIS4*$^+$ *leu2*$^-$ transformants were crossed with a *LEU2*$^+$ strain carrying the two *his4A*$^-$ mutations, *his4-260* and *his4-39*. His$^-$ progeny that gave rise to His$^+$ segregants were presumed to be duplicated for the *his4* region with the general structure *his4A*$^-$ · *his4C*$^-$. This structure was confirmed in two ways: (1) crosses of these strains with wild type (*HIS4*$^+$) gave rise to recombinant progeny containing either the *his4A*$^-$ or *his4C*$^-$ component of the duplication and (2) Southern hybridization analysis showed that these strains carried the novel junction fragment and were duplicated for the *HIS4* region (Figure 3; Table 2). Crosses of individual His$^+$ transformants, T12 and T2, gave rise to recombinant His$^-$ duplication strains of two orientations: orientation I (strain 347-27A), *his4A*$^-$ · *his4C*$^-$ *leu2*$^-$, and orientation II (strain 330-33B), *his4C*$^-$ · *his4A*$^-$ *LEU2*$^+$.

## RESULTS

### *Crosses of duplication strains by strains of normal chromosome structure*

His$^-$ duplication strains of orientation I (*his4A*$^-$ *[] his4C*$^-$) and orientation II (*his4C*$^-$ *[] his4A*$^-$) were constructed by crossing individual *HIS4*$^+$ transformants duplicated for the *HIS4* region (*HIS4*$^+$ *[] his4C*$^-$) by a *his4A*$^-$ strain of normal chromosome structure (Figure 4). Crosses of the direct duplications separated by plasmid sequences (pBR313, pBR322 or YIp5) and of the tandem duplications, without plasmid sequences, by the *his4A*$^-$ strain give similar results (Table 3). Tetrad analysis of these crosses indicates that neither the duplication plus plasmid sequences nor the duplication alone has a large effect on meiotic recombination in the *HIS4-LEU2* interval on chromosome *III*. The *HIS4-LEU2* distances we observed in these crosses are very similar to that found in crosses in which both parents had the normal chromosome structure. FINK and STYLES (1974) found a *HIS4-LEU2* distance of 15 cM (404 tetrads) in crosses of strains of normal chromosome structure closely related to the strains used in this study. MORTIMER and SCHILD (1980) report a *HIS4-LEU2* distance of 17.4 cM (4131 tetrads).

Crosses of duplication strains by strains of normal chromosome structure show that the intervening plasmid sequences in the direct duplications have no

TABLE 3

*Crosses of duplication strains by a strain of normal chromosome structure*

| Cross | Plasmid between elements of duplication | Total tetrads | *HIS4-LEU2*[a] distance (cM) | % tetrads carrying recombinant His$^-$ duplications[b] |
|---|---|---|---|---|
| Orientation I: | | | | |
| *HIS4$^+$ [] his4C$^-$ leu2-3* / *his4A$^-$ Leu2$^+$* | | | | *hisA$^-$ [] his4C$^-$* |
| 7217 | pBR313 | 117 | 16.8 | 14 |
| X150 | pBR322 | 36 | 17 | 14 |
| X138 | YIp5 | 34 | 17 | 15 |
| X330 | None | 45 | 16 | 18 |
| Orientation II: | | | | |
| *his4C$^-$ [] HIS4$^+$ leu2-3* / *his4A$^-$ LEU2$^+$* | | | | *his4C$^-$ [] his4A$^-$* |
| 7083 | pBR313 | 56 | 15 | 10 |
| X152 | BR322 | 29 | 17 | 17 |
| X137 | YIp5 | 38 | 20 | 13 |
| X347 | None | 40 | 14 | 15 |

His$^+$ transformants of genotype *HIS4$^+$ [] his4C$^-$ leu2-3* were crossed by a *his4A$^-$ Leu2$^+$* strain of normal chromosome structure. The orientation of the *HIS$^+$* and *his4C$^-$* sequences depends upon the site of integration of the transforming DNA (Figure 2). Results from crosses of transformants of both orientation I and II are shown.

[a] *HIS4-LEU2* distance was determined by the formula: map distance in centimorgans (cM) = $\frac{100\ (TT + 6\ NPD)}{2\ (PD + NPD + TT)}$, where PD = parental ditype, NPD = nonparental ditype, TT = tetratype asci (PERKINS 1949).

[b] % tetrads carrying recombinant His$^-$ duplications is a measure of the recombination between *HIS4* regions in the duplication strain and the *HIS4* region in the strain of normal chromosome structure.

pronounced effect on meiotic recombination between *HIS4* regions. His$^-$ duplication strains are the product of a meiotic crossover event between the single *HIS4* region on one chromosome and one of the duplicated *HIS4* regions on the other chromosome (diagrammed in Figure 4). Crosses in which the duplicated parent has a direct duplication separated by plasmid sequences (pBR313, pBR322 or YIp5) yield approximately the same frequency of meiotic recombination between *HIS4* regions as crosses in which the duplicated parent has no integrated plasmid sequences (Table 3).

*Crosses of duplication strains*

To study meiotic recombination between duplicated genetic elements, crosses were made between two strains, each of which carries a duplication of the 18.6-kb *Bam*HI fragment on chromosome *III*. The crosses analyzed in this study are listed in Table 4. These crosses involved four types of duplication strains that differ from each other with respect to the intervening sequences between the duplicated yeast DNA. Three types of these duplication strains carry a direct repeat of the *Bam*HI fragment separated by the *E. coli* plasmid sequences, pBR313, pBR322 or YIp5. The fourth type of duplication strain

TABLE 4

*Crosses of duplication strains*

| Integrated plasmid sequence | Cross | Haploid parents | Diploid genotype |
|---|---|---|---|
| pBR313 | X118 | 7168-44C | **a** *his4C⁻ pBR313 his4A⁻ leu2-3* |
| | | 7217-30D | α *his4A⁻ pBR313 his4C⁻ Leu2⁺* |
| | X176 | 118-84A | **a** *his4A⁻ pBR313 his4A⁻ leu2-3* |
| | | 118-84D | α *his4C⁻ pBR313 his4C⁻ Leu2⁺* |
| pBR322 | X158 | 152-9B | **a** *his4C⁻ pBR322 his4A⁻ Leu2⁺* |
| | | 150-32C | α *his4A⁻ pBR322 his4C⁻ leu2-3* |
| YIp5[a] | X142 | 138-25D | **a** *his4A⁻ YIp5 his4C⁻ leu2-3 ura3-52* |
| | | 137-47B | α *his4C⁻ YIp5 his4A⁻ LEU2⁺ura3-52* |
| | X144 | 142-47D | **a** *his4C⁻ YIp5 his4C⁻ leu2-3 ura3-52* |
| | | 142-47B | α *his4A⁻ YIp5 his4A⁻ LEU2⁺ura3-52* |
| | X209 | 193-3D | **a** *his4C⁻ YIp5 his4C1⁻ ura3-52* |
| | | 205-1A | α *his4A⁻ YIp5 his4B⁻ ura3-52* |
| None | X354 | 347-27A | **a** *his4A⁻ · his4C⁻ leu2-3* |
| | | 330-33B | α *his4C⁻ · his4A⁻ LEU2⁺* |

[a] YIp5 is *E. coli* plasmid pBR322 with the *URA3*⁺ gene inserted into the *Ava*I site (BOTSTEIN *et al.* 1979). *his4A⁻ = his4-260, his4-39; his4B⁻ = his4-147; his4C⁻ = his4-1176, his4-864; his4C1⁻ = his4-280.*

carries a tandem duplication of the *Bam*HI fragment with no intervening plasmid sequences. For each type of duplication, an orientation I (*his4A⁻ [] his4C⁻*) strain was crossed by an orientation II (*his4C⁻ [] his4A⁻*) strain of the same type resulting in a diploid of *HIS4* genotype $\frac{his4A^- \; [] \; his4C^-}{his4C^- \; [] \; his4A^-}$ (Table 4). For the pBR313 and YIp5 direct duplications an additional cross was made, resulting in diploids (1) X176 of *HIS4* genotype $\frac{his4A^- \; pBR313 \; his4A^-}{his4C^- \; pBR313 \; his4C^-}$ and (2) X144 of *HIS4* genotype $\frac{his4A^- \; YIp5 \; his4A^-}{his4C^- \; YIp5 \; his4C^-}$. Four-spored tetrads were analyzed as described in MATERIALS AND METHODS. The number of four-spored tetrads in all crosses ranged between 80 and 90%.

Colony hybridization is sensitive enough to indicate when a haploid spore has more than one copy of the integrated plasmid and, therefore, more than two copies of the *HIS4* region. For example, we identified a triplication of the *HIS4* region (two copies of the plasmid sequences) by colony hybridization and subsequently verified the structure by Southern hybridization analysis (Figure 5). Southern hybridization was performed on representative tetrads of each recombinant class in order to determine precisely the number of *HIS4* regions in each spore. Southern analysis was performed on a total of 29 tetrads from

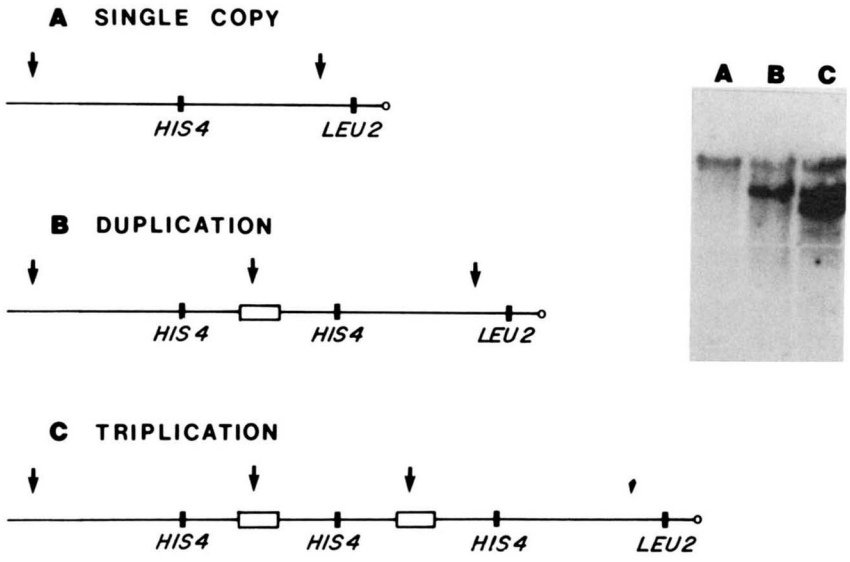


FIGURE 5.—Quantitation of *HIS4* regions using *HIS4 Bam*HI fragment as probe. The diagram shows the configuration of chromosome *III* in a strain carrying a single copy, a duplication and a triplication of the *HIS4* region. The thin lines represent yeast DNA, the boxes represent plasmid sequences (pBR313 or YIp5) and the arrows represent *Sma*I endonuclease sites. An autoradiogram of the hybridization profile of *Sma*I digested DNA from (A) a single copy, (B) a duplication (with intervening pBR313 sequences) and (C) a triplication (with intervening pBR313 sequences) carrying strain using labeled YIp300 as the probe. YIp300 consists of the *HIS4 Bam*HI fragment in pBR313. There is a single fragment that hybridizes to the *HIS4* probe in the single copy. This fragment is extremely large (>50 kb) and comigrates with DNA uncut with *Sma*I endonuclease. The two *Sma*I fragments in the duplication migrated together on the agarose gel. These two fragments are found in the triplication that has an additional smaller *Sma*I fragment. The duplication and the triplication also show a band that migrates in a position corresponding to the single-copy fragment. We believe that this band is due to *HIS4* sequences in DNA uncut by *Sma*I. YIp5, like pBR313, has a single *Sma*I site. Autoradiograms of the hybridization profile of *Sma*I-digested DNA from strains carrying a duplication or triplication of the *HIS4* region with intervening YIp5 sequences are qualitively the same as that shown in lanes B and C.

crosses of direct duplication-carrying strains. The results confirmed that there were no false positives or negatives in the colony hybridization analysis of these 116 spores. The only errors occurred in determining whether there was more than one copy of the plasmid sequences. Three spores that were thought to have only one copy of the plasmid were shown to have two by Southern analysis. Since both *HIS4* genotype and colony hybridization were used to classify the tetrads, this type of error would only be a problem in distinguishing tetrads resulting from unequal sister-strand exchange from those resulting from intrastrand reciprocal exchange [see next section and Figures 6 (3) and 10]. For this reason all tetrads from crosses X176 and X144 with this genotype were subjected to Southern analysis. For the rest of the tetrads we believe that this type of error would only lead to a slight underestimate of reciprocal exchange between unequally paired *HIS4* regions and would have no effect on the conclusions of this paper.

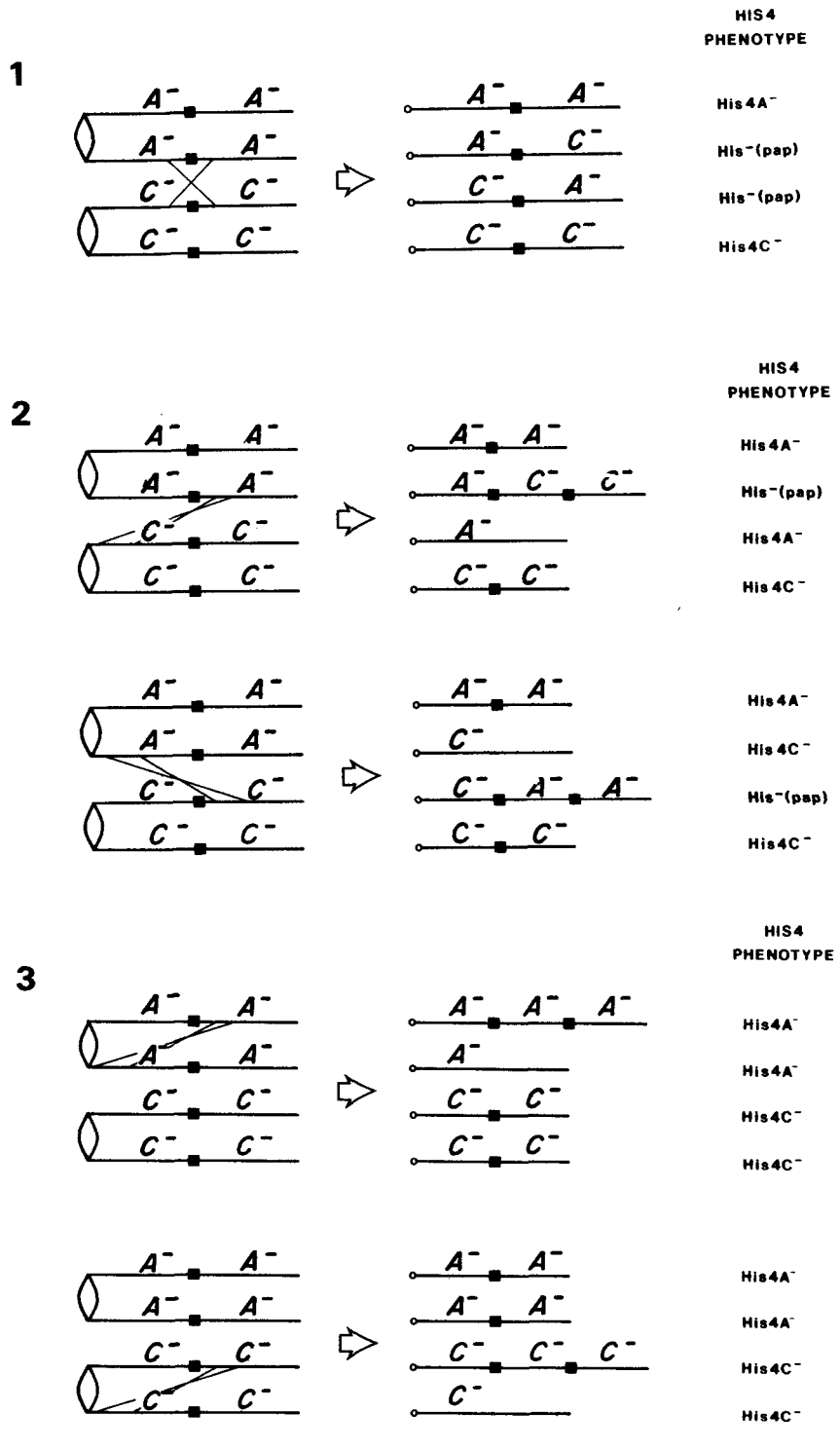
HIS4 PHENOTYPE
1
A⁻ A⁻
A⁻ A⁻
C⁻ C⁻
C⁻ C⁻
A⁻ A⁻
A⁻ C⁻
C⁻ A⁻
C⁻ C⁻
His4A⁻
His⁻(pap)
His⁻(pap)
His4C⁻
HIS4 PHENOTYPE
2
A⁻ A⁻
A⁻ A⁻
C⁻ C⁻
C⁻ C⁻
A⁻ A⁻
A⁻ C⁻ C⁻
A⁻
C⁻ C⁻
His4A⁻
His⁻(pap)
His4A⁻
His4C⁻
A⁻ A⁻
A⁻ A⁻
C⁻ C⁻
C⁻ C⁻
A⁻ A⁻
C⁻
C⁻ A⁻ A⁻
C⁻ C⁻
His4A⁻
His4C⁻
His⁻(pap)
His4C⁻
HIS4 PHENOTYPE
3
A⁻ A⁻
A⁻ A⁻
C⁻ C⁻
C⁻ C⁻
A⁻ A⁻ A⁻
A⁻
C⁻ C⁻
C⁻ C⁻
His4A⁻
His4A⁻
His4C⁻
His4C⁻
A⁻ A⁻
A⁻ A⁻
C⁻ C⁻
C⁻ C⁻
A⁻ A⁻
A⁻ A⁻
C⁻ C⁻ C⁻
C⁻
His4A⁻
His4A⁻
His4C⁻
His4C⁻

TABLE 5

*Summary of meiotic recombination events*

| Event | X176 | | X144 | | X209 | |
|---|---|---|---|---|---|---|
| | No. of tetrads (total 580) | % | No. of tetrads (total 106) | % | No. of tetrads (total 277) | % |
| Class I: interchromosomal reciprocal recombination | 237 | 41 | 33 | 31 | | |
| Class II: unequal interchromosomal reciprocal exchange | 99 | 17 | 18 | 17 | | |
| Class III: unequal intrachromosomal reciprocal exchange | 9 | 1.5 | 1 | 0.9 | | |
| Class IV: interchromosomal gene conversion | 14 | 2 | 3 | 2.8 | | |
| Class V: intrachromosomal gene conversion | | | | | 3 | 1.1 |
| Class VI–VIII: multiple recombination events | 21 | 3.6 | 3 | 2.8 | | |

*Reciprocal recombination between duplicated elements*: Reciprocal exchange is the most frequent recombination event occurring between the duplicated elements in meiosis. Tetrad analysis of the crosses of duplication strains reveals three major classes of recombinant tetrads that arise from single reciprocal exchange events. Class I results from interchromosomal reciprocal exchange between the two *HIS4* genes [Figure 6 (1); Table 5]. The other two classes involve unequal pairing of the duplicated regions. Class II results from an unequal interchromosomal reciprocal exchange [Figure 6 (2); Table 5], and Class III results from an unequal intrachromosomal (sister-chromatid) reciprocal exchange [Figure 6 (3); Table 5]. Each of these classes will be discussed in detail.

*Class I: interchromosomal reciprocal exchange*: The most frequent recombinant tetrad observed results from an interchromosomal reciprocal recombination

FIGURE 6.—Formation of class I, class II and class III tetrads by reciprocal exchange. 1, Class I. An interchromosomal reciprocal exchange event giving rise to a class I tetrad is shown. There are two parental and two recombinant spores. The recombinant spores are heteroallelic for the two *HIS4* genes in the duplication. 2, Class II. Unequal interchromosomal reciprocal exchange events giving rise to two types of class II tetrads are shown. There are two parental and two recombinant spores. One of the recombinants carries a triplication and the other carries a single copy of the *HIS4* region. 3, Class III. Unequal intrachromosomal reciprocal exchange events on chromosome *III* giving rise to two types of class III tetrads are shown. There are two parental and two recombinant spores. One recombinant carries a triplication of the *HIS4* region; the other carries a single copy of the *HIS4* region. Since the recombination event occurs between sister chromatids, the two recombinant spores have the same phenotype. The His phenotype of the four spores in class I, class II and class III tetrads are shown. The phenotypic designation, $His^-$(pap), indicates that these recombinant spores give rise to $His^+$ papillae by mitotic recombination. $A^-$ symbolizes *his4-260, his4-39*. $C^-$ symbolizes a *his4-1176, his4-864*. The thin lines represent yeast DNA and the boxes represent plasmid sequences.

event between the two *HIS4* genes (Table 5). This recombination event [diagrammed in Figure 6 (1)] yields a tetrad with two parental and two recombinant spores. In class I tetrads from cross X176, in which the parental diploid has the genotype $\frac{his4A^- \; pBR313 \; his4A^- \; leu2\text{-}3}{his4C^- \; pBR313 \; his4C^- \; LEU2}$, one recombinant spore has the genotype *his4A⁻ pBR313 his4C⁻ LEU2⁺* and the other recombinant spore has the genotype *his4C⁻ pBR313 his4A⁻ leu2-3*. Further confirmation of the structure deduced by these studies comes from Southern analysis of several class I tetrads.

*Unequal reciprocal exchange events*

Both class II and class III tetrads result from unequal reciprocal exchanges: class II from an interchromosomal event [Figure 6 (2)] and class III from an intrachromosomal event [Figure 6 (3)]. These two types of unequal reciprocal exchanges are differentiated unambiguously in crosses X176 and X144 in which the parental diploid has the genotype $\frac{his4A^- \; [] \; his4A^-}{his4C^- \; [] \; his4C^-}$ (Table 4).

*Class II: unequal interchromosomal reciprocal exchange*: Class II tetrads result from an unequal interchromosomal reciprocal exchange between *HIS4* regions [Figure 6 (2)]. This type of exchange, the second most frequent event, occurs in 17% of the tetrads in cross X176 and X144 (Table 5). This class of tetrad can be identified easily because unequal crossing over causes a change in gene number in two recombinant spores. Southern analysis of several representative class II tetrads confirmed the presence of a triplication and indicates that these tetrads are the result of interchromosomal reciprocal recombination between unequally paired *HIS4* regions.

Two types of class II tetrads can be generated by unequal exchange depending upon which *HIS4* regions pair and where the recombination event occurs [Figure 6 (2)]. These two types are present in equal frequency. This result suggests that each *HIS4* region has the same probability of unequal pairing. In addition, the high percentage of class II tetrads indicates that unequal pairing is a frequent event.

*Class III: unequal intrachromosomal exchange*: Class III tetrads result from an unequal intrachromosomal (sister-chromatid) reciprocal exchange [Figure 6 (3)]. This event occurs in 1% of the tetrads, which means that unequal intrachromosomal events are 10% as frequent as unequal interchromosomal exchange. Analysis of class III tetrads from cross X176 indicates that unequal intrachromosomal exchange is just as likely to occur between sister chromatids on one homologue (*his4A⁻ [] his4A*, four tetrads) as on the other (*his4C⁻ [] his4C⁻*, five tetrads).

*Gene conversion*

*Class IV: interchromosomal gene conversion*: Interchromosomal gene conversion between *HIS4* genes can be observed in crosses X176 and X144 in which the parental diploid has the genotype $\frac{his4A^- \; [] \; his4A^-}{his4C^- \; [] \; his4C^-}$. As shown in Figure 7, the

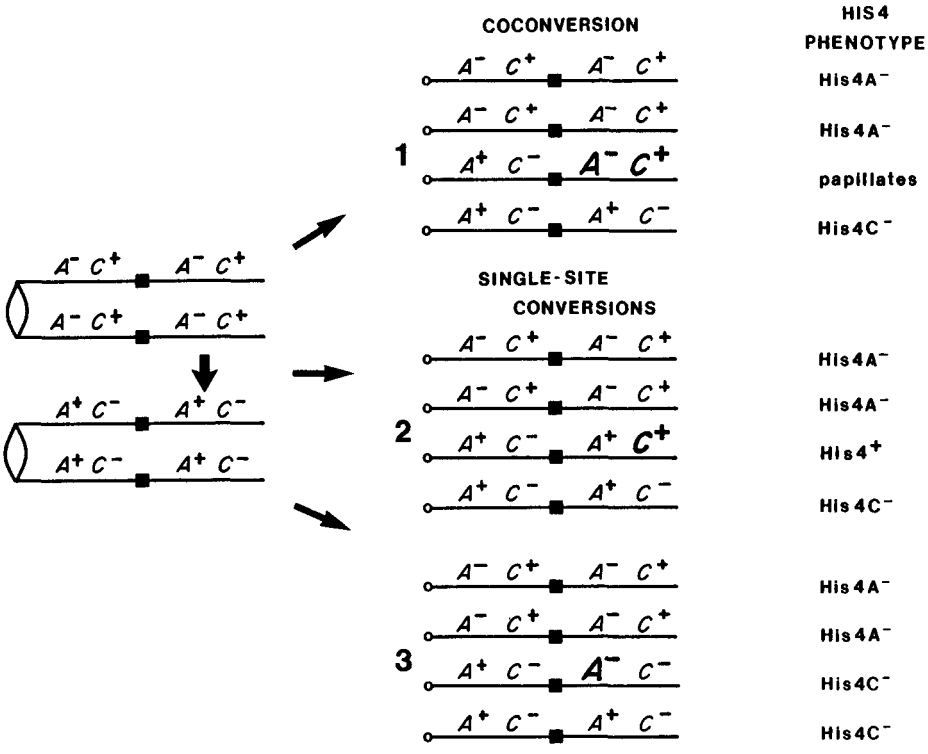


FIGURE 7.—Formation of class IV tetrads by interchromosomal gene conversion. Interchromosomal gene conversion events giving rise to three types of class IV tetrads are shown. Class IV tetrads have three parental and one recombinant spore. 1, A class IV tetrad resulting from a coconversion of the *HIS4A*$^+$ and *his4C*$^-$ sequences in one *HIS4* gene. 2, A class IV tetrad resulting from a single-site conversion of the *his4C*$^-$ sequences. 3, A class IV tetrad resulting from a single-site conversion of the *his4A*$^-$ sequences. Only tetrads of types 1 and 2 can be differentiated from nonrecombinant tetrads. A$^-$C$^+$ symbolizes a *his4A*$^-$ (*his4-260*, *his4-39*) gene that carries the wild-type sequence in the *HIS4C* region. A$^+$C$^-$ symbolizes a *his4C*$^-$ (*his4-1176*, *his4-864*) gene that carries the wild-type sequence in the *HIS4A* region. The His phenotype of the four spores is shown. The phenotypic designation, His$^-$(pap), indicates that the recombinant gives rise to His$^+$ papillae by mitotic recombination. The thin lines represent yeast DNA and the boxes represent plasmid sequences.

spore array in certain of the tetrads is unique and diagnostic. Two groups of class IV tetrads can be generated by an interchromosomal gene conversion, depending upon which homologue acts as the donor of genetic information. Analysis of class IV tetrads from cross X176 shows that both groups occur with approximately equal frequency. In one group (6/14) the *his4A*$^-$ sequences act as the donor of genetic information, and in the other group (8/14) the *his4C*$^-$ sequences act as the donor of genetic information. Figure 7 illustrates only the conversions where *his4A*$^-$*C*$^+$ sequences served as donor.

Class IV tetrads include those that have undergone a coconversion of two *HIS4* alleles (Figure 7), *i.e.*, *his4A*$^-$*C*$^+$ to *his4A*$^+$*C*$^-$ or *his4A*$^+$*C*$^-$ to *his4A*$^-$*C*$^+$ (1/3 class IV tetrads in cross X144, 3/14 class IV tetrads in cross X176). Single-site conversion tetrads have a *HIS4*$^+$ recombinant gene or a *his4A*$^-$*C*$^-$

recombinant gene. The single-site conversion events that give a *HIS4*$^+$ gene can be easily identified; those with the *his4A*$^-$*C*$^-$ configuration cannot because the response in the complementation test is not unique. For example, a duplication of genotype *his4A*$^-$*C*$^-$ *[] his4A*$^+$*C*$^-$ is phenotypically His4C$^-$. For this reason, we have underestimated the single-site conversion frequency. If parity in gene conversion is assumed, the number of *his4A*$^-$*C*$^-$ convertants should equal the number of *HIS4*$^+$ convertants. In cross X176 this assumption would add 11 conversion tetrads, resulting in an interchromosomal gene conversion frequency of 4.3%. By the same reasoning the frequency for cross X144 would increase to 4.7%.

Rare intragenic crossovers between a *his4A*$^-$ gene on one homologue with a *his4C*$^-$ gene on the opposite homologue could give rise to a phenotypically class IV tetrad with one His$^+$ spore associated with a crossover between *His4* and *LEU2*. One of three (33%) His$^+$ recombinants in cross X144 and three of 11 (27%) His$^+$ recombinants in cross X176 are associated with a crossover between *HIS4* and *LEU2*. Since previous studies have shown that gene conversion is associated with reciprocal exchange of flanking markers an average of 32% of the time (FOGEL, MORTIMER and LUSNAK 1981), it is unlikely that intragenic reciprocal exchange makes a significant contribution to the number of class IV tetrads.

*Class V: intrachromosomal gene conversion*: The frequency of intrachromosomal (sister-chromatid and intrachromatid) gene conversion at *HIS4* was determined by crossing a haploid strain of genotype *his4C*$^-$ *YIp5 his4C1*$^-$ (*his4-1176, his4-864 YIp5 his4-280*) by a haploid of genotype *his4A*$^-$ *YIp5 his4B*$^-$ (*his4-260, his4-39 YIp5 his4-147*) to create diploid X209 $\frac{\textit{his4C}^-\ \textit{YIp5 his4C1}^-}{\textit{his4A}^-\ \textit{YIp5 his4B}^-}$. An intrachromosomal gene conversion event will produce a class V tetrad with one recombinant spore in which both copies of the *HIS4* gene carry the same *his4*$^-$ allele (Figure 8).

Three (1.1%) class V tetrads occurred out of a total of 277 tetrads (Table 5). One has a single recombinant spore of phenotype His4A$^-$, one has a single recombinant spore of His4B$^-$ phenotype and the third has a single recombinant spore of His4C$^-$ phenotype. The scoring procedure makes it impossible to tell whether a recombinant spore is the result of a single-site conversion or a coconversion event. However, the three types of class V tetrads observed suggest that each *His4* gene is equally likely to act as the donor of genetic information in an intrachromosomal gene conversion event.

The actual frequency of intrachromsomal gene conversion may be higher than 1.1% because the scoring protocol misses some events. The single tetrad with a His$^+$ recombinant spore cannot be counted as class V because it could result from interchromosomal gene conversion. Comparison of interchromosomal (2.0–2.8%) and intrachromosomal (1.1%) gene conversion suggests that these two types of events occur with approximately equal frequencies (Table 5). Even when the interchromosomal gene conversion frequency is theoretically adjusted for the missed single-site conversions (4.3–4.8%, see previous section), the frequencies of these two types of conversions is not significantly different.

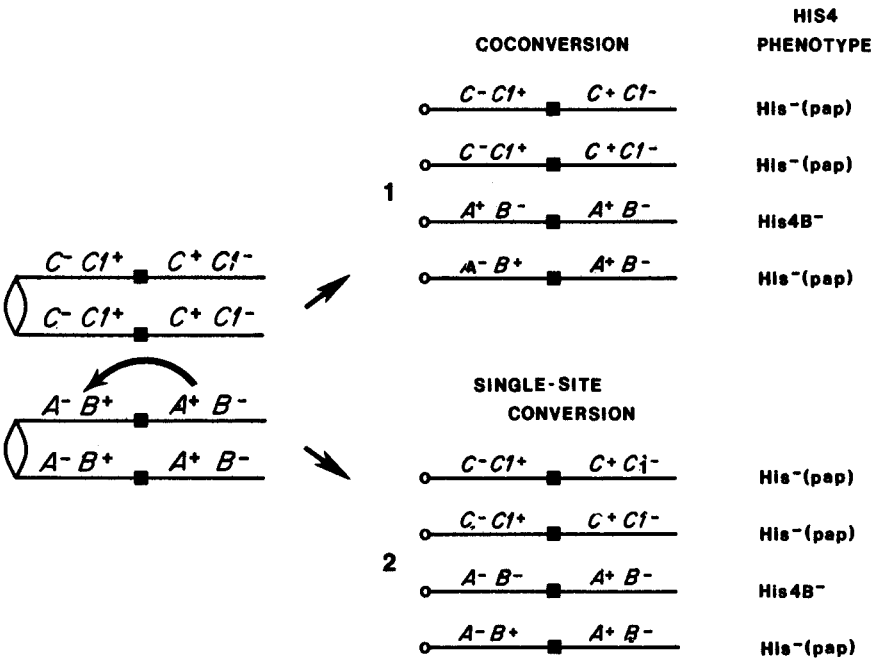


FIGURE 8.—Formation of class V tetrads by intrachromosomal gene conversion. Intrachromosomal gene conversion giving rise to class V tetrads is shown. Class V tetrads have three parental and one recombinant spore. 1, A class V tetrad resulting from a coconversion of the *his4A*$^-$ and *HIS4B*$^+$ sequences of one *HIS4* gene. 2, A class V tetrad resulting from a single-site conversion of the *his4B*$^-$ sequences of one *HIS4* gene. The curved arrow indicates that the $A^+B^-$ sequences act as the donor of genetic information and the $A^-B^+$ sequences act as the recipient in the gene conversion event. $A^+B^-$ symbolizes a *his4B*$^-$ (*his4*-147) gene that carries the wild-type *HIS4A* sequence. $A^-B^+$ symbolizes a *his4A*$^-$ (*his4-260*, *his4-39*) gene that carries the wild-type *HIS4B* sequence. $C^-Cl^+$ symbolizes a *his4C*$^-$ (*his4-1176*, *his4-864*) gene that carries the wild-type *HIS4C1* sequence. $C^+Cl^-$ symbolizes a *his4C1* (*his4-280*) gene that carries a wild-type *HIS4C* sequence. The His phenotype of the four spores is shown. The phenotypic designation, His$^-$(pap), indicates that the parental spores give rise to His$^+$ papillae by mitotic recombination. The thin lines represent yeast DNA and the boxes represent plasmid sequences.

*Multiple recombination events*

Tetrad classes I–V each result from a single meiotic recombination event and together account for almost 70% of the unselected tetrads from cross X176 (Table 5). Approximately 4% of the tetrads from cross X176 appear to result from multiple recombination events. Allele testing and colony hybridization identify these tetrads by the fact that three or four spores are recombinant for His4 phenotypes or chromosome structure. Figure 9 diagrams the events leading to class VI and class VII tetrads and is not meant to imply that we can tell in all cases which chromatids are involved.

*Class VI*: The largest phenotypic class of tetrads (12/580) due to multiple recombination events is class VI. [Figure 9(1)]. As shown in Figure 9(1), the class VI tetrads can be explained by an uneven reciprocal recombination event leading to the formation of recombinant spores 1 and 2. The third recombi-

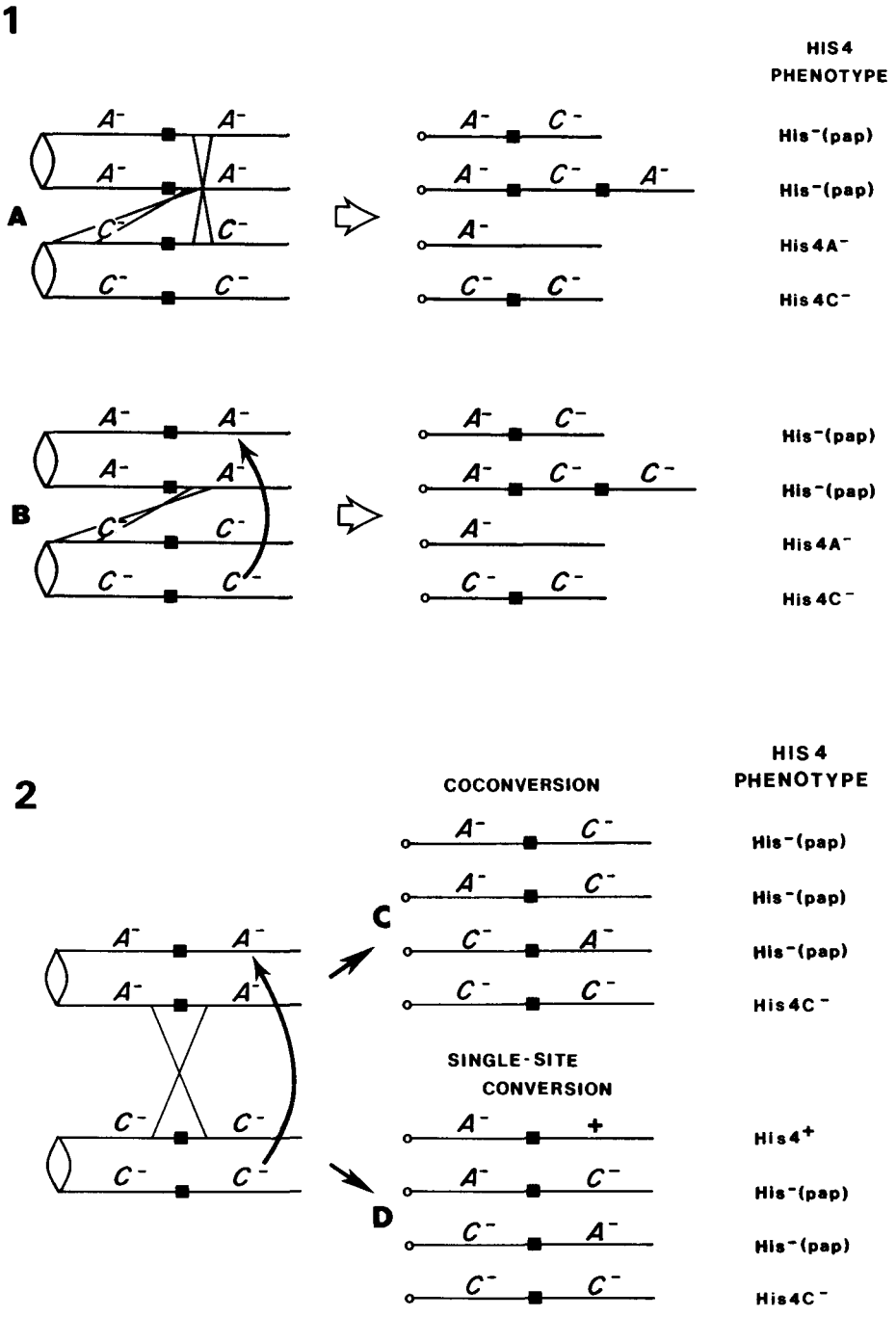


FIGURE 9.—Class VI and class VII tetrads. 1, Formation of class VI tetrads by a double recombination event is shown. A, The formation of a class VI tetrad by a three-strand double crossover. B, The formation of a class VI tetrad by an unequal reciprocal exchange event and a gene conversion event. 2, Formation of class VII tetrads by an interchromosomal reciprocal exchange

nant spore is formed by a simultaneous standard reciprocal recombination or gene conversion event.

*Class VII*: Class VII tetrads like those of class VI also contain three recombinant spores, but unlike those of Class VI all four spores in class VII tetrads have a single copy of the plasmid sequences and, thus, retain the duplication. Class VII tetrads can be explained by an even reciprocal exchange between *HIS4* genes on two of the chromatids and an intragenic reciprocal recombination or gene conversion event involving a third chromatid [diagrammed in Figure 9(2)].

*Class VIII*: All four spores in a class VIII tetrad are recombinant. We observed class VIII tetrads at a frequency of 0.3% (2/580 tetrads). Two recombinant spores carry three copies of the *HIS4* region. The other two spores carry one copy of the *HIS4* region. Class VIII tetrads could have resulted from two simultaneous unequal interchromosomal reciprocal exchange events. Unequal interchromosomal reciprocal exchange occurs at a frequency of 17% in crosses X176 and X144 (Table 5), so the frequency with which a double event of this type should occur is 3%. Perhaps there is interference with the unequal pairing of all four chromatids in meiosis. Alternatively, these tetrads could have been formed by a single premeiotic, mitotic recombination event. Unequal mitotic reciprocal exchange at *HIS4* in a haploid duplication-carrying strain occurs at an approximate frequency of $2.1 \times 10^{-5}$ (JACKSON and FINK 1981).

*Tetrads with a change in* HIS4 *copy number*: An unusual class of recombination events are those that result in tetrads with a change in the number of copies of *HIS4* regions. Crosses between two strains, both of which carry a duplication, should produce tetrads in which there are eight copies of the *HIS4* region distributed among the four spores. We observed rare recombinants that do not meet these expectations; one has seven *HIS4* regions and two appear to have nine.

One tetrad from cross X176 has seven *HIS4* regions. This tetrad has three parental spores and one recombinant spore. Southern hybridization analysis confirmed this interpretation. The simplest recombination event that could give rise to this tetrad is an intrachromosomal reciprocal recombination event. Such an event, diagrammed in Figure 10, would result in the loss of the circular recombination product, because this molecule would have no yeast origin of replication.

Southern hybridization analysis revealed two tetrads that appears to have at

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accompanied by a gene conversion event is shown. C, A class VII tetrad where the gene conversion event converted the *his4A*$^-$ gene to a *his4C*$^-$ gene. D, A class VII tetrad where the gene conversion event converted the *his4A*$^-$ gene to *HIS4*$^+$. A$^-$ symbolizes *his4-260*, *his4-39*. C$^-$ symbolizes *his4-1176*, *his4-864*. The curved arrow indicates that the C$^-$ sequences act as the donor of genetic information and the A$^-$ sequences as the recipient in the gene conversion event. The His phenotype of the four spores of each tetrad is shown. The phenotypic designation, His$^-$(pap), indicates that the recombinant spores give rise to His$^+$ papillae by mitotic recombination. The thin lines represent yeast DNA and the boxes represent plasmid sequences.

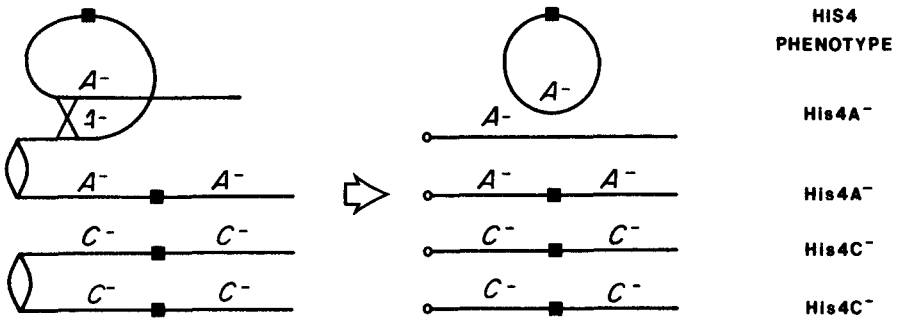


FIGURE 10.—Intrastrand reciprocal exchange. An intrastrand reciprocal exchange giving rise to a tetrad with seven *HIS4* regions is shown. The circular DNA molecule formed by the recombination event lacks an origin of replication and is lost on cell division. $A^-$ symbolizes *his4-260*, *his4-39* and $C^-$ symbolizes *his4*-1176, *his4-864*. The His phenotype of the four spores of each tetrad is shown. The thin lines represent yeast DNA and the boxes represent plasmid sequences.

least nine copies of the *HIS4* region. The simplest explanation of these recombinant tetrads is that mitotic unequal reciprocal exchange is followed by a meiotic intrachromatid reciprocal recombination event. This scheme is diagrammed for the tetrad from X176 in Figure 11. An alternative explanation is that the mitotic unequal reciprocal recombination event is followed by a meiotic unequal interchromosomal reciprocal exchange (Figure 11). This series of events results in a tetrad with ten copies of the *HIS4* region. To be consistent with this mechanism one of the spores that appears to have three copies of the *HIS4* region must have four. The Southern hybridization pattern is the same for a spore with three or more copies of the *HIS4* region, so we could not differentiate between three or four copies of *HIS4*.

The mitotic exchange followed by meiotic unequal interchromosomal reciprocal recombination event is more likely if we take into account our observations on the frequencies of various types of reciprocal exchange. Intrachromatid reciprocal exchange occurs in one of 686 tetrads (0.01%), whereas unequal interchromosomal reciprocal exchange occurs in 17% of the tetrads.

*Effect of plasmid sequences on yeast recombination*

The frequency of class I reciprocal exchanges is a reflection of the map distance between *HIS4* genes. The map distance, in centimorgans, between *HIS4* genes in the direct duplications separated by the plasmids pBR313, pBR322 and YIp5, is 22, 16.5 and 19.2 cM, respectively (Table 6), using the formula of PERKINS (1949). Since the physical distance between *HIS4* genes in these strains corresponds to the size of the plasmid used to create the duplication by transformation, the genetic distance and physical length of this chromosomal region can be compared for each construction. For the direct duplication strains with intervening plasmid sequences, 1 cM corresponds to 1.3–1.4 kb of DNA (Table 6). This figure, 1.3–1.4 kb/cM is approximately half that found by other workers for several regions of chromosome *III*. Cloning experiments have demonstrated that the *HIS4* gene has approximately 2.3 kb/cM (P. FARABAUGH and G. R. FINK, personal communication) and that the

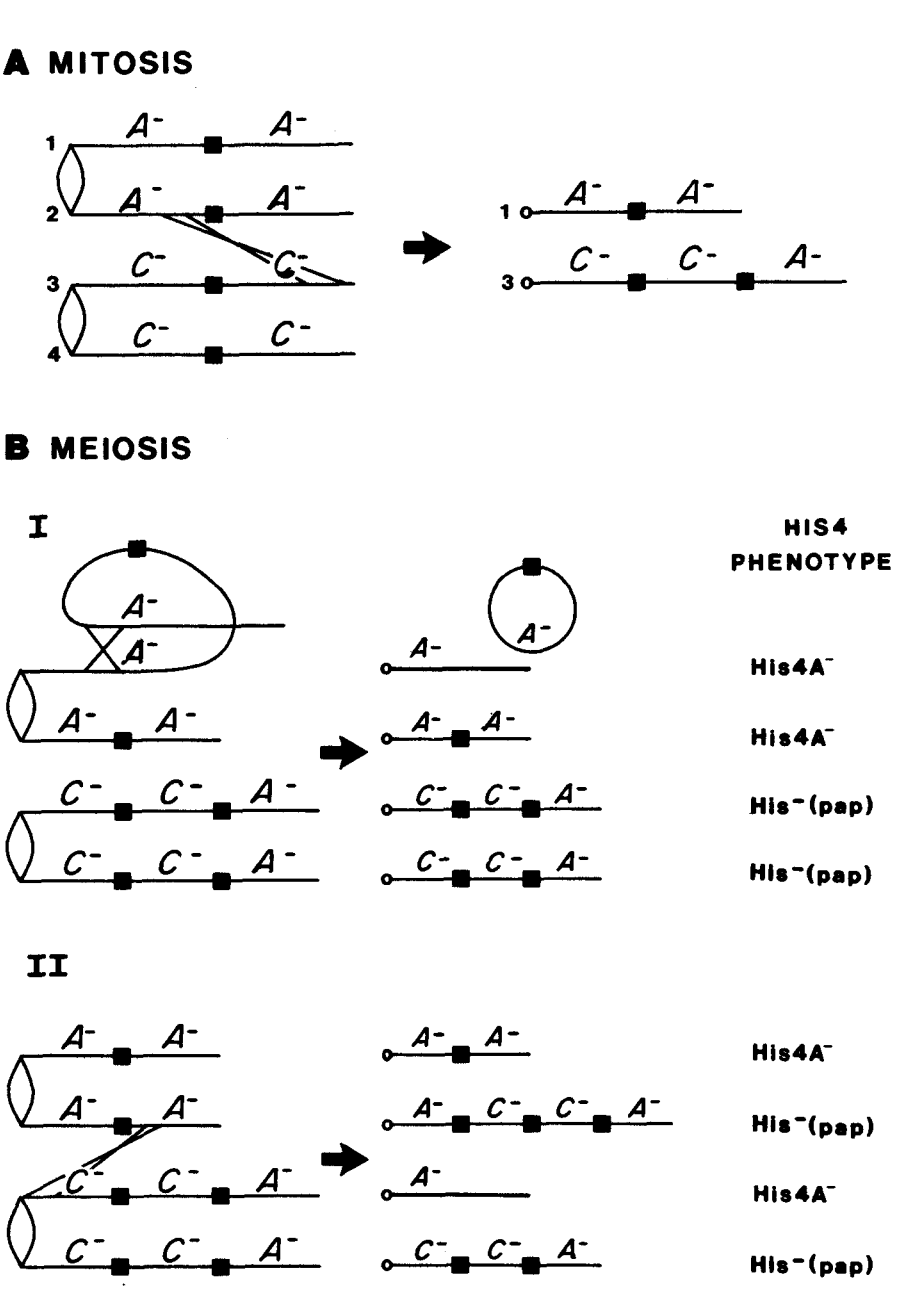


FIGURE 11.—Formation of tetrads with an extra copy of the *HIS4* region is explained by a mitotic exchange followed by a meiotic exchange. A, A mitotic unequal interchromosomal reciprocal exchange followed by segregation of chromatids 1 and 3 to create the diploid that is shown going through meiosis in B. I, A meiotic intrachromatid reciprocal exchange event giving rise to tetrad with nine copies of the *HIS4* region and a circular DNA molecule. II, An unequal interchromosomal reciprocal exchange event giving rise to a tetrad with ten copies of the *HIS4* region. Both recombinant tetrads have two His4A$^-$ spores and two spores that give rise to His$^+$ segregants by mitotic recombination. A$^-$ symbolizes *his4-260*, *his4-39* and C$^-$ symbolizes *his4-1176*, *his4-864*. The His phenotype of the four spores of each tetrad is shown. The phenotypic designation, His$^-$(pap), indicates that the recombinant spores give rise to His$^+$ papillae by mitotic recombination. The thin lines represent yeast DNA and the boxes represent plasmid sequences.

TABLE 6

*Map distance between* HIS4 *genes*

| | Cross | PD | NPD | TT | Map distance ± SE (cM) | kb/cM |
|---|---|---|---|---|---|---|
| Duplications + plasmid | | | | | | |
| *HIS4 pBR313 HIS4* | X118 | 100 | 1 | 66 | 21.6 ± 2.0 | |
| ← 28 kb → | X176 | 343 | 4 | 233 | 22.2 ± 1.0 | |
| | | Total 443 | 5 | 299 | 22.0 ± 0.5 | 1.3 |
| *HIS4 pBR322 HIS4* ← 22.9 kb → | X158 | 76 | 1 | 29 | 16.5 ± 3.0 | 1.4 |
| *HIS4 YIp5 HIS4* | X142 | 40 | 1 | 20 | 21.3 ± 4.0 | |
| ← 24 kb → | X144 | 73 | 1 | 32 | 17.9 ± 4.0 | |
| | | Total 103 | 2 | 52 | 19.2 ± 2.0 | 1.3 |
| Duplications − plasmid | | | | | | |
| *HIS4 · HIS4* ← 18.6 kb → | X354 | 238 | 1 | 33 | 7.2 ± 1.0 | 2.6 |

*LEU2*—to—*CDC10* region (Figure 2) has approximately 3 kb/cM (CLARKE and CARBON 1980). Electron microscopic analysis of a circular derivative of chromosome *III* has shown that the *HML*—to—*MAT* region (Figure 2), which includes the *HIS4* gene, has approximately 2.7 kb/cM (STRATHERN *et al.* 1979). Comparison of these figures suggests that a recombination event is twice as likely to occur, per kilobase, in the direct duplications of the *HIS4* region separated by plasmid sequences than in *HIS4* regions of normal chromosome structure.

One possible explanation for this increased recombination frequency is that all or part of the integrated plasmid sequences are recombinogenic when homozygous in yeast. To test this possibility, strains carrying tandem duplications of the *HIS4*-containing *Bam*HI fragment, without intervening sequences, were constructed. A haploid strain carrying a tandem duplication in orientation I (*his4A*$^-$ · *his4C*$^-$ *leu2-3*) was crossed by a haploid strain carrying a tandem duplication of orientation II (*his4C*$^-$ · *his4A*$^-$ *LEU2*$^+$) creating diploid X354 of genotype $\frac{his4A^- \cdot his4C^-\ leu2\text{-}3}{his4C^- \cdot his4A^-\ LEU2}$. An interchromosomal reciprocal recombination event in this cross will give a class I tetrade with two recombinant spores: one of genotype *his4A*$^-$ · *his4A*$^-$ *LEU2*$^+$ and the other of genotype *his4C*$^-$ · *his4C*$^-$ *leu2-3*. Unlike the parental strains, these His$^-$ recombinants are stable and their phenotypes can be determined by standard complementation tests (FINK and STYLES 1974).

Southern hybridization analysis of two tetrads from cross X354 that are phenotypically class I showed that one tetrad has four duplication-carrying spores and, thus, is of the class I type. The other tetrad is analogous to class VI [Figure 9(1)], in which two spores carry a duplication, one spore carries a triplication and one spore carries a single copy of the *HIS4* region. A class VI

tetrad is not formed by a reciprocal exchange between *HIS4* genes and should not be included in the calculation of map distance. It is not possible to know how many of the tetrads with the proper His phenotype from cross X354 are class I without performing Southern hybridization analysis on all 34 of these tetrads (Table 6). We can estimate the number by comparison with the results from direct duplication cross X176 in which 95% (237/249) of the tetrads with the class I His phenotype were class I tetrads (Table 5). The other 5% were class VI tetrads. However, the relative frequency of class I and class VI tetrads in cross X176 and X354 may not be the same. In determining the map distance between *HIS4* genes in cross X354, we assumed that all tetrads of class I phenotype were class I. This assumption probably leads to an overestimate of the genetic distance between *HIS4* genes in the construction devoid of vector sequence, and, therefore, we may have underestimated the effect of the plasmid sequences or recombination.

Tetrad analysis of cross X354 showed that fewer recombination events occur between *HIS4* genes, per kilobase, in a tandem duplication without intervening plasmid sequences, than in a direct duplication separated by plasmid sequences. The genetic distance between *HIS4* genes in X354 is 7.2 cM (Table 6). This figure is probably an overestimate. The physical distance between the two genes corresponds to the length of the *Bam*HI fragment, 18.6 kb. Thus, for the tandem duplication of the *HIS4* region has approximately 2.6 kb/cM (Table 6). This figure is twice as high as that observed in the direct duplications (1.3–1.4 kb/cM) and is very similar to the 2.3–3 kb/cM determined by other workers for chromosome *III* (P. FARABAUGH and G. R. FINK, personal communication; STRATHERN *et al.* 1979; CLARKE and CARBON 1980). If 7.2 cM were an overestimate of the map distance between *HIS4* genes, then the difference in recombination frequency between the two types of duplication-carrying strains could be even greater than two-fold. These results suggest that some sequences could be even greater than two-fold. These results suggest that some sequences on the plasmids used here are recombinogenic when homozygous in yeast. All three plasmids are related to each other and share substantial sequence homology (BOLIVAR *et al.* 1977a,b; BOTSTEIN *et al.* 1979).

## DISCUSSION

Meiotic recombination between duplicated genetic elements occurs at a high frequency. More than half of the tetrads from crosses of duplication strains are recombinant at or between the *HIS4* genes in each element of the duplication (Table 5). Several tetrads result from multiple recombination events. This high recombination frequency is to some extent a reflection of the large region of homology created by the duplication. An additional factor is that in this study, unlike previous studies of recombination between repeated sequences in yeast, no selection of particular classes of recombinants was imposed. The percentage of four-spored asci was high (80–90% in all crosses), suggesting that lethal recombination events were extremely rare or did not occur and that we detected most recombination events. These events include equal and unequal reciprocal exchanges between homologues, unequal sister-

chromatid exchange and intrachromatid reciprocal exchange. Inter- and intrachromosomal gene conversion events were also observed.

The most frequent recombination events observed are reciprocal exchanges between homologues. This interchromosomal reciprocal recombination occurs whether the duplicated elements are paired equally or unequally. The high frequency of unequal interchromosomal recombination (17%, Table 5) shows that unequal pairing of the duplicated sequences between homologues occurs often during meiosis.

Intrachromosomal reciprocal exchange, as measured by the occurrence of unequal sister-chromatid exchange and intrachromatid reciprocal exchange, is rare compared to interchromosomal reciprocal exchange. Unequal sister-chromatid exchange (0.9–1.5%, Table 5) is ten-fold less frequent than unequal interchromosomal reciprocal exchange (17%, Table 5). This result is interesting considering that interchromosomal reciprocal exchange is rare in the approximately 100 repeats of the 9-kb rDNA unit on chromosome *XII* (PETES and BOTSTEIN 1977; PETES 1979), although unequal sister-chromatid exchange is a frequent event (PETES 1980). It may be that the number, size or specific sequence of reiterated DNA sequences is important in determining whether the repeated sequences will be more likely to undergo inter- or intrachromosomal exchange.

Intrachromatid reciprocal exchange is even less frequent than unequal sister-chromatid exchange. An intrachromatid reciprocal exchange results in the excision of one copy of the duplicated element as well as the *E. coli* plasmid sequences (Figure 11). Of 11 phenotypically class III tetrads, colony hybridization and Southern analysis showed unambiguously that ten were the result of sister-chromatid exchange and one was the result of intrachromatid reciprocal exchange. Thus, it appears that the frequency of intrachromatid reciprocal exchange is extremely low (0.01%, 1/686 tetrads). This low frequency is striking considering the 18.6 kb of homology available for this recombination event between the elements of the duplication on each chromosome in the diploids studied here.

These data suggest that reciprocal exchange is far more likely to occur between regions of homology on homologues than between regions of homology on sister chromatids or on the same chromatid. This difference may simply reflect the fact that sister chromatids are more likely to be equally paired along their length than are homologous chromosomes. A reciprocal recombination event between equally paired sister chromatids would not be detected and equally paired sister chromatids would preclude the intrachromatid synapsis of the duplicated *HIS4* genes. This explanation seems unlikely due to the fact that inter- and intrachromosomal gene conversion between duplicated *HIS4* genes in this study occurred at approximately equal frequencies (Table 5). Interchromosomal gene conversion can occur whether the *HIS4* regions are paired equally or unequally. However, intrachromosomal gene conversion must occur during unequal sister-chromatid or intrachromatid pairing of the two *HIS4* genes on the same chromosome.

Our results suggest that only reciprocal exchange is suppressed between regions of homology on the same chromosome. This observation is consistent

with the results of KLEIN and PETES (1981) who studied the meiotic recombination behavior of a direct duplication of the *LEU2* gene separated by plasmid sequences and found that intrachromosomal gene conversion was the only meiotic recombination event detected between the two copies of the *LEU2* gene, again suggesting that sister-chromatid and intrastrand reciprocal exchange were suppressed. In addition, KLEIN (1984) has shown that intrachromosomal gene conversion between inverted *HIS3* sequences is frequent and not associated with reciprocal exchange. The reason we see some of these intrachromosomal reciprocal exchanges at *HIS4* may be due to differences in the size, sequence or chromosomal positions of the duplicated elements at *HIS4*, *HIS3* and *LEU2*.

The paucity of intrachromosomal reciprocal exchange events is probably not specific to meiosis. Our previous studies of mitotic recombination between duplicated *HIS4* genes in haploids showed that a gene conversion unassociated with reciprocal exchange event was responsible for 72–88% of the $His^+$ recombinants (JACKSON and FINK 1981). Thus, intrachromosomal reciprocal exchange is also a rare event between direct duplications in mitosis. The precise fraction due to sister-chromatid or intrachromatid reciprocal exchange cannot be determined because these events do not yield unique recombinants (see figure 4 of JACKSON and FINK 1981).

The suppression of intrachromosomal reciprocal exchange may have a selective advantage for yeast, because it minimizes the chances of chromosomal deletions or inversions produced by intrastrand exchange between direct or inverted repeated sequences on the same chromosome. This phenomenon is particularly important in the case of mating type switching in yeast. Although mating type switching has some unique features, such as the unidirectionality of the transfer of genetic information from *HMR* or *HML* to the *MAT* locus and the high frequency of this event in homothallic strains (HICKS and HERSKOWITZ 1977; STRATHERN and HERSKOWITZ 1979), it is similar to recombination between duplicated *HIS4* genes in important ways. Mating type switching in yeast occurs by the replacement of the Mat-**a**- or Mat-$\alpha$-specific information at the *MAT* locus by information from the *HML* or *HMR* locus by a gene conversion event (HABER, ROGERS and MCCUSKER 1980; KLAR *et al.* 1980; MALONE and ESPOSITO 1980; HERSKOWITZ and OSHIMA 1981). The *MAT*, *HML* and *HMR* loci are direct repeats of common DNA sequences on chromosome *III* (HICKS, STRATHERN and KLAR 1979; NASMYTH and TATCHELL 1980). Like recombination at *HIS4*, this intrachromosomal mating type conversion event is only rarely accompanied by reciprocal exchange of outside markers (HAWTHORNE 1963; STRATHERN *et al.* 1979; KLAR and STRATHERN 1984), but conversion forced to occur between two *MAT* loci on homologous chromosomes shows a significant association with reciprocal exchange (KLAR and STRATHERN 1984). It is possible that the low frequency of intrachromosomal reciprocal exchange associated with mating type switching is not a special feature of the mating type system but rather is a reflection of the infrequent occurrence of intrachromosomal reciprocal exchange between any pair of duplicated genetic elements in yeast.

Current models of recombination assume a mechanistic association of gene

conversion with reciprocal exchange (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). The failure to observe a significant amount of intrachromosomal reciprocal exchange in yeast may mean that there are topological restraints on intrachromosomal DNA interactions that preclude the resolution of recombination events by reciprocal exchange. Alternatively, it is possible that gene conversion and reciprocal exchange are not the outcome of the same event and are only coincidentally associated.

*Effect of plasmid sequences on meitoic recombination*: Meiotic mapping data presented in Table 6 suggest that the *E. coli* plasmids pBR313, pBR322 and YIp5 are recombinogenic when integrated into homologous yeast chromosomes. The data indicate that a recombination event is twice as likely to occur, per kilobase, in the direct duplications of the *HIS4* region separated by plasmid sequences than in tandem duplications with no intervening plasmid sequences or in *HIS4* regions of normal chromosome structure. This increased recombination may be due to a site that stimulates recombination in yeast that is carried on the plasmids or created by the joining of plasmid and yeast DNA. All three plasmids are related to each other and share substantial sequence homology (BOLIVAR *et al.* 1977a,b; BOTSTEIN *et al.* 1979). Moreover, the plasmid-yeast junction sequences formed by inserting the yeast *Bam*HI fragment into the *Bam*HI site on the plasmids are the same in all three cases. The existence of sites that stimulate recombination in yeast has been suggested by the observation of polarity of gene conversion events (FOGEL and MORTIMER 1971; FOGEL, MORTIMER and LUSNAK 1981). For example, at the *HIS1* and *ARG4* loci, mutations at one end of the gene undergo many more gene conversion events than mutations at the other end of the gene where recombination events are thought to be initiated or resolved (FOGEL, MORTIMER and LUSNAK 1981).

The increased recombination may be due to a specific site of action of a single-strand or double-strand nuclease within the plasmid sequences. Current models of recombination postulate that a single-strand (MESELSON and RADDING 1975) or double-strand (SZOSTAK *et al.* 1983) DNA break are required for initiation of recombination events and that a nuclease of some type is required for resolution of recombination intermediates. In addition, normal mating type gene conversion requires a specific double-strand break within the *MAT* locus (STRATHERN *et al.* 1982).

If a sequence in the plasmids or at the plasmid-yeast junctions is a specialized site of general recombination, it is probably not analogous to the *cog* sequences of *Neurospora crassa* (CATECHESIDE and ANGEL 1974) or the Chi sequences of *E. coli* (STAHL 1979) because the *cog* and Chi sequences have a dominant effect. Chi increases recombination even when the $Chi^-$ parent is deleted for the Chi site (STAHL and STAHL 1975). The recombination enhancement observed by plasmid sequences in our study is not dominant. Crosses of direct duplications and tandem duplications by strains of normal chromosome structure gave almost the same recombination frequencies (Table 3). Another feature of the *cog* and Chi sequences is that they affect recombination locally along the chromosome. The recombination frequency at the *HIS4* genes, as measured by the

TABLE 7

*His$^+$ recombinants from crosses of duplication strains*

| Integrated plasmid sequence | Cross | HIS$^+$ recombinants/total tetrads | % HIS$^+$ |
|---|---|---|---|
| pBR313 | X118 | 5/167 | 3 |
| pBR313 | X176 | 11/580 | 2 |
| pBR322 | X158 | 4/106 | 4 |
| YIp5 | X142 | 3/61 | 5 |
| YIp5 | X144 | 5/106 | 5 |
| None | X354 | 8/272 | 3 |

production of His$^+$ spores, was the same in all duplication by duplication crosses examined (Table 7). It is possible that the *HIS4* genes are too far away from the putative sequence to be affected.

An alternative model for the difference in recombination frequencies between the direct and tandem duplications is that the novel junction sequences created by the fusion of the two yeast *Bam*HI fragments in the tandem duplications are responsible for lowering the recombination frequency. This model appears unlikely due to the good correspondence between the number of kilobases per centimorgan in the tandem duplications with that found for segments of chromosome *III* by other laboratories (P. FARABAUGH and G. R. FINK, personal communication; STRATHERN *et al.* 1979; CLARKE and CARBON 1980). The mechanism for depression of recombination by a specialized site is difficult to imagine unless the junction sequences create a recognition site for a repressor of recombination. Repressors of recombination have been proposed in Neurospora (CATECHESIDE 1977).

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