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Meiotic Exchange within and between Chromosomes Requires a Common Rec Function in *Saccharomyces cerevisiae*

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We used haploid yeast cells that express both the *MAT*a and *MAT*α mating-type alleles and contain the *spo13-1* mutation to characterize meiotic recombination within single, unpaired chromosomes in Rec$^+$ and Rec$^-$ *Saccharomyces cerevisiae*. In Rec$^+$ haploids, as in diploids, intrachromosomal recombination in the ribosomal DNA was detected in 2 to 6% of meiotic divisions, and most events were unequal reciprocal sister chromatid exchange (SCE). By contrast, intrachromosomal recombination between duplicated copies of the *his4* locus occurred in approximately 30% of haploid meiotic divisions, a frequency much higher than that reported in diploids; only about one-half of the events were unequal reciprocal SCE. The *spo11-1* mutation, which virtually eliminates meiotic exchange between homologs in diploid meiosis, reduced the frequency of intrachromosomal recombination in both the ribosomal DNA and the *his4* duplication during meiosis by 10- to >50-fold. This Rec$^-$ mutation affected all forms of recombination within chromosomes: unequal reciprocal SCE, reciprocal intrachromatid exchange, and gene conversion. Intrachromosomal recombination in *spo11-1* haploids was restored by transformation with a plasmid containing the wild-type *SPO11* gene. Mitotic intrachromosomal recombination frequencies were unaffected by *spo11-1*. This is the first demonstration of a gene product required for recombination between homologs as well as recombination within chromosomes during meiosis.

Intrachromosomal recombination, that is, recombination between sister chromatids or between repeated sequences on a single chromatid, occurs during mitotic growth and during meiosis in the yeast *Saccharomyces cerevisiae* (16, 21, 28, 32). This form of recombination, encompassing both gene conversion and reciprocal exchange, has been detected between naturally occurring tandemly repeated genes such as the ribosomal DNA (rDNA) cistrons (28, 32) and between repeats created by transformation of *S. cerevisiae* with hybrid plasmid DNA that integrates at sites of homology (16, 21). Recombination within chromosomes, in principle, can change the number of copies of genes in repeated gene families, eliminate variant forms of genes from repeated families, and create new variants by intragenic exchange.

Little is known at present about the wild-type gene products that are required for intrachromosomal recombination or about the extent to which intrachromosomal recombination and recombination between homologs are dependent on common gene products. In *S. cerevisiae*, studies directed toward these issues have focused primarily on the role of the *RAD52* gene in genetic exchange. Mutations in this gene confer extreme sensitivity to ionizing radiation (10), a deficiency in double strand break repair (14, 31), and a failure to recover recombinant progeny during meiosis (11, 30). Several types of mitotic exchange events are also reduced or absent in *rad52* mutants. These include spontaneous gene conversion and reciprocal exchange between homologs (23), mating-type switching (23), intrachromosomal gene conversion (16), repair of gapped plasmids and integration of linear fragments during transformation (27), and δ-δ recombination leading to reversion of Ty-induced mutations (34). In contrast, *rad52* mutations have little effect on spontaneous mitotic intrachromatid exchange and unequal sister-chromatid exchange (SCE) (16, 29, 35) or integration of circular plasmids (27). These observations suggest that although *RAD52* is required for a number of forms of recombination during mitosis and meiosis, some types of mitotic intrachromosomal exchange occur by *RAD52*-independent mechanisms.

We recently have developed a genetic system that provides a straightforward means of analyzing the genetic control of intrachromosomal recombination during meiosis in *S. cerevisiae* (33). This system takes advantage of the meiotic behavior of haploid *S. cerevisiae* strains with a mutation in a gene required for the meiosis I division. Normally, *MAT*a or *MAT*α haploid cells are unable to initiate the earliest events of meiosis. Under conditions in which both mating-type alleles are expressed (e.g., in the presence of a *MAT*a/*MAT*α chromosome III disome or the *mar1* mutation which allows expression of the silent mating-type cassettes), haploids can execute premeiotic DNA synthesis and the meiosis I and meiosis II divisions. In these cases, immature spores are formed, presumably because of extensive aneuploidy resulting from random segregation of unpaired chromosomes at meiosis I. In contrast to wild-type strains, haploids bearing the *spo13-1* mutation bypass the meiosis I division. Premeiotic DNA synthesis, genetic recombination, and meiosis II chromatid separation appear to occur normally, resulting in the production of dyad asci containing two viable haploid spores. By using appropriately marked *spo13-1* haploids, it therefore is possible to assay meiotic recombination within single chromosomes in the absence of their normal homologs.

In addition to permitting haploids to sporulate, *spo13-1* allows diploids homozygous for certain Rec$^-$ mutations to sporulate and produce viable diploid meiotic products (19,

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TABLE 1. Strains used in experiments

Strain	Genotype (chromosome III; chromosome VIII; chromosome XII; other markers)	Source or reference
K355-9C	*MATa*; *spo11-1 spo13-1 spo12-1*; *URA3* in rDNA; *MATα* *ade2 cyh2 leu1 lys2 trp5 tyr1-1 ura3*	This study
K355-13A	*LEU2 MATa*; *spo13-1*; *URA3* in rDNA; *leu2 MATα* *ade2 cyh2 his7-2 leu1 lys2 met13 trp5 tyr1-1 ura3*	33
K355-24C	*MATa*; *spo11-1 spo13-1 spo12-1*; *URA3* in rDNA; *MATα* *ade2 cyh2 leu1 lys2 met13-c trp5 ura3*	This study
JW165-19D	*his4 leu2 MATa*; *spo13-1*; *URA3* in rDNA; *HIS4 LEU2 MATα* *ade2 his7 leu1 met13 trp5 ura3*	This study
JW165-37D	*his4 leu2 MATa*; *spo13-1*; *URA3* in rDNA; *HIS4 LEU2 MATα* *ade2 his7 leu1 lys2 lys11 met13 trp5 ura3*	This study
JW167-171B	*his4B-331 leu2-1 cry1 MATα*; *spo13-1*; *URA3* in rDNA; *his4A-25 leu2-27 CRY1 MATa* *ade2 cyh2 lys2-2 met13-c tyr1-2 ura3*	This study
JW168-7B	*his4B-331 leu2-1 cry1 MATα*; *spo13-1*; *URA3* in rDNA; *his4A-25 leu2-27 CRY1 MATa* *ade2 cyh2 lys2 tyr1 ura3*	This study
JW194-64B	*his4B-331 leu2-27 cry1 MATα*; *spo11-1 spo13-1*; *URA3* in rDNA; *his4A-25 leu2-27 CRY1 MATa* *ade2 can1 lys2-2 tyr1 ura3*	This study
JW194-67D	*his4B-331 leu2-1 CRY1 MATa*; *spo11-1 spo13-1*; *URA3* in rDNA; *his4A-25 leu2-27 cry1 MATα* *ade2 can1 lys2-2 met13-c tyr1 ura3*	This study
JW201-4B	*his4-39,260 pBR313 his4-1176,864 MATa*; *spo13-1*; *ade2 can1 lys1-1 mar1 ura3*	This study
JW201-22B	*his4-39,260 pBR313 his4-1176,864 leu2-3,112* colE1 *leu2-101 MATa*; *spo11-1 spo13-1*; *ade2 ade5 can1 lys1-1 mar1 trp1 ura3*	This study
TP406pSS31(1)	*leu2 MATa*; *URA3* in rDNA; *gal2 his5 lys11 ura3-52*	T. Petes

24). In the absence of *spo13-1*, Rec⁻ diploids sporulate poorly and produce almost no viable meiotic products, again because of meiosis I nondisjunction.

The *spo13-1* mutation permits sporulation not only of *MATa/MATα* haploids and of certain Rec⁻ diploids, but also of haploids bearing Rec⁻ mutations (33; J. E. Wagstaff, Ph.D. dissertation, University of Chicago, Chicago, Ill., 1983). In this study, we exploited this property of *spo13-1* to investigate the effects of the *spo11-1* mutation, which virtually eliminates reciprocal exchange and gene conversion between homologs during diploid meiosis (18, 19), on unequal reciprocal sister-chromatid exchange SCE, reciprocal intrachromatid exchange, and intrachromosomal gene conversion.

MATERIALS AND METHODS

Strains. The genotypes of the *S. cerevisiae* strains used in this study are shown in Table 1. Several of the *spo13-1* haploid strains contain the *spo12-1* mutation as well. Results of previous studies (33; S. Klapholz, Ph.D. dissertation, University of Chicago, Chicago. Ill.) have shown that the phenotype of *spo12-1 spo13-1* double mutants is indistinguishable from that of *spo13-1* mutants; the epistasis of *spo13-1* to *spo12-1* is confirmed in this study. The strains containing *URA3* integrated in the rDNA were derived from crosses involving TP406pSS31(1), which was kindly provided by T. Petes (35). The marked *his4* duplication in orientation I (16) was derived from strain 7313-10A,

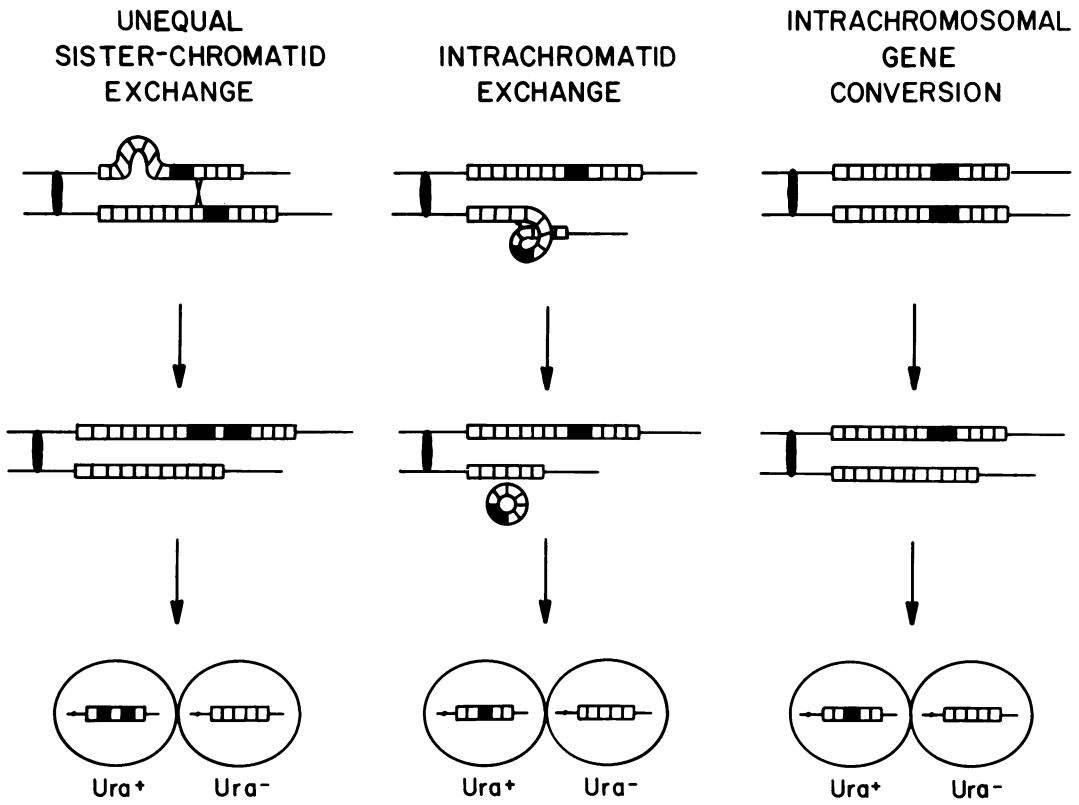

FIG. 1. Mechanisms of segregation of Ura⁻ spore clones from haploids with *URA3* in rDNA. Haploids contain a mutant *ura3* gene on chromosome V. The wild-type *URA3* gene integrated in the rDNA is designated by a shaded rectangle; rDNA repeat units are represented by open squares. Dyad genotypes shown are based on the assumption that rDNA circles produced by excision are unstable.

which was generously provided by S. Roeder and G. Fink.

Media and genetic techniques. Growth and sporulation media have been described previously (18). Standard methods for crosses, ascus dissection, and other genetic procedures were used (25).

Mitotic recombination experiments were done by inoculating 10 ml of YPD liquid medium with a single unbudded cell of a haploid yeast strain that had been micromanipulated onto an agar block. The culture was shaken vigorously at 30°C until it reached 1×10^7 to 2×10^7 CFU/ml. Cells in the culture were then collected by centrifugation, washed twice with water, suspended in water, and then sonicated briefly to disperse clumps of cells. Appropriate dilutions of the sonicated suspension were plated on selective medium or on synthetic complete medium.

Mitotic segregation of Ura⁻ cells from haploids containing the *URA3* gene inserted into their rDNA was assayed as follows. Cells from cultures grown as described above were dispersed on the surface of YPD plates at 50 to 75 CFU per plate and then incubated at 30°C for 2 days until colonies were approximately 2 to 3 mm in diameter. They were then replica plated to synthetic complete medium and to synthetic medium lacking uracil. After incubation at 30°C for 2 days, the plates were scored for colonies that grew on complete medium but not in the absence of uracil.

Transformation of JW201-22A with p(SPO11)9 was done as described by Beggs (1).

Hybridization analysis of restriction digests. DNA was prepared for purposes of restriction and hybridization analysis by the rapid technique of Davis et al. (5), followed in some cases by extraction with 50 μl of chloroform-isoamyl alcohol (24:1) and precipitation with 5 μl of 3 M sodium acetate and 33 μl of isopropanol. Restriction enzyme cleavage, gel electrophoresis, transfer of DNA to nitrocellulose, nick translation, hybridization, and autoradiography were carried out as described by Davis et al. (4). For strains containing *URA3* integrated into rDNA, DNA was cleaved sequentially with *Bgl*II and *Eco*RI and then separated on 0.7% agarose gels. The hybridization probe was fd6 replicative form DNA, which contains the *S. cerevisiae URA3* gene on a 1.2-kilobase pair (kbp) *Hin*dIII fragment inserted into the fd106 vector (15). For strains derived from haploids containing the *his4* duplication, DNA was cleaved with *Sma*I, separated on 0.5% agarose gels run at 40 V for 48 h, and probed with nick-translated pBR322 DNA. Autoradiograms were traced with a Joyce-Loebl microdensitometer.

RESULTS

Recombination in rDNA during Rec⁺ haploid meiosis. We used genetically marked strains of two types to characterize intrachromosomal recombination during haploid meiosis and to investigate the effects of the *spo11-1* mutation on the process. In strains of the first type (Fig. 1), the wild-type *URA3* gene was inserted by integrative recombination into the rDNA tandem array on chromosome XII of a strain containing a *ura3* mutation on chromosome V (35). These strains are disomic for chromosome III and heterozygous at the *MAT* locus and contain the *spo13-1* mutation. Either unequal reciprocal SCE or reciprocal recombination within a chromatid can yield a dyad with one Ura⁺ spore and one Ura⁻ spore. In the case of unequal reciprocal SCE, the Ura⁺

TABLE 2. Meiotic intrachromosomal recombination in rDNA

Genotype and strain[a]	No. of dyads	No. (%) of Ura segregants (+:−)[b]			No. (%) of MAT segregants (a: α: N)[c]			
		2:0	1:1	0:2	0:0:2	1:1:0	1:0:1	0:1:1
SPO11								
K355-13A	128	118	2	8	69	27	17	15
JW165-19D	115	99	7	9	61	22	13	19
JW165-37D	127	119	2	6	70	33	14	10
JW167-171B	441	426	8	7	243	103	50	45
Totals	811	762 (94)	19 (2.3)	30 (3.7)	443 (55)	185 (23)	94 (12)	89 (11)
spo11-1								
K355-9C	140	132	1	7	139	0	0	1
K355-24C	128	123	0	5	125	0	0	3
JW194-64B	509	453	1	55	502	1	1	5
JW194-67D	310	302	0	8	294	0	9	7
Totals	1,087	1,010 (93)	2 (0.2)	75 (6.9)	1,060 (98)	1 (0.1)	10 (0.9)	16 (1.5)

[a] All strains are *spo13-1* a/α disomic haploids.
[b] Ura 1^+:1^- segregation is due to meiotic intrachromosomal recombination on chromosome XII (Fig. 1).
[c] Mating phenotype segregation of 1a:1α:ON, where a = a-mating, α = α-mating, and N = nonmating, results from either genetic exchange between chromosome III homologs or reductional segregation of chromosome III (33).

spore contains two *URA3* copies in its rDNA; reciprocal intrachromatid exchange, by contrast, yields a Ura^+ spore with one *URA3* copy in its rDNA. For both mechanisms, the Ura^- spore contains no *URA3* sequences in its rDNA. Gene conversion of an rDNA repeat containing *URA3* to the sequence of a normal rDNA repeat generates products identical to those of reciprocal intrachromatid exchange.

Intrachromosomal recombination produced dyads with one Ura^+ spore and one Ura^- spore in 2.3% of Rec^+ haploid meiotic divisions (Table 2). This is almost certainly an underestimate of the probability per meiosis of intrachromosomal exchange in the rDNA, because only events involving repeat units on opposite sides of the *URA3* insert are detected. Dyads with two Ura^- spores, which constitute about 4% of the total, are likely to reflect mitotic recombination events that occurred prior to meiosis, rather than two distinct events during a single meiosis.

Hybridization analysis (Fig. 2) indicated that most dyads with one Ura^+ spore and one Ura^- spore arose via unequal reciprocal SCE. *URA3* DNA labeled with ^{32}P was hybridized to nitrocellulose filters containing *Bgl*II-*Eco*RI-digested total DNA from JW167-171B and from five 1^+:1^- dyads from JW167-171B. The ratio of hybridization to the chromosome XII (rDNA) copy to hybridization to the chromosome V copy reflects the number of *URA3* copies integrated into the rDNA. For JW167-171B, the hybridization ratio was 2.7 (Table 3), indicating that there were three *URA3* copies in its rDNA. This result is consistent with the inference by Zamb and Petes (35) that TP406pSS31(1), the Ura^+ transformant from which the *URA3* insert in JW167-171B was derived, has three (or an integral multiple of three) copies of the *URA3*-containing plasmid pSS31 in its rDNA, probably arranged in tandem.

In four of the five 1^+:1^- dyads analyzed from JW167-171B, the hybridization ratio for the Ura^+ spore was approximately 6 (Table 3); these spores had twice as many *URA3* copies in their rDNA as their parent JW167-171B and arose by unequal reciprocal SCE. The Ura^+ spore from the fifth 1^+:1^- dyad analyzed showed a hybridization ratio of 1, indicating the presence of only one *URA 3* copy in its rDNA. This result is not anticipated for either unequal reciprocal SCE or reciprocal intrachromatid exchange and is likely due to either multiple meiotic recombination events or a mitotic intrachromsomal exchange event followed by meiotic ex-

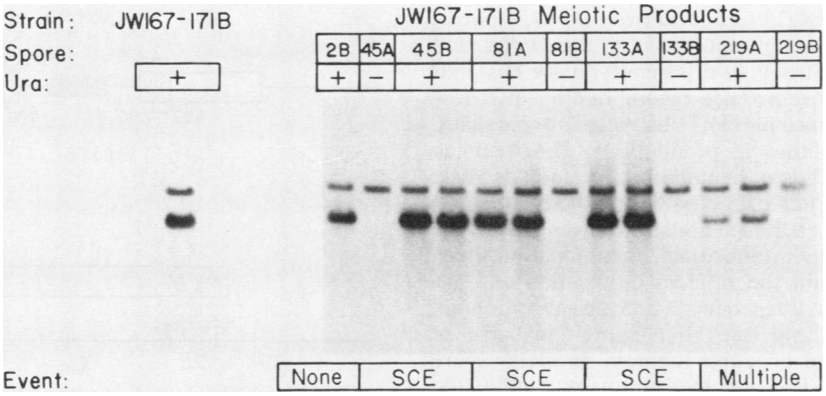

FIG. 2. Analysis of *URA3* copy number by Southern hybridization in dyads from Rec^+ haploids. Hybridization was performed as described in the text. The upper band of hybridization corresponds to the *ura3* gene on chromosome V; the lower band represents *URA3* copies integrated into the rDNA. Duplicate lanes contain DNA from two different DNA preparations from the same strain. All spore clones examined were from one Ura^+:1 Ura^- dyads except JW167-171B-2B, which was from a 2 Ura^+:0 Ura^- dyad.

TABLE 3. Analysis of *URA3* copy number in meiotic segregants by hybridization

Genotype and strain	Ura phenotype	Hybridization band[a]		Hybridization ratio (II/I)[b]	Inferred mechanism
		I	II		
SPO11					
TP406pSS31(1)	+	+	+	3.4	Parent strain
JW167-171B	+	+	+	2.7	Parent strain
JW167-171B-2A	+	+	+	3.0	No exchange
JW167-171B-2B	+	+	+	2.5	
JW167-171B-45A	−	+	−		Unequal SCE
JW167-171B-45B	+	+	+	6.5	
JW167-171B-81A	+	+	+	5.3	Unequal SCE
JW167-171B-81B	−	+	−		
JW167-171B-133A	+	+	+	5.8	Unequal SCE
JW167-171B-133B	−	+	−		
JW167-171B-219A	+	+	+	1.0	Intrachromatid exchange[c]
JW167-171B-219B	−	+	−		
JW167-171B-370A	+	+	+	5.5	Unequal SCE
JW167-171B-370B	−	+	−		
spo11-1					
JW194-64B	+	+	+	2.1	Parent strain
JW194-64B-387A	+	+	+	2.0	No exchange
JW194-64B-387B	+	+	+	3.7	
JW194-64B-395A	+	+	+	2.4	No exchange
JW194-64B-395B	+	+	+	3.4	
JW194-64B-398A	−	+	−		Unequal SCE
JW194-64B-398B	+	+	+	6.5	

[a] Hybridization band I corresponds to the *ura3* gene on chromosome V; band II corresponds to *URA3* integrated into the rDNA on chromosome XII.
[b] The hybridization ratio (band II/band I) provides an estimate of the number of copies of *URA3* integrated into the rDNA of a particular haploid meiotic segregant. All ratios are based on at least two independent determinations.
[c] This dyad probably resulted from a mitotic exchange event which produced a cell with only one copy of *URA3* in its rDNA (as suggested by Zamb and Petes [35]), followed by meiotic intrachromatid exchange in that cell or in one of its mitotic progeny. We cannot, however, rule out multiple meiotic exchange events.

change or gene conversion. The Ura$^-$ spores from all five dyads showed no hybridization at the position corresponding to the *URA3* copy integrated in rDNA, as expected.

Recombination in a *his4* duplication during Rec$^+$ haploid meiosis. The second type of strain used in this study contains duplicate copies of the *his4* locus on chromsome III, separated by the plasmid pBR313 (16). The two *his4* loci contain different noncomplementing mutations in the *his4A* and *his4C* regions (Fig. 3). Strains of this genotype are His$^-$ but undergo mitotic intrachromsomal recombination at a low ratio to yield His$^+$ colonies in a His$^-$ background (papillae). This phenotype which allows the production of His$^+$ papillae during vegetative growth is designated in this study as His^P. The various types of dyads expected to result from meiosis with or without genetic exchange are shown in Fig. 4. In the absence of meiotic intrachromosomal recombination, both spores of a dyad contain the original duplication and are His^P. If recombination does occur, a dyad may contain either one His^P spore and one His$^-$ spore incapable of producing His$^+$ papillae, or it may contain one His^P spore and one His$^+$ spore. The mechanism of recombination yielding a $0^+:1^-:1^P$ dyad or a $1^+:0^-:1^P$ dyad can be inferred from the number of *his4* gene copies in each spore. Unequal reciprocal SCE gives one spore with one copy and one spore with three copies, reciprocal intrachromatid exchange yields one spore with one copy and one spore with two copies, and gene conversion (between or within chromatids) produces two spores each with two copies of the *his4* locus (Fig. 4).

In a Rec$^+$ strain containing the *his4* duplication, detectable

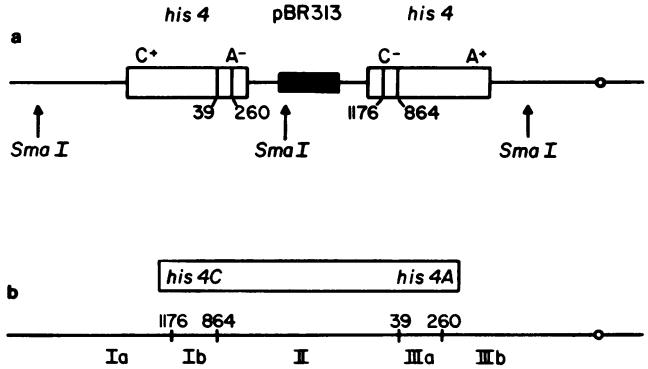

FIG. 3. (a) The *his4* duplication derived from strain 7313-10A (orientation I [16]). (b) Subdivisions of *his4* and the surrounding region in which recombination may occur. Region I is centromere distal to *his4-864*, region II is between *his4-864* and *his4-39*, and region III is centromere proximal to *his4-39*.

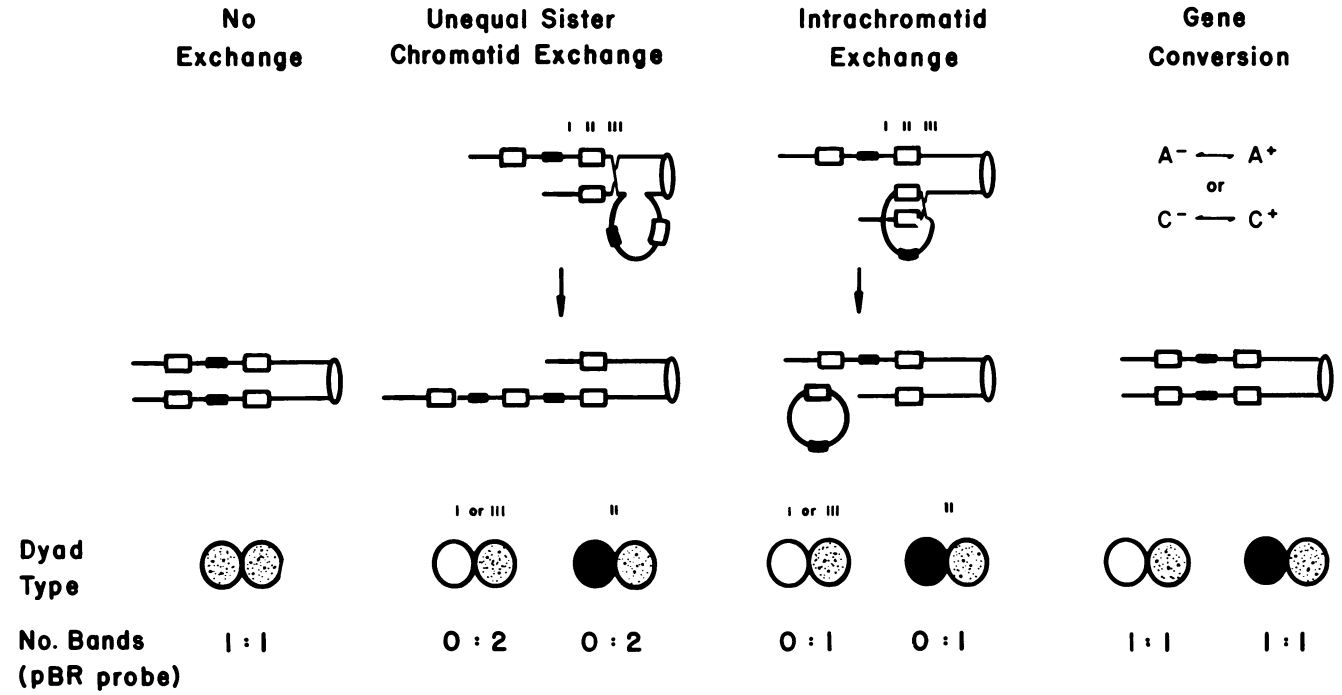

FIG. 4. Mechanisms of intrachromosomal recombination in the *his4* duplication. Open rectangles symbolize the 18.6-kb cloned region that contains *his4*; shaded rectangles represent plasmid pBR313. Roman numerals I, II, and III designate regions within the duplication in which genetic exchange may occur (Fig. 3). Open ovals represent His^- spores, shaded ovals represent His^+ spores, and ovals containing dots represent His^P (papilla-producing) spores. The number of bands refers to the number of hybridizing bands in *Sma*I-cleaved DNA when probed with pBR322; this number is one less than the number of *his4* copies for strains containing duplications, triplications, or single copies of *his4*.

intrachromosomal recombination events occurred at an unexpectedly high frequency: 31% of meiotic divisions (Table 4). Most of these events (43 of 49) generated dyads containing one His^P spore and one His^- spore. Three mechanisms can produce this type of dyad (Fig. 3 and 4): (i) unequal reciprocal SCE with the exchange located centromere distal to *his4-864* (region I) or centromere proximal to *his4-39* (region III); (ii) reciprocal intrachromatid exchange centromere distal to *his4-864* or centromere proximal to *his4-39*; (iii) gene conversion of any wild-type site to the corresponding mutation (e.g., *HIS4A* → *his4-260*).

Only five dyads contained one His^+ spore and one His^P spore and arose from either unequal reciprocal SCE or reciprocal intrachromatid exchange in the region between the *his4A* and *his4C* alleles (region II) or gene conversion (i.e., *his4-1176,864* → *HIS4C* or *his4-39,260* → *HIS4A*). The remaining recombinant dyad, phenotypically $0^+:2^-:0^P$, is difficult to explain on the basis of a single meiotic recombination event; its origin is discussed below.

Recombinant dyads due to unequal reciprocal SCE can be distinguished from dyads due to reciprocal intrachromatid exchange and from dyads due to gene conversion by hybridization with ^{32}P-labeled pBR322 DNA. As shown by Jackson and Fink (16), when DNA from *his4* duplication-bearing strains is cleaved with endonuclease *Sma*I, two fragments are produced that hybridize to pBR322 plasmid DNA and that comigrate in agarose gels, yielding a single band on an autoradiogram (Fig. 4 and 5). Strains with *his4* triplications, analyzed in the same way, give the same band together with a new smaller (approximately 28-kb) *Sma*I fragment. Strains with only a single copy of *his4*, on the other hand, contain no sequences that hybridize to pBR322 DNA.

Hybridization analysis of 43 $0^+:1^-:1^P$ dyads from JW201-4B (Table 5) indicated that 24 (56%) arose from unequal reciprocal SCE, 16 (37%) from reciprocal intrachromatid exchange, and 3 (7%) from gene conversion. Dyads of the first group, due to unequal reciprocal SCE, contained a His^- spore that gave no hybridization to pBR322 DNA and a His^P spore that had two hybridizing DNA bands and that therefore bears a *his4* triplication. By determining which *his4* alleles are present in the His^- spores from these dyads, the region of the *his4* gene and surrounding sequences in which exchange has occurred can be localized. For instance, JW201-4B-22A is His^- and contains the

TABLE 4. Meiotic intrachromosomal recombination between duplicated *his4* genes

Genotype	Strain[a]	No. of dyads	No. (%) of *His* segregants (+:−:P)[b]			
			0:0:2	0:1:1	1:0:1	0:2:0
SPO11	JW201-4B	157	108 (69)	43 (27)	5 (3)	1 (0.6)
spo11-1	JW201-22A	177	176 (99)	0 (<0.6)	0 (<0.6)	1 (0.6)

[a] Both strains are *spo13-1 mar1* haploids.

[b] Spore clones with the His^P phenotype were His^- but gave rise to His^+ papillae during mitotic growth. Spore clones with the His^- phenotype gave no His^+ papillae. Dyads showing $0^+:1^-:1^P$ and $1^+:0^-:1^P$ segregation arose from meiotic intrachromosomal recombination (Fig. 4).

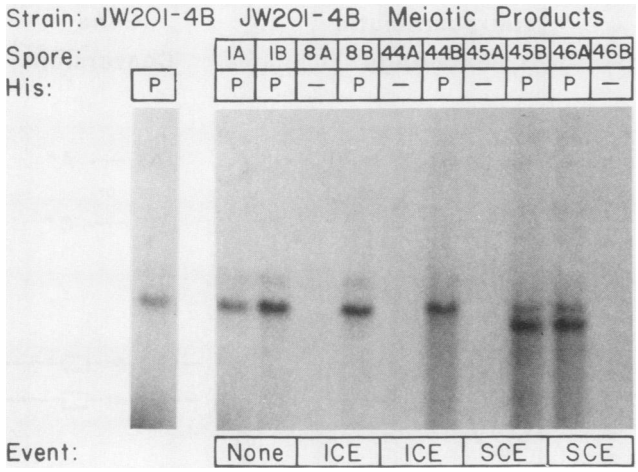

FIG. 5. Hybridization analysis of dyads exhibiting recombination in the *his4* duplication. Hybridization was performed as described in the text. The upper band of hybridization is actually due to two distinct DNA fragments found in *his4* duplication- and triplication-bearing strains that comigrate in agarose gels; the lower band is found only in triplication (and presumably higher repeat) strains. Some lanes contain a very faint high-molecular-weight band that comigrates with uncut DNA; we believe that this is due to incomplete restriction endonuclease digestion of our DNA samples. Mechanisms by which dyads arose were inferred as described in Fig. 4; ICE, reciprocal intrachromatid exchange. Dyads resulting from gene conversion (data not shown) give the same hybridization pattern as dyad 1 (spores 1A and 1B).

his4-39 and *his4-260* mutations but not *his4-1176* or *his4-864*. The dyad containing this spore is most easily explained by unequal reciprocal SCE occurring centromere proximal to the *his4-260* mutant site (region IIIb), generating one spore with a single *his4-39,260* copy and one spore with three copies of *his4* (*his4-39,260*, *his4-1176,864*, and *his4-1176,864*). The spore with the *his4* triplication generates His^+ papillae by intrachromosomal recombination during vegetative growth following germination. It should be pointed out that this analysis does not allow one to distinguish between a single reciprocal exchange event, on the one hand, and a reciprocal exchange centromere proximal to *his4-260* associated with, for example, adjacent gene conversion of *HIS4A* → *his4-260*. Both of these events would be classified as unequal reciprocal SCE by our criteria. Also undetected by this analysis would be gene conversion of any one of the mutant sites to the wild type without associated reciprocal exchange. The mechanisms listed in Table 5 are the simplest mechanisms consistent with the hybridization and allele testing results.

The majority of the 24 unequal reciprocal SCE events (15 of 24) occurred centromere proximal to *his4-260* in region IIIb; 5 occurred centromere distal to *his4-1176* (region Ia), 1 occurred in the interval between *his4-1176* and *his4-864* (region Ib), and 2 occurred between *his4-39* and *his4-260* (region IIIa). One dyad in which unequal reciprocal SCE apparently occurred, dyad 124, had a His^- spore that contained the *his4-260*, *his4-1176*, and *his4-864* alleles (Table 5). This genotype is not compatible with a single unequal reciprocal SCE, but could be generated by reciprocal exchange together with nonadjacent gene conversion (for example, unequal reciprocal SCE in region Ia with conversion of *HIS4A* → *his4-260*).

Localization of the exchange events in the 16 reciprocal intrachromatid exchange dyads by allele testing yielded a distribution similar to that found in the reciprocal SCE dyads: most of the events (10 of 16) were in region IIIb. Dyad 193 had a His^- spore that contained only *his4-260*; this genotype cannot be explained by a single recombination event but could arise from reciprocal exchange in region IIIb associated with *his4-39* → + conversion or from exchange in region II associated with *HIS4A* → *his4-260* conversion.

The three $0^+:1^-:1^P$ dyads that hybridization indicates arose from gene conversion alone include two cases of *HIS4A* → *his4-39,260* conversion and one case of *HIS4C* → *his4-1176,864* conversion. No cases of single-site conversion (e.g., *HIS4A* → *his4-39*) were detected in this class, indicating that as in meiotic recombination between homologs (9), physically close heterozygous sites coconvert more frequently than would be predicted on the basis of random independent conversion.

In addition to the 43 $0^+:1^-:1^P$ dyads, 5 other recombinant dyads had one His^+ spore and one His^P spore. Hybridization analysis of these indicated that one was due to unequal reciprocal SCE in region II, two were due to reciprocal intrachromatid exchange in region II, and two were due to gene conversion. The rarity of this dyad class is due to the fact that region II constitutes less than 2.4 kb of the entire 18.6-kb duplication (6).

Finally, dyad 186 contained two His^- spores. Spore 186A contained a *his4* duplication, with each copy bearing the *his4-1176* and *his4-864* mutant alleles. Spore 186B had only a single copy that was *his4-39,260*. This dyad clearly cannot be attributed to a single recombination event, and is most likely due to a gene conversion (*HIS4C* → *his4-1176,864*) on one chromatid, together with a reciprocal intrachromatid exchange in region IIIb involving the other chromatid.

In summary, among 49 recombinant dyads, 46 can be attributed to a single recombination event: 24 to unequal reciprocal SCE, 17 to reciprocal intrachromatid exchange, and 5 to gene conversion. The genotypes of three dyads from JW201-4B reflect the occurrence of both an intrachromosomal gene conversion and a (nonadjacent) reciprocal exchange event during the same meiosis. Other dyads may have been produced by gene conversion with adjacent reciprocal exchange but would not be distinguishable from reciprocal exchange alone by our analysis. Are the apparent double recombination dyads more numerous than would be predicted if the two events were statistically independent? The expected incidence of independent gene conversion and unequal reciprocal SCE is 1.3 dyads among the 157 dyads examined ($0.159 \times 0.051 \times 157$). (The probabilities used to make this calculation are the probability of unequal reciprocal SCE with or without gene conversion and the probability of gene conversion with or without reciprocal exchange.) We found one dyad in which both gene conversion and unequal reciprocal SCE must have occurred. Independent gene conversion and reciprocal intrachromatid recombination would be expected in $0.121 \times 0.051 \times 157 = 1.0$ dyad, and we found two dyads of this type. These data indicate that there is not a large excess of double events over the number predicted from independent recombination. We wish to emphasize that these calculations do not bear directly on the association of gene conversion with adjacent reciprocal exchange (8) because such events would not be differentiated from reciprocal exchange alone by the criteria used here.

Intrachromosomal recombination is virtually absent in *spo11-1 spo13-1* haploid meiosis. The *spo11-1* mutation eliminates almost all meiotic recombination between homologous chromosomes during diploid meiosis (18, 19) and be-

TABLE 5. Recombinant dyads from a haploid containing a *his4* duplication

Mechanism	No. of dyads[a]	Spore clone	His phenotype	No. of bands hybridizing to pBR322[b]	*his4* alleles[c]			
					-39	*-260*	*-1176*	*-864*
Unequal SCE								
Region Ia	5	A	P	2				
		B	−	0	+	+	−	−
Region Ib	1	A	P	2				
		B	−	0	+	+	+	−
Region II	1	A	P	2				
		B	+	0				
Region IIIa	2	A	P	2				
		B	−	0	−	+	+	+
Region IIIb	15	A	P	2				
		B	−	0	−	−	+	+
Intrachromatid exchange								
Region Ia	3	A	P	1				
		B	−	0	+	+	−	−
Region Ib	2	A	P	1				
		B	−	0	+	+	+	−
Region II	2	A	P	1				
		B	+	0				
Region IIIa	0							
Region IIIb	10	A	P	1				
		B	−	0	−	−	+	+
Gene conversion								
+,+→	2	A	P	1				
39,260		B	−	1	−	−	+	+
+,+→	1	A	P	1				
1176,864		B	−	1	+	+	−	−
−,−→+,+	2	A	P	1				
		B	+	1				
Multiple events								
Dyad 124	1	A	−	0	+	−	−	−
		B	P	2				
Dyad 186	1	A	−	1	+	+	−	−
		B	−	0	−	−	+	+
Dyad 193	1	A	P	1				
		B	−	0	+	−	+	+

[a] Dyads analyzed are products of strain JW201-4B.

[b] The number of bands hybridizing to pBR322 DNA was determined as shown in Fig. 5. In cases in which there was one hybridizing band, that band was in all cases the lower mobility band seen in Fig. 5.

[c] Designations of genetic regions in the vicinity of *his4* are shown in Fig. 3. All His$^-$ spore clones were crossed to haploid testers containing *his4-39*, *his4-260*, *his4-1176*, or *his4-864*. If the resulting diploid produced His$^+$ papillae following sporulation, the allele test was scored +; if no His$^+$ papillae were seen, the test was scored −.

tween chromosome III homologs during *spo13-1* haploid meiosis (33). To determine whether the wild-type *SPO11* gene is a necessary prerequisite for intrachromosomal recombination as well, we examined strains closely related to the Rec$^+$ strains described above for evidence of meiotic SCE or intrachromatid recombination in the rDNA and between duplicated *his4* genes.

In four *spo11-1* haploids containing *URA3* inserted into the rDNA, the average frequency of dyads with one Ura$^+$ spore and one Ura$^-$ spore was 0.2%, or approximately

TABLE 6. Meiotic intrachromosomal recombination in the *his4* duplication in a *spo11-1* strain transformed with the cloned *SPO11* gene[a]

Dyad type (Trp^+ Ura^+:Trp^- Ura^-)[b]	No. of dyads	No. (%) of His segregants (+:−:P)			
		0:0:2	0:1:1	1:0:1	0:2:0
2:0	86	57 (66)	24 (28)	3 (3)	2 (2)
0:2	68	68	0	0	0
1:1	5	5	0	0	0

[a] JW201-22A[p(SPO11) 9] is a *spo11-1 trp1 ura3* haploid transformed with p(SPO11) 9, a centromere plasmid containing the wild-type *SPO11*, *TRP1*, and *URA3* genes. The strain was grown on synthetic medium lacking uracil before being replica plated to SPIII-22, a uracil-containing sporulation medium.
[b] Trp^+ Ura^+ spores contain p(SPO11) 9; Trp^- Ura^- spores do not.

one-tenth the average frequency in *SPO11* haploids (Table 2). The frequency of dyads with one *a*-mating spore and one α-mating spore, which are the consequence of genetic exchange between the chromosome III homologs in these disomic haploids or of reductional division of chromosome III (33), was also reduced by over 200-fold. Hybridization analysis of one 1^+:1^- dyad from JW194-64B showed that the Ura^+ spore contains six copies of *URA3* in its rDNA (Table 3), indicating that the dyad arose from unequal reciprocal SCE rather than from reciprocal intrachromatid exchange.

The reduction by *spo11-1* of meiotic intrachromosomal recombination frequency assayed with the *his4* duplication system is even more dramatic: <1% (0 of 177) of dyads in the *spo11-1* haploid, versus 30% in the *SPO11* haploid, showed 0^+:1^-:1^P or 1^+:0^-:1^P segregation (Table 4). The only dyad from the *spo11-1* haploid that was not 0^+:0^-:2^P had two His^- spores; hybridization analysis showed that neither spore clone contained pBR313 sequences, indicating that both spores must have contained only a single *his4* gene. This dyad was probably the product of a mitotic intrachromosomal recombination event prior to meiosis. The *spo11-1* mutation, then, reduces meiotic intrachromosomal recombination between the *his4* genes to less than 2% of its normal frequency.

A cloned *SPO11* gene restores high levels of intrachromosomal recombination in a *spo11-1* haploid. To confirm that the nearly complete absence of meiotic recombination in *spo11-1* haploids is, in fact, due to the *spo11-1* mutation, we transformed JW201-22A (*spo11-1 trp1 ura3*) with a plasmid containing the *SPO11* gene, which was cloned on the basis of its ability to complement the homolog exchange defect of *spo11-1* diploids (B. DiDomenico, R. Elder, J. Kowalisyn, and R. E. Esposito, manuscript in preparation). The plasmid used here [p(SPO11)9] contains sequences from the centromere of chromosome IV, as well as the wild-type *SPO11*, *TRP1*, and *URA3* genes, and is maintained as a circular minichromosome in yeast strains.

Among 159 dyads from the transformed strain JW201-22A[p(SPO11) 9], 86 (54%) had two spores that each retained the plasmid and therefore were phenotypically Trp^+ Ura^+ (Table 6). In this group of dyads, which maintained the centromere plasmid through both mitotic growth and meiotic division, the frequency of meiotic intrachromosomal recombination producing 0^+:1^-:1^P or 1^+:0^-:1^P dyads was 31% (27 of 86), which is almost identical to the frequency of 30% observed in JW201-4B (*SPO11*).

In 68 of the remaining dyads, neither spore clone contained the *SPO11* plasmid, as judged by the Trp^- Ura^- phenotype of both spores. These dyads are likely to be due to mitotic loss of the plasmid prior to meiosis, although the possibility that the plasmid was present during meiosis but was not packaged into either spore cannot be ruled out on the basis of these data. All of the dyads in this group showed a 0^+:0^-:2^P His segregation; i.e., there is no evidence of meiotic intrachromosomal recombination, as was the case in the untransformed parent JW201-22A.

The remaining five dyads each contained one Trp^+ Ura^+ spore and one Trp^- Ura^- spore, reflecting either meiotic nondisjunction of the *SPO11* plasmid or meiotic loss of one plasmid copy after replication. Each of these dyads showed 0^+:0^-:2^P segregation for growth on histidineless medium, indicating an absence of intrachromosomal exchange events in this small class as well.

In summary, wild-type levels of meiotic intrachromosomal recombination cosegregate with plasmid p(SPO11)9. The *SPO11* gene present in the plasmid is sufficient to correct the intrachromosomal recombination defect of *spo11-1* haploids, and loss of the plasmid results in a rapid and complete loss of the ability to undergo meiotic unequal reciprocal SCE, reciprocal intrachromatid recombination, and gene conversion.

***SPO11* is not required for mitotic intrachromosomal recombination.** The evidence presented here and in our previous reports indicates that *SPO11* is required for all types of homologous recombination during meiosis in *S. cerevisiae*: reciprocal and nonreciprocal events between homologs, between sister chromatids, and within chromatids. Does *SPO11* also play a role in recombination during the mitotic cell cycle? Results of experiments with *a*/α diploids (19) have shown no difference in the frequencies of mitotic gene conversion between *SPO11* and *spo11-1* diploids. Also, no difference was found in the frequencies of mitotic segregation of drug-resistant clones from diploids heterozygous for recessive drug resistance alleles, which can result from either gene conversion or reciprocal exchange.

We used *a*/α disomic and *mar1* haploids to determine whether *SPO11* is required for mitotic intrachromosomal recombination within the rDNA and between duplicated copies of *his4*. Exchange was assayed in strains with *URA3* inserted into the rDNA by determing (i) the frequency of Ura^- colonies and (ii) the frequency of sectored Ura^+/Ura^- colonies which reflect recombination events that occur near the time of plating. The analysis of these sectored colonies is important because their frequency provides an estimate of the probability of mitotic intrachromosomal recombination per cell division and, more importantly, because they can be analyzed by hybridization in much the same way as meiotic 1^+:1^- dyads to determine whether they are due to unequal reciprocal SCE or to reciprocal intrachromatid exchange or gene conversion.

Cultures of two independent *SPO11* haploids contained from 0.5% to 2.5% Ura^- cells, as did cultures of the two *spo11-1* haploids K355-24C and JW194-67D (Table 7). The mean Ura^- frequency for the two *SPO11* haploids was 1.0%, whereas that for K355-24C and JW194-67D was 1.3%. The third *spo11-1* strain, JW194-64B, actually showed Ura^- frequencies 5- to 10-fold higher than those of the other strains examined. These results indicate that (i) *SPO11* is not essential for intrachromosomal recombination within the rDNA during mitosis and (ii) the *spo11-1* mutation has no consistent effect on mitotic intrachromosomal recombination, either inhibitory or stimulatory. The elevated exchange frequency in JW194-64B therefore must be due to genetic differences at loci other than *SPO11* that are segregating in these strains.

Data in Table 7 also demonstrate that *spo11-1* has no effect on the frequency of mitotic recombination between chromo-

some III homologs in disomic haploids, yielding Leu$^+$ or Cry^r (cryptopleurine-resistant) colonies. In both the *SPO11* and *spo11-1* haploids, most Leu$^+$ colonies arose from intragenic recombination between *leu2-1* and *leu2-27*, while most Cry^r colonies arose from crossing over between the centromere of chromosome III and the *cry1* locus (data not shown).

Five sectored Ura$^+$/Ura$^-$ colonies from the *SPO11* haploid JW167-171B and five from the *spo11-1* haploid JW194-64B were analyzed by hybridization with a ^{32}P-labeled *URA3* probe to determine the mechanism by which they arose (Table 8). In three of the five *SPO11* sectored colonies and in all five of the *spo11-1* colonies, the Ura$^+$ portion of the colony contained approximately six copies of *URA3* in the rDNA, indicating that the colonies arose from unequal reciprocal SCE. Hybridization results from the other two *SPO11* sectored colonies are consistent with reciprocal intrachromatid exchange or gene conversion. (It should be noted that in four of the sectored colonies attributed to unequal reciprocal SCE, at least one subclone of the Ura$^+$ portion contained only three copies of *URA3*, suggesting that the recombination events detected may have occurred after the first division on the plating medium.)

TABLE 7. Mitotic intrachromosomal recombination in rDNA

Genotype and strain	Culture no.	Ura$^-$/CFU ($\times 10^2$)	Ura$^+$/Ura$^-$ sectors/CFU ($\times 10^2$)[a]	Leu$^+$/CFU ($\times 10^6$)[b]	Cry^r/CFU ($\times 10^4$)[b]
SPO11					
JW167-171B	1	0.7	0.08	8.5	5.8
	2	1.9	0.05	12.4	3.1
	3	1.0	0.03	7.3	3.2
	4	1.2	0.07	8.0	25.7
	5	0.6	0.08	10.0	2.6
Mean		1.1	0.06	9.2	8.1
JW168-7B	1	—[c]	—	7.4	2.6
	2	1.2	0.20	9.6	2.2
	3	0.5	<0.06	6.4	7.4
Mean		0.9	0.09	7.8	4.1
spo11-1					
K355-24C[d]	1	2.2	0.27	ND[e]	ND
	2	1.9	0.16	ND	ND
	3	2.4	0.37	ND	ND
	4	1.6	0.59	ND	ND
	5	—	—	ND	ND
Mean		2.0	0.29		
JW194-64B[f]	1	6.4	1.48	ND	4.1
	2	5.9	3.07	ND	2.7
	3	5.5	1.66	ND	6.3
	4	5.1	1.98	ND	4.0
	5	7.4	2.43	ND	4.2
Mean		6.1	2.06		4.3
JW194-67D	1	0.5	0.12	13.2	5.0
	2	0.6	<0.05	9.5	4.7
	3	1.3	0.08	6.8	6.6
	4	0.5	0.05	5.9	3.9
	5	0.7	0.14	7.7	3.8
Mean		0.7	0.09	8.6	4.8

[a] Mean values for Ura$^+$/Ura$^-$ sectored colony frequencies were determined by dividing the total number of sectored colonies observed for each strain by the total number of colonies screened.
[b] Leu$^+$ and Cry^r colonies reflect the occurrence of mitotic exchange between chromosome III homologs.
[c] —, All colonies plated from a culture were Ura$^-$ because of mitotic recombination prior to inoculation of the culture.
[d] K355-24C is neither heteroallelic at *leu2* nor heterozygous for *cry1*.
[e] ND, Not determined.
[f] JW194-64B contains a *leu1* mutation in addition to *leu2* heteroalleles.

TABLE 8. Hybridization analysis of *URA3* copy number in mitotic Ura$^+$/Ura$^-$ sectored colonies

Genotype and strain	Sectored colony no.	Ura phenotype	Hybridization band I	Hybridization band II	Hybridization ratio (II/I)[a]	Inferred Mechanism
SPO11						
JW167-171B	1	+	+	+	5.6	Unequal SCE
		−	+	−		
	2	+	+	+	3.3	Intrachromatid exchange
		−	+	−		
	3	+	+	+	5.5[b]	Unequal SCE
		−	+	−		
	4	+	+	+	3.8	Intrachromatid exchange
		−	+	−		
	5	+	+	+	6.0	Unequal SCE
		−	+	−		
spo11-1						
JW194-64B	1	+	+	+	5.6[c]	Unequal SCE
		−	+	−		
	2	+	+	+	7.0	Unequal SCE
		−	+	−		
	3	+	+	+	5.8	Unequal SCE
		−	+	−		
	4	+	+	+	6.3[d]	Unequal SCE
		−	+	−		
	5	+	+	+	6.0[e]	Unequal SCE
		−	+	−		

[a] All ratios are based on analysis of at least two subclones. In cases in which one subclone of a sector gave a ratio of approximately 6 and others gave ratios of approximately 3 or less, we list the larger ratio in the table. We assume that the heterogeneity is due to exchange events occurring after the first cell division on plating medium.
[b] One subclone from the Ura$^+$ portion of this sectored colony gave a hybridization ratio of 3.8.
[c] In addition to two subclones from the Ura$^+$ portion of this sectored colony that gave a mean ratio of 5.6, three other subclones gave ratios of 1.0, 1.1, and 2.5.
[d] One subclone from the Ura$^+$ portion of this sectored colony gave a hybridization ratio of 3.2.
[e] One subclone from the Ura$^+$ portion of this sectored colony gave a hybridization ratio of 2.4.

Data from mitotic cultures of strains containing duplicate copies of *his4* confirm that *spo11-1* has no effect on rates of intrachromosomal recombination during vegetative growth (Table 9). The mean frequency of His$^+$ recombinants in five *SPO11* cultures was 7.6×10^{-5}/CFU; in five *spo11-1* cultures the mean was 7.1×10^{-5}/CFU. Hybridization analysis with pBR322 DNA as a probe indicated that 8 of 10 His$^+$ clones from the *SPO11* strain contained the *his4* duplication and therefore arose via gene conversion. The other two His$^+$ clones included one that contained no pBR313 sequences, which therefore arose from reciprocal exchange, and one with three copies of the *his4* gene, which likely arose from gene conversion associated with unequal reciprocal SCE. Among 10 His$^+$ clones from the *spo11-1* haploid, 7 contained

TABLE 9. Mitotic intrachromosomal recombination in the *his4* duplication

Genotype	Strain	Culture no.	His^+/CFU ($\times 10^5$)
SPO11	JW201-4B	1	8.4
		2	6.7
		3	7.8
		4	7.1
		5	7.9
Mean			7.6
spo11-1	JW201-22A	1	9.8
		2	7.4
		3	6.0
		4	6.8
		5	5.7
Mean			7.1

the *his4* duplication and were due to gene conversion, and 3 contained a single *HIS4* copy and arose from reciprocal exchange, either between sister chromatids or within a chromatid.

DISCUSSION

Genetic exchange occurs between homologous chromosomes in *spo13-1 MAT***a**/*MAT*α disomic haploids at rates almost identical to those observed in typical *SPO13* diploid meiosis (33). In this study we showed that recombination within chromosomes also occurs at high levels during haploid meiosis. We characterized the mechanisms of this intrachromosomal recombination and investigated the effects of a meiotic Rec^- mutation, *spo11-1* on the process.

In strains containing *URA3* integrated into the rDNA, phenotypically detectable intrachromosomal exchange events occurred at rates of 1.6 to 6.1% per meiosis. Most of the recombinant dyads characterized by Southern hybridization (four of five) were shown to be due to unequal reciprocal SCE. In a strain containing a duplication of the *his4* gene, 31% of dyads showed evidence of intrachromosomal recombination during meiosis. Of these dyads, 49% were due to unequal reciprocal SCE, 35% to reciprocal intrachromatid exchange, 10% to gene conversion without associate reciprocal exchange, and the remaining 6% to multiple recombination events.

How do these results compare with those from studies of meiotic intrachromosomal recombination in diploid strains? In the first such study in *S. cerevisiae*, Petes (28) examined two diploids which were formed by mating haploids with independently derived *LEU2* insertions in their rDNA and found intrachromosomal exchange frequencies of 10.2 and 3.3%. Analysis of 10 tetrads from one of these diploids by Southern hybridization indicated that all 10 arose from unequal reciprocal SCE rather than from reciprocal intrachromatid exchange or gene conversion. Because these events were observed in diploids containing inserts in each chromosome XII homolog, the frequencies must be divided by two to give mean per chromosome frequencies (5.1 and 1.7%, respectively) for comparison to those found in haploid meiosis.

In a second study, Klein and Petes (21) constructed a diploid heterozygous for a direct repeat of the *LEU2* locus on chromosome III and found evidence of intrachromosomal recombination in 2% (6 of 306) of tetrads. In contrast to the results of the previous study of exchange in the rDNA, all of these events were cases of intrachromosomal gene conversion, with there being no evidence of either reciprocal intrachromatid exchange or unequal reciprocal SCE. Klein (20) investigated further the types of events that can occur within a chromosome in a strain heterozygous for an inverted repeat of the *HIS3* locus on chromosome XV. In this diploid, intrachromosomal gene conversion also occurred at a rate of 2% per meiosis. None of the gene conversion events (zero of six) was found to be associated with reciprocal exchange. In this system, associated reciprocal intrachromatid exchange would simply flip the interstitial sequences; unequal reciprocal SCEs are not detected as they will produce a dicentric chromosome and an acentric fragment.

Finally, Jackson and Fink (17), using diploids in which both chromosome III homologs contained *his4* duplications, found evidence of meiotic intrachromosomal reciprocal exchange in 0.9 to 1.5% of tetrads and intrachromosomal gene conversion in 1.1% of tetrads. Among 11 reciprocal exchange events, 10 were unequal SCE and 1 was an intrachromatid exchange. They concluded that, whereas unequal pairing and reciprocal exchange within a chromosome occurs only 10% as frequently as unequal pairing and crossing over between homologs, gene conversion between and within chromosomes occurs at roughly equal frequencies.

The results obtained in this study of haploid strains are consistent in several respects with those from diploid strains. First, the frequency of events in haploids with *URA3* inserted into the rDNA (~2%) is similar to the frequencies observed in the rDNA and on chromosomes III and XV in diploids. Second, almost all of the events that occurred in the rDNA of the haploids examined here were cases of unequal reciprocal SCE, as in diploids. These similarities suggest that the factors regulating the frequency and mechanism of intrachromosomal recombination in the rDNA are not strikingly different in *spo13-1* haploid meiosis from those in *SPO13* diploid meiosis.

The genetic behavior of the *his4* duplication on chromosome III during haploid meiosis, on the other hand, differs markedly from that seen in other systems during diploid meiosis. Almost one-third of dyads showed evidence of intrachromosomal recombination (in contrast to 2 to 5%), and among the recombination events approximately 40% were reciprocal intrachromatid exchange events resulting in the deletion of genetic material from a single chromatid. Such a high fraction of excision has not been observed in any previous studies on meiotic intrachromosomal recombination. An overall frequency of approximately one event per three dyads was found not only in the *spo13-1* strain originally examined but also in a *spo11-1 spo13-1* strain that was transformed with a plasmid containing the wild-type *SPO11* gene, so it is unlikely to be strain specific.

How can we explain this dramatic difference in chromosome behavior between diploid and haploid meiosis? A simple hypothesis is that exchange between homologs in a diploid competes with intrachromosomal recombination for limiting quantities of one or more components of the genetic exchange machinery. Thus, the absence of the competing homolog could be responsible for the very high intrachromosomal recombination frequencies seen during haploid meiosis. Recently, Jackson and Fink (17), on the basis of studies in diploid cells, have also proposed that homologous chromosomes act to suppress exchange within chromosomes. We suggest that the limiting component is the

synaptonemal complex, a structure thought to play a role in establishing or maintaining homolog pairing during meiotic prophase in diploid cells (for a review, see references 13, 26, and 32a). Several lines of evidence are consistent with the view that pairing within single chromosomes mediated by synaptonemal complexes is responsible for the high level of intrachromosomal recombination between the duplicated *his4* genes in haploid meiosis. First, intrachromosomal pairing via synaptonemal complexes has been observed during haploid meiosis in several plant species, including barley (12), and in haploid *Physarum polycephalum* (22). Second, Byers and Goetsch (3) have found that in diploid *S. cerevisiae* cells, synaptonemal complexes cannot be visualized in the nucleolus, in which most or all of the rDNA sequences are located. This observation is intriguing in light of our finding that the elevation in intrachromosomal recombination due to haploidy is limited to non-rDNA regions. Our proposal that haploid *S. cerevisiae* cells that lack paired homologs can form synaptonemal complexes that promote high levels of recombination within single chromosomes can be tested experimentally in at least two ways. First, *spo13-1* haploid cells should contain synaptonemal complexes during meiotic prophase, and second, mutants that lack synaptonemal complexes should have levels of intrachromosomal recombination at loci such as *his4* as low in haploids as those seen in diploids. We are in the process of testing both of these predictions.

Using the results obtained from Rec$^+$ *spo13-1* haploid meiosis as a base line, we have shown that the *spo11-1* mutation reduces the frequency of meiotic intrachromosomal recombination by 10-fold (in the *URA3* rDNA system) to >50-fold (in the *his4* duplication system). Haploids containing *spo11-1* lack meiotic unequal reciprocal SCE, reciprocal intrachromatid exchange, and intrachromosomal gene conversion. *SPO11* is therefore the first gene product to be implicated in all forms of intrachromosomal recombination.

Results of previous studies have shown that *SPO11* is not required for early sporulation events such as premeiotic DNA synthesis, but is required for meiotic gene conversion and reciprocal exchange between homologs in both diploids and disomic haploids and for the formation of cytologically normal asci in otherwise wild-type cells (7, 18, 19, 33). Since *spo11-1* mutant diploids form synaptonemal complexes that are normal in morphology and in their time of appearance during sporulation, we have proposed previously that the wild-type *SPO11* gene functions after homologous chromosome pairing (19).

What does the fact that *SPO11* is also required for meiotic recombination within chromosomes add to our understanding of its role in normal meiosis? *SPO11* must act at an early step in genetic exchange that is common to homolog recombination, SCE, and intrachromatid exchange and that is a necessary prerequisite to both reciprocal exchange and gene conversion events. A similar suggestion that *SPO11* functions early in meiotic recombination comes from studies which have shown that *spo11-1* is epistatic to other meiotic Rec$^-$ mutants such as *rad52-1* for production of viable ascospores in *spo13-1* diploids (S. Klapholz, C. Waddell, L. Jensen, and R. E. Esposito, manuscript in preparation).

The *spo11-1* mutation has no effect on the frequency of intrachromosomal recombination during mitotic division (this study) or on mitotic recombination between homologs (19), supporting the conclusion that the action of the wild-type *SPO11* gene product is meiosis specific. Results of recent molecular studies (B. DiDomenico, S. Frackman, L. Jensen, R. E. Esposito, and R. Elder, unpublished data) have indicated that *SPO11* encodes a developmentally regulated transcript, present in less than one copy per 20 cells in mitosis, that is induced 50- to 100-fold during meiosis. One *spo11-1* strain examined in this study showed a rate of mitotic intrachromosomal recombination in the rDNA approximately 10-fold higher than that in closely related strains. This variation among *spo11-1* strains must be due to genetic differences other than the *spo11-1* mutation; similar genetic modifiers may be responsible for the slightly elevated frequencies of mitotic recombination between homologs reported in *spo11-1* diploids by Bruschi and Esposito (2).

Almost all mitotic His$^+$ segregants in both *SPO11* and *spo11-1* haploids containing the *his4* duplication arose from gene conversion rather than reciprocal exchange. This confirms the results of Jackson and Fink (16) and may be contrasted with the meiotic data, which show that among five His$^+$ segregants, one arose from unequal reciprocal SCE, two arose from reciprocal intrachromatid exchange, and only two arose from gene conversion. The relative rarity of reciprocal exchange among intrachromosomal recombination events involving the *his4* duplication therefore is a phenomenon limited to mitosis.

In conclusion, using the *spo13-1* haploid meiosis system, we characterized the first mutation causing a defect in all types of intrachromosomal recombination: unequal reciprocal SCE, reciprocal intrachromatid recombination, and intrachromosomal gene conversion. The mutation *spo11-1* also prevents meiotic recombination between homologs but has no effect on mitotic exchange between or within chromosomes. The phenotype of *spo11-1* indicates that intrachromosomal recombination is a dispensible feature of *S. cerevisiae* meiosis. The characterization of this and other mutations by haploid meiosis promises to permit a distinction between those gene products that are required specifically for synapsis and recombination involving homologs and those required for meiotic recombination in general.

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