RECOMBINATION BETWEEN GENES LOCATED ON NONHOMOLOGOUS CHROMOSOMES IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

We constructed strains of *Saccharomyces cerevisiae* that contained two different mutant alleles of either the *leu2* gene or the *ura3* gene. These repeated genes were located on nonhomologous chromosomes; the two *ura3*$^-$ alleles were located on chromosomes *V* and *XII* and the two *leu2*$^-$ alleles were located on chromosomes *III* and *XII*. Genetic interactions between the two mutant copies of a gene were detected by the generation of either Leu$^+$ or Ura$^+$ revertants. Both spontaneous and ultraviolet irradiation-induced revertants were examined. By genetic and physical analysis, we have shown that Leu$^+$ or Ura$^+$ revertants can arise by a variety of different genetic interactions. The most common type of genetic interaction is the nonreciprocal transfer of information from one repeat to the other. We also detected reciprocal recombination between repeated genes, resulting in reciprocally translocated chromosomes.

MOST recombination studies in eukaryotes are done with single-copy genes. The type of interaction that is examined, therefore, is the recombination between allelic pairs of genes on homologous chromosomes. Other types of genetic events in eukaryotes can be detected by examining the interactions between genes that are repeated. Several of these events have been demonstrated in *Saccharomyces cerevisiae*. For example, tandemly repeated genes can pair out of alignment, resulting in unequal sister strand recombination (PETES 1980; SZOSTAK and WU 1980). Nonreciprocal genetic interactions (gene conversion events) can also occur between two copies of a gene located on the same chromosome (KLEIN and PETES 1981; JACKSON and FINK 1981). Unequal sister strand exchange and intrachromosomal gene conversion are thought to contribute to the maintenance of sequence homogeneity between different copies of tandemly repeated genes (SZOSTAK and WU 1980; KLEIN and PETES 1981; NAGYLAKI and PETES 1982).

Interactions between genes repeated on different chromosomes can also take place. SCHERER and DAVIS (1980) showed that in yeast, gene conversion events occurred between two mutant *his3* genes located on nonhomologous chromosomes. ERNST, STEWART and HERMAN (1981) demonstrated that mutations in the yeast gene for iso-1-cytochrome *c* could revert due to a nonreciprocal inter-

action with the partially homologous iso-2-cytochrome *c* gene; these two genes are located on nonhomologous chromosomes. In addition, MUNZ and LEUPOLD (1981) showed that gene conversion could occur between tRNA genes located on nonhomologous chromosomes.

As described below, we looked at mitotic interactions between two copies of a gene located on different chromosomes. For this analysis, we used recombinant DNA techniques and the yeast transformation procedure to construct yeast strains that have either two different mutant alleles of the *leu2* gene (one allele on chromosome *III*, the other on chromosome *XII*) or two mutant alleles of the *ura3* gene (one allele on chromosome *V*, the other on chromosome *XII*). Genetic and physical analysis of Leu^+ or Ura^+ revertants showed that both nonreciprocal and reciprocal interactions could occur between the genes repeated on nonhomologous chromosomes. Some of the reciprocal recombination events generated translocations. Interactions between repeated genes on nonhomologous chromosomes are sufficiently frequent that these events are likely to be important in the generation of genetic diversity.

MATERIALS AND METHODS

Strains: Many of the yeast strains used in this study were derived from SSU10, a diploid constructed by S. SMOLIK-UTLAUT, (1982) which has the following genotype:

α *HIS5 HIS4 LYS11 leu2-3,112 ura3-50 can1-101 ASP5 gal2* form I rDNA
a *his5 his4 lys11 leu2-3,112 ura3-50 CAN1 asp5 gal2* form II rDNA

Form I and form II refer to an EcoRI restriction enzyme polymorphism in the ribosomal DNA (rDNA) described previously (PETES, HEREFORD and SKRYABIN 1978). SSU10 was transformed (HINNEN, HICKS and FINK 1978) with the plasmid pSS2. This recombinant plasmid, constructed by T. PETES and S. SMOLIK-UTLAUT, contains yeast rDNA, the wild-type *LEU2* and *URA3* genes and pMB9 bacterial plasmid sequences. Using the heterozygous form I/form II rDNA as a genetic marker, SMOLIK-UTLAUT (1982) mapped the $LEU2^+$ and $URA3^+$ genes into the rDNA in the transformant, SSU10-T7. SSU10-T7-2d is a haploid spore from dissection of SSU10-T7. The genotype of this strain is α *his5 leu2-3,112 ura3-50 CAN1 asp5 gal2* (form II rDNA::$LEU2^+$ $URA3^+$); the symbol "::" indicates an insertion of $LEU2^+$ and $URA3^+$ into the rDNA. SSU10-T7-2d was treated with ethylmethane sulfonate (EMS) and the following strains isolated: 1) EMS60, which has the same genotype as SSU10-T7-2d except it has a $ura3^-$ allele in the rDNA instead of $URA3^+$; 2) EMS219, which has the same genotype as SSU10-T7-2d except it has a $leu2^-$ allele in the rDNA instead of $LEU2^+$. Spontaneous and ultraviolet irradiation-induced (UV-induced) revertants of EMS60 to Ura^+ or EMS219 to Leu^+ were isolated and analyzed. The following three strains were crossed to the revertants to map the $LEU2^+$ and $URA3^+$ genes: 1) DBY689 (**a** *leu2-3,112 ura3-50 can1-101* form I rDNA) (from D. BOTSTEIN); 2) SSU10-T7-2a (**a** *leu2-3,112 ura3-50 can1-101 his4* [form II rDNA::$LEU2^+$ $URA3^+$]); 3) X2180-1a (**a** *gal2* form I rDNA). SSU10-T7-2a is another spore from dissection of SSU10-T7.

Genetic analysis: Methods of tetrad dissection and media used were standard (MORTIMER and HAWTHORNE 1969). YPD media was used for nonselective growth (SHERMAN, FINK and LAWRENCE 1974). Nutritional markers were scored by growth on synthetic complete media plates lacking one amino acid. Canavanine resistance was detected by growth on synthetic complete plates lacking arginine and containing canavanine (60 μg/ml).

Isolation of EMS-induced mutants: We used a modification of the procedure employed by LINDEGREN *et al.* (1965) and SHERMAN, FINK and LAWRENCE (1974). Cells were grown to stationary phase (about 3×10^8 cells/ml) in rich broth (YPD). The culture was spun down and the cell pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.0) and 40% glu-

cose. The cells were shaken in 3% EMS at 30° for 45 min to 1 hr (which gave 48% and 41% survivors, respectively). The EMS was inactivated by a hundredfold dilution in 6% sodium thiosulfate followed by incubation at room temperature for 10 min. Dilutions were made in sterile water and plated onto rich media to give approximately 50–100 colonies per plate. These plates were incubated at 32° for 2 days then replica-plated onto minimal media lacking either leucine or uracil. Colonies that were either Leu^-/Ura^+ or Leu^+/Ura^- were picked and purified by restreaking onto rich media. Standard complementation tests were done to determine the Leu^- mutants that were *leu2*$^-$ and the Ura^- mutants that were *ura3*$^-$.

Isolation of Ura$^+$ *and Leu*$^+$ *revertants*: Thirteen single colonies from both EMS60 and EMS219 were inoculated into liquid YPD and grown to stationary phase. The media were removed and the cells plated onto plates lacking uracil (for EMS60) or leucine (for EMS219). Several plates from each culture were either untreated or irradiated with 1000 ergs/mm^2 of UV light. From each culture, no more than one revertant of a given phenotype was used; all revertants, therefore, were independent.

Isolation of DNA: Yeast DNA was isolated from CsCl gradients that contained Hoechst dye 33258 (PETES, HEREFORD and BOTSTEIN 1977 and D. H. WILLIAMSON, personal communication). Plasmid DNA was isolated from CsCl gradients containing ethidium bromide (CLEWELL and HELINSKI 1970). DNA fragments were recovered from agarose gels as described by CHEN and THOMAS (1980).

Yeast colony hybridization: Colony hybridization was performed as described (HINNEN, HICKS and FINK 1978).

Southern analysis: Restriction enzyme digests were carried out under the reaction conditions recommended by the supplier. Following agarose gel electrophoresis, DNA fragments were transferred to nitrocellulose (SOUTHERN 1975). These filters were hybridized to DNA probes which had been labeled with ^{32}P by nick translation (SCHACHAT and HOGNESS 1973). As a probe for ura3 sequences, we used ^{32}P-labeled MB1068 DNA (strain provided by S. FALCO). This recombinant plasmid was constructed by insertion of a HindIII fragment containing the yeast *URA3*$^+$ gene into the HindIII site of pBR322 (BACH, LACROUTE and BOTSTEIN 1979). The plasmid pYI16H1, a recombinant plasmid from the collection of PETES (PETES, HEREFORD and SKRYABIN 1978; PETES *et al.* 1978), contains the yeast *leu2-1* mutant gene (HICKS, HINNEN and FINK 1979). We determined that the *leu2-1* sequences were located on a 2.2 kb KpnI fragment of pYI16H1 (MIKUS and PETES, unpublished data). This KpnI fragment was labeled with ^{32}P and used as a probe for the *leu2* genes. The hybridization conditions used were similar to those described by BOTCHAN, TOPP and SAMBROOK (1976), except that some reactions included dextran sulfate (WAHL, STERN and STARK 1979).

RESULTS

To examine the types of genetic interactions that could occur among repeated yeast genes, we constructed haploid strains that had two mutant copies of a gene that is normally present in one copy. Since each copy of the gene contained a different noncomplementing mutant allele, these strains expressed a mutant phenotype. By selecting for wild-type revertants of these strains, we selected for genetic interactions between the repeats. As described in detail below, we detected by this technique both reciprocal recombination events (generating translocations) and nonreciprocal recombination events between repeated yeast genes. Before discussing these results in detail, we will describe the construction of the yeast strains containing the duplicated mutant alleles.

Construction of haploid yeast strains that have two mutant alleles

HINNEN, HICKS and FINK (1978) showed that when a yeast strain was transformed with a recombinant plasmid containing the wild-type *LEU2* gene, the plasmid integrated into the genome by sequence homology. This integration

event usually established a tandem duplication of the *leu2* gene. A variant of this procedure can also create a nontandem duplication. It has been shown (SZOSTAK and WU 1979; PETES 1980) that a recombinant plasmid containing the yeast *LEU2* gene and yeast ribosomal DNA sequences usually integrates into the tandem array of chromosomal rRNA genes following transformation. Since the ribosomal RNA genes are on chromosome *XII* (PETES 1979) and the normal location of the *leu2* locus is chromosome *III*, the net result of this transformation is a duplication of the *leu2* locus on nonhomologous chromosomes.

For the studies described below, we needed duplicated loci containing different mutant alleles. The initial experiments toward this goal involved transformation of the diploid yeast strain SSU10 with the recombinant plasmid pSS2 (SMOLIK-UTLAUT 1982). The complete genotype of SSU10 is described in MATERIALS AND METHODS. The most important features of the strain for these experiments are that it was homozygous for mutant alleles at the *leu2* and *ura3* loci, and was heterozygous for form I and form II ribosomal DNA (rDNA). Form I and form II are rDNA variants defined by a restriction site polymorphism. These variants behave as single alleles in most crosses (PETES and BOTSTEIN 1977). The pSS2 plasmid (Figure 1) contains an intact form II rRNA gene, a wild-type *LEU2* gene, a wild-type *URA3* gene and pMB9 bacterial plasmid sequences. When pSS2 was used to transform SSU10, more than 90% of the Ura^+ Leu^+ transformants were integrated in the rDNA (SMOLIK-UTLAUT 1982). The preference for integration into the rDNA rather than at the *leu2* or *ura3* loci presumably reflects the 100-fold duplication of the rDNA sequences.

One transformant SSU10-T7 was characterized in considerable detail. When the transformed diploid was sporulated, in seven of eight tetrads, two spores were Leu^+ Ura^+ and two were Leu^- Ura^-. In four of four tetrads, the form II rDNA cosegregated with the Leu^+ Ura^+ phenotype (SMOLIK-UTLAUT 1982). These segregation patterns are those expected for integration of pSS2 into the form II rRNA gene cluster of SSU10. The haploid spore SSU10-T7-2d derived from SSU10-T7 contained a form II rDNA cluster with an insertion of the wild-type *LEU2* and *URA3* genes.

To get mutant alleles derived from these wild-type genes, we treated the strain SSU10-T7 with the mutagen EMS. Strains that were either *$LEU2^+$ $ura3^-$* (EMS60) or *$leu2^-$ $URA3^+$* (EMS219) were isolated. We used EMS60 (*$LEU2^+$ $ura3^-$*) to look for interactions between the EMS-induced *ura3* mutant allele on chromosome *XII* and the pre-existing *ura3-50* mutant allele on chromosome *V*. EMS219 (*$leu2^-$ $URA3^+$*) was used to look for interactions between the EMS-induced *leu2* mutant allele on chromosome *XII* and the pre-existing *leu2-3,112* mutant gene on chromosome *III*. Although similar types of interactions were found for both strains, we will discuss each strain separately.

Interactions between duplicated mutant ura3 *genes*

Genetic interactions between *ura3* genes were detected by analyzing Ura^+ revertants derived from the *$LEU2^+$ $ura3^-$* haploid strain EMS60. Twenty-five independent Ura^+ revertants were isolated and characterized; 12 were sponta-

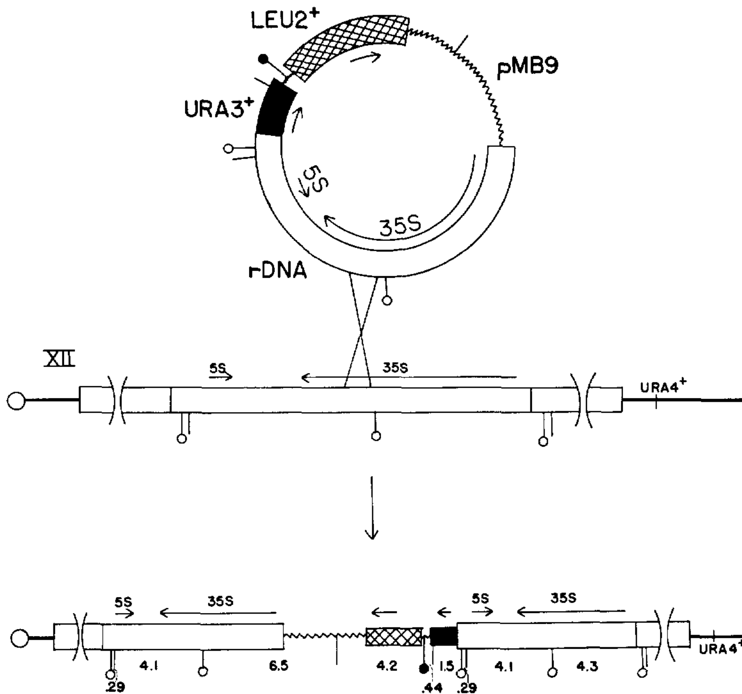

FIGURE 1.—Integration of the recombinant plasmid, pSS2, into the form II rDNA tandem array on chromosome *XII*. pSS2 contains the yeast $LEU2^+$ and $URA3^+$ genes, yeast rDNA and pMB9 bacterial plasmid sequences, as indicated. Each arrow shows the direction of transcription for the gene ($URA3^+$, M. ROSE and D. BOTSTEIN, personal communication; $LEU2^+$, P. SCHIMMEL, personal communication; 35S rRNA, PHILIPPSEN *et al.* 1978; 5S rRNA, KRAMER, PHILIPPSEN and DAVIS 1978; rRNA gene cluster orientation, ZAMB and PETES 1982). Restriction sites for Bgl II ($\overset{\circ}{|}$), SmaI (|) and BamHI ($\overset{\circ}{|}$) are designated. Molecular weights are given in kilobases. The scale for the lower drawing is half that of the upper one.

neous and 13 were induced by UV irradiation. The frequency of spontaneous reversion to Ura^+ (4×10^{-8}) was not much less than the UV-induced reversion frequency (1×10^{-7}). Most of the Ura^+ revertants examined were also Leu^+ but four of 25 were Leu^-.

We genetically analyzed the revertants by three types of crosses. The revertants were first crossed to the $leu2^-$ $ura3^-$ strain DBY689 (complete genotype in MATERIALS AND METHODS). The purpose of this cross was to determine the number of $LEU2^+$ and $URA3^+$ alleles in the revertant and to find out whether the $LEU2^+$ and $URA3^+$ alleles cosegregated. The second cross was to a haploid strain (SSU10-T7-2a) that had a $LEU2^+$ gene and a $URA3^+$ gene integrated in the rDNA. The purpose of this cross was to find out whether either the $LEU2^+$ or $URA3^+$ genes of the revertant were in the rDNA. In the third cross, the revertant was mated to X2180-1a, a strain that had the $LEU2^+$ and $URA3^+$ alleles at their normal positions (chromosome *III* for *LEU2* and chromosome *V* for *URA3*). This cross was used to test allelism of the $LEU2^+$ and $URA3^+$ genes

in the revertants to the same genes in their normal location. For all three crosses, the spore viability was monitored. All revertants were crossed to X2180-1a and SSU10-T7-2a; at least one revertant from each class was crossed to DBY689.

The data from these crosses is summarized in Tables 1–3. On the basis of this analysis, we classify the Ura^+ revertants into six classes:

Class I revertants: Class I revertants were Leu^+ Ura^+ and showed normal spore viability in all crosses. In crosses with the *leu2*$^-$ *ura3*$^-$ strain, DBY689, all tetrads segregated two Leu^+ Ura^+ spores to two Leu^- Ura^- spores. Thus, in the Class I revertants, there is one copy of the wild-type *LEU2* and *URA3* genes, and the two genes are linked. In crosses with SSU10-T7-2a (*LEU2*$^+$ *URA3*$^+$ in rDNA), most tetrads segregated 4 Leu^+ Ura^+ spores. This result indicates that the wild-type genes in the Class I revertants are located in the rDNA. In confirmation of this conclusion, when Class I revertants are crossed to X2180-1a (*LEU2*$^+$ on chromosome *III*, *URA3*$^+$ on chromosome *V*), 2^+:2^-, 3^+:1^- and 4^+:0^- segregation patterns are found for *LEU2*$^+$ and *URA3*$^+$. This is the result expected for unlinked genes that give the same phenotype.

Class I revertants can be explained as either a reversion of the EMS-induced *ura3* mutant allele to *URA3*$^+$ or as a nonreciprocal recombination event (gene conversion) between the EMS-induced *ura3* mutant allele and *ura3-50* allele on chromosome *V*. Although we cannot determine which of these alternatives explains the Class I revertants, for reasons stated below, we believe that the second mechanism is more likely.

Class II revertants: Class II revertants were also Leu^+ Ura^+ and showed normal spore viability in all crosses. In diploids derived from crosses with the *leu2*$^-$ *ura3*$^-$ strain DBY689, all tetrads segregated 2^+:2^- spores for both *LEU2* and *URA3*; however, the *LEU2*$^+$ and *URA3*$^+$ alleles did not cosegregate. When Class II revertants were crossed to SSU10-T7-2a (*LEU2*$^+$ and *URA3*$^+$ alleles in rDNA), the diploids segregated 4 Leu^+:0 Leu^- spores, but 4^+:0^-, 3^+:1^-, and 2^+:2^- for Ura. The Ura^+ phenotype segregated 4^+:0^- in crosses in which Class II revertants were crossed to X2180-1a (*URA3*$^+$ allele at normal chromosome *V* location). These results show that Class II revertants retain the *LEU2*$^+$ allele in the rDNA and that the reverted *URA3*$^+$ allele is on chromosome *V*. As with Class I revertants, there are two possible interpretations of Class II revertants, either the *ura3-50* mutation reverted to wild type or a conversion event occurred between the *ura3-50* allele and the EMS-induced *ura3* mutant on chromosome *XII*. For Class II revertants, the second explanation is far more likely than the first. We have detected no revertants of the *ura3-50* allele, either spontaneous or UV-induced in more than 10^{10} cells.

To confirm that Class II revertants were the result of a gene conversion event rather than a reciprocal double recombination, we analyzed one of these Ura^+ revertants (EMS60-7-S1) in more detail. If the *URA3*$^+$ gene in EMS60-7-S1 was formed by a gene conversion event, the mutant *ura3*$^-$ gene on chromosome *XII* would be likely to have only the original EMS-induced *ura3*$^-$ mutation. Alternatively, if the *URA3*$^+$ was formed by a double recombination event, the

TABLE 1

Ura+ revertants of EMS60 × X2180-1a (LEU2+ *on chromosome* III, URA3+ *on chromosome* V)

Strain	Class	Phenotype	Viable spores per tetrad					Segregation of Leu+				Segregation of Ura+			
	UV induced		4	3	2	1	0	4+:0-	3+:1-	2+:2-	1+:3-	4+:0-	3+:1-	2+:2-	1+:3-
60-1-U1	I	Leu+, Ura+	10	1	0	0	0	4/10	6/10	—	—	3/10	6/10	1/10	—
60-5-U5			10	1	0	0	0	1/10	7/10	—	2/10	—	7/10	2/10	1/10
60-6-U10			8	3	0	0	0	2/8	4/8	2/8	—	1/8	4/8	3/8	—
60-8-U1			11	0	0	0	0	3/11	5/11	3/11	—	1/11	5/11	5/11	—
60-11-U9			9	2	0	0	0	2/9	5/9	2/9	—	3/9	5/9	1/9	—
60-3-U3	II	Leu+, Ura+	9	1	1	0	0	2/9	6/9	1/9	—	9/9	—	—	—
60-9-U5			9	0	1	0	0	2/9	5/9	2/9	—	9/9	—	—	—
60-10-U38			9	2	0	0	0	5/9	3/9	1/9	—	9/9	—	—	—
60-UVR-18	III	Leu+, Ura+	15	6	1	0	0	6/15	3/15	6/15	—	15/15	—	—	—
60-1-U7	IV	Leu-, Ura+	10	1	0	0	0	—	—	10/10	—	9/10	1/10	—	—
60-3-U6			9	1	0	0	0	—	—	8/9	1/9	9/9	—	—	—
60-UVR-12	V	Leu+, Ura+	11	0	23	2	7	6/11	1/11	4/11	—	11/11	—	—	—
60-7-U1	VI	Leu+, Ura+	11	9	2	0	0	5/11	6/11	—	—	5/11	1/11	4/11	1/11
	Spontaneous														
60-3-S1	I	Leu+, Ura+	9	1	1	0	0	3/9	4/9	2/9	—	1/9	6/9	2/9	—
60-11-S2			7	2	2	0	0	—	5/7	2/7	—	1/7	5/7	1/7	—
60-1-S4	II	Leu+, Ura+	10	1	0	0	0	1/10	8/10	1/10	—	9/10	1/10	—	—
60-5-S1			10	0	1	0	0	2/10	6/10	2/10	—	10/10	—	—	—
60-6-S7			10	0	1	0	0	3/10	4/10	3/10	—	9/10	1/10	—	—
60-7-S1			7	3	1	0	0	4/7	1/7	2/7	—	7/7	—	—	—
60-8-S1			11	0	0	0	0	2/11	4/10	5/11	—	11/11	—	—	—
60-9-S6			7	2	0	0	0	1/7	4/7	2/7	—	7/7	—	—	—
60-10-S2			8	3	0	0	0	1/8	6/8	1/8	—	8/8	—	—	—
60-23-S1			9	1	1	0	0	3/9	4/9	2/9	—	8/9	1/9	—	—
60-5-S7	IV	Leu-, Ura+	9	0	2	0	0	—	—	9/9	—	8/9	1/9	—	—
60-6-S5			10	1	0	0	0	—	—	10/10	—	9/0	1/10	—	—

TABLE 2

Ura$^+$ revertants of EMS60 × SSU10–T7–2a (LEU2$^+$/URA3$^+$ *on chromosome* XII)

Strain	Class	Phenotype	Viable spores per tetrad					Segregation of Leu$^+$			Segregation of Ura$^+$		
	UV induced		4	3	2	1	0	4$^+$:0$^-$	3$^+$:1$^-$	2$^+$:2$^-$	4$^+$:0$^-$	3$^+$:1$^-$	2$^+$:2$^-$
60–1–U1	I	Leu$^+$ Ura$^+$	11	0	0	0	0	9/11	1/11	1/11	9/11	1/11	1/11
60–5–U5			10	1	0	0	0	9/10	1/10	—	9/11	1/11	—
60–6–U10			7	2	2	0	0	5/7	—	2/7	5/7	—	2/7
60–8–U1			4	5	2	0	0	4/4	—	—	4/4	—	—
60–11–U9			7	3	0	0	1	6/7	—	1/7	6/7	—	1/7
60–3–U3	II	Leu$^+$ Ura$^+$	5	4	1	1	0	4/5	—	1/5	—	5/5	—
60–9–U5			8	1	2	0	0	8/8	—	—	—	7/8	1/8
60–10–U38			5	3	0	1	2	5/5	—	—	—	4/5	1/5
60–UVR–18	III	Leu$^+$ Ura$^+$	16	8	5	1	0	3/16	7/16	6/16	3/16	7/16	6/16
60–1–U7	IV	Leu$^-$ Ura$^+$	7	2	1	0	0	—	—	7/7	1/7	4/7	2/7
60–3–U6			5	3	3	0	0	—	—	5/5	1/5	3/5	1/5
60–UVR–12	V	Leu$^+$ Ura$^+$	8	0	21	2	11	7/8	1/8	—	7/8	1/8	—
60–7–U1	VI	Leu$^+$ Ura$^+$	9	16	5	2	1	7/9	2/9	—	1/9	5/9	3/9
	Spontaneous												
60–3–S1	I	Leu$^+$ Ura$^+$	6	3	0	0	0	5/6	—	1/6	5/6	—	1/6
60–11–S2			6	4	0	1	0	5/6	—	1/6	4/6	—	2/6
60–1–S4	II	Leu$^+$ Ura$^+$	6	2	4	0	0	6/6	—	—	1/6	4/6	1/6
60–5–S1			8	9	1	1	2	7/8	1/8	—	1/8	4/8	3/8
60–6–S7			7	1	7	1	5	7/7	—	—	2/7	4/7	1/7
60–7–S1			8	1	1	0	1	7/8	—	1/8	1/8	6/8	1/8
60–8–S1			6	4	1	0	0	6/6	—	—	—	5/6	1/6
60–9–S6			7	1	3	0	0	7/7	—	—	—	5/7	2/7
60–10–S2			6	4	1	0	0	5/6	—	1/6	1/6	3/6	2/6
60–23–S1			7	4	0	0	0	7/7	—	—	3/7	3/7	1/7
60–5–S7	IV	Leu$^-$ Ura$^+$	5	3	3	0	0	—	—	5/5	1/5	4/5	—
60–6–S5			6	3	1	1	0	—	—	6/6	—	5/6	1/6

TABLE 3

Ura+ revertants of EMS60 × DBY689 (leu2⁻ ura3⁻)

Strain	Class	Phenotype	Viable spores per tetrad					Segregation of Leu+				Segregation of Ura+	Cosegregation Leu+/Ura+
	UV induced		4	3	2	1	0	4+:0-	3+:1-	2+:2-	0+:4-	2+:2-	
60-5-U5	I	Leu+ Ura+	6	1	0	0	1	—	—	6/6	—	6/6	+
60-11-U9			10	5	0	0	0	—	—	10/10	—	10/10	+
60-3-U3	II	Leu+ Ura+	6	0	0	0	0	—	—	6/6	—	6/6	—
60-UVR-18*	III	Leu+ Ura+	16	4	1	1	0	—	—	16/16	—	16/16	+
60-1-U7	IV	Leu- Ura+	8	0	0	0	0	—	—	—	8/8	8/8	
60-UVR-12	V	Leu+ Ura+	0	1	10	0	2	—	—	10/10†	—	10/10†	+
60-7-U1	VI	Leu+ Ura+	6	12	1	1	2	2/6	2/6	2/6	—	6/6	—
	Spontaneous												
60-3-S1	I	Leu+ Ura+	5	2	1	0	0	—	—	5/5	—	5/5	+
60-11-S2			7	0	0	0	0	—	—	7/7	—	7/7	+
60-7-S1	II	Leu+ Ura+	7	1	0	0	0	—	—	7/7	—	7/7	—
60-6-S5	IV	Leu- Ura+	6	0	1	0	0	—	—	—	6/6	6/6	

* Crossed to SSU10-T7-2a-2D (lost *LEU2+/URA3+* insert in rDNA).
† 10/10 dyads 1+:1-.

mutant gene on *XII* should contain both the EMS-induced mutation and the mutant substitution from chromosome *V* (*ura3-50*). Thus the *ura3*$^-$ on *XII* formed by conversion should be revertible in a strain carrying a *ura3-50* mutation on *V*. A *ura3*$^-$ gene formed by recombination should be nonrevertible in this background. In a cross of EMS60-7-S1 to DBY689, we isolated a spore that was Leu$^+$ Ura$^-$. Such spores should contain a *ura3-50* mutation on chromosome *V* and the *ura3*$^-$ mutant being tested for reversion on chromosome *XII*. We found that such spores revert to Ura$^+$ at frequencies similar to those obtained with EMS60. If mitotic recombination is restricted to G1, this result suggests that Class II revertants arise by conversion rather than by double exchanges.

Our results, therefore, confirm earlier studies that showed that gene conversion can occur between repeated genes on nonhomologous chromosomes (SCHERER and DAVIS 1980; MUNZ and LEUPOLD 1981; ERNST, STEWART and SHERMAN 1981). Since Class II revertants are due to gene conversions, it seems reasonable that at least some of the Class I revertants also result from gene conversion events.

Class III revertants: Class III revertants were Leu$^+$ Ura$^-$ and showed normal spore viability in all crosses. By analysis of the crosses to DBY689, SSU10-T7-2a, and X2180-1a, we found that Class III revertants have a single copy each of the *LEU2*$^+$ and *URA3*$^+$ alleles. These wild-type alleles cosegregate and are allelic to the normal *URA3*$^+$ gene on chromosome *V*. In crosses of Class III revertants to X2180-1a, we observed 4 Ura$^+$:0 Ura$^-$ segregation in 15 of 15 tetrads. Thus, Class III revertants contain both a *LEU2*$^+$ allele and a *URA3*$^+$ allele integrated on chromosome *V* tightly linked to the normal *ura3* locus.

Class III revertants are likely to be the result of a two-step process. The first step is the excision of the pSS2 plasmid from the rRNA gene cluster. This excision event could be the exact reversal of the original integration event. Alternatively, a recombination event between any two rRNA genes that flank the pSS2 insertion would result in excision of a circular plasmid containing the pSS2 sequences. Following the excision of the pSS2 sequences from the rDNA, the same sequences could reinsert in the genome as the result of a homologous reciprocal recombination event between the *ura3-50* mutation on chromosome *V* and the EMS-induced *ura3* mutation on the plasmid. This reciprocal recombination event (or an associated gene conversion event) could generate a *URA3*$^+$ allele. If these events occurred as described above, the pMB9 sequences of the plasmid should map to chromosome *V* in Class III revertants. To test this prediction, we did colony hybridization (HINNEN, HICKS and FINK 1978) of spore colonies derived from crossing the Class III revertant (EMS60-UVR-18) to the *leu2*$^-$ *ura3*$^-$ DBY689 strain. We found that in five of five tetrads the ability to hybridize with ^{32}P-labeled pMB9 DNA cosegregated with the Leu$^+$ Ura$^+$ phenotype. In summary, Class III revertants are formed by the excision of sequences containing pSS2 from chromosome *XII* and the reinsertion of these sequences at the *ura3* locus of chromosome *V*.

Class IV revertants: This class of revertants is Ura^+ but Leu^-, and shows normal spore viability in all crosses. The genetic analysis indicates the presence of a single $URA3^+$ allele and this allele is located at the normal chromosome *V* locus. Both colony hybridization and Southern blotting showed that Class IV revertants lacked pMB9 sequences. We suggest, therefore, that Class IV revertants arise as the result of excision of the pSS2 sequences from chromosome *XII*, followed by conversion of the *ura3-50* mutation on chromosome *V* and loss of the plasmid. We hypothesize a conversion event rather than reversion because *ura3-50* is nonrevertible. There are also more complicated pathways than can explain Class IV revertants. For example, the plasmid excised from chromosome *XII* could transiently insert into chromosome *V*. This plasmid could then be excised. The conversion of *ura3-50* to $URA3^+$ could be associated with either the entry or the exit of the plasmid from chromosome *V*.

Class V revertants: This class of revertants was Leu^+ Ura^+ and showed poor spore viability in all crosses. The particular pattern of spore inviability was similar to that seen for yeast or Neurospora strains that were heterozygous for a reciprocal translocation (PERKINS and BARRY 1977; SHERMAN and HELMS 1978; CHALEFF and FINK 1980). In such strains, most of the tetrads segregate two live to two dead spores and the number of four live to zero dead, and zero live to four dead tetrads are approximately equal. One obvious way to get a reciprocal translocation is to have a recombination event between the *ura3* gene on chromosome *XII* and the *ura3* insertion on chromosome *V* (Figure 2).

In a diploid heterozygous for this translocation, the pairing configurations shown in Figure 3 are expected to occur. With no meiotic recombination, three different segregation patterns are possible. Segregation of chromosomes *1* and *3* to one pole and *2* and *4* to another (adjacent-*1* segregation) would be expected to produce four inviable spores since all spores would have deletions and duplications. Segregation of chromosomes *1* and *4* to one pole and *2* and *3* to the other (alternate segregation) should result in four viable spores, two containing the wild-type chromosomes and two containing the balanced translocation. In the last type of segregation (adjacent-*2*), chromosomes *1* and *2* go to one pole and 3 and *4* go to the other. In yeast, almost all segregation appears to be adjacent-*1* or alternate (SHERMAN and HELMS 1978).

If a single recombination event occurs between the translocation breakpoint and the centromere, the expected spore viability pattern is two live to two dead spores because duplications and deficiencies will be created at the second mitotic division. This viability pattern would be obtained regardless of whether segregation was alternate or adjacent-*1*. In summary, if a strain is heterozygous for a reciprocal translocation, then the predominant classes of tetrads will be those with four live spores, those with four dead spores and those with two live and two dead spores. The fraction of tetrads with two live and two dead spores will depend on the distance between the centromeres and the translocation breakpoints. It should also be pointed out that since the products of recombination between the centromere and the translocation breakpoint segregate into the inviable spores, in the viable spores, "pseudo-linkage" can be detected between heterozygous markers located distal to the breakpoints.

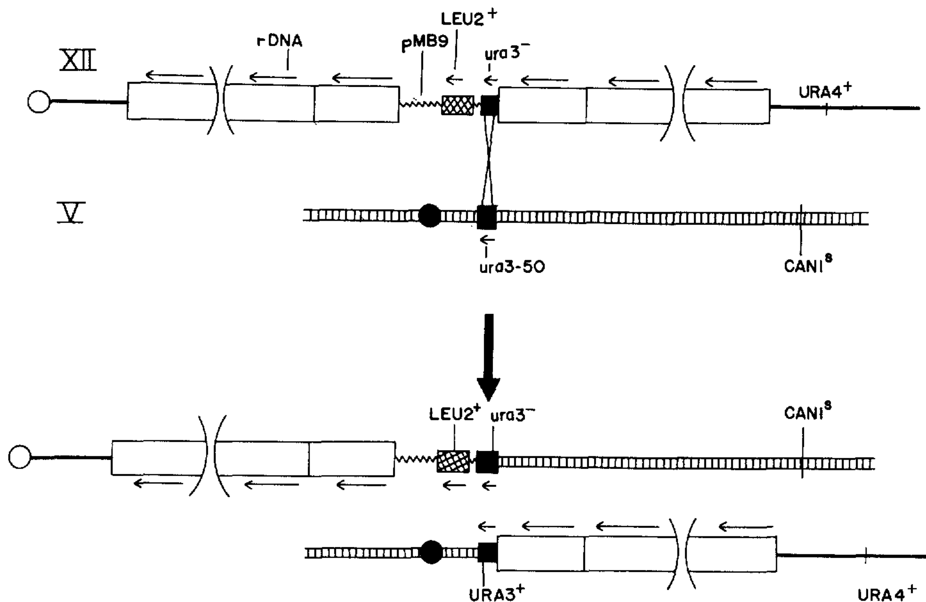

FIGURE 2.—Reciprocal recombination within the mutant *ura3* genes located on chromosomes *V* and *XII*, resulting in a translocation and a *URA3*$^+$ gene. The normal chromosomes are shown at the top of the figure and the translocated chromosomes are depicted at the bottom. The directions of transcription of the *LEU2* and *ura3* genes and the 35S precursor rRNA are designated by the arrows. The orientation of *ura3* on chromosome *V* was inferred from our data. Two alternate possibilities for the position of the *ura3* alleles on the translocation chromosomes are that the wild-type *URA3* gene could be located on the same chromosome as the *LEU2*$^+$ gene or both *URA3* genes could be wild type.

The rDNA of yeast is unlinked to the centromere of chromosome *XII* (PETES and BOTSTEIN 1977; PETES 1979). If a strain is heterozygous for a translocation involving a breakpoint in the rDNA, therefore, tetrads with two live spores should be more common than those with four live or four dead spores. We found that when the data on the spore viability of the Class V revertant (EMS60-UVR-12) crossed to all nontranslocated strains were summed (Tables 1, 2 and 3), 19 of the tetrads had four live spores, one tetrad had three live and one dead spore, 54 tetrads had two live and two dead spores, four had one live and three dead spores and 20 had four dead spores (Figure 4b). Thus, the spore viability pattern of this Class V revertant is consistent with that expected for a translocation heterozygote in which one of the breakpoints is unlinked to the centromere.

We also observed pseudolinkage between chromosome *XII* and chromosome *V* genes. The genotype of the Class V revertant EMS60-UVR-12 was: *LEU2*$^+$ *URA3*$^+$ *CAN1*^{S} form II rDNA. The rDNA is located on chromosome *XII* (PETES 1979). The gene *CAN1* is located on chromosome *V* centromere-distal to *ura3* (MORTIMER and SCHILD 1980). The genotype of DBY689 was: *leu2*$^-$ *ura3*$^-$ *can1*^{R} form I rDNA. Diploids resulting from the cross of these two

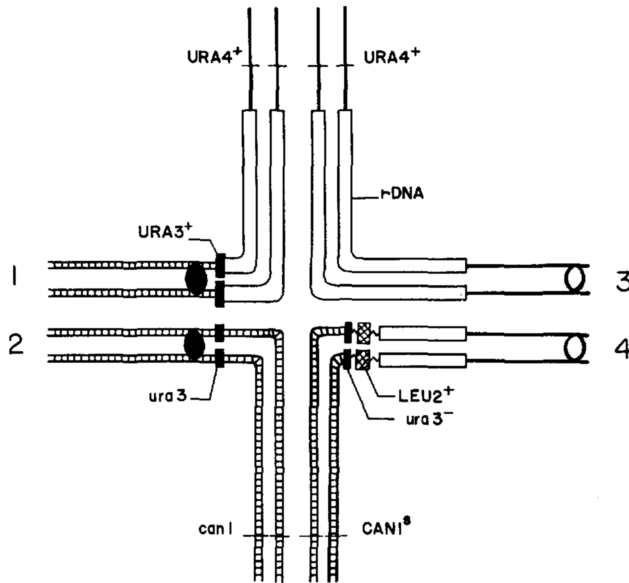

FIGURE 3.—Meiotic pairing configurations for a diploid that is heterozygous for a translocation between chromosomes *V* and *XII* (translocation breakpoints within the two *ura3* genes). Sequences from chromosome *V* are indicated by the hatched lines. The rRNA gene cluster is indicated by the open boxes and other sequences from chromosome *XII* are designated by heavy lines. The chromosome *XII* centromeres are depicted as open ovals and the chromosome *V* centromeres are dark ovals.

strains were dissected and analyzed. In seven of eight tetrads in which there were two viable spores, one spore was form I $can1^R$ $leu2^-$ $ura3^-$ and the other spore was form II $CAN1^S$ $LEU2^+$ $URA3^+$. This pattern of cosegregation strongly indicates a *V-XII* translocation. In one of eight tetrads in which there were two live spores, one spore was form I $CAN1^S$ $leu2^-$ $ura3^-$ and the other was form II $can1^R$ $LEU2^+$ $URA3^+$. This exceptional tetrad presumably reflects a second recombination event between the translocation breakpoint and the *can1* locus.

The expected chromosome structures are indicated in Figure 2. If the translocation breakpoint is located within the *ura3* genes, then the DNA sequences that flank both *ura3* genes should be different in the Class V revertants relative to the unreverted parental strain. To test this prediction, we isolated DNA from the parental strain EMS60 and the Class V revertant EMS60-UVR-12. The DNA was treated with BamHI and BglII, and a Southern blot analysis was done. We hybridized the resulting filter to a ^{32}P-labeled plasmid (MB1068) that contained a cloned yeast *ura3* gene. As shown in Figure 5, when DNA isolated from the unreverted strain EMS60 was analyzed, two bands of hybridization were observed, representing molecular weights of approximately 1.9 kb and 3.7 kb. Since the *ura3* gene used as a hybridization probe contains no BglII or BamHI sites, these bands represent the two different *ura3* genes. When the Class V revertant was examined, both these bands were missing and two new

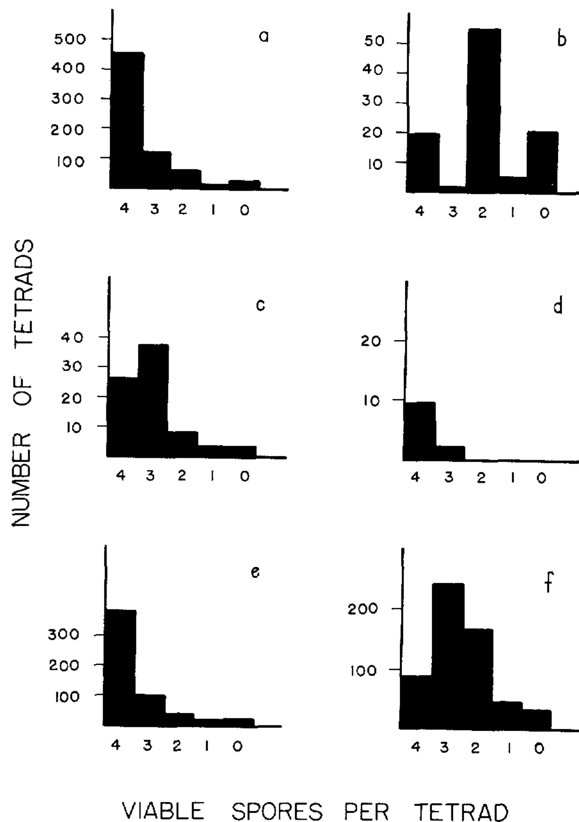

FIGURE 4.—Pattern of spore viability for revertants of EMS60 and EMS219. The total number of tetrads is plotted against the number of viable spores per tetrad. The spore viability data were taken from Tables 1 to 6. Each histogram represents the sum of the spore viability data for all crosses done with that type of revertant. The following spore viability profiles are depicted in (a)-(f): (a) EMS60 Classes I-IV revertants (normal spore viability); (b) EMS60 Class V revertant; (c) EMS60 Class VI Ura$^+$ revertant; (d) EMS60 Class VI Ura$^-$ isolate; (e) EMS219 Classes I, II, and IV (normal spore viability); (f) EMS219 Class V revertants.

bands (3.3 and 2.4 kb) appeared. These observations support the conclusion that the strain EMS60-UVR-12 contains a reciprocal translocation between the *ura3* genes on chromosomes *V* and *XII*. In particular, the observation that the sum of the molecular weights of the *ura3* bands in the unreverted strain equals the sum of the molecular weights of the *ura3* bands in the Class V revertants strongly indicates a translocation in which the breakpoints are within the *ura3* genes.

Recombination between repeated genes located on nonhomologous chromosomes should generate a viable translocation only if the repeats are oriented in the same direction relative to the centromere. Recombination between repeats oriented in opposite directions should generate dicentric chromosomes and acentric fragments. The transcriptional orientation of the *LEU2*, *URA3* and

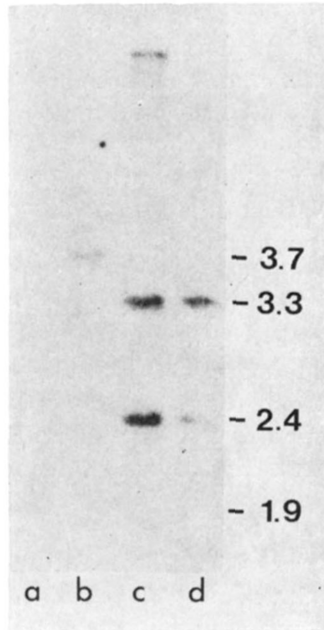

FIGURE 5.—Southern blot analysis of the *ura3* genes present in EMS60 (the original *ura3⁻* strain) compared to the *ura3* genes present in the *URA3⁺* Class V revertant, EMS60-UVR-12. DNA samples were digested with Bgl II and BamHI. The DNA fragments were analyzed by gel electrophoresis, transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled MB1068, a plasmid which contains the *URA3⁺* gene inserted into pBR322 (BACH, LACROUTE and BOTSTEIN 1979). Since Bgl II and BamHI do not cut within the *ura3* insert on chromosome *XII*, the two *ura3* genes can be seen as two different bands after hybridization, one band for each copy of the gene. Lanes (a) and (b) show the hybridization patterns for rDNA and non-rDNA, respectively, of EMS60. The 1.9 kb band in lane (a) represents the *ura3* gene on chromosome *XII*, and in lane (b) the 3.7 kb band is the *ura3* gene on chromosome *V*. rDNA and non-rDNA samples from EMS60-UVR-12 are shown in lanes (c) and (d), respectively. The 3.3 and 2.4 kb bands are the two *ura3* genes on the translocated chromosomes. The higher molecular weight band in lane (c) is due to cross hybridization of the pBR322 sequences of MB1068 to the pMB9 sequences of pSS2 that are inserted in the rDNA. This band can also be seen in lane (a) in longer exposures.

rRNA genes of pSS2 is known and is indicated in Figure 1. The transcriptional orientation of the rRNA genes on chromosome *XII* is also known (ZAMB and PETES 1982). In the EMS60 strain, therefore, both the *leu2* and the *ura3* genes in the rDNA are transcribed toward the centromere. The existence of a viable translocation formed by recombination between the *ura3* genes on chromosomes *XII* and *V* strongly suggests that the *ura3* gene on chromosome *V* is transcribed toward the centromere.

Class VI revertants: The last of the Ura⁺ revertants was Leu⁺ Ura⁺ and showed lower than normal spore viability in all crosses. In 77 tetrads derived from three crosses, 26 had 4 viable spores (34%), 37 had 3 viable spores (48%), eight had 2 viable spores (10%), three had 1 viable spore (4%) and three had no viable spores (4%) (Figure 4c). For all other classes (except Class V),

the tetrads with four viable spores were the most common class (71%) (Figure 4a). Our analysis, described in detail below, suggests that Class VI revertants contain a *LEU2*$^+$ *ura3*$^-$ insertion on chromosome *XII*, a *LEU2*$^+$ *URA3*$^+$ insertion associated with a recessive lethal on chromosome *V* and a duplication of chromosome *V*.

The most informative cross in support of these conclusions is the cross of the Class VI revertant EMS60-7-U1 to the *leu2*$^-$ *ura3*$^-$ strain DBY689. In six tetrads that had four viable spores, all six segregated 2 Ura$^+$ to 2 Ura$^-$ spores; this result indicates the presence of a single *URA3*$^+$ gene in EMS60-7-U1. In contrast, in the same cross, the segregation of Leu$^+$ phenotype indicated the presence of 2 *LEU2*$^+$ genes (2/6 tetrads 4 Leu$^+$:0 Leu$^-$, 2/6 3 Leu$^+$:1 Leu$^-$, 2/6 2 Leu$^+$:2 Leu$^-$). In addition, colony hybridization with ^{32}P-labeled pMB9 DNA was done with five tetrads from the cross of DBY689 with EMS60-7-U1. In all tetrads, the pMB9 sequences cosegregated with the Leu$^+$ phenotype. This result shows that Class VI revertants have two copies of the pMB9 portion of pSS2.

Several other interesting observations were made in the DBY689 × EMS60-7-U1 cross. The DBY689 strain is *can1*^{R} whereas EMS60-7-U1 is *CAN1*^{S}. In Class VI revertants, unlike all the other classes of revertants, the *can1* alleles in the cross showed a high frequency of departures from 2 to 2 segregation. (2/6 tetrads were 2 *CAN1*^{S}:2 *can1*^{R}, 2/6 were 3 *CAN1*^{S}:1 *can1*^{R}, 2/6 were 2 *CAN1*^{S}: 1 *can1*^{R}:1 *CAN1*^{S} with *can1*^{R} papillations.) One simple interpretation of this result is that chromosome *V* is disomic in EMS60-7-U1. We also observed that in 11 of 12 tetrads from this cross in which there were three viable spores, one spore was Ura$^+$ and two spores were Ura$^-$. This result suggests a specific association between the spore lethality and the Ura$^+$ phenotype. Since the spore lethality evident in crosses of all strains with EMS60-7-U1 is not expressed in the parental EMS60-7-U1 strain, the lethal event must be recessive. The simplest interpretation of the data described above is that the *URA3*$^+$ gene present in EMS60-7-U1 is located on chromosome *V* and is associated with a recessive lethal. In EMS60-7-U1, the lethality is not expressed because the strain is disomic for chromosome *V*.

The location of the two *LEU2*$^+$ genes in EMS60-7-U1 is straightforward. In the cross of EMS60-7-U1 to DBY689, we observed that all *URA*$^+$ spores were also *LEU*$^+$ (23 of 23 spores). Of all viable spores (Ura$^+$ and Ura$^-$) examined from this same cross, 43 of 61 were Leu$^+$ and 18 of 61 were Leu$^-$. A contingency χ^2 analysis of these data showed that the Ura$^+$ phenotype was specifically associated with the Leu$^+$ phenotype ($\chi^2 = 6.97$). One of the *LEU2*$^+$ genes, therefore, is on chromosome *V*, and the other is likely to be on chromosome *XII*. When EMS60-7-U1 was crossed to SSU10-T7-2a (*LEU2*$^+$ *URA3*$^+$ insertion in rDNA), we found that seven of nine tetrads had 4 Leu$^+$ spores:0 Leu$^-$ spores. Two of the nine tetrads had 3 Leu$^+$:1 Leu$^-$ segregation, possibly as the result of meiotic unequal sister strand recombination. In crosses of Class VI revertants to X2180-1a, the Ura$^+$ phenotype segregated 3^+:1^- or 2^+:2^- or 1^+:3^- in six of 11 tetrads (Table 1). These departures from 4^+:0^- seg-

regation suggest that one copy of chromosome *V* in Class revertants contains the *ura3* mutant allele.

These conclusions were tested by an additional experiment. When EMS60-7-U1 is grown nonselectively, single colonies can be isolated that are Ura^- but still Leu^+. One of these strains ($LEU2^+$ $ura3^-$ $CAN1^S$) was crossed to DBY689 ($leu2^-$ $ura3^-$ $can1^R$) and tetrads analyzed. We found that the spore viability in this strain was restored to normal; nine of 11 tetrads had four viable spores (Figure 4d). In addition, the *can1* gene segregated 2 $can1^R$:2 $CAN1^S$ spores in nine of nine tetrads examined. The Leu^+ segregated $2^+:2^-$ in seven of nine tetrads and $0^+:4^-$ in two of nine tetrads. The $0^+:4^-$ segregation of the *leu2* gene may reflect unequal sister strand recombination. In summary, Class VI strains occasionally show simultaneous mitotic loss of one of two chromosome *V*s (containing the $CAN1^S$ gene), a $URA3^+$ gene, a $LEU2^+$ gene and a spore lethality effect. This simultaneous loss suggests a causal association of these events and strongly supports our previous interpretation of Class VI strains.

In summary, EMS60-7-U1 (the only Class VI revertant) contains a $URA3^+$ $LEU2^+$ insertion which is associated with a recessive lethal on chromosome *V*. Chromosome *V* is disomic in the revertant. The revertant contains an additional $LEU2^+$ gene on chromosome *XII*, possibly associated with $ura3^-$ gene. It seems unlikely that this class VI revertant was the result of a single event. The revertant may have arisen in a cell that was disomic for chromosome *V*. The initial event in the reversion was probably an excision of pSS2 from chromosome *XII* followed by an insertion of the plasmid into one of the two copies of chromosome *V*. If this excision–insertion occurred in G2 of the cell cycle, a cell could be segregated that retained the pSS2 insertion on chromosome *XII*, in addition to having an additional copy of the plasmid on chromosome *V*.

Why the insertion event of chromosome *V* generates a recessive lethal is not clear. The data on Class III revertants indicate that the insertion of pSS2-derived sequences into chromosome *V* is not necessarily a lethal event. One possibility is that the excision event removing the plasmid DNA from chromosome *XII* may include more rDNA in the Class VI revertant than in the Class III revertant. This extra rDNA might have caused a position effect that shuts off some vital sequence on chromosome *V*. Alternatively, the insertion of DNA into chromosome *V* may have been an imprecise event, resulting in partial deletion of information on chromosome *V* that is required for cell viability. Greer and Fink (1979) found that transposition of the *his4C* gene (which is normally on chromosome *III*) into chromosome *XII* was also associated with a recessive lethal.

Summary of Ura^+ revertant data: When two different mutant *ura3* alleles are present on nonhomologous chromosomes of a haploid, a number of different types of interactions can result in Ura^+ revertants. The most common classes (eight of 13 UV-induced revertants and ten of 12 spontaneous revertants) were the result of reversion or gene-conversion events (Classes I and II). The next most common class (two of 13 UV-induced revertants and two of 12 spon-

taneous revertants) was that in which the plasmid excised itself from one location and converted the mutant allele on the nonhomologous chromosome (Class IV). For the UV-induced Ura$^+$ revertants, we found three other classes, each represented by a single revertant. These classes included: 1) an excision of sequences from one chromosome, followed by insertion in another (Class III); 2) a translocation generated by recombination between the two mutant alleles (Class V); and 3) an excision of sequences from one copy of chromosome *XII*, and insertion of those sequences into chromosome *V* (generating a recessive lethal covered by chromosome *V* disomy). Although UV treatment did not greatly increase the frequency of Ura$^+$ reversion (4×10^{-8} spontaneous, 10^{-7} after UV treatment), more classes of revertants are observed in UV-treated cells.

Analysis of Leu$^+$ revertants: To generalize our analysis, we also examined reversion events between a *leu2* mutant allele located in the rDNA and a different *leu2* mutant allele (*leu2-3,112*) located at the normal location on chromosome *III*. The strain used in these studies (EMS219) is similar to the strain used for the Ura$^+$ reversion studies except the *LEU2$^+$ URA3$^+$* genes in pSS2 were mutagenized to *leu2$^-$ URA3$^+$*. The Leu$^+$ reversion data can be briefly summarized by stating that similar classes of revertants were found as for the Ura$^+$ revertants. The Leu$^+$ revertants arose at similar frequencies (spontaneous reversion, 2×10^{-8}; UV-induced, 10^{-7}). The data from the genetic analysis are summarized in Tables 4 to 6. The Roman numerals for the classes of Leu$^+$ revertant correspond to the comparable Ura$^+$ revertant class. Since the genetic evidence for these classifications is similar to that used earlier for the Ura$^+$ revertants, we have abbreviated the description of the evidence.

The most frequently observed class of Leu$^+$ revertants (7/14 UV-induced, 8/10 spontaneous) was Class I. In this class, the *leu2$^-$* allele located in the rDNA changed to *LEU2$^+$*. This change represents a reversion of the original mutation or a conversion event between the mutant *leu2* allele on chromosome *III* and the mutant allele in the rDNA. Class II revertants (two of 14 UV-induced revertants) have the *LEU2$^+$* allele on chromosome *III* and retain the *URA3$^+$* gene in the rDNA. Since the *leu2-3,112* allele on chromosome *III* is a double mutation and has never been observed to revert (HINNEN, HICKS and FINK 1978), the Class II revertants almost certainly represent interchromosomal gene conversion events.

Class III revertants would have resulted from excision of pSS2 sequence from the rDNA and reinsertion into chromosome *III*. This class was not observed among the Leu$^+$ revertants. Class IV revertants involve the excision of the *leu2* and *URA3* alleles from the rDNA and conversion of *leu2-3,112*. The conversion event occurs without insertion of *URA3$^+$* or pMB9 sequences.

The second most common class of Leu$^+$ revertants (three of 14 UV-induced revertants; two of ten spontaneous) was Class V. This class represents translocations between the *leu2* insertion in the rDNA and the *leu2* on chromosome *III*. Since these strains containing *III–XII* translocations have some interesting properties that are distinct from the strains with *V–XII* translocations (Class V

of the Ura$^+$ revertants), the genetic analysis of these strains will be discussed in detail.

The Leu$^+$ and Ura$^+$ segregation data for Class V revertants are consistent with the results expected for translocations. As shown in Table 4, when Class V revertants were crossed to X2180-1a (*LEU2*$^+$ on *III*, *URA3*$^+$ on *V*), the Leu$^+$ phenotype showed predominately $4^+:0^-$ segregation. The *URA3*$^+$ allele of the Class V revertant segregated independently of the *URA3*$^+$ allele on chromosome *V*. In the cross of Class V revertants to SSU10-T7-2a (*LEU2*$^+$ and *URA3*$^+$ on chromosome *XII*), both the Leu$^+$ and Ura$^+$ phenotypes showed predominately $4^+:0^-$ segregation. When Class V strains were crossed with DBY689 (*leu2*$^-$ *ura3*$^-$), all tetrads with four viable spores segregated 2 Ura$^+$ Leu$^+$ spores to 2 Ura$^-$ Leu$^-$ spores. As explained below, this pattern is expected if Class V revertants represent a reciprocal translocation between chromosomes *III* and *XII*.

The three possible consequences of a reciprocal translocation between the *leu2* alleles on chromosomes *III* and *XII* are shown in Figure 6. In Class Va revertants, there is a single *LEU2*$^+$ allele, and *LEU2*$^+$ and *URA3*$^+$ are on different chromosomes. In Class Vb revertants, there is a single *LEU2*$^+$ allele, and *LEU2*$^+$ and *URA3*$^+$ are on the same chromosome. In the third putative class (Vc), two *LEU2*$^+$ alleles and one *URA3*$^+$ allele are located on the translocated chromosomes. Since Class Vc requires a double-conversion event, it is possibly less likely than the Va and Vb classes.

The meiotic pairing configuration of chromosomes in a diploid heterozygous for the Class Va translocation is shown in Figure 7. As with the Ura$^+$ translocations described previously, four viable spores will be recovered if there is no recombination between the translocation breakpoints and the centromere *and* if alternate segregation is observed. If a Class Va strain were crossed to X2180-1a (*LEU2*$^+$ on *III* and *URA3*$^+$ on *V*), two spores should contain the balanced translocations and, therefore, be *LEU*$^+$ *URA*$^+$; the other two spores should contain the normal chromosomes *III* and *XII*. Since the normal chromosome *III* contains a *LEU2*$^+$ allele, the expected segregation pattern for the Leu$^+$ phenotype is $4^+:0^-$. Since chromosome *V* is not involved in the translocation, the *URA3*$^+$ allele on chromosome *V* (from strain X2180-1a) and the *URA3*$^+$ allele on the translocated chromosome should segregate independently. The data of Table 4 are consistent with these predictions.

Similarly, if Class Va revertants are crossed with SSU10-T7-2a (*LEU2*$^+$ *URA3*$^+$ on chromosome *XII*), asci with four viable spores should have two Leu$^+$ Ura$^+$ spores containing the balanced translocation and two Leu$^+$ Ura$^+$ spores containing the normal *III* and *XII* chromosomes. The data in Table 5 are consistent with this expected pattern. If Class Va revertants are crossed with DBY689 (*leu2*$^-$ *ura3*$^-$), asci with four viable spores will have two Leu$^+$ Ura$^+$ spores that have the balanced translocation and two Leu$^-$ Ura$^-$ spores with the normal *III* and *XII* chromosomes. This pattern is consistent with that shown in Table 6. In summary, the segregation of the *LEU2*$^+$ and *URA3*$^+$ genes observed in crosses with the Class V revertants is consistent with the pattern expected

TABLE 4

Leu+ revertants of EMS219 × X2180–1a (LEU2+ *on chromosome* III, URA3+ *on chromosome* V)

Strain	Class	Phenotype	Viable spores per tetrad					Segregation of Leu+			Segregation of Ura+			
	UV induced		4	3	2	1	0	4+:0-	3+:1-	2+:2-	4+:0-	3+:1-	2+:2-	1+:3-
219–UVR–1	I	Leu+ Ura+	8	2	0	0	0	1/8	5/8	2/8	1/8	6/8	1/8	—
219–2–U2			10	1	0	0	0	2/10	3/10	5/10	5/10	4/10	1/10	—
219–5–U3			10	1	0	0	0	2/10	3/10	5/10	2/10	5/10	3/10	—
219–6–U1			10	0	1	0	0	1/10	5/10	4/10	3/10	3/10	4/10	—
219–8–U1			9	1	1	0	0	2/9	4/9	3/9	2/9	4/9	3/9	—
219–9–U2			9	2	0	0	0	—	6/9	3/9	1/9	6/9	2/9	—
219–10–U1			9	2	0	0	0	1/9	4/9	4/9	—	4/9	5/9	—
219–1–U2	II	Leu+ Ura+	10	1	0	0	0	10/10	—	—	4/10	5/10	1/10	—
219–UVR–4			7	3	0	0	1	7/7	—	—	—	6/7	1/7	—
219–5–U10	IV	Leu+ Ura-	9	1	1	0	0	9/9	—	—	—	—	9/9	—
219–8–U2			11	0	0	0	0	11/11	—	—	—	—	11/11	—
219–3–U3	V	Leu+ Ura+	5	11	24	13	22	5/5	—	—	3/5	1/5	1/5	—
219–7–U10			5	6	6	4	0	5/5	—	—	2/5	2/5	1/5	—
219–11–U1			7	4	7	3	1	6/7	1/7	—	—	4/7	3/7	—
	Spontaneous													
219–1–S1	I	Leu+ Ura+	11	0	0	0	0	2/11	6/11	3/11	1/11	9/11	1/11	—
219–2–S1			11	0	0	0	0	2/11	7/11	2/11	2/11	7/11	2/11	—
219–6–S1			8	3	0	0	0	—	5/8	3/8	1/8	6/8	1/8	—
219–7–S1			10	1	0	0	0	2/10	7/10	1/10	2/10	6/10	2/10	—
219–8–S1			9	1	1	0	0	3/9	6/9	—	—	8/9	1/9	—
219–9–S1			6	4	0	0	0	—	4/6	2/6	2/6	3/6	1/6	—
219–10–S1			9	2	0	0	0	—	4/9	5/9	2/9	3/9	3/9	1/9
219–11–S1			11	0	0	0	0	2/11	5/11	4/11	1/11	7/11	3/11	—
219–3–S1	V	Leu+ Ura+	5	4	11	1	1	4/5	1/5	—	1/5	1/5	3/5	—
219–5–S1			7	16	13	3	2	6/7	1/7	—	2/7	1/7	3/7	1/7

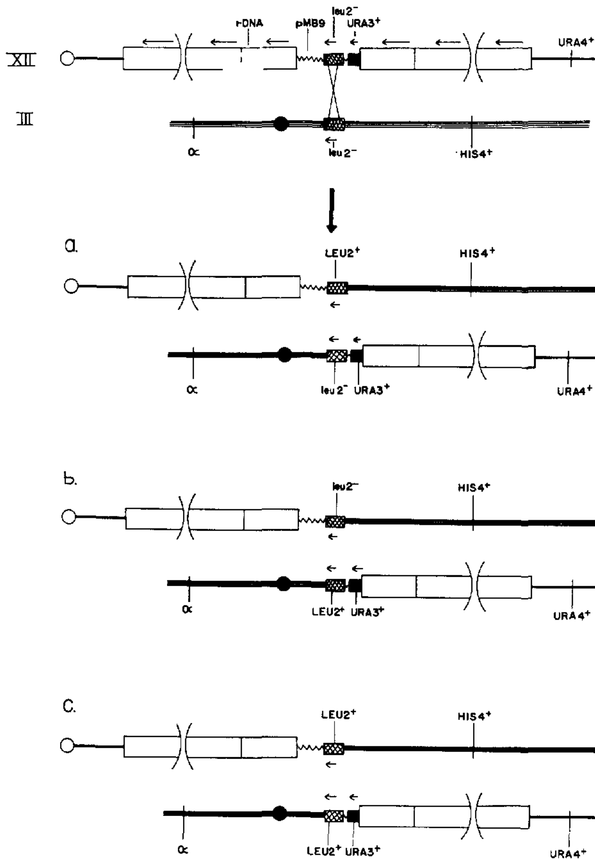

FIGURE 6.—Three possible consequences of a reciprocal translocation between *leu2* genes located on chromosomes *III* and *XII* generating a *LEU2*$^+$ strain. At the top of the figure are drawn the normal *III* and *XII* chromosomes. The letters (a), (b), and (c) refer to Class Va, b, or c as described in the text. Thin arrows refer to the direction of transcription of the gene indicated; for the rDNA, the arrow shows the direction of the 35S transcript. The orientation of *leu2* on chromosome *III* was previously determined (P. SCHIMMEL, personal communication; KINGSMAN *et al.* 1981).

from a strain heterozygous for the Class Va translocation. A similar analysis of the expected segregation patterns of Classes Vb and Vc demonstrates that revertants of these classes would show exactly the same properties as Class Va revertants. Therefore, although the results shown in Tables 4 to 6 are consistent with the hypothesis that a translocation occurred between chromosomes *III* and *XII*, the number and location of the *LEU2*$^+$ alleles in Class V revertants cannot be determined by the data analyzed thus far.

To obtain additional evidence for a reciprocal translocation in the Class V Leu$^+$ revertants, we did a physical analysis similar to that described previously for the Class V revertants. DNA was isolated from the unreverted EMS219 strain as well as from Class V revertants. This DNA was treated with the re-

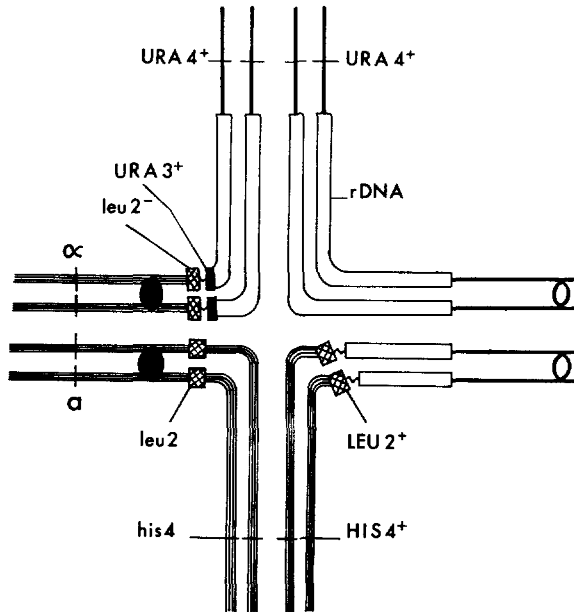

FIGURE 7.—Meiotic pairing configurations for a diploid that is heterozygous for a Class Va translocation between chromosomes *III* and *XII*. Sequences from chromosome *III* are indicated by lined areas. The rRNA gene cluster is shown as open boxes and other sequences from chromosome *XII* are designated by heavy dark lines. The chromosome *III* centromeres are shown as dark ovals and the chromosome *XII* centromeres as open ovals. The *leu2* and *his4* genes on the normal chromosome *III* can be either wild type or mutant depending on the particular cross.

striction enzymes Sma I and Bgl II, which do not cut within the *leu2* gene. Restriction fragments were then separated by gel electrophoresis, transferred to nitrocellulose and hybridized to a ^{32}P-labeled *leu2* probe (SOUTHERN 1975). As expected, the unreverted strain EMS219 had two bands of hybridization (Figure 8) at positions representing molecular weights of 4.7 and 3.0 kb. The Class V revertant was missing both these bands and had two new bands at positions corresponding to 3.4 and 4.4 kb (Figure 8). The result is precisely that expected for a reciprocal translocation involving the two *leu2* alleles. Of the four Class V revertants examined (EMS219-11-U1 was not checked), one (EMS219-3-U3) showed this hybridization pattern. In the other three (EMS219-7-U10, EMS 219-5-S1 and EMS219-3-S1), in addition to the 3.4 and 4.4 kb bands, the 4.7 kb band existing in the unreverted strain was also present. It is likely, although not proven, that in these strains the original pSS2 insertion was duplicated prior to the reversion event by unequal sister strand exchange. Such events are frequent in yeast in both meiosis (PETES 1980) and mitosis (SZOSTAK and WU 1980).

Although both the physical analysis and the Leu$^+$ Ura$^+$ segregation patterns are consistent with the hypothesis that Class V revertants represent reciprocal translocations, the spore viability observed in crosses with Class V revertants is

TABLE 5

Leu+ revertants of EMS219 × SSU10-T7-2a (LEU2+/URA3+ *on chromosome* XII)

Strain	Class	Phenotype	Viable spores per tetrad					Segregation of Leu+			Segregation of Ura+		
	UV induced		4	3	2	1	0	4+:0-	3+:1-	2+:2-	4+:0-	3+:1-	2+:2-
219-UVR-1	I	Leu+ Ura+	6	3	0	0	0	4/6	—	2/6	5/6	—	1/6
219-2-U2			7	2	0	1	1	6/7	1/7	—	7/7	—	—
219-5-U3			8	1	1	0	0	8/8	—	—	8/8	—	—
219-6-U1			6	2	3	0	0	6/6	—	—	6/6	—	—
219-8-U1			10	5	0	3	2	8/10	—	2/10	9/10	1/10	—
219-9-U2			10	6	3	1	0	7/10	—	3/10	10/10	—	—
219-10-U1			8	2	1	0	0	6/8	1/8	1/8	6/8	1/8	1/8
219-1-U2	II	Leu+ Ura+	9	6	3	2	1	—	5/9	4/9	6/9	2/9	1/9
219-UVR-4			9	—	—	—	—	—	6/9	3/9	9/9	—	—
219-5-U10	IV	Leu+ Ura-	9	2	0	0	0	1/9	7/9	1/9	—	—	9/9
219-8-U2			8	2	0	0	1	3/8	2/8	3/8	—	—	8/8
219-3-U3	V	Leu+ Ura+	6	25	15	7	1	5/6	—	1/6	5/6	—	1/6
219-7-U10			8	11	6	7	1	6/8	2/8	—	6/8	2/8	—
219-11-U1			8	24	9	0	0	7/8	1/8		7/8	1/8	
	Spontaneous												
219-1-S1	I	Leu+ Ura+	7	1	0	1	1	6/7	1/7	—	6/7	1/7	—
219-2-S1			6	3	1	0	1	5/6	1/6	—	6/6	—	—
219-6-S1			8	7	3	2	1	7/8	1/8	—	8/8	—	—
219-7-S1			9	4	2	2	4	7/9	1/9	1/9	9/9	—	—
219-8-S1			9	6	3	1	1	7/9	1/9	1/9	8/9	1/9	—
219-9-S1			6	4	1	0	0	5/6	1/6	—	6/6	—	—
219-10-S1			7	8	5	2	0	6/7	—	1/7	7/7	—	—
219-11-S1			6	2	1	0	1	6/6	—	—	6/6	—	—
219-3-S1	V	Leu+ Ura+	5	17	7	1	1	4/5	—	1/5	4/5	—	1/5
219-5-S1			3	15	12	2	0	3/3	—	—	3/3	—	—

TABLE 6

Leu+ revertants of EMS219 × DBY689 (leu2⁻, ura3⁻)

Strain	Class	Phenotype	Viable spores per tetrad					Segregation of Leu+			Segregation of Ura+			Cosegregation of Leu+/Ura+
	UV induced		4	3	2	1	0	2+:2-	1+:3-	0+:4-	2+:2-	1+:3-	0+:4-	
219-2-U2	I	Leu+ Ura+	8	0	1	0	0	5/8	2/8	1/8*	5/8	3/8•	—	+*
219-5-U3			8	1	1	0	1	6/8	1/8	1/8†	7/8†	—	1/8	+†
219-1-U2	II	Leu+ Ura+	11	5	0	1	3	11/11	—	—	10/11	1/11	—	—
219-8-U2	IV	Leu+ Ura-	5	1	0	1	0	5/5	—	—	—	—	5/5	
219-3-U3	V	Leu+ Ura+	2	14	5	0	0	2/2	—	—	2/2	—	—	+
219-7-U10			11	22	12	1	1	11/11	—	—	11/11	—	—	+
219-11-U1			4	33	13	0	1	4/4	—	—	4/4			+
	Spontaneous													
219-1-S1	I	Leu+ Ura+	8	1	0	0	0	7/8	—	1/8	7/8	—	1/8	+
219-2-S1			7	1	0	0	1	6/7	1/7	—	6/7	1/7	—	+
219-7-S1			7	0	0	0	0	7/7	—	—	7/7	—	—	+
219-3-S1	V	Leu+ Ura+	6	13	10	0	0	6/6	—	—	6/6	—	—	+
219-5-S1			4	27	6	1	0	4/4	—	—	4/4	—	—	+

* One tetrad has one spore sectored for Ura; 1 Leu⁻ Ura+:1 Leu⁻ Ura+/Ura⁻:2 Leu⁻ Ura⁻.

† One tetrad is 1 Leu+ Ura+:1 Leu⁻ Ura+: 2 Leu⁻ Ura⁻.

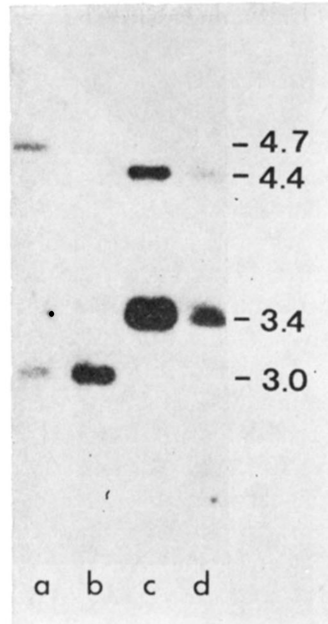

FIGURE 8.—Southern blot analysis of the *leu2* genes present in EMS219 (the original *leu2*⁻ strain) compared to the *leu2* genes present in the Leu$^+$ Class V revertant, EMS219-3-U3. DNA samples were cleaved with Bgl II and SmaI. The DNA fragments were then analyzed by gel electrophoresis, transferred to a nitrocellulose fiber and hybridized to a ^{32}P-labeled DNA fragment which contains the *leu2* gene (described in MATERIALS AND METHODS). Since SmaI and Bgl II do not cut in the *leu2* insert on chromosome *XII*, each band on the autoradiogram represents one of the two *leu2* genes. Lanes (a) and (b) show the hybridization patterns for rDNA and non-rDNA, respectively, from EMS219. The 4.7 kb band is the *leu2* gene on chromosome *XII*; the rDNA samples are contaminated with some non-rDNA. The 3.0 kb band is the *leu2* gene on chromosome *III*. Hybridization to rDNA and non-rDNA samples of EMS219-3-U3 are shown in lanes (c) and (d); the two *leu2* genes on the translocated chromosomes are the 4.4 and 3.4 kb bands.

not typical of translocation heterozygotes. In all crosses, all five Class V strains had poor spore viability (Figure 4f). In crosses of the Class V Ura$^+$ revertants, tetrads with two live spores were the predominant class. In crosses of Class V Leu$^+$ revertants, the spore viability, although poorer than that found for other Leu$^+$ classes, was considerably better than for the Ura$^+$ Class V crosses. Since the Southern analysis strongly suggests that the Class V Leu$^+$ revertants are translocation-containing strains, an apparent inconsistency in the data exists. By considering the causes of spore inviability in translocation heterozygotes, we found an obvious resolution of the inconsistency.

The spore inviability in translocation heterozygotes is the result of segregation events that produce spores containing one wild-type chromosome and one translocated chromosome. Such spores would have large deletions and duplications. Since yeast is tolerant of duplications (PARRY and COX 1971), it is likely that spore inviability is the consequence of deletions. One obvious explanation

of the spore viability patterns observed with Class V Leu$^+$ revertants is that, in some cases, spores that have deletions of genetic information are viable. Below, we present evidence that *in some genetic backgrounds* a spore is viable if it contains a normal chromosome *XII* and a translocated chromosome with the right half of *III* and the right half of *XII*. Such strains should be deleted for the left arm of chromosome *III* distal to the *leu2* locus and should be duplicated for a portion of the right arm of chromosome *XII*.

Much of the evidence for the conclusion stated above was obtained by examining the Leu$^+$ Ura$^+$ segregation patterns in tetrads with only three viable spores. The crosses between Class V revertants and DBY689 were particularly informative. These crosses showed that Class V revertants were of two different types (Table 7). EMS219-3-U3 and EMS219-7-U10 segregated, in 32 of 36 tetrads, 1 Leu$^+$ Ura$^+$ spore: 1 Leu$^-$ Ura$^+$ spore: 1 Leu$^-$ Ura$^-$ spore. Revertants EMS219-5-S1, EMS219-3-S1 and EMS219-11-U1 segregated, in 54 of 58 tetrads, 2 Leu$^+$ Ura$^+$ spores to 1 Leu$^-$ Ura$^-$ spore. Both these types of segregation indicate a specific association between the lethal event and the *leu2* and *ura3* genes. In the first type of segregation, the lethal event is specifically associated with the *LEU2*$^+$ and *ura3*$^-$ genes; in the second type, the lethality is specifically associated with the *leu2*$^-$ and *ura3*$^-$ mutant alleles.

Both of the above types of segregation are explicable in terms of *III–XII* translocation chromosomes. As described previously, three types of *III–XII* translocations are possible (Figure 6). The segregation patterns of EMS219-3-U3 and EMS219-3-U10 when crossed to DBY689 are consistent with the Class Va translocation. As shown in Figure 9, a recombination event between the rDNA and the centromere followed by adjacent-*1* or alternate segregation should produce one spore each of the classes: Leu$^+$ Ura$^+$, Leu$^-$ Ura$^+$, Leu$^+$ Ura$^-$ and Leu$^-$ Ura$^-$. The Leu$^+$ Ura$^+$ spore should contain the balanced translocation and, therefore, be viable. The Leu$^-$ Ura$^-$ spore should have the normal *III* and *XII* chromosomes and be viable. The Leu$^-$ Ura$^+$ spore should have the right arm of chromosome *III* (shown on the left in Figure 9) and part of the left arm (up to *leu2*), and should have the right arm of *XII*. In addition, a normal chromosome *XII* should be present. The Leu$^+$ Ura$^-$ spore should have an intact

TABLE 7

Class V Leu$^+$ *revertants of EMS219* (leu2$^-$ URA3$^+$ *in the rDNA*) *crossed to DBY689* (leu2$^-$ ura3$^-$): *phenotypes of tetrads with three viable spores*

Stain	Class	Phenotypes			
		1 Leu$^+$ Ura$^+$: 1 Leu$^-$ Ura$^+$: 1 Leu$^-$ Ura$^-$	2 Leu$^+$ Ura$^+$: 1 Leu$^-$ Ura$^-$	1 Leu$^+$ Ura$^+$: 2 Leu$^-$ Ura$^-$	2 Leu$^+$ Ura$^+$: 1 Leu$^+$ Ura$^-$
219-3-U3	Va	12/14	2/14	—	—
219-7-U10	Va	20/22	1/22	1/22	—
219-3-S1	Vb(c)	—	13/13	—	—
219-5-S1	Vb(c)	—	15/18	2/18	1/18
219-11-U1	Vb(c)	—	26/27	1/27	—

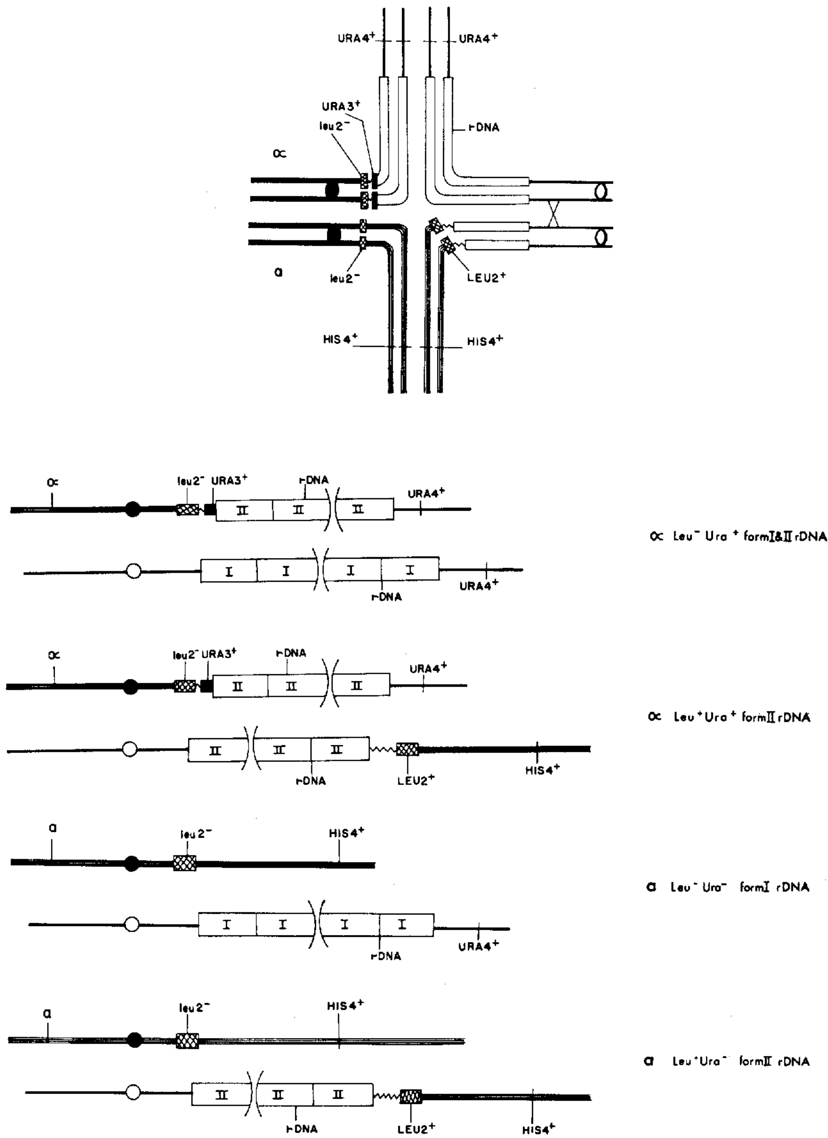

FIGURE 9.—Chromosome segregation pattern of EMS219 Class Va revertants crossed to DBY689. This figure shows the segregation pattern following a single crossover between the chromosome *XII* centromere and the translocation breakpoint. The phenotypes of the spores and their form of rDNA are indicated on the right. The genetic right arm of chromosome *III* is depicted on the left of the figure.

chromosome *III* and a translocation with the left arm of *XII*, part of the right arm of *XII* and part of the left arm of *III*; in Figure 9, the left arm of *III* is shown on the right. In crosses of EMS219-3-U3 and EMS219-7-U10, Leu$^-$ Ura$^+$ spores are viable but Leu$^+$ Ura$^-$ spores are not. This result argues that the left arm of chromosome *III* distal to *leu2* is dispensable for spore viability

(at least in some strain backgrounds) but that the left arm of chromosome *XII* distal to the rDNA is not. There are two other results that support this interpretation of the data. Since the mating-type locus is linked to the translocation breakpoint (Figure 9), we examined the segregation of the mating-type locus with Leu^+ and Ura^+ in tetrads with three viable spores. The results summarized in Table 8 demonstrate a significant association of the following sort: Leu^+ Ura^+ (α), Leu^- Ura^- (**a**), Leu^- Ura^+ (α) and, by inference, the Leu^+ Ura^- inviable spore should be mating type **a**. Thus, one of the two spores that get the normal chromosome *III* is inviable. A second line of evidence concerns the segregation of ribosomal DNA. EMS219 contains form II rDNA and DBY-689 has form I rDNA; these variants can be distinguished by EcoRI treatment of rDNA, followed by agarose gel electrophoresis. If our interpretation of the Class Va segregation pattern is correct, the Leu^- Ura^+ spore should have both form I and II rDNA, the Leu^+ Ura^+ spore should have only form II and the Leu^- Ura^- spore should have only form I. As shown in Table 9, this expected

TABLE 8

EMS219-7-U10 × DBY689: segregation of mating type in tetrads with three viable spores

Phenotype of spore		Number of spores of each mating type		χ^2
Leu	Ura	**a**	α	
—	—	49	12	21.25
+	+	15	46	14.75
—	+	17	44	11.08
+*	—	42	19	7.93

*This line of data refers to the dead spore. The Leu and Ura phenotype and mating type are inferred, assuming 2^+:2^- (or 2**a**:2α) segregation would have occurred if the tetrads had four viable spores.

TABLE 9

EMS219-7-U10 × DBY689: Analysis of tetrads with three live:one dead spore

Spore number	Phenotype Leu	Phenotype Ura	Form of rDNA
6a	—	+	I and II
6b	—	—	I
6c	+	+	II
8a	—	+	I and II
8b	—	—	I
8c	+	+	II
10a	+	+	II
10b	—	+	I and II
10c	—	—	I
11a	—	—	I
11b	+	+	II
11c	—	+	I and II

pattern was observed. Thus, the expected segregation pattern for Class Va clearly explains all the observed data for EMS219-3-U3 and EMS219-7-U10. The results also indicate that the DNA distal to the *leu2* locus on chromosome *III*, in some strain backgrounds, is dispensable.

Similar arguments can be used to explain the segregation data of EMS219-3-S1, EMS219-5-S1 and EMS219-11-U1. For these strains, either Class Vb or Class Vc translocations would give the expected segregation pattern of 2 Leu^+ Ura^+ spores to 1 Leu^- Ura^- spore. The summary of the expected chromosomes in each spore is shown in Figure 10. We have not done as complete an analysis with the Class Vb (Vc) revertants as with the Class Va. The strikingly similar spore viability patterns for all Class V revertants as well as the similar results in Southern analysis, suggest that all Class V revertants have similar origins and properties. All Class V revertants also show similar Leu^+ Ura^+ segregation patterns in asci with four viable spores.

The evidence described above is consistent with the conclusion that the genetic information centromere-distal to the *leu2* locus is dispensable for cell viability. This finding is very unexpected. The genetic distance between the *leu2* locus and the most distal chromosome marker on the same chromosome arm (HMLα) is about 40 cM (MORTIMER and SCHILD 1980). Assuming 2.7 kb per centimorgan (STRATHERN *et al.* 1979), we calculate that approximately 100 kb of DNA are missing in these strains. It is surprising that such a large region contains no indispensable genes. We believe, in fact, that this region of chromosome *III* is dispensable only in strain backgrounds in which a fraction of the DNA sequences from chromosome *III* has been transposed to a different chromosomal location. Several of the strains used in this study were provided by D. BOTSTEIN and were derived from strains of F. LACROUTE. The haploid strains DBY689, DBY613, DBY746 and SSU10-T7-2d in crosses with *his4*$^-$ strains, instead of showing $2^+:2^-$ segregation for *his4*, have frequent $4^+:0^-$ and $3^+:1^-$ segregations (M. ROSE, S. FALCO and D. BOTSTEIN, personal communication; PETES and MIKUS, unpublished observations). This aberrant pattern of segregation is not due to a suppressor mutation since crosses to deletions show the same property. Southern blotting analysis (M. ROSE, S. FALCO and D. BOTSTEIN, personal communication) shows that the haploid strain DBY613 contains a duplication of the *his4* locus. The exact extent of the duplication is not known but it involves at least 40 kb; the duplication does not include the *leu2* locus. The extra *his4* information has recently been mapped to chromosome *I*, approximately 25 cM from *ade1* (A. VINCENT and T. PETES, unpublished data).

Since EMS219 was derived by mutagenesis from SSU10-T7-2d, this strain also contains the duplicated *his4* information. We believe that it is this duplicated region that is responsible for the increased spore viability of the Leu^+ Class V translocation strains. T. DONAHUE and J. FINK (personal communication) have shown that a function centromere-distal to *his4 is* required for cell viability although the DNA sequences between *leu2* and *his4* are not required (S. ROEDER and J. FINK, personal communication). Our results suggest that the transposed *his4* region must also contain the indispensable sequence identified by DONAHUE and FINK.

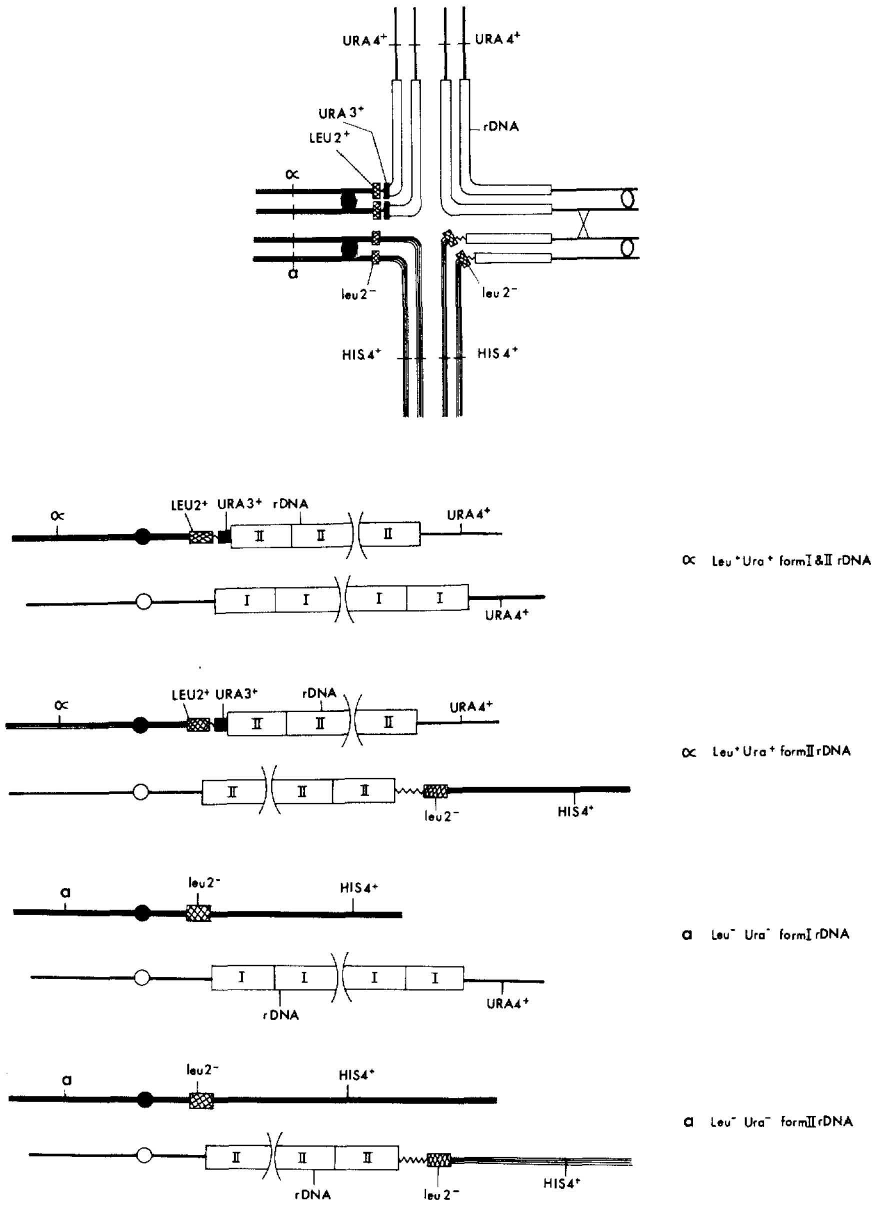

FIGURE 10.—Chromosome segregation pattern of EMS219 Class Vb revertants crossed to DBY689. In this diagram, the segregation pattern following a single crossover between the chromosome *XII* centromere and the translocation breakpoint is shown. The phentoypes of the spore and their form of rDNA are indicated on the right. The genetic right arm of chromosome *III* is drawn on the left of the figure.

Summary of Leu$^+$ revertant data: The classes of events observed in the UV-induced Leu$^+$ revertants are very similar to those seen with the Ura$^+$ revertants. As observed with the Ura$^+$ revertants, spontaneous Leu$^+$ revertants could be classified with a smaller number of classes than the UV-induced revertants. Reciprocal translocations, however, were observed among both spontaneous and UV-induced Leu$^+$ revertants. It is also interesting that the reversion to Leu$^+$ was similar to the Ura$^+$ reversion frequency. This result suggests that the frequency of interactions between repeated genes on nonhomologous chromosomes may be similar for different chromosomes.

DISCUSSION

We have shown that repeated genes located on nonhomologous chromosomes can interact in either reciprocal or nonreciprocal mitotic recombination events. The frequency of these interactions is comparable to the expected frequency of spontaneous mutations. Such interactions, therefore, are likely to be extremely important in the generation of genetic diversity in eukaryotes. The different classes of interactions and the implications of these interactions will be described in detail below.

The most common class of both Ura$^+$ and Leu$^+$ revertants consisted of interchromosomal gene conversion events, the nonreciprocal transfer of information from one repeat to the other. Similar conversion events have been detected in *Saccharomyces cerevisiae* by SCHERER and DAVIS (1980) and ERNST, STEWART and SHERMAN (1981) and in *Schizosaccharomyces pombe* by MUNZ and LEUPOLD (1981). Gene conversion events between repeated genes on a single chromosome (intrachromosomal gene conversion) have also been detected in *S. cerevisiae* (KLEIN and PETES 1981; JACKSON and FINK 1981; S. FALCO and D. BOTSTEIN, personal communication). It is likely, although not proven, that both intra- and interchromosomal gene conversion involve the same molecular interactions that are postulated for "classical" gene conversion, heteroduplex formation followed by mismatch repair (HOLLIDAY 1964).

The frequency of interchromosomal gene conversion events was approximately 2.5×10^{-8} for spontaneous events and 5×10^{-8} for UV-induced events. This estimate of the frequency is a maximum assuming both Class I and Class II revertants were generated by conversion events. As discussed previously, a fraction of the Class I revertants could be due to reversion without gene conversion. UV irradiation does not greatly increase the frequency of this type of gene conversion; a similar lack of effect has also been seen for intrachromosomal gene conversion between repeated genes (S. FALCO and D. BOTSTEIN, personal communication). In our experiments, the amount of UV used was about 1000 ergs/mm^2. In "classical" mitotic studies (DAVIES, EVANS and PARRY 1975; DAVIES, TIPPINS and PARRY 1978) this amount of irradiation would increase the frequency of gene conversion approximately 30-fold. The lack of effect of UV irradiation in our experiments suggests that the rate-limiting step of interchromosomal gene conversion might be a process (such as a diffusion-limited nucleation between the two repeats) that is independent of DNA damage and inducible repair systems.

The frequency of conversion in our experiments can be compared to the frequency of conversion events between repeated genes located on the same chromosome and the frequency of conversion between alleles in a diploid. JACKSON and FINK (1981) observed a frequency of 1.5×10^{-4} for intrachromosomal mitotic conversion between duplicated *his4* genes. The frequency of spontaneous recombination between allelic *his4* genes in a **a**/α diploid was about 6×10^{-6}. In our experiments (in a haploid strain), the observed frequency of spontaneous conversion was about 2.5×10^{-8}. Although there may be some variation in recombination frequencies because of the sequence differences between the *his4* gene and the genes used in our studies, the length of the repeat or other factors, these results suggest that mitotic interactions between genes located on nonhomologous chromosomes are approximately 100-fold less frequent than interactions between genes located on homologs and are about 10,000-fold less frequent than intrachromosomal interactions. Our results also indicate that the frequency with which two genes on nonhomologs interact may be similar for any two chromosomes.

Another interesting feature of the frequency data is that the spontaneous frequency of gene conversion is of a similar order of magnitude as the frequency of spontaneous mutations in yeast. Thus, changes in DNA sequences within repeated genes can occur either by spontaneous mutations or by gene conversion events. The likelihood of a diffusion-limited reaction should be proportional to N^2 (N equals the number of repeats). Therefore we believe that the probability that variants arise by conversion will also be a function of N^2, whereas the probability that variants arise by mutation will be independent of N. Finally, we should point out that gene conversion between repeated genes can have two effects. First, repeated conversion events can result in sequence homogeneity for a family of repeats (EDELMAN and GALLY 1970; KLEIN and PETES 1981; JACKSON and FINK 1981; NAGYLAKI and PETES 1982). Second, conversion between two repeats that differ by more than one base change can result in new combinations of base sequences (ERNST, STEWART and SHERMAN 1981; PETES 1982). In either case, gene conversion events among repeated genes are likely to be important in evolution.

The second most common class of genetic interactions represented reciprocal recombination events between repeated genes, resulting in reciprocal translocations. The approximate frequencies of reciprocal translocation events were 2×10^{-9} (spontaneous) and 1.4×10^{-8} (UV-induced). In several studies in which interactions between repeated genes on nonhomologous chromosomes were examined (SCHERER and DAVIS 1980; ERNST, STEWART and SHERMAN 1981; MUNZ and LEUPOLD 1981), no reciprocal recombination events were detected. One obvious reason that such events could be missed, as suggested by SCHERER and DAVIS (1980), is that reciprocal recombination between repeated genes oriented in opposite directions with respect to their centromeres would generate dicentric chromosomes and acentric fragments. Such events would probably be lethal in a haploid. In two other studies, reciprocal translocations have been detected. CHALEFF and FINK (1980) showed that some revertants of

a *Ty1*-induced mutant of *his4* were reciprocal translocations. One possible explanation of this observation is that the translocation was formed by reciprocal recombination between *Ty1* elements located on nonhomologous chromosomes. N. SUGAWARA and J. SZOSTAK (personal communication), using methods similar to those employed in our studies, have demonstrated that reciprocal translocations can be generated by recombination between a *his3* gene inserted in the rDNA and a *his3* at the normal locus on chromosome *XV*.

The spontaneous frequency of translocations that we observed (2×10^{-9}), although low, is likely to be high enough to account for the generation of spontaneous translocations in yeast. The rate that we measured represents the frequency of interactions generated between a single pair of repeated genes. Since yeast has a large number of repeated elements located on nonhomologous chromosomes, the expected frequency with which translocations could be generated by homologous recombination is obviously much higher than 2×10^{-9}. For example, there are about 35 *Ty1* elements in the yeast genome (CAMERON, LOH and DAVIS 1979). If all repeats interact with equal probability, the number of different types of interactions is (35)(34)/2 or 595. Thus, the expected frequency of translocations generated by homologous recombination between different *Ty1* elements would be about 10^{-6}. Obviously, when other repeated elements are taken into account, the frequency with which translocations could be generated by homologous recombination increases. Although more data are required to prove the hypothesis, we believe that in yeast most spontaneous translocations are the result of reciprocal recombination between repeated genes on nonhomologous chromosomes. It has previously been suggested (LEE 1975) that X-ray-induced translocations in Drosophila have breakpoints in repetitious DNA.

The reciprocal translocations described in our experiments have a property that is different from most reciprocal translocations. Since one of the breakpoints occurred within an inserted sequence that was located in the middle of the rRNA gene tandem array, both translocation chromosomes should contain rDNA sequences. Thus, the two translocation chromosomes share significant sequence homology. A recombination event between the rDNA sequences on the two translocated chromosomes could "reverse" the translocation. We have preliminary evidence for one such event. In crosses of the Class V strain EMS-219-11-U1 to DBY689 and X2180-1a, the diploids showed poor spore viability in the pattern expected for a translocation. In the cross of EMS219-11-U1 with SSU10-T7-2a, however, the first diploid analyzed showed very good viability (nine tetrads with four viable spores, two with three viable spores). When we dissected two other diploids isolated from the same cross, we found the poor spore viability expected for a translocation (Table 5). The simplest interpretation of this result is that translocation events are capable of reversal. Reversals of this sort have also been noted by other workers (N. SUGAWARA and J. SZOSTAK, personal communication).

A third type of genetic interaction we observed was the excision of the inserted *leu2* and *ura3* genes from the rDNA. This excision event was sometimes

accompanied by reinsertion of the sequences elsewhere (Class III). In some other revertants, the excised sequences were apparently used as a substrate for gene conversion and were then lost (Class IV). The excision event was presumably the result of a homologous recombination between rDNA repeats that flanked the *leu2* and *ura3* insertions. A recombination event of this sort would produce a circular DNA molecule and a tandem array with a deletion. The existence of these excision events suggests the possibility that the extra-chromosomal rDNA molecules which have been observed in some strains of yeast (MEYERINK *et al.* 1979; CLARK-WALKER and AZAD 1980) arise by intrachromosomal recombination.

The last class of revertants that were described (Class VI) involved multiple events, including an excision, an insertion and a chromosomal nonjunction. CHALEFF and FINK (1980), in studies of reversion of a *Ty1*-induced mutation, noted that revertant strains often contained multiple genomic rearrangements and suggested that concerted changes could occur. Class VI revertants may represent another example of concerted alterations.

In summary, we have shown that repeated genes on nonhomologous chromosomes can interact in a number of ways. The predominant mode of interaction is a gene conversion between repeats; however, reciprocal recombination events generating translocations have also been detected. These interactions are sufficiently frequent that it is likely that these events are important in the evolution of the eukaryotic genome.

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