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Mitotic and Meiotic Gene Conversion of Ty Elements and Other Insertions in *Saccharomyces cerevisiae*

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ABSTRACT

We examined meiotic and mitotic gene conversion events involved in deletion of Ty elements and other insertions from the genome of the yeast *Saccharomyces cerevisiae*. We found that Ty elements and one other insertion were deleted by mitotic gene conversion less frequently than point mutations at the same loci. One non-Ty insertion similar in size to Ty, however, did not show this bias. Mitotic conversion events deleting insertions were more frequently associated with crossing over than those deleting point mutations. In meiosis, conversion events duplicating the element were more common than those that deleted the element for one of the loci (*HIS4*) examined.

THE yeast *Saccharomyces cerevisiae* has about 30–40 copies per haploid genome of a 5.9-kb repeated sequence, Ty (reviewed by ROEDER and FINK 1983). Ty elements contain a 5.2-kb region (epsilon) flanked by 330-bp direct repeats (delta elements) and transpose through an RNA intermediate (BOEKE *et al.* 1985) at a minimum frequency of 10^{-8} to 10^{-9} insertions per cell at a single genetic locus (ROEDER, SMITH and LAMBIE 1984; PAQUIN and WILLIAMSON 1984). Once Ty elements are inserted into the haploid genome, the elements do not perfectly excise themselves at detectable (10^{-10}) frequencies (ROEDER and FINK 1983), although recombination between the flanking delta sequences (resulting in retention of a solo delta) occurs at a frequency of about 10^{-6} (CIRIACY and WILLIAMSON 1981; WINSTON *et al.* 1984; ROTHSTEIN 1979). There are approximately 100 solo deltas in the haploid genome (CAMERON, LOH and DAVIS 1979; EIBEL *et al.* 1980). Since Ty elements transpose, different haploid strains have at least some Ty elements located at different positions. A cross between two distantly related strains will produce a diploid that is heterozygous for multiple insertions. In the study described below, we examine the properties of meiotic and mitotic gene conversion events involving heterozygous Ty elements and other insertions.

Gene conversion is the nonreciprocal transfer of information between homologous sequences, usually located on homologous chromosomes. If a diploid strain is heterozygous (*A* and *a*) at a single genetic locus, meiotic gene conversion is detected as departures from 2*A*:2*a* segregation, yielding either 3*A*:1*a* or 1*A*:3*a* tetrads. It has been suggested that this transfer of information may be the result of mismatch repair of heteroduplexes formed between regions of sequence homology (HOLLIDAY 1964; MESELSON and RADDING 1975), repair of a double-strand gap that deletes one allele (SZOSTAK, ORR-WEAVER and ROTHSTEIN 1983) or replication of a heteroduplex formed in G_1 between homologous chromosomes (ESPOSITO 1978; BRUSCHI and ESPOSITO 1983).

Most studies of meiotic gene conversion in yeast were done with mutant alleles that were point mutations (FOGEL, MORTIMER and LUSNAK 1981) and, in general, such alleles showed approximate parity (equal proportions of 3*A*:1*a* and 1*A*:3*a* tetrads). Of 30 different mutant alleles examined, however, 10 showed significant (but usually less than twofold) departures from parity. In yeast, mutant alleles generated by deletions and insertions have rates of meiotic conversion similar to those observed for point mutations (FINK and STYLES 1974; FOGEL, MORTIMER and LUSNAK 1981; MCKNIGHT, CARDILLO and SHERMAN 1981; PUKKILA *et al.* 1986). Of three different deletions that have been examined, one converted with approximate parity (FOGEL, MORTIMER and LUSNAK 1981) whereas two showed deviations from parity in the direction leading to loss of the deletion (FOGEL, MORTIMER and LUSNAK 1981; MCKNIGHT, CARDILLO and SHERMAN 1981; PUKKILA *et al.* 1986). In meiotic gene conversion studies in Ascobolus, insertions and deletions of single base pairs often result in large departures from parity (LEBLON 1972a, b); conversion preferentially duplicates single base pair insertions while single base pair deletions are predominantly converted to wild type. Most large deletions exhibit parity. However, a large insertion showed a threefold

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excess of conversion events that removed the insertion (HAMZA, NICOLAS and ROSSIGNOL 1987).

Gene conversion events in yeast can occur in mitosis as well as meiosis (reviewed by ESPOSITO and WAGSTAFF 1981). Mitotic gene conversion events are usually detected as prototrophs arising in diploid strains that are heteroallelic for noncomplementing auxotrophic mutations (*m1-1+/+m1-2*). Parity of mitotic gene conversion is difficult to measure in this system since, although the rate of deletion of the mutant allele by conversion (resulting in a wild-type gene) can be estimated, the rate of duplication of the mutant allele (resulting in a double mutant) is not easily measured. In general, when the heteroalleles are both point mutations, the two different alleles are depleted by conversion with equal frequency (*m1-1*+/++ equal to ++/+*m1-2*). In a study in which one heteroallele was a point mutation and the second was a deletion, CHERNOFF *et al.* (1984) found that deletions were less frequently converted than point mutations.

In this paper, we investigated meiotic and mitotic conversion events involving Ty elements. We found that in meiosis, at some loci, conversion events tend to duplicate rather than delete Ty insertions. In mitosis, Ty elements were removed less easily than point mutations by gene conversion. Although one non-Ty insertion has similar mitotic conversion properties as Ty, a second non-Ty insertion behaves differently.

MATERIALS AND METHODS

Media and growth conditions: Standard media and growth conditions were used in these experiments (SHERMAN, FINK and HICKS 1986). Yeast colonies were grown nonselectively at 32° on YPD media. Nutritional markers were scored on omission plates (synthetic complete [SC] minus amino acid or nitrogen base). Canavanine resistance was scored on SC-Arg plates supplemented with 50 μg/ml of canavanine. Ura⁻ strains were selected on 5-fluoro-orotic acid (5-FOA) plates (SC plus 1 mg/ml 5-FOA and 50 μg/ml uracil) (BOEKE, LACROUTE and FINK 1984). Lys⁻ strains were selected on α-amino-adipate plates (CHATTOO *et al.* 1979).

Sporulation and tetrad dissection: Strains were sporulated on plates or in 5 ml liquid cultures at approximately 20° for 3–5 days. Strains were grown on YPD overnight and transferred to sporulation plates (1% potassium acetate, 0.1% yeast extract, 0.05% dextrose). For sporulation in liquid medium, strains were grown at 32° to mid-log phase on YPA (1% yeast extract, 2% Bacto-peptone, 1% potassium acetate) harvested, washed and transferred to SM (2% potassium acetate supplemented with appropriate amino acids). Tetrads were dissected after 3–5 days.

Plasmid construction: Plasmids used in this study are described in Table 1. *Escherichia coli* strains HB101 (r_B m_B *recA13*) or RK1448 (*thr⁻ leuB6 thi⁻ thyA trpC1117 hsdR12 hsdM12* str^R *recA13 argH*) were used as hosts in all cloning experiments. Standard cloning procedures were used (MANIATIS, FRITSCH and SAMBROOK 1982). The plasmids pLS12 and pAV17 were constructed by linearizing pLS11 with *Stu*I, which cuts at position 663 to create blunt ends in the *URA3 Hin*dIII fragment. YIp333 was digested with *Eco*RI and *Hin*dIII to produce a 5-kb fragment containing *LYS2*. The 3′ recessed ends of this fragment were filled in with deoxynucleotides by the Klenow fragment of *E. coli* DNA polymerase I. This fragment was ligated into the *Stu*I site of pLS11 with T_4 DNA ligase to produce the plasmids in pLS12 and pAV17.

The plasmid pAV23 is derived from pAV17; it lacks the promoter for the *LYS2* gene inserted in the *URA3 Hin*dIII fragment. The plasmid was constructed by digesting pAV17 with *Bgl*II and *Eco*RI (partial). The 3′ recessed ends of the resulting fragments were filled in with deoxynucleotides by the Klenow fragment of *E. coli* DNA polymerase I. The appropriate fragment, which lacked 630 bp of the 5′ end of the *LYS2* gene, was isolated on a 0.6% low melting temperature agarose gel and ligated to produce pAV23.

The plasmid pAV21 contains the Ty element from pGN821 cloned into the 5′ proximal *Bgl*II site of the *HIS3* gene in pLS10. First, pSR7, which has a 1.7-kb *Bam*HI *HIS3* fragment cloned into the *Bam*HI site of pBR322 (JINKS-ROBERTSON and PETES 1986), was digested with *Eco*RI to linearize the plasmid in pBR322 sequences. A 1.1-kb fragment of *URA3* flanked by *Eco*RI linkers (from pSR9 courtesy S. JINKS-ROBERTSON) was ligated into the linearized pSR7 to produce pLS10. Purified linear derivatives of pLS10 produced by partial digestion with *Bgl*II were ligated to a 6-kb *Bam*HI fragment containing the Ty element from pGN821.

Yeast strain construction: Strains used in this study are described in Table 2. The alleles *his4-912, lys2-128*, and *ura3-52* are due to the insertion of a Ty element into or near the gene. The Ty insertion in *his4-912* (ROEDER and FINK 1980) creates a promoter mutation. The Ty insertions in *lys2-128* and *ura3-52* disrupt the coding sequence of the genes (CLARK-ADAMS and WINSTON 1987; ROSE and WINSTON 1984). Yeast transformation was performed by spheroplasting or by treatment with lithium acetate (SHERMAN, FINK and HICKS 1986). AV50 was constructed by transforming 9665-10B (**a** *his4-912 lys2-128 ura3-52*) to Ura⁺ with the *URA3 Bam*HI fragment of pSR13 (JINKS-ROBERTSON and PETES 1986). The *URA3* gene of AV50 was replaced with *ura3::LYS2* disruptions (*LYS2* in different orientations) to produce AV51 and AV52. Similarly, AV54 was constructed by transforming AV50 with the *ura3::lys2 Hin*dIII fragment from pAV23. Ura⁻ transformants were selected on 5-FOA plates. AV53 was isolated from 9665-10B by selecting for weakly His⁺ revertants at 37°. Previous studies (CHALEFF and FINK 1980; ROEDER and FINK 1980; FARABAUGH and FINK 1980; SILVERMAN and FINK 1984) have indicated that many His⁺ revertants of *his4-912* are due to delta-delta recombination events that remove most of the Ty element, leaving behind a solo delta sequence. A revertant containing a *his4-912δ* was identified by Southern analysis and renamed AV53.

AV165 was constructed by mating SJR42 to SK124. The construction of SJR42 was previously described (JINKS-ROBERTSON and PETES 1986). This strain contains a 5.5-kb *ura3-3 Bam*HI fragment inserted into the 5′ proximal *Bgl*II site of the *HIS3* gene. AV16 [**a**/α *his3::ura3-3/his3 lys2-128/lys2-2 ura3-50/ura3-50 TYR1/tyr1-2 SUP* (amber)] was constructed through a series of crosses. SK124 was mated with strains containing the *lys2-2* allele derived from AV7-15D to isolate one haploid parent of AV16 containing *his3 lys2-2* and *ura3-50* alleles. SJR42 was crossed to strains containing the *lys2-128* allele derived from 9665-10B to produce the other haploid parent with *his3::ura3-3 lys2-128* and *ura3-50* alleles. AV16-H1 is a *HIS3* convertant of AV16. AV160 was constructed by transformation of AV16-H1 with pAV21, selecting for Ura⁺ transformants. pAV21 was linearized by digestion with *Bss*HII in the 3′ end of the *his3*

TABLE 1

Plasmids

Plasmid	Relevant allele	Description
B66	*HIS4*	1.6-kb *Sal*I fragment of *HIS4* cloned into *Sal*I site of pBR322 (from F. WINSTON)
YIp333	*LYS2*	7-kb *Eco*RI-*Pst*I fragment of *LYS2* cloned into *Eco*RI and *Pst*I sites of pBR322 (from R. ROTHSTEIN)
pLS11	*URA3*	1.1-kb *Hin*dIII *URA3* fragment cloned into *Hin*dIII site of pBR322 (from L. SYMINGTON)
pSR13	*URA3*	5.5-kb *Bam*HI *URA3* fragment cloned into *Bam*HI site of pBR322 (JINKS-ROBERTSON and PETES 1986)
pRB388	*URA3*	13-kb *Eco*RI *URA3* fragment cloned into *Eco*RI site of pBR322 (from ROSE 1982)
pSR7	*HIS3*	1.7-kb *Bam*HI *HIS3* fragment cloned into *Bam*HI site of pBR322 (JINKS-ROBERTSON and PETES 1986)
pGN821		TyH3 with *Bam*HI linkers cloned into *Bam*HI site of SP65 (from J. BOEKE)
pLS12	*ura3::LYS2*	5-kb *Eco*RI-*Hin*dIII *LYS2* fragment cloned into *Stu*I site of *URA3* gene in pLS11, *LYS2* transcript same strand as *URA3* transcript (from L. SYMINGTON)
pAV17	*ura3::LYS2*	5-kb *Eco*RI-*Hin*dIII *LYS2* fragment cloned into *Stu*I site of *URA3* gene in pLS11, *LYS2* transcript opposite strand to *URA3* transcript
pLS10	*URA3* and *HIS3*	1.1-kb *Eco*RI *URA3* fragment cloned into *Eco*RI site of pSR7 (from L. SYMINGTON)
pAV21	*his3::Ty* and *URA3*	6-kb *Bam*HI fragment containing Ty element from pGN821 cloned into 5′ proximal *Bgl*II site of *HIS3* in pLS10, Ty transcript opposite strand to *HIS3* transcript
pAV23	*ura3::lys2*	4.4-kb *Bgl*II-*Hin*dIII *lys2* fragment cloned into the *Stu*I site of *URA3* gene; derived from pAV17

gene to target the plasmid to genomic *HIS3* sequences (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983). Recombination events (SHERER and DAVIS 1979), which removed vector sequences and replaced the *HIS3* sequences in AV16-H1 with the *his3::Ty* disruption of pAV21, were detected by plating several independent transformants to 5-FOA to select for Ura$^-$ colonies (BOEKE, LACROUTE and FINK 1984). The desired strain (AV160) became simultaneously *ura3* and *his3* as expected.

Physical analysis: Yeast DNA was prepared by the 5-ml mini-prep procedure described by SHERMAN, FINK and HICKS (1986). Restriction enzyme digestions were performed as recommended by the manufacturer. The resulting fragments were separated by agarose gel electrophoresis and transferred by the method of SOUTHERN (1975) to either nitrocellulose or Hybond-N (Amersham). Plasmid DNA molecules used as hybridization probes were labeled by nick translation with ^{32}P-labeled nucleotides (SCHATCHET and HOGNESS 1973) and purified by a spin-column procedure (MANIATIS, FRITSCH and SAMBROOK 1982). Radioactively labeled fragments were visualized by autoradiography.

Mitotic experiments: The heteroallelic diploid strains described in Table 2 were used to isolate independent mitotic gene conversion events in the following manner. Two-day-old single colonies of a diploid yeast strain were picked from YPD plates and spread to appropriate media to screen for prototrophic convertants. For each colony plated, a single convertant was picked after 2 days and streaked to YPD for purification. A single purified colony of each convertant was used for all subsequent analysis. Southern analysis as described above was used to identify those convertants still containing an insertion. Initially, tetrads were dissected from many convertants to assure that the selected phenotype was not due to suppression. Additional genetic markers distal to the conversion event were analyzed where available to determine if recombination was associated with conversion. Prototrophs that had converted both heteroalleles were identified by several methods. Homozygous prototrophs produce 4$^+$:0$^-$ spores at the converted locus. Homozygous *LYS2* or *URA3* convertants were also identified by their inability to papillate on α-amino-adipate or 5-FOA plates, respectively.

For those experiments in which the rate of conversion was to be measured, 20 2-day-old diploid colonies were resuspended in sterile water. A fraction of each resuspended colony was plated to appropriate media to screen for convertants; another fraction was diluted and used for a cell count to estimate the size of the colony. All plates were analyzed after 2 days in order to count only those conversion events that existed in the colony prior to plating or that occurred shortly after plating. The rate of mitotic conversion was calculated by the method of the median (LEA and COULSON 1948; ARMITAGE 1952). The mean of the 20 cell counts from each experiment was used as an estimate of n in this formula. The rate of mitotic conversion events that remove mutations to restore a gene to wild-type function was measured using heteroallelic diploid strains.

UV-stimulated mitotic conversion: Experiments were as described above except that each single colony was resuspended in sterile water and evenly spread to an appropriate medium. Half of the selection plate was shaded and the other half was UV-irradiated at a rate of 2 J/m^2/sec for 10 sec. The plates were immediately wrapped in aluminum foil following irradiation and were incubated at 32° for 2 days. Ultraviolet light stimulated mitotic conversion approximately 5–10-fold. A single convertant from the UV-stimulated half of each plate was purified and analyzed as described above.

RESULTS

In the studies described below, we examined the meiotic and mitotic gene conversion of Ty elements and other insertions in yeast. The type of conversional interactions analyzed were those that resulted in deletion or duplication of the Ty element rather than ectopic conversional events between different Ty elements.

Meiotic gene conversion of Ty elements: The diploid strains PD1 and TP704 are heterozygous for

TABLE 2

Yeast strains

Strain	Origin	Genotype
Haploids:		
α131-20	(from B. BYERS)	*αade2-R8 leu1 ura3 can1 cyh2 MALX*
α131-20(U$^+$1)	Ura$^+$ revertant of α131-20	*α ade2-R8 leu1 can1 cyh2 MALX*
SK124	(from R. E. ESPOSITO)	*α ade2 asp5 his2 his3 lys7 trp1*
AV7-15D		*α his4C-864 his7 lys2-2 met13-c tyr1-2 ura3-18 can1*
L154	(from G. FINK)	*α SPT2-1 his4Δ29 HOL1-1*
SJR42	(JINKS-ROBERTSON and PETES 1986)	**a** *ade2 his3::ura3-3*$_a$ *his4 leu2-3,112 met8-1*$_a$ *trp1*$_a$ *SUP* (amber)
9665-10B		**a** *his4-912 lys2-128 ura3-52*
AV50	*ura3-52* in 9665-10B replaced with *URA3* from pSR13	**a** *his4-912 lys2-128*
AV51	*URA3* in AV50 replaced with *ura3::LYS2* from pLS12	**a** *his4-912 lys2-128 ura3::LYS2* (transcript same orientation as *ura3*)
AV52	*URA3* in AV50 replaced with *ura3::LYS2* from pAV17	**a** *his4-912 lys2-128 ura3::LYS2* (flip)
AV53	*his4-912* delta-delta recombination in 9665-10B	**a** *his4-912δ lys2-128 ura3-52*
AV54	*URA3* in AV50 replaced with *ura3::lys2* from pAV23	**a** *his4-912 lys2-128 ura3::lys2*
Diploids:		
PD1	α131-20(U$^+$1) × 9665-10B	α/**a** *ade2-R8/ADE2 HIS4/his4-912 leu1/LEU1 LYS2/lys2-128 URA3/ura3-52 can1/CAN1*^{s} *cyh2/CYH2 MALX/malx*
TP704	α131-20(U$^+$1) cross to spore of PD1	α/**a** *ade2-R8/ADE2 HIS4/his4-912 leu1/LEU1 LYS2/lys2-128 URA3/ura3-52*
AV100	α131-20 × 9665-10B	α/**a** *ade2-R8/ADE2 HIS4/his4-912 leu1/LEU1 LYS2/lys2-128 ura3/ura3-52 can1/CAN1*^{s} *cyh2/CYH2 MALX/malx*
AV101	α131-20 × AV51	α/**a** same as above except *ura3/ura3::LYS2*
AV102	α131-20 × AV52	α/**a** same as above except *ura3/ura3::LYS2* (flip)
AV90	AV7-15D × 9665-10B	α/**a** *his4C-864/his4-912 his7/HIS7 lys2-2/lys2-128 met13-c/MET13 tyr1-2/TYR1 ura3-18/ura3-52 can1/CAN1*^{s}
AV90-H1	*HIS4* convertant of AV90	α/**a** same as above except *HIS4/his4-912*
AV91	AV7-15D × AV51; *HIS4* convertant	α/**a** same as above except *ura3-18/ura3::LYS2*
AV92	AV7-15D × AV52; *HIS4* convertant	α/**a** same as above except *ura3-18/ura3::LYS2* (flip)
AV93	AV7-15D × AV53	α/**a** *his4C-864/his4-912δ his7/HIS7 lys2-2/lys2-128 met13-c/MET13 tyr1-2/TYR1 ura3-18/ura3-52 can1/CAN1*^{s}
AV94	AV7-15D × AV54	α/**a** *his4C-864/his4-912 his7/HIS7 lys2-2/lys2-128 met13-c/MET13 tyr1-2/TYR1 ura3-18/ura3::lys2 can1/CAN1*^{s}
AV94-H1	*HIS4* convertant of AV94	α/**a** same as above except *HIS4/his4*
AV16		α/**a** *his3::ura3-3*$_a$*/his3 lys2-128/lys2-2 ura3-50/ura3-50 TYR1/tyr1-2 SUP* (amber)
AV16-H1	*HIS3* convertant of AV16	α/**a** same as above except *HIS3/his3*
AV16-H3	*HIS3* convertant of AV16	α/**a** same as above except *his3::ura3-3/HIS3*
AV160	*HIS3* in AV16-H1 replaced with *his3::Ty* from pAV21	α/**a** same as above except *his3::Ty/his3*
AV165	SK124 × SJR42	α/**a** *ade2/ade2 asp5/ASP5 his2/HIS2 his3/his3::ura3-3*$_a$ *HIS4/his4 LEU2/leu2-3,112 lys7/LYS7 MET8/met8-1*$_a$ *trp1/trp1*$_a$ *URA3/ura3-50 SUP* (amber)
AV11		α/**a** *HIS4/his4-912 lys2-2/lys2-128 trp5-c/TRP5 tyr1-2/TYR1 URA3/ura3-52*
AV19		α/**a** *his3::ura3-3*$_a$*/his3-5 lys2-128/lys2-2 TYR1/tyr1-2 ura3-50/ura3-50 SUP* (amber)
AV20		α/**a** *his3::ura3-3*$_a$*/his3 lys2-128/lys2-2 TYR1/tyr1-2 ura3-52/ura3-50 SUP* (amber)
AV21		α/**a** *his3::ura3-3*$_a$*/his3-7 lys2-128/lys2-2 TYR1/tyr1-2 ura3-50/ura3-50 CAN1*^{s}*/can1 SUP* (amber)

mutations at the *HIS4*, *LYS2* and *URA3* loci that are caused by insertion of a Ty element. These strains were sporulated and tetrads were dissected in order to examine meiotic gene conversion of Ty elements. TP704 was held at 37° for 21 hr on sporulation medium and then shifted to 20° to increase the level of meiotic recombination and gene conversion in the strain (DAVIDOW and BYERS 1984). The data are

TABLE 3

Meiotic gene conversion of heterozygous Ty insertions

Strain	Alleles analyzed	No. of 3⁺:1⁻ conversions	No. of 1⁺:3⁻ conversions
PD1 (total of 240 tetrads)	*his4-912/HIS4*	1	2
	lys2-128/LYS2	1	0
	ura3-52/URA3	0	0
TP704 (total of 532 tetrads)	*his4-912/HIS4*	3	16[a]
	lys2-128/LYS2	4	7[b]
	ura3-52/URA3	1	1

[a] In the strain TP704, 3 of the 16 1^+:3^- conversions at the *his4* locus are due to the insertion of a solo delta into the *his4* gene instead of a complete Ty element.

[b] In the strain TP704, 1 of the 7 1^+:3^- conversions at the *lys2* locus is due to the insertion of a solo delta into the *lys2* gene.

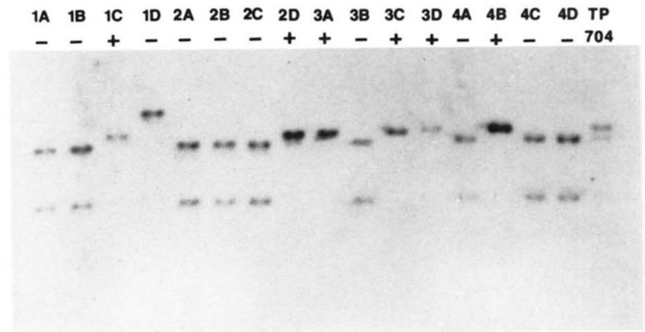

FIGURE 1.—Southern blot of total yeast DNA isolated from four TP704 tetrads with gene conversion events at the *HIS4* locus. The *HIS4* genotype of spore clones is indicated. The rightmost lane contains a control digest from the diploid strain TP704. The DNA from each spore was digested with *Bgl*II and analyzed on a 0.7% agarose gel. Fragments were transferred to Hybond-N and hybridized to the ^{32}P-labeled plasmid B66 (1.6-kb *Sal*I fragment of *HIS4*). The *HIS4* gene is on a fragment of approximately 3 kb. His⁻ spores that contain Ty912 produce two fragments (approximately 2.9 kb and 2.4 kb) that hybridize to *HIS4* sequences. One His⁻ spore (1D) produces a fragment of approximately 3.3 kb, which corresponds to the fragment size predicted for a *his4-912δ* allele. Additional experiments as described confirmed that this band was due to the presence of a solo delta in the *HIS4* gene.

summarized in Table 3. DNA was isolated from all the spores of a tetrad with a conversion event. These DNAs were digested with restriction enzymes and examined by Southern analysis for the presence or absence of the Ty element associated with the conversion event. In all but four cases (exceptions to be discussed below), mutant spores contained a complete Ty element.

As indicated in Table 3, at the *HIS4* locus, meiotic conversion preferentially duplicates the Ty element. Not all of the 1^+:3^- conversion events, however, are the result of a duplication of the complete Ty element. In three of the 16 1^+:3^- conversion tetrads, one of the mutant spores did not contain a complete Ty element (Figure 1). The sizes of the hybridizing bands (using the restriction enzymes *Bgl*II, *Sal*I, *Sau*3A, *Pvu*II and *Xho*I) indicated that these spores contained an insertion of about 300 bp, the size expected for a delta element. Genetic characterization of these inserts as described below indicates that they are probably *his4-912δ* alleles (a solo delta located at the point of Ty insertion in *his4-912*). Meiotic conversion events at the *LYS2* and *URA3* loci did not deviate significantly from parity, although it should be noted that the number of conversion events at these loci was small. One of the mutant spores in one of the 1^+:3^- convertant tetrads at *LYS2* did not contain a complete Ty element; instead it had the hybridization pattern (using restriction enzymes *Pst*I, *Bgl*II, *Eco*RI and *Kpn*I) expected for a solo delta replacing the original Ty.

To confirm the Southern analysis, which indicated the presence of a solo delta replacing the Ty element in some of the convertant tetrads, we did several genetic tests. Previously, mitotic revertants of *his4-912* had been isolated that had lost Ty912 but retained the solo delta (CHALEFF and FINK 1980; ROEDER and FINK 1980; FARABAUGH and FINK 1980; SILVERMAN and FINK 1984). Such revertants are weakly His⁺ but readily revert to strains that grow well in medium lacking histidine by accumulating mutations in unlinked genes (the *spt* genes) (WINSTON *et al.* 1984). We found that the His⁻ mutant spores that had hybridization patterns suggesting the presence of a delta element readily reverted to His⁺ at 32°, whereas the His⁻ spores containing Ty elements did not revert to His⁺ at detectable frequencies. We also crossed the putative *his4-912δ* strains to a strain (L154, provided by G. FINK) that contained the dominant *SPT2-1* mutation as well as a nonsuppressible *his4* mutant allele (*his4Δ29*). As a control, a strain containing *his4-912* was also crossed to L154. As expected, diploids with the putative *his4* solo delta were His⁺ and diploids with *his4-912* were His⁻. Finally, we constructed diploids by crossing the strains with the putative *his4-912δ* to a strain containing *his4-912*. No His⁺ mitotic recombinants could be obtained from such diploids, indicating that the delta element and the Ty element are located at the same position.

Similar experiments were done with the strain that appeared to have a solo delta at the *LYS2* locus. The Lys⁻ phenotype in this strain, however, was not suppressed by *SPT2-1* nor did the Lys⁻ phenotype readily revert to Lys⁺. This result is not completely unexpected since different insertions have different patterns of suppression (WINSTON *et al.* 1984; SIMCHEN *et al.* 1984). By allelism tests described above, the strain with the putative solo delta insertion at *LYS2* has a mutation at the same place as the Ty element of *lys2-128*.

It was unexpected to find convertant tetrads in which one of the mutant spores was different than the preexisting mutant allele, since meiotic gene conversion does not normally generate new mutant alleles

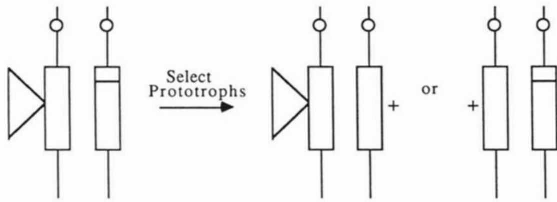

FIGURE 2.—Experimental design to examine mitotic conversion. Several diploid strains were constructed such that one of the two alleles of each gene was due to a Ty insertion into or near the gene. The other allele was a point mutation. (Triangles indicate insertions; lines across a chromosome indicate point mutations.) Prototrophs were isolated for analysis by plating independent diploid colonies to appropriate omission media.

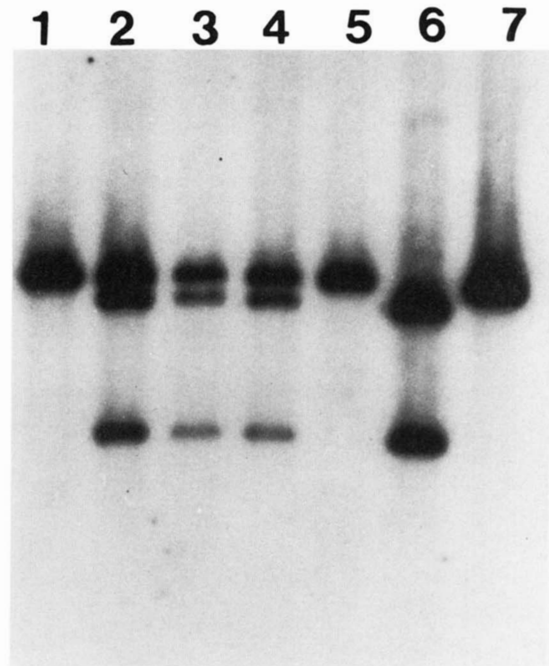

FIGURE 3.—Southern blot analysis of DNA isolated from AV9 *HIS4* diploids. DNAs were digested with *Bgl*II and analyzed on a 1% agarose gel. Fragments were transferred to Hybond-N and hybridized to the ^{32}P-labeled plasmid B66 (1.6-kb *Sal*I fragment of *HIS4*). DNAs from the haploid parents of AV9 (lanes 6 and 7) were run to indicate the positions of the fragments expected with each allele. The *HIS4* gene is on a fragment approximately 3 kb in size. The *his4-912* allele (created by the insertion of Ty912) produces two fragments (approximately 2.9 kb and 2.4 kb) that hybridize to *HIS4* sequences. The *his4C-864* allele, a point mutation, produces a single fragment that comigrates with *HIS4* sequences. Prototrophic diploids that lacked the bands indicative of a Ty insertion were assumed to have removed the element to produce a His$^+$ phenotype (lanes 1 and 5). Prototrophic diploids that had the Ty insertion pattern were assumed to have converted the point mutation (lanes 2, 3 and 4).

(FOGEL, MORTIMER and LUSNAK 1981). One obvious issue is whether the loss of the intact Ty element (generation of the solo delta) is associated with transfer of information between the homologous chromosomes or whether the loss of the Ty element occurs frequently in meiosis. We, therefore, looked for solo delta elements at the *HIS4* locus in nonconvertant (2^+:2^-) tetrads by examining the ability of the His$^-$ strains to revert to His$^+$. Only one of the 513 tetrads contained a mutant spore that was capable of reversion. Southern analysis of this spore confirmed that it contained a solo delta. Since 3 of 16 *his4* convertant but only 1 of 513 noncovertant tetrads contained a solo delta, we conclude that loss of the Ty element (presumably by delta-delta recombination) is associated with conversion between homologous chromosomes. In studies involving meiotic conversion of an insertion of Tn*9* (which also has a direct repeat) in yeast, R. MALONE, S. CRAMTON and R. GEHRHARDT (personal communication) have observed similar events. This class of convertant tetrad can be explained as the result of concerted double events (conversion of the intact Ty, followed by recombination between the flanking delta elements) or as the consequence of slippage during the replication event required to repair a double-strand gap.

In summary, meiotic gene conversion events in some loci lead to net duplication of Ty elements. In addition, some conversion events involving Ty elements result in insertion of a delta element into the homologous chromosome rather than the intact Ty.

Mitotic gene conversion in diploid strains with Ty insertion/point mutation heteroalleles: Strains in which mitotic conversion events involving Ty elements had occurred were isolated as prototrophs arising from auxotrophic diploid strains initially containing heteroalleles. Several diploid strains were constructed (Figure 2) in which one mutant allele was the result of a Ty insertion into or near the gene and the other allele was a point mutation (as defined by its ability to revert at a low frequency $\leq 10^{-7}$). Prototrophs were isolated after two days of growth on the appropriate omission medium. DNA was isolated from independent prototrophs and examined by Southern analysis to determine if the conversion event removed the point mutation or the Ty element to produce a wild-type gene (Figure 3).

Most prototrophs contained one wild-type and one mutant allele (Table 4). Prototrophs in which both alleles were wild-type, however, were also observed. Although such strains have Southern hybridization patterns identical to those convertants that remove only the Ty element, these strains can be distinguished by two tests. First, when strains homozygous for the wild-type alleles were sporulated, we observed 4^+:0^- spores. Second, strains that are heterozygous for mutant alleles at either the *LYS2* or *URA3* loci will form papillations on α-amino-adipate or 5-FOA plates, respectively (CHATTOO *et al.* 1979; BOEKE, LACROUTE and FINK 1984). These papillations arise as the result of mitotic recombination in the heterozygote, resulting in Lys$^-$ or Ura$^-$ derivatives that can grow on the selective medium. Strains that are homozygous for

TABLE 4

Mitotic gene conversion of Ty insertion/point mutation heteroalleles

Strain	Relevant genotype	Conversion of point mutation	Conversion of Ty element	Others	Convertants with assoc. reciprocal recombination
AV100	*ura3/ura3-52*	67% (40/60)	28% (17/60)	5% (3/60)[a]	8% pt. (3/40); [67% *CAN1*^{S}][b] 35% Ty (6/17); [83% *can1*^{r}] 50% +/+ (1/2); [100% *can1*^{r}] 0% +/pt-Ty (0/1)
AV90	*his4C-864/his4-912*	85% (51/60)	13% (8/60)	2% (1/60)	—
AV90-H1	*ura3-18/ura3-52*	70% (42/60)	28% (17/60)	2% (1/60)	17% pt. (7/42); [14% *CAN1*^{S}] 47% Ty (8/17); [75% *can1*^{r}] 100% +/+ (1/1); [100% *can1*^{r}]
AV11	*lys2-2/lys2-128*	63% (76/120)	22% (26/120)	15% (18/120)	2% pt. (1/41); [0% *TYR1/TYR1*] 23% Ty (3/13); [100% *tyr1/tyr1*] 67% +/+ (4/6); [75% *tyr1/tyr1*]
AV16-H3	*lys2-128/lys2-2*	73% (44/60)	25% (15/60)	2% (1/60)	Not done
AV160	*his3::Ty/his3*	67% (78/117)	32% (37/117)	2% (2/117)[c]	—
AV93	*his4C-864/his4-912δ*	68% (41/60)	32% (19/60)	0%	—

The percentage of conversion for each allele was calculated as the number of each type of conversion event isolated, divided by the total number of prototrophs analyzed for each strain. The actual number of conversions of each allele over the total number of prototrophs analyzed is indicated in parentheses next to the percentages. In some of the strains, prototrophs were isolated that had converted both heteroalleles (+/+ diploids). Such convertants were classified in the "Others" category. All convertants classified as "Others" are +/+ diploids except as indicated in the footnotes. A standard chi-square test of the data from each pair of heteroalleles indicates that all the results are significantly different from a 1:1 ratio at a 5% level of significance. Reciprocal recombination associated with conversion was also scored where possible using heterozygous distal markers. Dashes indicate strains in which such events could not be scored. The percentage of recombination associated with each conversion category was calculated as the number of events homozygous for the distal markers divided by the total number of conversions of each allele.

[a] One prototroph has both mutations in the mutant copy of *ura3*.

[b] Percentage of the total reciprocal recombinants with genotype as indicated. The genotype shown is that predicted by crossing-over at G_2 (see text for discussion). Remaining reciprocal recombinants have the opposite configuration of distal markers. For those prototrophs in which both chromosomes have wild-type alleles, the major configuration of distal markers is shown.

[c] Two prototrophs had an extra copy of the 5′ end of the *HIS3* gene (see text for details).

wild-type alleles at these loci papillate much less frequently. All *LYS2* and *URA3* prototrophs were checked by one of these tests. Most *HIS3* and *HIS4* prototrophs that had removed the Ty insertion (as determined by Southern analysis) were examined by the first test.

The results of conversion events at different heteroallelic loci in several strains are summarized in Table 4. All the heteroallelic combinations examined exhibit a significant bias in their relative frequencies of mitotic gene conversion. A standard χ^2 test indicates that the conversion pattern of each allele combination is different from a 1:1 ratio at a 5% level of significance. Conversion events remove point mutations 2 to 6 times more frequently than Ty insertions. The variation in the degree of the bias observed may be due to differences in the genes analyzed, differences in the relative proximity of the heteroalleles, or differences in the strain background.

Two unusual convertants were isolated from AV160 as *HIS3* prototrophs. Southern analysis of these two prototrophs indicated that they contained a partial extra copy of *HIS3* sequences. Tetrads dissected from these strains segregated 2 *HIS3*:2 *his3* spores and Southern analysis of spore DNA revealed that the extra *HIS3* homology was linked to the *HIS3* gene. One tetrad out of eight, however, was tetratype for the *HIS3* gene and the extra *HIS3* homology. In addition, one tetrad which contained a 1 *HIS3*:3 *his3* conversion event was also isolated; the extra *HIS3* homology was not altered by the conversion event. These results indicate that the His$^+$ phenotype is not due to the extra *HIS3* homology. Additional Southern analysis of the spore DNA indicated that the extra *HIS3* sequence was homologous only to *HIS3* sequences 5′ of the Ty insertion in the *his3::Ty* allele of AV160 and restriction sites flanking these sequences were consistent with the presence of a Ty2 element. One explanation of these results is given in Figure 4. We suggest that some small fraction of AV160 diploid cells had a recombination event between non-sister chromatids involving the *his3::Ty* element and a closely linked Ty2 element on chromosome *XV*. Such an event generates a partial duplication of the *HIS3* gene. Similar recombination events between different Ty elements have been previously demonstrated (Roeder 1983). Subsequent intrachromosomal conversion (Jackson and Fink 1981) between the 5′ duplication and the *his3* gene removes the point mutation to generate a wild-type gene.

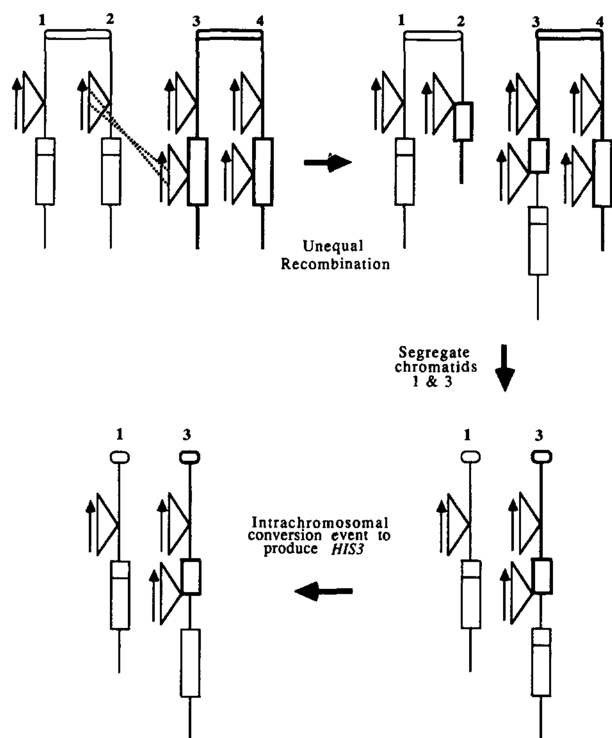

FIGURE 4.—Mechanism for the formation of the two unusual AV160 *HIS3* prototrophs. Some small fraction of AV160 cells are postulated to have a recombination event between non-sister chromatids involving the *his3*::*Ty* element and a centromere proximal Ty2 element on chromosome *XV*. Such an event generates a partial duplication of the 5′ end of the *HIS3* gene. Segregation of chromatids 1 and 3 together produces a His$^-$ diploid with a partial extra copy of *HIS3* linked to the *his3* point mutation. Subsequent intrachromosomal conversion between the 5′ duplication and the *his3* gene removes the point mutation to generate a wild-type gene.

Mitotic gene conversion of a diploid strain with delta insertion/point mutation heteroalleles: The results described above indicate that Ty elements are more difficult to remove by mitotic gene conversion than point mutations. In order to determine whether this bias was proportional to the size of the insertion, we examined mitotic gene conversion in a diploid strain (AV93) in which one heteroallele was a delta element and the other was a point mutation. This strain is isogenic to AV90 except at the *HIS4* locus. As in previous experiments, AV93 was plated to SC-his plates to isolate His$^+$ convertants. After 2 days, both large and small His$^+$ colonies were seen. Tetrad dissection indicated that the large colonies represented mitotic gene convertants (2 His$^+$:2 His$^-$ spores) and the small colonies represented extragenic suppressors (presumably *spt* mutations) of the *his4-912δ* mutation. Southern analysis of the His$^+$ convertants indicated that the point mutation rather than the delta insertion was preferentially converted to wild-type information (Table 4), although the bias is not as severe as that observed for the *his4-912* Ty element in this strain. Since the delta element is about 20 times smaller than the Ty, this result indicates that the bias is not directly proportional to the size of the insertion.

Mitotic gene conversion in diploid strains with non-Ty insertion/point mutation heteroalleles: To determine if the bias observed in mitotic gene conversion of Ty/point mutation heteroalleles is Ty-specific, we examined mitotic gene conversion of other yeast DNA insertions using similar combinations of heteroalleles in strains isogenic to those used in the Ty studies (Table 5). We found that mutations caused by the insertion of a 5-kb fragment containing the *LYS2* gene or a 4.4-kb promoterless *lys2* gene showed approximately the same degree and direction of bias as mutations induced by Ty elements in equivalent strains. However, mutations caused by insertion of a 5.5-kb fragment containing *ura3-3$_a$* were converted as frequently as point mutations. In strains with mutations induced by insertion of *LYS2*, the bias was not greatly affected by different genetic backgrounds and different point mutations (compare the results obtained with AV101 and AV102 with AV91 and AV92). In addition, the bias was similar for different orientations of the insert. In strains AV91 and AV101, the *LYS2* insert is in the same transcriptional orientation as *ura3*, the gene into which it is inserted; in strains AV92 and AV102, the *LYS2* insert is in the opposite orientation.

The lack of bias shown for conversion of mutations generated by insertion of the fragment containing *ura3-3$_a$* indicates that the bias is not an inherent feature of mutations generated by large insertions. The clearest evidence that the bias is the result of the particular insertion is the comparison between strains AV16 and AV160. These strains are isogenic except for the particular insertion within the *HIS3* locus. In AV16, one of the mutant *his3* genes is the result of insertion of the 5.5 kb *ura3-3$_a$* fragment into a *Bgl*II restriction site near the 5′ end of the *HIS3* gene. In AV160, instead of an insertion of *ura3-3$_a$*, there is an insertion of a Ty element at exactly the same position. Since equal frequencies of conversion of the heteroalleles are observed in AV16 (Table 5) but not in AV160 (Table 4), an unbiased conversion pattern (or a biased one) must be related to the sequence of the specific insertion. A conversion pattern consistent with a 1:1 ratio was observed for the *ura3-3$_a$* insertion with several different point mutations and in both orientations (Table 5).

One of the constant features of insertions that are difficult to remove by mitotic gene conversion is the presence of a strong promoter near one end of the insertion. Both the Ty and *LYS2* insertions have a promoter within 200 bp of the end of the insertion; the solo delta element at *his4-912δ* also has promoter activity (SILVERMAN and FINK 1984). The weak promoter of the *URA3* gene (LOSSON and LACROUTE 1979) in the 5.5-kb *Bam*HI fragment is more than 2

TABLE 5

Mitotic gene conversion of non-Ty insertion/point mutation heteroalleles

Strain	Relevant genotype	Conversion of point mutation	Conversion of non-Ty insert	Convertants with associated reciprocal recombination	Deviation from 1:1 ratio
AV101	*ura/ura3::LYS2*	68% (41/60)	32% (19/60)	15% pt. (6/41); [50%][a] 47% insert (9/19); [78%]	Yes
AV102	*ura3/ura3::LYS2*(flip)	68% (41/60)	32% (19/60)	2% pt. (1/41); [0%] 26% insert (5/19); [100%]	Yes
AV91	*ura3-18/ura3::LYS2*	78% (77/99)	22% (22/99)	0% pt. (0/77) 32% insert (7/22); [100%]	Yes
AV92	*ura3-18/ura3::LYS2*(flip)	87% (86/99)	13% (13/99)	3% pt. (3/86); [67%] 23% insert (3/13); [100%]	Yes
AV94-H1[b]	*ura3-18/ura3::lys2*	68% (41/60)	30% (18/60)	0% pt. (0/41) 28% insert (5/18); [80%]	Yes
AV165	*his3/his3::ura3-3*	34% (17/50)	66% (33/50)		No
AV16	*his3::ura3-3$_a$/his3*	50% (30/60)	50% (30/60)		No
AV19	*his3::ura3-3$_a$/his3-5*	51% (44/86)	49% (42/86)		No
AV20	*his3::ura3-3$_a$/his3*	41% (37/90)	59% (53/90)		No
AV21	*his3::ura3-3$_a$/his3-7*	41% (32/79)	59% (47/79)		No

The percentage of conversion for each allele was calculated for each strain as the number of each type of conversion event isolated, divided by the total number of prototrophs analyzed. The actual number of conversions of each allele over the total number of prototrophs analyzed is indicated in parentheses next to the percentages. A standard χ^2 test of the data from each pair of heteroalleles was used to determine if conversion differed significantly from a 1:1 ratio at a 5% level of significance. The results of this test for each strain are indicated. Reciprocal recombination associated with conversion was analyzed where possible as described in Table 4.

[a] Percentage of the total reciprocal recombinants with the configuration of distal markers expected from a crossover event in a G_2 model of recombination. The remaining reciprocal recombinants have the opposite configuration of distal markers.

[b] One prototroph that had converted both heteroalleles (+/+ diploid) was isolated from AV94-H1.

kb from the end of the insertion, although we cannot rule out the possibility of unmapped additional promoters near the ends.

In order to test the hypothesis that a strong promoter contributes to the bias observed in mitotic gene conversion, we deleted the promoter of the *LYS2* fragment inserted in *URA3*. A strain, AV94-H1, which contained this deletion but was otherwise equivalent to AV92, was constructed. As noted in Table 5 this strain still exhibited a bias in its mitotic conversion pattern. However, the extent of this bias is significantly less (contingency chi-square < 3%) than that observed in AV92. As these two strains are derived from essentially the same parental strains (two different transformations of AV50), we suggest the difference in the degree of bias results from the presence or absence of the 600 bp of the *LYS2* fragment containing the *LYS2* promoter. Obviously, since the pattern observed for AV94-H1 is different from a 1:1 ratio, other factors must also affect mitotic gene conversion of insertions. In summary, although we cannot exclude several other possibilities (similar phasing of nucleosomes in the Ty and *LYS2* insertions, for example), we favor the idea that insertions with strong promoters near one end are difficult to remove by mitotic gene conversion.

UV-stimulated mitotic gene conversion in heteroallelic diploids: Many previous experiments that study mitotic gene conversion have used UV-irradiation to increase the frequency of conversion events. As in spontaneous conversion in heteroallelic diploids, most heteroalleles are converted with equal frequency after treatment with UV (reviewed by ESPOSITO and WAGSTAFF 1981). Consequently, we investigated whether UV-irradiation affected the conversion bias in diploids with insertion/point mutation heteroalleles. Several of the heteroallelic strains used in the previous experiments were plated onto appropriate omission medium and UV-irradiated to stimulate mitotic conversion 5–10-fold. Control experiments indicated that this treatment did not lead to a significant increase in prototroph formation as a result of reversion or suppression of point mutations (data not shown). Prototrophs were isolated and analyzed as described previously. The results are summarized in Table 6. The UV-stimulated mitotic conversion patterns of the strains with the Ty/point mutation heteroalleles (AV100 and AV11) are significantly different from the spontaneous results observed with these strains (Table 4). The bias against removal of Ty elements by conversion previously demonstrated in these strains is more severe with UV stimulation. The

TABLE 6

UV-stimulated mitotic gene conversion of heteroalleles

Strain	Relevant genotype	Conversion of point mutation	Conversion of insertion	Others	Convertants with associated reciprocal recombination	Spontaneous and UV conversion different
AV100	*ura3/ura3-52*	93% (56/60)	7% (4/60)	0%	4% pt. (2/56); [100%][a] 50% Ty (2/4); [100%]	Yes
AV11	*lys2-2/lys2-128*	85% (51/60)	12% (7/60)	3% (2/60)	Not Done	Yes
AV101	*ura3/ura3::LYS2*	78% (47/60)	22% (13/60)	0%	9% pt. (4/47); [50%] 23% insert (3/13); [67%]	No

The percentage of conversion for each allele was calculated as the number of each type of conversion event isolated, divided by the total number of prototrophs analyzed for each strain. The actual number of conversions of each allele over the total number of prototrophs analyzed is indicated in parentheses next to the percentages. In some of the strains, prototrophs were isolated that had converted both heteroalleles (+/+ diploids). Such convertants were classified in the "Others" category. A chi-square test of the data using a 2 × 3 (or 2 × 2) contingency table was done to determine if spontaneous conversion (Tables 4 and 5) differed significantly from UV-stimulated conversion at a 5% level of significance. The results of this test for each strain are indicated. Reciprocal recombination associated with conversion was analyzed as described in Table 4.

[a] Percentage of the total reciprocal recombinants with the configuration of distal markers expected from a crossover event in a G_2 model of recombination. The remaining reciprocal recombinants have the opposite configuration of distal markers.

strain isogenic to AV100, AV101, which has the *ura3/ura3::LYS2* heteroalleles, does not exhibit this difference. These results indicate that the types of events resulting from UV-stimulated mitotic conversion are not always comparable to those isolated spontaneously and that different insertions are affected differently by UV treatment. It should also be pointed out that UV treatment increases the transcription of Ty elements (ROLFE, SPANOS and BANKS 1986). Thus, the observation that UV increases the bias in Ty conversion is consistent with our suggestion that the bias is related to transcription of the insertion.

Association of mitotic conversion and reciprocal recombination: Approximately 10–55% of mitotic gene conversion events in previous studies were associated with reciprocal exchange (reviewed by ESPOSITO and WAGSTAFF 1981). To monitor this association in our study, we examined the configuration of markers distal to the heteroallelic loci either by tetrad dissection of the prototrophs or by tests of drug resistance (if the distal heterozygous mutations were *CAN1^S/can1^r*). If gene conversion associated with a crossover occurs in G_2 and segregation of the recombined chromatids is random, then the distal marker will become homozygous about half the time (Fig. 5A). The percentage of reciprocal recombination associated with each type of conversion event is summarized in Tables 4 and 5. At the *URA3* locus, reciprocal recombination events were significantly more frequently associated with conversion of the insertions (both Ty and *LYS2* insertions) than with conversion of the point mutations. At the *LYS2* locus, a similar preference is observed, although the difference in the association for insertions and point mutations at this locus is not statistically significant.

As shown in Figure 5A, if conversion and associated crossovers occur in G_2, the particular distal marker that becomes homozygous should be related to the particular mutant allele that is converted. For example, in Figure 5A, if the insertion is lost by gene conversion, the *can1^r* allele will become homozygous in the strain that contains the wild-type *URA3* allele. If the point mutation is lost, the *CAN1^S* allele should become homozygous. From the data shown in Tables 4, 5 and 6, it is clear that frequently the "wrong" allele becomes homozygous. One interpretation of this result is that the conversion event and the crossover (although they occur in a concerted manner) are not mechanistically related and, therefore, need not involve the same two chromatids (ROMAN and FABRE, 1983). Alternatively, as diagrammed in Figure 5B, mitotic gene conversion may occur as the result of repair of a heteroduplex formed in G_1 and crossing over as a consequence of replication of the HOLLIDAY intermediate, as suggested by ESPOSITO (1978).

Rate of mitotic gene conversion: Prototroph formation in heteroallelic strains reflects the rate at which mitotic conversion replaces a mutation in a gene with wild-type information from homologous sequences. The rate of mitotic gene conversion in the heteroallelic strains was estimated as described in MATERIALS AND METHODS. The results of these calculations are presented in Table 7. The rates of conversion vary by approximately an order of magnitude depending upon the locus measured. The rate of conversion at the *HIS3* locus in all the heteroallelic strains examined is low relative to the rate of conversion at the other loci.

DISCUSSION

Our conclusions from this study are: (1) meiotic gene conversion of Ty insertions at some loci tend to duplicate the Ty element, (2) mitotic gene conversion events in diploids with Ty/point mutation heteroalleles preferentially remove the point mutation, (3) the frequency with which an insertion is removed by

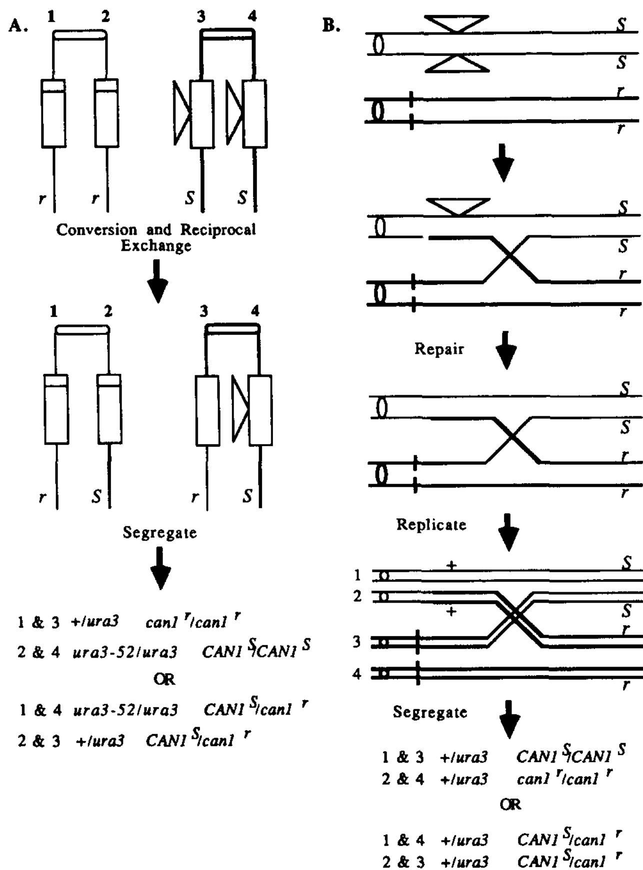

FIGURE 5.—Association of mitotic conversion and reciprocal recombination. (A) G_2 model of association between conversion and crossing over. Lines indicate replicated chromatids (double-stranded) joined by their centromeres (ovals) to their sisters. Mitotic conversion of the Ty insertion in *ura3-52* with an associated crossover produces a *URA3* gene linked to the *can1^r* allele. If segregation is random, half of the *URA3* diploids will be *can1^r/can1^r*; the other *URA3* diploids will be heterozygous at the *can1* locus. (B) G_1 model of association between conversion and crossing over. Lines indicate single strands of DNA attached to their centromeres (ovals). Mitotic conversion of the Ty insertion in *ura3-52* with replication through the HOLLIDAY intermediate can produce two copies of the *URA3* gene, one linked to the *can1^r* allele and one linked to *CAN1^S*. If segregation is random, one-quarter of the *URA3* diploids will be *can1^r/can1^r*, one-quarter will be *CAN1^S/CAN1^S*, and the other half will be heterozygous at the *can1* locus.

mitotic gene conversion relative to a point mutation is related to the sequence (and possibly the transcription) of the insertion, and (4) mitotic reciprocal recombination appears preferentially associated with conversion of insertions rather than with conversion of point mutations. These conclusions will be discussed in more detail below.

Most mutant alleles are deleted or duplicated in meiosis with approximately equal frequencies (equal numbers of $3^+:1^-$ and $1^+:3^-$ tetrads; FOGEL, MORTIMER and LUSNAK 1981). We found that a mutation induced by a Ty insertion at *HIS4* was more frequently duplicated than deleted by meiotic gene conversion. Although we are not aware of any previous studies of meiotic conversion of insertions in yeast, there have been several studies of conversion of deletions (FINK and STYLES 1974; FOGEL, MORTIMER and LUSNAK 1981; MCKNIGHT, CARDILLO and SHERMAN 1981; PUKKILA *et al.* 1986). Although approximate parity was observed for most deletions, in two cases, *his4-15* (FOGEL, MORTIMER and LUSNAK 1981) and *CYC7-H3* (MCKNIGHT, CARDILLO and SHERMAN 1981; PUKKILA *et al.* 1986), gene conversion events more frequently duplicated wild-type information than mutant information. Since the chromosome containing the wild-type allele can be considered to have an insertion relative to the chromosome containing the deletion mutation, this disparity in conversion is in the same direction as we observed at the *HIS4* locus. In Ascobolus, large deletions also either show parity or disparity favoring the wild-type allele (HAMZA, NICOLAS and ROSSIGNOL 1987).

Any explanation of the effect of some insertions and deletions on the parity of conversion must be discussed in the context of the two most popular recombination models. In the MESELSON-RADDING model (1975), which is based on an earlier model of HOLLIDAY (1964), conversion is initiated by a nick on the donor chromosome (Figure 6A). The displaced strand from the donor forms a heteroduplex with the recipient chromosome. If a mismatch exists within the heteroduplex, it is corrected using information derived from either the donor or recipient strands of the heteroduplex. Only correction using the donor information will result in a gene conversion event. RADDING (1978) has suggested that correction of mismatches can occur even when the mismatch is a large insertion loop rather than a single base pair mismatch (Figure 6B). Thus, for this model, the parity of conversion can be affected by two parameters, a preference for nicking one of the interacting alleles or a preference in the repair (or resolution) of the heteroduplex. Thus, our meiotic results could be explained by the hypothesis that certain insertions increase the probability of nicking the chromosome adjacent to the insertion or that heteroduplexes containing certain insertions are preferentially resolved to duplicate the insertion.

The alternative model of recombination (Figure 6C) involves a double-strand break in the recipient DNA that is extended into a gap (SZOSTAK, ORR-WEAVER and ROTHSTEIN 1983). This gap is repaired using the other homologous chromosome and the resulting double HOLLIDAY junction is resolved by strand cleavage. By this model, our data could be explained by hypothesizing that certain insertions result in a decreased probability of double-strand breakage in flanking sequences.

In our experiments, only the Ty element at *HIS4* showed disparity in meiotic gene conversion. Although the Ty elements at *LYS2* and *URA3* did not

TABLE 7

Rate of mitotic gene conversion of heteroalleles

Strain	Heteroalleles	Rate of conversion (× 10^{-6} convertants/division)
AV100	*ura3/ura3-52*	7.2 ± 1.0 (20 colonies)
		4.7 ± 0.8 (20 colonies)
AV101	*ura3/ura3::LYS2*	3.3 ± 0.6 (20 colonies)
		6.8 ± 1.8
AV102	*ura3/ura3::LYS2* (flip)	3.6 ± 0.6 (20 colonies)
AV91	*ura3-18/ura3::LYS2*	1.9 ± 0.4 (20 colonies)
AV92	*ura3-18/ura3::LYS2* (flip)	2.5 ± 0.5 (20 colonies)
AV94-H1	*ura3-18/ura3::lys2*	2.2 ± 0.6
AV93	*his4c-864/his4-912δ*	4.3 ± 1.0 (large colonies only)
AV11	*lys2-2/lys2-128*	6.6 ± 1.7
AV16	*his3::ura3-3$_a$/his3*	0.65 ± 0.24
AV160	*his3::Ty/his3*	0.63 ± 0.26
AV19	*his3::ura3-3$_a$/his3-5*	0.60 ± 0.23
AV20	*his3::ura3-3$_a$/his3*	1.3 ± 0.4
AV21	*his3::ura3-3$_a$/his3-7*	0.88 ± 0.33

Prototrophs and the total number of cells in 10 independent colonies (or 20 where indicated) were counted. A rate of conversion (convertants/division) and a standard deviation was calculated from this data by the method of the median from LEA and COULSON (1948). All experiments measure spontaneous conversion rates.

show disparity, the number of conversion events involving these genes was small. Previously, PUKKILA *et al.* (1986) showed that the *CYC7-H3* had approximately the same disparity at two different chromosomal locations whereas a different deletion at approximately the same site showed no disparity.

In contrast to the meiotic results, we found that all Ty elements behaved similarly during mitotic gene conversion. More specifically, Ty elements were more difficult to remove than point mutations in the same gene. R. MALONE, examining the mitotic conversion of the *ura3-52* Ty element, has obtained similar results (personal communication). In the context of the MESELSON-RADDING model, this result can be interpreted as indicating that certain insertions (those that contain a strong promoter) increase the probability of nicks in flanking sequences, have a smaller likelihood of being included within a heteroduplex, or, when included in a heteroduplex, affect the resolution of the structure. Alternatively, applying the double-strand break model, it is possible that certain insertions have a decreased probability of loss by conversion because they are more difficult to degrade by double-stranded DNases or because they decrease the probability of a double-strand break in flanking sequences. In experiments that were similar to ours, CHERNOFF *et al.* (1984) found that large deletions were harder to remove than point mutations and suggested that deletions might impede the formation of heteroduplex DNA. Our results, however, indicate that not all deletions are likely to have this effect.

Mitotic crossing over in heteroallelic diploids appears preferentially associated with conversion of insertions rather than conversion of point mutations, indicating the possibility of a difference in the mechanism by which these different changes are removed. In three of the five heteroallelic combinations examined, the insertion was located distal to the point mutation (in two heteroallelic combinations, the relative orientation of the mutant alleles was not known). An alternative (and less likely) explanation of the data is, therefore, that conversion of the distal allele is more frequently associated with crossing over regardless of the physical nature of the mutation. Our results agree with those of CHERNOFF *et al.* (1984) who showed that mitotic conversion of a deletion was more frequently associated with crossing over than conversion of a point mutation.

Although we cannot exclude other explanations of our mitotic data, we propose the following interpretation. Heteroduplexes are an intermediate in mitotic gene conversion; DNA sequences with insertions that are actively transcribed are more difficult to include within a heteroduplex than DNA sequences with point mutations. In addition, such insertions may require formation of a more stable (longer) heteroduplex than those that involve point mutations. These longer tracts are preferentially resolved as crossovers.

HOLLIDAY (1982) suggested that gene conversion events may act to remove transposable elements from the genome. Our results indicate gene conversion in yeast can remove heterozygous insertions. Since we do not have an estimate of the rate of duplication of insertions by gene conversion, it is not clear that this mechanism (in the absence of selection) will lead to a net loss of transposable elements.

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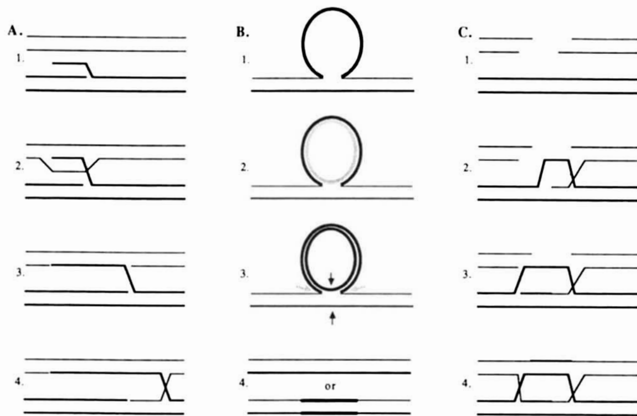

FIGURE 6.—Models for recombination (lines represent single strands of DNA). (A) MESELSON-RADDING model (MESELSON and RADDING 1975). 1, Recombination is initiated by a single-stranded nick. The resulting free end serves as a primer for DNA synthesis, displacing a single strand. 2, The displaced strand invades homologous sequences forming a D-loop. 3, The D-loop is degraded. The asymmetric heteroduplex is extended by DNA synthesis on the donor chromatid and degradation on the recipient chromatid. 4, Ligation of strands produces a HOLLIDAY junction which may branch-migrate to generate a region of symmetric heteroduplex. Resolution of the HOLLIDAY junction by cleavage of the crossed strands or cleavage of the top and bottom strands (as drawn) yields a noncrossover or crossover product, respectively. Repair of mismatches in the heteroduplex produces conversion to either wild-type or mutant information. (B) Mechanism of conversion of insertion by a heteroduplex (RADDING 1978). 1, Insertion becomes part of a heteroduplex region as in MESELSON-RADDING model. 2, DNA synthesis makes the single-stranded loop double-stranded. The structure formed is similar to a HOLLIDAY junction. 3, Strand cleavage as indicated by the solid arrows converts the insertion into the DNA sequence. Cleavage as indicated by the checkered arrows removes the insertion from the DNA sequence. (C) Double-strand break model (SZOSTAK, ORR-WEAVER and ROTHSTEIN 1983). 1, A double-strand break is made in one DNA duplex and enlarged to a gap with 3′ overhangs by nucleases. 2, A 3′ end invades homologous sequences and forms a D-loop. 3, DNA synthesis enlarges the D-loop until the other 3′ overhang can pair with complementary displaced sequences. 4, The other 3′ end serves as a primer for DNA synthesis to repair the gap. Ligation of the nicks forms two HOLLIDAY junctions that may branch migrate. Resolution of both junctions in the same sense (crossed strands or outer strands cut) produces a noncrossover product; resolution of the junctions in the opposite sense produces a crossover product.

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