Analysis of the Mechanism for Reversion of a Disrupted Gene

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ABSTRACT

A positive selection system for intrachromosomal recombination in *Saccharomyces cerevisiae* has been developed. This was achieved by integration of a plasmid containing an internal fragment of the *HIS3* gene into its chromosomal location. This resulted in two copies of the *HIS3* gene one with a terminal deletion at the 3′ end and the other with a terminal deletion at the 5′ end. Reversion of the gene disruption could be brought about by plasmid excision, unequal sister chromatid exchange or sister chromatid conversion. The purpose of this study was to define the mechanisms involved in reversion of the gene disruption. The frequency of plasmid excision could be determined by placing a yeast sequence bearing an origin of replication onto the plasmid that was subsequently integrated into the yeast genome. Unequal sister chromatid exchange and conversion could be distinguished by determining the nature of the reciprocal product by Southern blotting. The results indicate that reversion might occur mainly by conversion between sister chromatids. This is because the frequency of plasmid excision was about two orders of magnitude lower than the overall frequency of reversion and no reciprocal product indicative of sister chromatid exchange was found. The findings of this presentation suggest that conversion might be an important mechanism for recombination of sister chromatids and possibly for repair of damaged DNA in S or G_2.

MEIOTIC recombination occurs after DNA replication when four DNA duplexes are present. Most mitotic gene conversion, however, occurs in the G_1 stage of the cell cycle before DNA replication (FABRE 1978; ESPOSITO 1978).

Recombination between homologs is called interchromosomal. Intrastrand and sister chromatid recombination (SCR) is called intrachromosomal recombination. Recombination between homologs have been extensively studied (reviews: ESPOSITO and WAGSTAFF 1981; KUNZ and HAYNES 1981; ORR-WEAVER and SZOSTAK 1985). It has been demonstrated that heteroallelic mitotic recombination occurs predominantly by gene conversion (ROMAN 1956).

Reciprocal exchange between sister chromatids has been observed in Drosophila (TARTOFF 1973), mammalian cells (PERRY and EVANS 1975; reviewed by LATT and SCHRECK 1980) and in yeast (PETES 1980; SZOSTAK and WU 1980). Conversion events between sister chromatids on the other hand have so far not been positively identified.

To study recombination in yeast new approaches using recombinant DNA techniques have recently been applied. These studies have the advantage that depending on the system used specific events can be analyzed. For example plasmid integration (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; FALCO, ROSE and BOTSTEIN 1983) meiotic recombination (KLEIN and PETES 1981; KLEIN 1984; JACKSON and FINK 1985; LICHTEN, BORTS and HABER 1987) and mitotic recombination (JACKSON and FINK 1981; SUGAWARA and SZOSTAK 1983) were studied. The type of recombination event one can study depends on the nature of the recombining alleles and their genomic position. When point mutations at the *his4* locus were used, reversion events in mitosis included predominantly gene conversion not associated with crossing over and less frequently crossing over (JACKSON and FINK 1981). Reversion of *his3* mutant alleles in inverted orientation occurs mainly by gene conversion in meiosis (KLEIN 1984); and when *his3* alleles were placed on different chromosomes about 10% of the conversion events were associated with reciprocal translocation (SUGAWARA and SZOSTAK 1983).

Previous studies on unequal SCR were done with plasmids integrated at the ribosomal DNA (rDNA) locus (SZOSTAK and WU 1980; PETES 1980; PRAKASH and TAILLON-MILLER 1980). Because screening rather than selection methods were applied these studies were time consuming. It also has been shown that rDNA contains a hotspot for recombination (KEIL and ROEDER 1984). Therefore studies on SCR at the rDNA locus might not reflect the features of SCR at other loci in yeast.

A positive selection system to study intrachromosomal recombination in yeast similar to the construct of SHORTLE, HABER and BOTSTEIN (1982) has been constructed. Recombination occurred between two deletion alleles of *his3*. The purpose of the present

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study was to define the mechanism by which the gene disruption used can revert in a haploid. The finding of the present study which show that the gene disruption reverts at high frequency and does so probably by a conversional mechanism between sister chromatids might stress the importance of a usually silent, error free and very efficient recombinational DNA repair mechanism between sister chromatids in S or G_2.

MATERIALS AND METHODS

Strains: *Escherichia coli* strain HB101 (*hsdr*$^-$, *hsdm*$^-$, *recA1, supE44, lacz4, leuB6, proA2, thi1*) was used. Yeast strain S35/2-10C: *MAT***a**; *ura3-52*; *leu2-3,112*; *trp5-27*; *arg4-3*; *ade2-40*; *ilv1-92* was constructed by RS. Yeast strains RSY6, RSY11 and RSY11FRT$^-$ have plasmids pRS6, pRS11 and pRS11FRT$^-$ (Figure 2) respectively integrated into the *HIS3* gene of strain S35/2-10C thus disrupting the *HIS3* gene. Strain 294 (*MAT*α; *his3*; *leu2*; *trp1*; *ura3*) was obtained from JEFF STRATHERN.

Media, genetic analyses, plasmids and Southern blotting: Growth, minimal and sporulation media were prepared as have been described previously (SCHIESTL and WINTERSBERGER 1982). Isolation, sporulation, dissection of asci, and testing of spore colonies for markers and mating type were performed according to standard methods (*cf.* SHERMAN, FINK and HICKS 1986). Large scale plasmid isolation from *E. coli, E. coli* transformation and electrophoresis were performed according to MANIATIS, FRITSCH and SAMBROOK (1982). Isolation of DNA fragments from agarose gels was carried out according to DRETZTEN *et al.* (1981). Small scale plasmid preparations from *E. coli* were carried out as a modification of the boiling method (HOLMES and QUIGLEY 1981). A rapid procedure for the preparation of small amounts of high molecular weight yeast DNA was used (C. DENIS, unpublished; published in CIRIACY and WILLIAMSON 1981). pBR322 DNA was radioactive labeled (FEINBERG and VOGELSTEIN 1983) Southern transfer and hybridization procedures were used as described in WILLIAMSON, YOUNG and CIRIACY (1981). Transformation of yeast was carried out by treating intact cells with lithium acetate (ITO *et al.* 1983) or spheroplasts were prepared with glusulase (HINNEN, HICKS and FINK 1978) to promote DNA uptake. A modification of the HIRT (1967) procedure was used for purification of plasmid DNA from yeast.

GeneScreen membrane (NEN) was used as indicated by the supplier. Restriction enzymes were purchased from BRL.

Construction of a positive selection system for intrachromosomal recombination: *pRS6*: Plasmid pSZ515 obtained from JACK SZOSTAK (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983) was cut with *Bam*HI and the *HIS3* fragment (Figure 1) was isolated. This fragment was further cut with *Hpa*II and the large fragment containing most of the *HIS3* gene with a deletion of the promoter of the gene (STRUHL and DAVIS 1980) was isolated. Plasmid pBR322 was cut with *Cla*I and *Bam*HI and the large fragment isolated. This fragment was ligated with the *HIS3* gene containing the promoter deletion to produce pRS5 (Figure 2). Plasmid pRS5 was cut with *Kpn*I and *Sal*I to produce a second deletion at the 3′ end of the *HIS3* gene. Plasmid YEp13 (BROACH, STRATHERN and HICKS 1979) was cut with *Sal*I and partially with *Kpn*I—the partial containing the entire *LEU2* gene was isolated and ligated with the above isolated fragment of pRS5 to yield pRS6 (Figure 2).

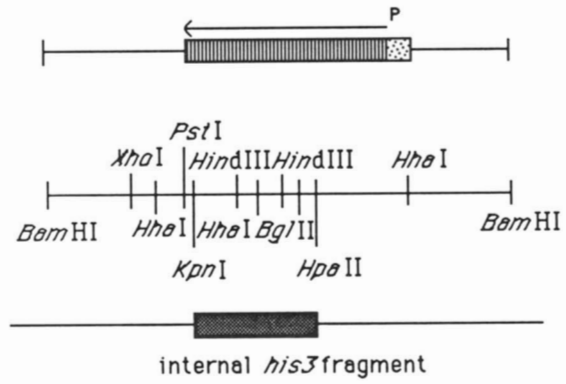

FIGURE 1.—Restriction map of the *HIS3* gene. The internal *HIS3 Kpn*I *Hpa*II fragment used for disruption of the gene is shown. The fragment was cut with *Hin*dIII to produce a gap and target the plasmid to integrate at the *HIS3* locus (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983).

pRS11: Plasmid YEp13 was cut with *Hin*dIII, the restriction sites filled with Klenow polymerase and the blunt ends ligated to yield pRS10 which lacks the *Hin*dIII site of YEp13. pRS10 was cut with *Kpn*I and *Sal*I and the fragment containing the 2-μm ARS (BROACH and HICKS 1980) was isolated. Plasmid pRS6 was cut with *Sal*I and partially with *Kpn*I and the large partial containing the entire *LEU2* gene was isolated and ligated with the pRS10 fragment containing the ARS.

pRS11FRT$^-$: To construct plasmid pRS11 lacking the FLP recombination target site (BROACH, GUARASCIO and JAYARAM 1982; ANDREWS *et al.* 1985) pRS11 was cut with *Xba*I, sticky ends filled with Klenow polymerase and religated (VOLKERT and BROACH 1986). The final product was identified by its lack of the *Xba*I site.

Plasmids pRS11 and pRS11FRT$^-$ transformed yeast at high efficiency and DNA isolated from those transformants was used to retransform *E. coli* demonstrating the free replicating nature of the plasmids. To produce the *HIS3* gene disruption plasmids pRS6, pRS11 and pRS11FRT$^-$ were cut with *Hin*dIII to produce a gap within the internal fragment of *HIS3* (Figure 1). Strain S35/2-10C was transformed with the gapped plasmid according to ORR-WEAVER, SZOSTAK and ROTHSTEIN (1983) to give raise to strains RSY6, RSY11 and RSY11FRT$^-$, respectively. LEU$^+$ colonies were isolated and checked for their histidine phenotype. HIS$^-$ colonies were picked and checked by SOUTHERN (1975) blotting for the copy number of the integrated plasmid. Colonies containing a single integrated plasmid were used for recombination experiments and colonies containing a double integration event were used as controls for Southern blots testing for the reciprocal products after sister chromatid exchange.

RESULTS

Plasmids were constructed as substrates to study intrachromosomal recombination. The plasmids pRS6 (Figure 2B) and pRS11 (Figure 2C) both contain an internal fragment of the *HIS3* gene as well as one selectable marker (*LEU2*). pRS11 in addition contains the 2-μm origin of replication to select for the presence of the plasmid after plasmid excision.

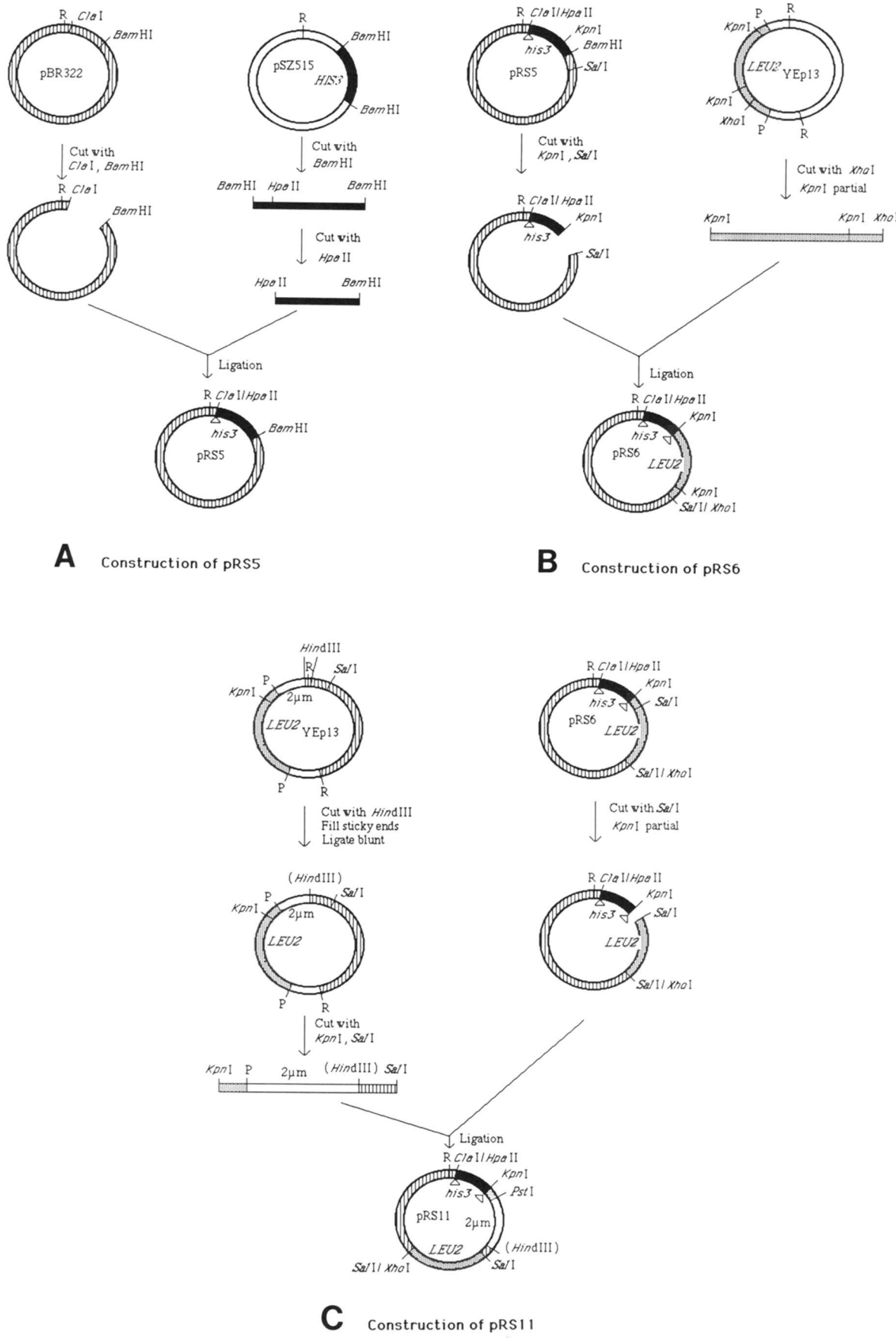

FIGURE 2.—Construction of the plasmids pRS6 and pRS11 used in this study. See MATERIALS AND METHODS section for details.

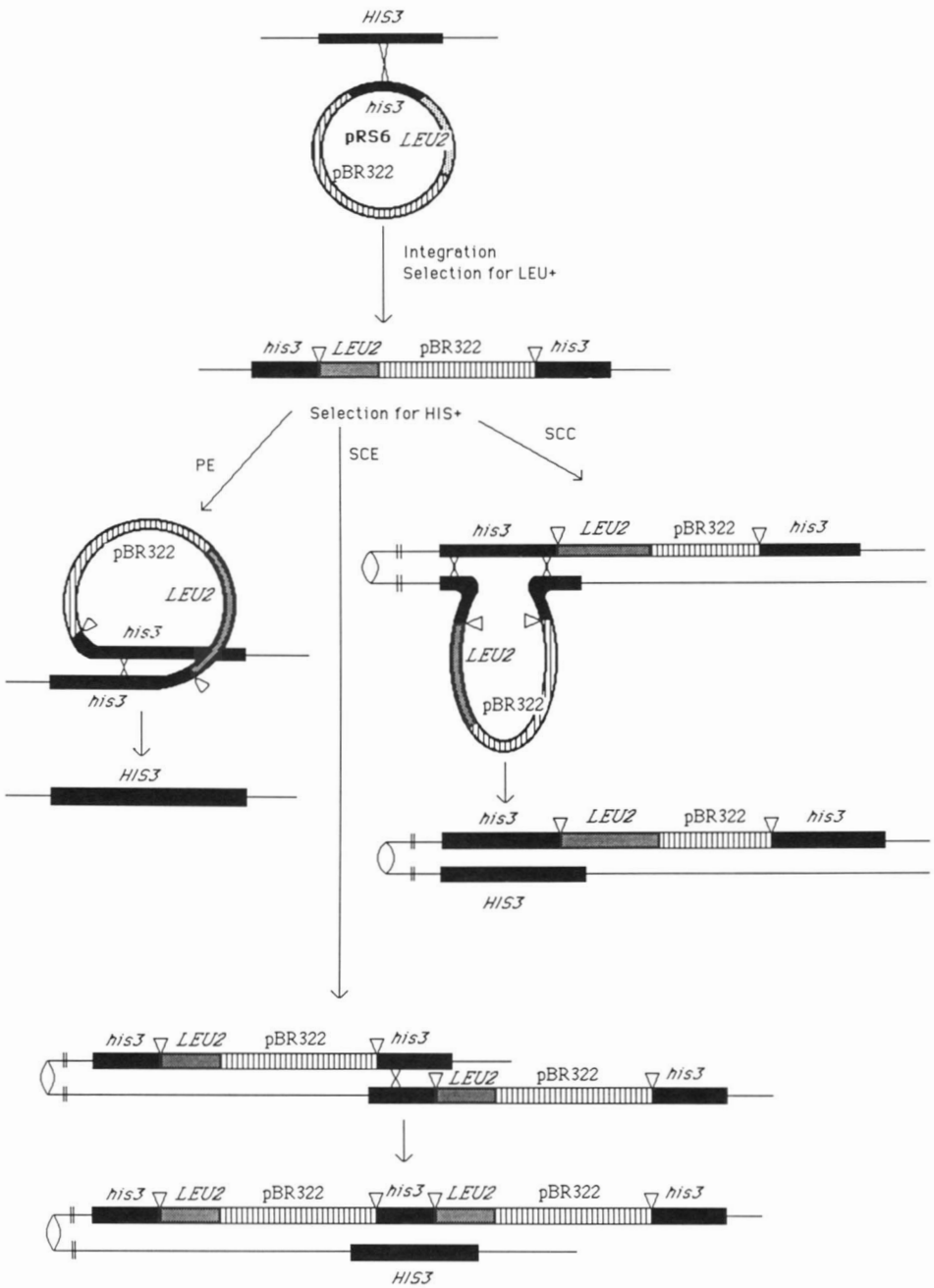

FIGURE 3.—Positive selection system for intrachromosomal recombination. The possible mechanisms plasmid excision (PE), sister chromatid exchange (SCE) and conversion (SCC) leading to reversion of the *his3* disruption are shown. The frequency of plasmid excision could be determined by putting an origin of replication onto the plasmid and plasmid could be reisolated from cells after plasmid excision events (Figure 4). SCE and SCC differ from each other in their reciprocal product, for SCE the reciprocal product is a duplication of the integration and for SCC it remains a single integration. Southern blotting was used to differentiate these two possibilities (Figure 5).

pRS11FRT$^-$ had the FLP recombination site disrupted. Yeast strains RSY6, RSY11 and RSY11FRT$^-$ were used. They contain the respective pRS plasmids integrated into the *HIS3* gene of strain S35/2-10C giving rise to disruptions of the *HIS3* gene (Figure 3). The two resulting *his3* deletion alleles (Figure 3) share about 400 basepairs of homology (SRUHL and DAVIS 1980) and thus can interact with each other by homologous recombination. Reversion of his$^-$ to HIS$^+$ occurred at a frequency of about 4×10^{-4} in all three strains. All of 100 tested HIS$^+$ revertants of strain RSY6 were leu$^-$. Because of this observation strains used for recombination studies were always pregrown on -LEU medium to prevent growth of HIS$^+$ revertants. Therefore the frequency of HIS$^+$ revertants is a measure of the recombination rate.

Reversion of the gene disruption produced by integration of pRS6 into the *HIS3* locus (Figure 3) can theoretically come about by three different mechanisms. The mechanisms include reciprocal crossing over between two *his3* deletion alleles in G_1 or on one chromatid in G_2 (PE) as well as unequal sister chromatid exchange (SCE) and conversion (SCC). The present study is aimed at defining which one of the above mechanisms is involved in reversion of the gene disruption at *HIS3*.

If plasmid excision (PE, Figure 3) were the main mechanism for reversion the frequency of PE should

be similar to the frequency of the overall reversion events. Because the integrated plasmid pRS11 contains an origin of replication as well as one selectable marker following excision the plasmid should become established in the cells in high copy number. Sister chromatid exchange (SCE) and conversion (SCC) differ from each other in their reciprocal product. After SCE the reciprocal product contains a duplication of the integrated plasmid whereas after SCC the reciprocal product remains a single integrant (Figure 3).

Excised plasmids are not recovered at high frequency: The 2-μm plasmid contains an origin of replication (BROACH and HICKS 1980) and is most likely located in the nucleus (review: BROACH 1981). Derivatives of the 2-μm plasmid were shown to interact with nuclear DNA (FALCO *et al.* 1982; FALCO, ROSE and BOTSTEIN 1983). Furthermore VOLKERT and BROACH (1986) have shown that the 2-μm plasmid can establish itself in high copy number in most of the cells after a single copy has been excised from the chromosome. They integrated one 2-μm circle into the 2-μm FLP site of a plasmid and the entire construction was integrated into a chromosome. Upon induction of the *FLP* gene under the *GAL1* promoter the 2-μm circle was excised from the chromosome and the free plasmid was amplified from one to more than 20 copies per cell (VOLKERT and BROACH 1986). Since this amplification was observed using the entire culture a large proportion of the cells should have amplified their 2-μm circle.

Since pRS11 contains the 2-μm ORI the plasmid should be able to establish itself in high copy number after excision especially because the uncut plasmid transforms yeast at high frequency and confers an unstable LEU^+ phenotype. Therefore one should be able to select for the presence of the plasmid after excision. If plasmid excision were the major event reverting the *his3* disruption one would expect similar frequencies of prototrophs after selection for HIS^+ (all recombination events) and HIS^+ LEU^+ (plasmid excision). Both strains RSY6 and RSY11 gave the same frequency of HIS^+ colonies of about 4×10^{-4} so that the frequency of the recombination events was not altered by the presence of the 2-μm ORI or the FLP site within the insert.

On -LEU-HIS selection medium again the same frequency of revertants were obtained for both strains RSY6 and RSY11 but this time the frequency was only 2×10^{-6}. To determine whether the *LEU2* marker in HIS^+ LEU^+ isolates is carried on a freely replicating plasmid the isolates were tested for the stability of their LEU^+ phenotype. Prototrophies carried on 2-μm based plasmids are lost from cells at about 1% per generation (BROACH, STRATHERN and HICKS 1979; review: STRUHL 1983). Ten HIS^+ LEU^+ isolates from strain RSY6 as well as RSY11 were examined for the stability of their LEU^+ marker after ten generations under nonselective conditions. Strain RSY6 only gave rise to colonies showing a stable LEU^+ phenotype. The frequencies of leu^- colonies given in Table 1 show that RSY11 yielded four colonies with an unstable LEU^+ phenotype. As further test for the presence of a free excised plasmid, DNA was isolated from all 20 HIS^+ LEU^+ isolates and *E. coli* was transformed and *E. coli* transformants were obtained only from the DNA of the four colonies showing an unstable LEU^+ phenotype. Figure 4 shows a restriction digest with representative plasmids from those *E. coli* transformants. Plasmids identical to pRS11 can be obtained as well as two larger plasmids as recombination products of the excised plasmid with both forms A and B of the yeast resident 2-μm plasmid (Figure 4). It is a salient feature of plasmids containing the FLP site to recombine with the 2-μm plasmid in yeast (BROACH and HICKS 1980; BROACH, GUARASCIO and JAYARAM 1982).

TABLE 1

Stability of the LEU^+ phenotype of HIS^+LEU^+ isolates

Isolate No.	No. of colonies tested	No. of leu^- colonies	Percent of leu^- colonies
1	243	26	10.7
2	107	0	<1.0
3	308	0	<0.3
4	338	35	10.4
5	270	0	<0.4
6	271	38	14.0
7	263	0	<0.4
8	150	0	<0.7
9	249	21	8.4
10	168	0	<0.6

HIS^+LEU^+ isolates of strain RSY11 were grown for ten generations on nonselective medium and plated onto nonselective medium. After 3 days of growth colonies were replica plated onto medium lacking histidine as well as medium lacking leucine. Numbers in the third column and percentages in the fourth column show colonies unable to grow on leucine omission medium and therefore reflect plasmid losses. Thus four colonies (1, 4, 6 and 9) show an unstable LEU^+ phenotype and plasmid DNA capable of transforming *E. coli* has been isolated from them (Figure 4).

To determine the nature of events that led to the occurrence of the 16 HIS^+ LEU^+ colonies with a stable LEU^+ phenotype from strain RSY6 as well as RSY11 meiotic segregation of the HIS^+ and LEU^+ phenotypes was examined (Table 2). Control his^- LEU^+ colonies always showed cosegregation of the two phenotypes as expected and the crosses showed high spore viability and mendelian segregation for all markers (Table 2). One third of the HIS^+ LEU^+ isolates from strain RSY11 showed high spore viability and nonmendelian segregation, either 4:0 or 0:4, for the LEU^+ phenotype which is consistent with carrying the LEU^+ phenotype on a free replicating plasmid (LIVINGSTON 1977; HICKS, HINNEN and FINK

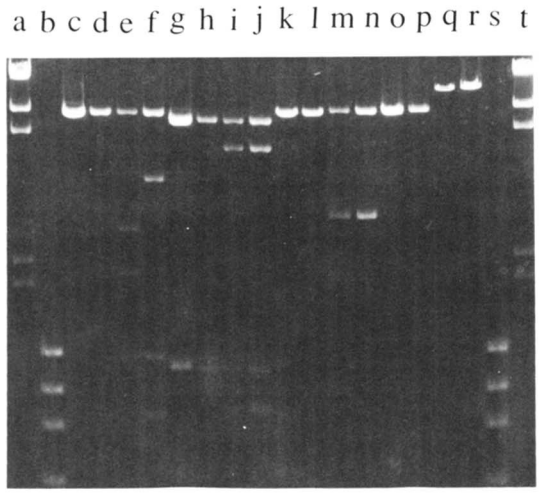

FIGURE 4.—EtBr-stained agarose gel showing plasmids from successful excision events cut with different restriction enzymes. Lanes a,t: *Hin*dIII digest of λ DNA; b,s: *Hae*III digest of θX174 DNA; c,g,k,o: pRS11 (Figure 2); d,h,l,p: isolate No. 1 from strain RSY11 after selection for plasmid excision; e,i,m,q: isolate No. 2; f,j,n,r: isolate No. 7 Lanes c,d,e,f: digested with *Hin*dIII; g,h,i,j: cut with *Pvu*I; k,l,m,n: cut with *Xba*I; o,p,q,r: cut with *Sal*I. Most isolates from plasmid excision events had the same size and restriction pattern as pRS11 (like isolates No. 1) proving that the same plasmid which has been integrated can be retrieved after PE. Isolates No. 2 and No. 7 were isolated from *E. coli* transformed with DNA from the same yeast colony showing that the restriction and size difference might have occurred after PE. It is shown that isolates No. 2 and No. 7 originate from recombination events of plasmid pRS11 with the two forms A and B of the 2-μm plasmid (BROACH 1981). A and B forms do have the two unique regions in inverted orientation with respect to each other. *Sal*I does not cut within 2 μm and shows the size difference of the recombinant plasmids to pRS11. *Xba*I cuts in both forms of 2μm once in the same position and gives equal fragments for both isolates, *Pvu*I cuts twice in one unique region of the 2-μm plasmid so it also gives equal sized fragments for both; *Hin*dIII cuts twice in the larger unique region and once in the smaller region which reveals the inversion of the two regions by the variation of two fragments, lanes e. and f.

1978, BROACH, STRATHERN and HICKS 1979). Four of these isolates have been shown to bear an unstable LEU^+ phenotype (Table 1) and free plasmid could be extracted from these yeast cells (Figure 4). As expected this class arises only in RSY11 which bears an origin of replication on the integrated plasmid. A second class giving high spore viability (four of 21 RSY11 isolates) shows cosegregation of HIS^+ and LEU^+. This class cannot be easily explained and has to await further analyses. The last class which represents all HIS^+ LEU^+ isolates from strain RSY6 as well as one half of the isolates from strain RSY11 (Table 2) shows very low spore viability and nonmendelian segregation for most markers including many nonmaters. This observation is consistent with triploid meiosis (CAMPBELL *et al.* 1981) caused by a cross of a diploid isolate with the tester strain. Duplication of the chromosome *XV* bearing the *his3* marker in a diploid or an aneuploid cell, followed by one of the events outlined in Figure 3 within or between sister chromatids or homologs might explain the origin of this class.

It has been reported that integration of the 2-μm plasmid into yeast chromosomes leads to loss of the affected chromosome as well as a high frequency of recombination in a diploid (FALCO *et al.* 1982). 2-μm circles contain the FLP gene which codes for a site-specific recombinase (BROACH and HICKS 1980; COX 1983; VETTER *et al.* 1983) which accepts as substrate the FLP recombination target (FRT) site (BROACH, GUARASCIO and JAYARAM 1982, ANDREWS *et al.* 1985). Since FLP promoted recombination might have an effect on the results obtained with strain RSY11 the FRT site of pRS11 was mutated before integration into the *HIS3* locus to construct strain $RSY11FRT^-$. This destroys the substrate for the FLP recombinase at the *HIS3* locus. Without integration plasmid $pRS11FRT^-$ proved to transform yeast with high frequency and conferred an unstable LEU^+ phenotype. This served as test for the replication ability of the plasmid and is in agreement with the work of MCLEOD, VOLKERT and BROACH (1984) that the 2-μm site specific recombination system is not essential for plasmid stability. With strain $RSY11FRT^-$ a frequency of HIS^+ leu^- colonies similar to strains RSY6 or RSY11 was found. HIS^+ LEU^+ colonies arose on the average at a frequency of 5×10^{-6}, which is about two times more frequent than with strain RSY6 or RSY11 but still 100 times less frequent than the occurrence of HIS^+ leu^- colonies.

The likeliness that the plasmid might not be able to replicate after excision is reduced by the fact that plasmids pRS11 as well as $pRS11FRT^-$ both transform yeast at high frequency and successful excision events were found bearing plasmids which do not differ in their stability to similar other 2-μm based plasmids (BROACH, STRATHERN and HICKS 1979; review: STRUHL 1983). It has further been shown that the 2-μm plasmid can replicate in the majority of the cells after excision from the genome (VOLKERT and BROACH 1986).

In summary plasmid excision could be demonstrated with strain RSY11 but it seems to occur 100 times less frequently than reversion of the duplication deletion at *HIS3*. These results are rather independent of an active FRT site on the integrated plasmid and indicate that a mechanism different than plasmid excision (see Figure 3) might be required to explain the majority of the reversion events.

Sister chromatid conversion seems to be mainly responsible for reversion of the deletion duplication: Since from the above experiments plasmid excision seems to be unlikely to be the major mechanism for reversion of the duplication deletion other possible mechanisms are SCE and/or SCC (Figure 3).

TABLE 2

Genetic analysis of HIS$^+$LEU$^+$ isolates

			No. of isolated colonies showing			
			>90% spore viability, cosegregation of		Nonmendelian segregation of	
Strain	Phenotype	Total	His$^-$LEU$^+$	HIS$^+$LEU$^+$	LEU$^+$	Most markers low spore viability
RSY6	HIS$^-$LEU$^+$	5	5	0	0	0
RSY6	HIS$^+$LEU$^+$	10	0	0	0	10
RSY11	HIS$^-$LEU$^+$	5	5	0	0	0
RSY11	HIS$^+$LEU$^+$	21	0	4	7	10

HIS$^+$LEU$^+$ colonies derived from strains RSY6 and RSY11 were crossed to strain 294 which is *his3*$^-$*leu2*$^-$ to test for segregation of the HIS$^+$ and LEU$^+$ phenotype. his$^-$LEU$^+$ colonies were crossed to HIS$^+$*leu2*$^-$ strains as controls. Nonmendelian segregation of LEU$^+$ can be explained by the presence of the *LEU2*$^+$ marker on a free replicating plasmid. Colonies showing nonmendelian segregation for most markers exhibit low spore viability and a large surplus of prototroph colonies for each auxotrophy. These features are characteristic for triploid meiosis (CAMPBELL *et al.* 1981). Therefore the isolated colonies were most likely diploid to start off with and recombination lead to HIS$^+$leu$^-$ on one homolog leaving his$^-$LEU$^+$ on the other homolog and might be explained by diploidy or aneuploidy of the isolated colonies.

TABLE 3

Isolation of HIS$^+$ sectored colonies

Exp. No.	UV irradiation	No. of colonies screened	Reversion rate $\times 10^4$	No. of sectors used
1	0	3000	2.5	3
2	0	1461	4.7	2
3	0	891	0.7	0
4	0	2284	3.8	4
5	40 J/m^2	2900	8.4	8
6	40 J/m^2	1109	18.1	8

Stationary cells without irradiation were used for experiments 1–4. Log phase cells were irradiated with 40 J/m^2 UV light for experiments 5–6. Cells were plated onto nonselective medium and after incubation for 3 days replica plated onto -HIS medium. 1/2, 1/4 and 1/8 sectored colonies were used for Southern blotting (Figure 5).

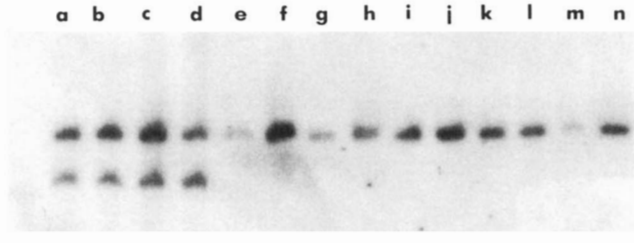

FIGURE 5.—Southern blot of DNA from strain RSY6 and different isolates digested with *Pst*I which cuts once in pRS6 and probed with P32 labeled pBR322. Lanes e: control with DNA from RSY6; d: control from a double integration event which shows a second fragment of about the size of the pRS6; c: ½; b: ¼; a: ⅛ of DNA from the double integrant added to DNA from RSY6 showing that the method used should easily detect a duplication of the integrated plasmid which would be characteristic for sister chromatid exchange (Figure 3). Lanes f–n: samples of isolates, entire colonies from nonselective medium which showed a HIS$^+$ sector of at least ⅛ of the colony size were used (for isolation of the colonies see Table 3) no fragments characteristic for SCE are visible.

These two possibilities can be distinguished by determining whether the reciprocal product of the recombination event contains a single copy of the integrated plasmid (as for SCC) or a duplication of the plasmid (as for SCE). Since the cells containing the reciprocal products are still his$^-$ LEU$^+$ (Figure 3), they cannot be selected for. Hence sectors adjacent to HIS$^+$ recombinant sectors were examined by Southern blotting.

Colonies sectored for their HIS$^+$ phenotype were isolated in six nonselective experiments using strain RSY6 (Table 3). The first four experiments were done with cells in stationary phase without irradiation and the last two experiments were done with log phase cells irradiated with 40 J/m^2 UV light giving about 40% survivors. The UV treatment caused about a threefold enhancement of the reversion rate (Table 3). Nine colonies from experiments 1–4 and 16 colonies from experiments 5 and 6 were used for Southern blotting (Figure 5). Entire colonies containing ½, ¼ or ⅛ HIS$^+$ sectors were used. HIS$^+$ sectors alone did not give any signal when probed with pBR322.

From the transformation of RS35/2-10C with pRS6 single as well as double integrants were obtained. In double integrants an additional fragment of about the size of the plasmid pRS6 hybridizes to pBR322 (lane d, Figure 5). These colonies served as control for the detection of the duplication of the integrated plasmid after SCE. After mixing DNA from a single integrant with ½, ¼ or ⅛ of DNA from a double integrant the band characteristic for the double integrant could still be easily identified (lanes c, b, and a, Figure 5). With the reasonable expectation that the cells containing the reciprocal product should encompass a sector of equal size to the ½, ¼ or ⅛ HIS$^+$ sector the method used should be valid for detection of a sector containing a duplication of the integrated plasmid. None of the 25 colonies examined

gave any indication of a duplication (Figure 5), suggesting that SCC might be the main mechanism responsible for reversion of the *his3* disruption.

DISCUSSION

A system to study intrachromosomal recombination has been constructed. It consists of two different deletion alleles of *HIS3* constructed by integration of a plasmid bearing an internal fragment of *HIS3* into the wildtype locus. The study was designed to uncover the mechanisms operating in reversion of the constructed gene disruption. Plasmid excision (PE), sister chromatid exchange (SCE) or conversion (SCC) have been evaluated as likely mechanism for reversion of the gene disruption (Figure 3). To determine the frequency of plasmid excision an origin of replication was put onto the integrated plasmid. Selection for the recombination event as well as for the maintenance of the plasmid was applied and in a portion of these isolates successful plasmid excision could be demonstrated by reisolation of the plasmid from the cells. Plasmid excision occurred 100-fold less frequently than the overall frequency of recombination and therefore is probably not likely to be the major mechanism for reversion. SCE and SCC differ from each other in their reciprocal product. Southern blotting was used to determine the structure of the reciprocal product and no fragments characteristic for SCE were found. Thus sister chromatid conversion is suggested to be the main mechanism responsible for reversion.

Theoretically there is yet another mechanism to explain the results, nonreciprocal crossing over in G_1 or G_2. There is some evidence that nonreciprocal recombination occurs in a *rad52*$^-$ background (HABER and HEARN 1985). The experiments of the present study were done with wild-type strains for which nonreciprocal recombination has not been used to explain recombination events. Furthermore nonreciprocal recombination has been suggested to be RAD52 independent (HABER and HEARN 1985) whereas the recombination events analyzed in the present study are reduced about fivefold in frequency in a *rad52*$^-$ background (our own unpublished results).

Intrachromosomal conversion, PE and, or SCE have been used to explain reversion of mutant alleles constructed by plasmid integration (SCHERER and DAVIS 1980; JACKSON and FINK 1981; GOLIN, FALCO and MARGOLSKEE 1986; KUNZ, TAYLOR and HAYNES 1986). Gene trans- or replacement of wild-type sequences with *in vitro* constructed mutant alleles has been suggested to be due to excision of the plasmid now bearing the wild-type sequence leaving behind in chromosomal position the constructed mutant sequence (SCHERER and DAVIS 1979; WINSTON, CHUMLEY and FINK 1983). In these studies it was supposed that the excised plasmid does not have any origin of replication and is therefore lost after several divisions. The results of the present study at the *HIS3* locus suggest that the integrated plasmid even with a functioning origin of replication cannot be retrieved at a frequency high enough to explain the majority of the reversion events. Furthermore the results indicate that not SCE but SCC might be the main mechanism for reversion of disrupted sequences. At this time it is not known whether the results are specific to the deletion alleles which were used or whether SCC is a more common mechanism. Since similar systems to the one discussed in the present work are currently widely used to test the influence of different genes on recombination and to screen for recombination deficient mutants it seems to be important to define the mechanism involved in these recombination events.

FASULLO and DAVIS (1987) recently constructed a similar system also testing for sister chromatid recombination using a complete deletion of the *HIS3* gene at the *HIS3* locus and a split *his3* gene on a plasmid integrated into various other positions. This creates a situation reversed to the one of the present study in that the deletions are present on the outside of the construction (compare to Figure 3). Therefore the HIS$^+$ product has to represent a duplication of the integrated structure instead of a deletion. Because their recombination events are X-ray and MMS but to a lesser extent UV inducible and because the frequency is decreased in a *rad52*$^-$ background, also a conversional mechanism between sister chromatids is proposed. The frequency of reversion measured is about two orders of magnitude lower than the frequency of reversion obtained in the present study. Unless this result is due to a position effect at different loci it might display a marked disparity in favour of a deletion versus the insertion which might indicate the presence of a mechanism specifically repairing recent duplications due to recombination or replication errors. Also in recombination involving δ sequences crossing over and conversion between sister chromatids has been recently suggested as one of the mechanisms (ROTHSTEIN, HELMS and ROSENBERG 1987).

Most studies on sister chromatid recombination in yeast have been done with the rDNA locus (PETES 1980; SZOSTAK and WU 1980; PRAKASH and TAILLON-MILLER 1980; ZAMB and PETES 1980). Recombinants were screened as losses of a plasmid integrated into the rDNA. The reciprocal products have been isolated and for spontaneously occurring recombinants the majority contained the duplicated plasmid. As mechanism to explain those recombinants SCE has been suggested (SZOSTAK and WU 1980). Furthermore recombination at the rDNA locus is *rad52* independent (PRAKASH and TAILLON-MILLER 1980;

ZAMB and PETES 1980) which does not seem to be true for SCR at other loci (FASULLO and DAVIS 1987; our own unpublished observation). A recombination hotspot *HOT1* has been found to exist at every repeat of rDNA (KEIL and ROEDER 1984). *HOT1* induced recombination might be different in mechanism from SCR at other loci. Alternatively, it might be possible that SCR at the rDNA locus is due to conversion initiated between the unequally paired plasmid insertions or that conversion is associated with crossing over.

Theoretically there are two mechanisms possible to explain conversion between sister chromatids: gene conversion involving double strand break repair (RESNICK 1976; SZOSTAK *et al.* 1983), or heteroduplex formation and repair in mitosis (GOLIN and ESPOSITO 1984; GOLIN, FALCO and MARGOLSKEE 1986). The latter interpretation would involve several kilobases long regions of unpaired single stranded DNA the occurrence of which is not proven. Furthermore GOLIN, FALCO and MARGOLSKEE (1986) find their own data better explained by a different mechanism not involving long heteroduplex DNA. Even in meiosis where there is compelling evidence for the occurrence of heteroduplex DNA (reviews: FOGEL, MORTIMER and LUSNAK 1981; HASTINGS 1987) postmeiotic segregation which would be evidence for the occurrence of heteroduplex DNA has not been observed for conversion of deletions (FOGEL *et al.* 1979). This would be required for the interpretation of the results of the present study as product of the repair of heteroduplex DNA. On the other hand there is ample evidence that double strand breaks can induce conversion (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981, 1983: ORR-WEAVER and SZOSTAK 1983; KOLODKIN, KLAR and STAHL 1986). Therefore a model involving SCC initiated by a double strand break (Figure 6) similar to models proposed by others (RESNICK 1976; SZOSTAK *et al.* 1983) is presented.

Theoretically in order for sister chromatid recombination to occur the entire chromosome does not have to be replicated so that it may occur in S or G_2 phase. Haploid yeast cells are most resistant to X-rays which produces single and double strand breaks in DNA in their S or G_2 phase (HATZFELD and WILLIAMSON 1974; BRUNBORG, RESNICK and WILLIAMSON 1980). FABRE, BOULET and ROMAN (1984) find that diploids blocked and irradiated in S or G_2 show a 10–20-fold lower inducibility for interchromosomal recombination than cells in G_1. They postulate that damage produced in G_2 is predominantly repaired by sister chromatid recombination thereby reducing the frequency of interchromosomal recombination. SCC if compared with PE and SCE is the primary mechanism for efficient DNA repair. In addition it has been shown that conversion is involved in repair of double strand breaks or gaps (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; ORR-WEAVER and SZOSTAK 1983). The high frequency at which SCC occurs in the system (4×10^{-4}) presented here might reflect a mechanism usually occurring during or after replication and might be an efficient DNA repair mechanism in S or G_2 (recombinational part of postreplication repair of UV damaged DNA) (PRAKASH 1981). The initiating double strand breaks might be spontaneously produced during replication or alternatively might be produced by a mechanism which scans newly replicated DNA and recognizes nonhomologous loop structures in sister chromatids (as in Figure 6).

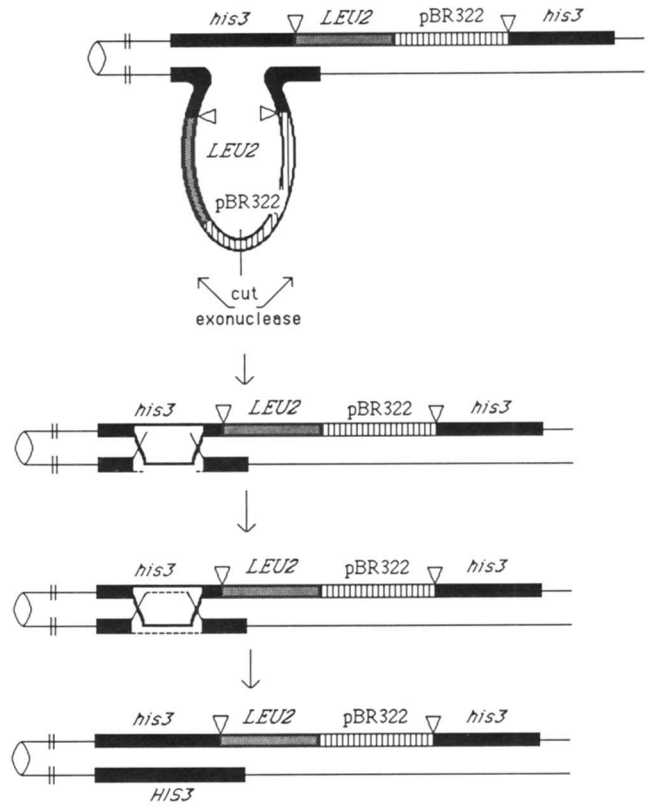

FIGURE 6.—Model for sister chromatid conversion by a double strand break repair mechanism similar to SZOSTAK *et al.* (1983). The initiating event is a double strand break in the *LEU2* or pBR322 region of the integrated plasmid. In the configuration shown the DNA has to be degraded up to the region of homology with the *his3* deletion allele on the sister chromatid. Invasion of the other duplex, D loop formation, DNA synthesis, ligation and resolution of the Holliday structure are shown according to the double-strand-break repair mechanism for recombination (SZOSTAK *et al.* 1983). Resolution can yield a crossover or noncrossover configuration which cannot be differentiated unless parts of the system would be placed within a multigene family.

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