The Behavior of Insertions Near a Site of Mitotic Gene Conversion in Yeast

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ABSTRACT

In yeast, coincident gene conversion events involving the *LEU1* and *TRP5* loci (16 cM apart) occur at frequencies that are far greater than is expected for two independent acts of recombination. When a large plasmid (pJM53) is placed between these genes so that a direct repeat is produced, there is frequent loss of the insert among coincident convertants. Previous results strongly suggest that this is due to a separate, intrachromosomal exchange between the direct repeats rather than to excision from an extensive region of heteroduplex DNA. In this paper, we extend our genetic and molecular analysis to a plasmid insertion (pKSH) which replaces rather than duplicates the chromosomal material. The relative stabilities of pKSH and pJM53 are compared among coincident *Leu*⁺*Trp*⁺ convertants and convertants involving only one locus (*LEU1*). The pKSH insertion is significantly more stable in the latter which constitute a large majority of the selectable recombinants. In the former, both insertions are lost with high frequency. These results are used to argue that, while most mitotic conversion does not result from long intermediates, coincident convertants may arise from either multiple intermediates or extensive heteroduplex regions.

IN a companion study (GOLIN and TAMPE 1988), we showed that coincident recombination events have a strong distance dependency. When heteroallelic mutations are present at relatively close sites, such as *LEU1* and *TRP5* (16 cM), double *Leu*⁺*Trp*⁺ convertants arise at frequencies that are far greater than expected for two independent acts of conversion. As the genetic distance between the loci increases or when two genes are unlinked, there is a decline in enhancement by about tenfold.

In this paper, we continue to investigate the mechanism of distance-dependent gene conversion. Initially, we proposed that it was most likely due to extensive recombination intermediates (GOLIN and ESPOSITO 1984) such as long heteroduplexes. Recently, however, we have found evidence suggesting that this might not be the explanation for most of the observed conversion (GOLIN, FALCO and MARGOLSKIE 1986). In these experiments, a large 5.5-kbp plasmid insertion flanked by a 6.1-kbp direct repeat was inserted between the *LEU1* and *TRP5* loci. When either coincident *Leu*⁺*Trp*⁺ or single site (*Leu*⁺) convertants were selected, the intervening insertion was frequently lost. Genetic experiments indicated that this was due to a separate, intrachromosomal recombination event rather than to preferential excision of the plasmid from a single, extensive region of heteroduplex DNA. For example, among *Leu*⁺*Trp*⁺ convertants, loss of plasmid was as frequent in insertion *homozygotes* (where no heteroduplex

existed) as in an insertion hemizygote. Therefore, the simplest model to account for the distance dependent *Leu*⁺*Trp*⁺ coincident convertants was to posit a series of multiple recombination events involving intrachromosomal exchange between the direct repeats.

The experiments described in this paper were designed to further distinguish between these models as explanations for mitotic gene conversion when convertants are selected at single (*LEU1*) or at multiple (*LEU1* or *TRP5*) loci. To do this, recombination was analyzed in a diploid strain hemizygous for a plasmid transplacement and thus lacking a direct repeat. This is located 8.8 kbp distal to *LEU1* and 27kbp proximal to *TRP5*. The long heteroduplex model predicts that this insertion will also be lost at high frequency during gene conversion. The discontinuous model predicts increased stability since the possibility of intrachromosomal exchange is gone. Plasmid stability was compared among coincident (*Leu*⁺*Trp*⁺) convertants which are relatively rare and among single locus *Leu*⁺ (*LEU1*) convertants which constitute a large (99%) majority of the selectable recombinants. Among the latter class, the transplacement is significantly (fivefold) more stable than the insertion that is flanked by a direct repeat. When coincident (*LEU1*, *TRP5*) convertants are analyzed, however, there is less disparity between the behavior of the two plasmid insertions. Our results therefore suggest that most mitotic intermediates are not ex-

TABLE 1
Yeast strains

Designation	Genotype	Comments
JG 33-18B	<i>HO a/α leu1-c trp5-c cyh2 met13c ade5 ade2 lys2-2 tyr1-2 his7-2 ura3-1</i>	GOLIN and ESPOSITO (1977)
JG 34-38A	<i>HO a/α leu1-12 trp5-d CYH2 met13d ADE5 ade2 lys2-1 tyr1-1 his1 ura3-313</i>	GOLIN and ESPOSITO (1977)
JG44	JG33-18B × JG 34-38A	GOLIN and ESPOSITO (1977)
JG 231	JG34-38A with pJM53 (<i>URA3</i>) integrated between <i>leu1-12</i> and <i>trp5-d</i>	GOLIN, FALCO and MARGOLSKEE (1986)
JG 300	JG 231 × JG 33-18B	
JG 267	JG 34-38A with pKSH (<i>URA3</i>) integrated between <i>leu1-12</i> and <i>trp5-d</i>	
JG 292	JG33-18B with pKSH integrated between <i>leu1-c</i> and <i>trp5-c</i>	
JG 303	JG 267 × JG 33-18B	
JG 403	JG 292 × JG 34-38A	
JG 305	JG 267 × JG 292	Homozygous for pKSH

FIGURE 1.—Fine structure of the *LEU1-TRP5* region. The diploids employed in this study carry a series of markers on chromosome VII used to monitor recombination. The site of pJM53 (and pKSH) integration is indicated by an arrow. In addition, there are three pairs of noncomplementing alleles *LEU1*, *TRP5* and *MET13*. The *ADE5* and *CYH2* loci are heterozygous for recessive mutations. Numerals indicate the distance in centi-Morgans. After GOLIN, FALCO and MARGOLSKEE (1986).

tensive, though multiple intermediates exist. In contrast, *Leu*⁺*Trp*⁺ convertants may be produced by both mechanisms.

MATERIALS AND METHODS

Plasmids: pJM53 was described previously (GOLIN, FALCO and MARGOLSKEE 1986). The construction and characterization of the related plasmid, pKSH, is described later.

Yeast strains: The yeast strains in this study are described in Table 1. A fine-structure map of the critical region is shown in Figure 1. Of note are the isogenic diploids JG300 and JG303. These strains are hemizygous for a plasmid insertion and are used in recombination analysis described below. In addition, both diploids are *heteroallelic* for the flanking markers *TRP5* and *LEU1*. These loci are used to monitor coincident gene conversion.

Media and culturing conditions: The media used to grow yeast and bacteria, as well as the culturing conditions employed to obtain recombinants, were described recently (GOLIN and ESPOSITO 1984; GOLIN, FALCO and MARGOLSKEE 1986).

Assay for gene conversion: The assay for gene conversion at *LEU1* and *TRP5* is as follows. All diploids used in this study contain pairs of noncomplementing (*heteroallelic*) mutations at the *LEU1* and *TRP5* loci. Gene conversion is assayed by plating a known concentration of cells on the appropriate omission media and counting the prototrophic colonies that arise. These prototrophs are analyzed further to determine whether they have retained a plasmid insertion by replica plating to media lacking uracil. The diploids

also contain heteroallelic or heterozygous sites distal to *TRP5*. These are used to establish whether gene conversion is associated with distal marker homozygosity. A more detailed description of this assay is published elsewhere (ESPOSITO 1978; GOLIN and ESPOSITO 1977, 1981, 1984).

Transformation: Yeast cells were transformed with plasmid DNA according to the procedure of HINNEN, HICKS and FINK (1978). Bacterial transformation was performed as described by MANIATIS, FRITSCH and SAMBROOK (1982).

DNA preparation: Yeast chromosomal DNA was prepared by the method of FALCO *et al.* (1982). Plasmid DNA was made as described by DAVIS, BOTSTEIN and ROTH (1980).

DNA hybridization: DNA hybridization was carried out according to the method of Southern (1975). The conditions for blotting, nick translation and hybridization are described elsewhere (GOLIN, FALCO and MARGOLSKEE 1986).

Enzyme reactions: Restriction enzymes and T4 DNA ligase were purchased from International Biotechnologies, Inc. (Boston). The assay conditions used were those described by MANIATIS, FRITSCH and SAMBROOK (1982). To analyze the products of the restriction digests, the reaction mixture was run on a 0.8% agarose gel (15 hr, 50 mA) and stained with ethidium bromide (10 mg/ml) prior to visualization with an ultraviolet source.

RESULTS

Construction of a plasmid insertion lacking direct

DNA repeats: The purpose of the experiments described below was to compare the stability of the two different plasmid insertions; one with and one without a 6-kbp duplication of chromosomal DNA, in otherwise isogenic diploids that had undergone gene conversion at the flanking sites *LEU1* and *TRP5*. Diploids bearing the pJM53 insertion contain a direct repeat; those with the pKSH insertion do not. The two plasmid insertions are related, however. Both are derivatives of the Ylp5 vector (BOTSTEIN *et al.* 1979) and contain a 6.1-kbp insert of yeast DNA from the region between *LEU1* and *TRP5*. The pKSH plasmid was constructed from pJM53 as shown in Figure 2. In pKSH, Ylp5 vector DNA was placed in the unique *Xho*I site present within the 6.1-kb repeat to create an interruption in the latter (ROTHSTEIN

FIGURE 2.—Construction of pKSH. The plasmid pKSH was constructed from Ylp5* and pJM53 as outlined. Ylp5* is identical to Ylp5, except that the *Hind*III site was removed by digesting the plasmid with *Hind*III, resynthesizing with the Klenow fragment of DNA polymerase I and carrying out blunt and ligation with T4 DNA ligase to re-seal. These procedures are described in MANIATIS, FRISCH and SAMBROOK (1982). The Ylp5* and pJM53 plasmids were cut with *Sal*I and *Xho*I, respectively, and ligated together (step A). As a result, these restriction sites are destroyed. The resulting plasmid was treated with *Hind*III followed by ligation (step B) to create pKSH. pKSH was integrated by integrative transformation (step C). To do this so that a transplacement rather than a duplication was created, the plasmid was first linearized with *Hind*III.

1983). When this plasmid was digested with *Hind*III and the linear fragment used to transform yeast cells, the resulting *Ura*⁺ transformants had a replacement of the chromosomal region with one containing the YIp5 generated interruption. In contrast, transformation of cells with circular pJM53 resulted in the integration of the plasmid so that the YIp5 vector was flanked by 6.1-kbp direct repeats (GOLIN, FALCO and MARGOLSKIE 1986).

The structure of the integrated pKSH plasmid was determined by DNA hybridization (Figure 3). DNAs extracted from the isogenic strains JG267 and JG34-38A were cut with *Hind*III and hybridized with nick-translated pKSH probe (Figure 3). The original transformant JG267, which is hemizygous for pKSH, shows two fragments that hybridize. The larger fragment is not found in the isogenic, nonplasmid-bearing control (JG34-38A).

The JG303 strain derived as described in Table 1 stably maintains the pKSH insertion. When the diploid was streaked out on nonselective (YEPD) media

FIGURE 3.—Verification of the integrated structure. Transplacement of the uninterrupted chromosomal region with pKSH in the homothallic (diploid) strain JG34-38A is expected to create an additional large fragment representing the transplaced region when *Hind*III-treated chromosomal DNA from transformants is prepared and hybridized to radiolabeled pKSH plasmid DNA as described in MATERIALS AND METHODS (lane 1, JG34-38A; lane 2, pKSH hemizygote).

and tested for its *URA3* phenotype, the frequency of *Ura*[−] colonies was less than 10^{−3} (0 in 1036).

Analysis of coincident gene conversion events in diploids hemizygous for pKSH or pJM53: The diploids JG300 and JG303 are isogenic, except that the former contains pJM53 inserted between *LEU1* and *TRP5*, while the latter has pKSH inserted in the same region. To determine whether the two strains differ in the rate of recombination at the critical sites, the rates of *Leu*⁺, *Trp*⁺ and *Leu*⁺*Trp*⁺ convertants were determined as described previously (GOLIN and ESPOSITO 1984; GOLIN, FALCO and MARGOLSKIE 1986). The results, presented in Table 2, indicate little, if any, difference between the two diploids analyzed. The rates obtained are also similar to those observed in the isogenic, nonplasmid (JG44)-bearing control (GOLIN and ESPOSITO 1984; GOLIN, FALCO and MARGOLSKIE 1986).

When independently derived *Leu*⁺ *Trp*⁺ recombinants were obtained from JG300 and analyzed, most (125 of 172) had lost the pJM53 insert and were *Ura*[−] (GOLIN, FALCO and MARGOLSKIE 1986). A comparative study of JG300 (pJM53) and JG303 (pKSH) was undertaken to determine whether the absence of the 6.1-kb direct repeat had any effect on recombination in the otherwise isogenic strains. The results of this experiment are found in Table 3. *Leu*⁺*Trp*⁺ coincident convertants derived from the JG300 diploid continued to show high frequency loss of the pJM53 insertion (71%). The JG303-derived convertants, in contrast, showed a significantly lower proportion of *Ura*[−] recombinants (39%). A similar result was found when pKSH was placed between the *leu1-c* and *trp5-c* alleles on the opposite homolog.

TABLE 2
Recombination rates

Diploid	No. cultures sampled	Rates of gene conversion			Comments
		TRP5/10 ⁷	LEU1/10 ⁷	LEU1, TRP5/10 ⁸	
JG 44	10	50	50	2.0	GOLIN, FALCO and MARGOLSKEE (1986)
	15	26	34	1.1	GOLIN and ESPOSITO (1984)
JG 300	9	24	40	1.3	This study
	10	20	40	1.0	GOLIN, FALCO and MARGOLSKEE (1986)
JG 303	9	26	35	1.1	This study

TABLE 3
Fraction of Ura⁻ recombinants among independently derived convertants

Diploid	No. convertants analyzed	No. Ura ⁻	Percent Ura ⁻ recombinants ^a	Comments
<i>A. Leu⁺ Trp⁺ Convertants</i>				
JG 300 (pJM53) (a)	172	125	73 (67-79)	GOLIN, FALCO and MARGOLSKEE (1986)
(pJM53) (b)	105	74	71 (61-80)	This study
JG 303 (pKSH)	274	107	39 (32-46)	pKSH in <i>leu1-12</i> homolog
JG 403 (pKSH)	125	41	33 (24-43)	pKSH in <i>leu1-c</i> homolog
<i>B. Leu⁺ convertants (single locus conversion)</i>				
JG 300	220	69	32 (25-38)	
JG 303	237	13	5.5 (3-10)	

^a The 95% confidence limits are given in parentheses.

Of 125 Leu⁺Trp⁺ recombinants analyzed, 41 were Ura⁻ (33%).

Seventy-four independently derived Leu⁺Trp⁺ convertants from JG303 which remained Ura⁺ were tested further to determine whether they were hemizygous (Ura⁺/Ura⁻) or homozygous (Ura⁺/Ura⁺) for pKSH. To do this, the convertants were sporulated and a random spore analysis was performed as previously described (GOLIN and ESPOSITO 1981). Twelve of the recombinants contained two copies of pKSH (about 10% of the total), while 62 retained the initial (one copy) hemizygous configuration.

An important feature of coincident conversion in both JG300 and JG303 is the high association of homozygosity for distal loci. Twenty-two percent (50 of 227) of the JG303 coincident convertants were either *cyh2/cyh2*, *ade5/ade5* or *CYH2/CYH2*, *ADE5/ADE5*. Initially, we thought that the high frequency of associated crossing over resulting in distal marker homozygosity could account for most of the plasmid loss in the pKSH hemizygotes. Two observations argue against this. First, crossing over should produce equal numbers of Ura⁻/Ura⁻ and Ura⁺/Ura⁺ recombinants. Instead, the latter were in a distinct minority. Second, we observed equal frequencies of Ura⁻/Ura⁻ *CYH2/CYH2 ADE5/ADE5* and Ura⁻/Ura⁻ *cyh2/cyh2 ade5/ade5* recombinants. If these were the result of crossing over, the latter could be produced by a single exchange. The former, however,

would require a presumably rarer four-strand double crossover. A more likely explanation is that pKSH is often lost by gene conversion.

Stability of pKSH/pKSH homozygotes: A striking feature of pJM53/pJM53 homozygous diploids is that when coincident convertants were selected and analyzed, many (62%) lost one or both copies of the insertion (GOLIN, FALCO and MARGOLSKEE 1986). This strongly suggested that pJM53 was removed by intrachromosomal recombination between the 6.1-kbp direct repeats. Since the insertion of pKSH results in a transplacement lacking a duplication of chromosomal DNA, we expected no loss to occur when it was present in homozygous configuration. To determine stability, we constructed the JG305 (pKSH/pKSH) diploid (Table 1). Thirty-five independently selected coincident convertants were sporulated. Ten random Lys⁺ spores were recovered from each [see GOLIN and ESPOSITO (1981) for a discussion of this method] and tested by replica plating to uracil omission media. Thirty-four of the Leu⁺Trp⁺ recombinants were *URA3/URA3* in phenotype. One recombinant was *URA3/ura3*. The *URA3/ura3* genotype might be a rare instance of conversion between the alleles on chromosome V and the *URA3* gene in pKSH. In any case, the pKSH insertion is not readily lost among coincident convertants derived from a homozygous strain.

Analysis of conversion at a single (LEU1) locus:

Leu⁺Trp⁺ convertants make up a very small proportion of the total selectable recombination events at either locus. Gene conversion resulting in a Leu⁺ cell that remains heteroallelic at *TRP5* is *ca.* 200 times more frequent than the coincident counterpart. To better understand the mechanism of mitotic gene conversion *vis-à-vis* intermediate length, we analyzed the behavior of both insertions among those single locus (*LEU1*) convertants that remained heteroallelic and heterozygous for the distal markers.

Independently derived Leu⁺ recombinants were selected and analyzed for their *URA3* phenotype in a strain containing pKSH (JG303) and in one containing pJM53 (JG300). The results, also found in Table 3, can be summarized as follows. First, among Leu⁺ convertants, loss of pKSH or pJM53 insertions is less frequent than among Leu⁺Trp⁺ recombinants. Second, the fraction of Leu⁺Ura⁻ recombinants was significantly reduced in the pKSH strain (0.06) when compared to the pJM53 bearing counterpart (0.32). Among 30 Leu⁺ convertants that remained Ura⁺, 29 contained one copy of pKSH and were therefore heterozygous. Only one was homozygous (*pKSH/pKSH*).

DISCUSSION

Summary of results: In a companion study (GOLIN and TAMPE 1988) we showed that coincident recombination has both distance-dependent and distance-independent components. A strong distance-dependent mechanism operates to produce Leu⁺Trp⁺ coincident gene convertants. The experiments in this paper were designed to further test the long heteroduplex and the multiple event models as explanations for such distance-dependent conversion. To do this, recombination was also analyzed in a diploid strain hemizygous for a plasmid transplacement and therefore lacking a direct repeat. The long heteroduplex model predicts that this insertion will be lost at high frequency during gene conversion. The discontinuous model predicts increased stability since the possibility for intrachromosomal exchange is gone. The behavior of the two different insertions (duplication and transplacement) was compared among various classes of conversion (Table 3). The results may be summarized as follows: (1) As was previously reported (GOLIN and ESPOSITO 1984; GOLIN and TAMPE 1988), Leu⁺Trp⁺ double convertants occur more frequently than expected for two independent acts; (2) a large plasmid insertion (pJM53) flanked by a direct repeat and located between *LEU1* and *TRP5* is lost with high frequency when coincident convertants are selected (GOLIN, FALCO and MARGOLSKIE 1986). When the insertion (pKSH) is a replacement (and no direct repeats are generated), the loss is reduced by approximately 50%;

and (3) the two insertions differ greatly in their stability when single (*LEU1*) locus Leu⁺ convertants are analyzed. The loss of pJM53 is 5 fold greater than that of pKSH.

Mechanism of gene conversion: Among Leu⁺ (single locus) conversions, the transplacement (pKSH) is considerably more stable than the insertion that is flanked by a direct repeat (pJM53). The simplest interpretation of the data is to conclude that loss of pJM53 occurs via an intrachromosomal exchange, while infrequent loss of pKSH occurs via gene conversion. Therefore, the possibility of long heteroduplexes or double-strand gaps frequently stretching from *LEU1* to pKSH (8.8 kbp) is small. In contrast, the possibility that several intermediates in a given region give rise to recombinants remains strong since 32% of the convertants lose the duplication.

Loss among coincident convertants: Leu⁺Trp⁺ coincident convertants are much less frequent than their single locus (LEU⁺) counterparts. Among the former, pKSH is lost with high frequency (40%), presumably by gene conversion. Furthermore, a significant proportion of *pKSH/pKSH* homozygotes are produced. Thus, these data argue that long heteroduplexes may indeed be prevalent in a subset of the convertants. This result appears to contradict previous findings with pJM53 hemi- and homozygotes which seemed to suggest that plasmid loss among Leu⁺Trp⁺ convertants was due to a second, intrachromosomal exchange (GOLIN, FALCO and MARGOLSKIE 1986). It may be that both mechanisms (multiple events *vs.* long intermediates) can operate to produce coincident Leu⁺Trp⁺ convertants. The two are not exclusive. Several intermediates could be initiated and then merge to create one extensive structure.

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