Long-Tract Mitotic Gene Conversion in Yeast: Evidence for a Triparental Contribution During Spontaneous Recombination

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ABSTRACT

In *Saccharomyces cerevisiae*, spontaneous mitotic gene conversion at one site is statistically correlated with recombination at other loci. In general, coincident conversion frequencies are highest for tightly linked markers and decline as a function of intermarker distance. Paradoxically, a significant fraction of mitotic gene convertants exhibits concomitant nonreciprocal segregation for multiple and widely spaced markers. We have undertaken a detailed genetic analysis of this class of mitotic recombinants. Our results indicate that mitotic gene conversion in yeast is frequently associated with nonreciprocal segregation of markers centromere-distal to the selected site of conversion. In addition, distal markers are often found to be mosaic within the product colonies. These observations, and others described here, suggest that a percentage of gene conversion in vegetative yeast cells is coupled to a chromosome break and repair mechanism. This hypothesis was further tested using a strain trisomic for chromosome *VII* which was specially marked to detect homolog-dependent repair events. An association between mitotic gene conversion events and the production of broken chromosomes which are repaired by a homologous-pairing-copy mechanism was supported.

IN yeast mitosis, a statistical correlation exists between gene conversion at one site and additional acts of conversion elsewhere along the same chromosome arm (GOLIN and ESPOSITO 1981, 1984; GOLIN and TAMPE 1988). When pairs of independent loci are monitored for gene conversion between heteroalleles, a distance-dependent distribution of coincident events is observed, with more tightly linked markers co-converting more frequently than less tightly linked markers (GOLIN and TAMPE 1988). Two distinct models have been proposed to account for coincident exchanges. One hypothesizes a single, long intermediate encompassing more than one locus (GOLIN and ESPOSITO 1981, 1984; MONTELONE *et al.* 1981). The other is based on the formation of discrete, concerted multiple intermediates (GOLIN and ESPOSITO 1984). Although these two explanations are not mutually exclusive (discrete intermediates can merge into one large structure), evidence suggests that most statistically correlated mitotic events spanning large distances are due to the latter (GOLIN and FALCO 1988; RAY *et al.* 1989).

A class of coincident convertants first described by ESPOSITO (1978) is not readily accounted for by a multiple-event model. He selected for prototrophic gene conversion events at the *TRP5* locus on chromosome *VII* using the recombination assay shown in Figure 1A. A subpopulation of the tryptophan prototrophs he recovered appeared to have an associated exchange involving the distal *ADE5* markers and therefore resulted

in red-white-sectored colonies (see MATERIALS AND METHODS for a discussion of this chromogenic screening method). Analysis revealed that although a majority of the sectored colonies displayed a genotype consistent with a classic reciprocal exchange (the white side was composed of cells that were *ade5/ade5*; the red side was composed of *ADE5/ADE5* cells), a significant proportion exhibited the nonreciprocal genotype shown in Figure 1B. In these cases, the white side was homozygous for the markers distal to *TRP5*, but the red side retained its heterozygosity for the same markers. BRUSCHI and ESPOSITO (1983) argued that these coincident gene convertants could arise if a long asymmetric heteroduplex intermediate spanned the distal sites and was often left unrepaired before DNA replication (except at the site of conversion, which was an infrequent and selected event). Because the observed convertants segregate all of the distal sites nonreciprocally, the proposal of a single intermediate is more satisfactory than a multiple-event model for purely statistical considerations. Concerted, yet distinct, intermediates are expected to involve a subset of widely spaced markers with greater frequency than all of the markers. The long heteroduplex model, although capable of accounting for the involvement of all distal sites, remains controversial due to the length of the hypothesized intermediates, which are unprecedented. The expanse of heteroduplex DNA required to account for ESPOSITO's (1978) observations is on the order of 200 kbp. JUDD and PETES (1988) estimated mitotic conversion tracts to average 4 kbp and project an upper limit of 10 kbp.

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FIGURE 1.—System used to monitor recombination. (A) Gene conversion at *TRP5*, which is accompanied by an associated crossover, is detected by using the distal *ADE5* locus. The diploid is normally red because it is *ade2/ade2*. Crossovers which segregate an *ade5/ade5* genotype result in a red-white-sectored colony if there is no prior division in culture. The illustration shows conversion at *TRP5* (resulting in two *Trp*⁺ strands—only one of several possibilities) associated with a reciprocal exchange. In addition to *ADE5*, all other markers (not shown) distal to *TRP5* are rendered homozygous. The conversion event is illustrated as resulting from heteroduplex DNA generated before, and being resolved by replication, as is well documented in the literature (WILDENBERG 1970; ESPOSITO 1978; FABRE 1978; GOLIN and ESPOSITO 1981; ROMAN and FABRE 1983). (B) Some *Trp*⁺-sectored colonies are not the result of reciprocal exchange. Rather, one side is homozygous for markers distal to the conversion site (*CYH2* and *ADE5*), while the other (the red side) is heterozygous for the same markers.

In order to disclose the true intermediates in the generation of these genotypes, we have undertaken an extensive analysis of this class of revertants through the genetic dissection of sectored recombinants. Observations which are not readily accountable by either the long heteroduplex or multiple-event model are reported: in particular, the differential behavior of markers centromere-proximal and distal to the selected site of conversion and the mosaic recovery of alleles. We suggest that these observations are consistent with gene conversion-associated chromosome breakage and subsequent repair using homologous chromatids as templates, and we provide support for this hypothesis using a specially marked trisomic strain.

MATERIALS AND METHODS

Yeast strains and plasmid pKSH: *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Diploid strain JG303 is hemizygous for the plasmid insertion pKSH. Both the strain and plasmid construction have been described previously (GOLIN and FALCO 1988). The use of this strain to evaluate gene conversion and crossing over is discussed below. A fine structure map of the chromosomal region critical for this analysis is shown in Figure 2. Strain JG419 is trisomic for chromosome VII.

Media and culturing conditions: Yeast strains were grown in YPGal medium (20 g/liter galactose, 20 g/liter peptone and 10 g/liter yeast extract) or in YPD (GOLIN and ESPOSITO 1977). The recipes for synthetic dextrose complete, amino acid omission media and cycloheximide medium were described previously (GOLIN and ESPOSITO 1981, 1984).

TABLE 1

Yeast strains

Strain	Genotype	Reference
Diploid analysis		
JG33-18B	<i>HO leu1-c trp5-c cyh2 met13-c ade5 lys2-2 tyr1-2 his7-2 ura3-1 ade2-1</i>	GOLIN and ESPOSITO (1977)
JG34-38A	<i>HO leu1-12 trp5-d CYH2 met13-d ADE5 lys2-1 tyr1-1 his7-1 ura3-313 ade2-1</i>	GOLIN and ESPOSITO (1977)
JG267	JG34-38A transformed with pKSH	GOLIN and FALCO (1988)
JG303	JG267 × JG33-18B	GOLIN and FALCO (1988)
Trisomic analysis		
x3371-1B	<i>ade6-21 LEU1 TRP5 cyh2 met13 aro2 lys5</i>	Yeast Genetics Stock Center
JG283	<i>ade6-23 leu1 trp5 CYH2 MET13 ARO2 LYS5</i>	DR. SUE KLAPHOLZ
JG314-16C	<i>ade6 LEU1 TRP5 CYH2 MET13 ADE5</i>	JG283x X3371-1B
JG324	<i>ADE6 leu1-12 TRP5 CYH2 MET13 ADE5</i>	DR. CARL FALCO
JG417-3A	<i>ade3 cyh4 ura1 leu1-c trp5-20</i>	JG324x JG314-16C
	<i>ade6 leu1-12 TRP5 CYH2 MET13 ade3</i>	
JG252	<i>ADE6 leu1-c trp5-20 CYH2 MET13 ADE5</i>	
JG252	<i>MATα leu1-12 trp5-d CYH2 met13-d ade5 ade2 lys2-1 tyr1-1 his7-2 ura3-13 can1-100</i>	DR. M. ESPOSITO
JG253	<i>MATα leu1-c trp5-c cyh2 met13-c ADE5 ade2 lys2-2 tyr1-2 his7-1 ura3-1</i>	DR. M. ESPOSITO
JG384	JG252 × JG253	This study
JG443	<i>MATα leu1-c,12 TRP5 cyh2 met13-c ade2 LYS2 tyr1 his7</i>	This study
JG382	JG443 × JG253	This study
JG382-7A	<i>MATα leu1-c,12 trp5-c, cyh2 met13-c ade2 lys2 tyr1 ura3 his7</i>	This study
Z506-13A	<i>MATα Ade gal1</i>	Yeast Genetics Stock Center
JG418	JG382-7A × Z506-13A	This study
JG418-6A	<i>MATα leu1-c,12 trp5-c cyh2 met13-c ade2 lys2 tyr1 ura3 Ade⁻</i>	This study
JG419	JG417-3A × JG418-6A	This study

FIGURE 2.—Fine structure map of the *LEU1-ADE5* region. The diploid (JG303) employed in this study carries a series of markers on chromosome VII used to monitor recombination. The site of 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 distance in cM. The physical distance of pKSH from *LEU1* and *TRP5* is ca. 8 and 27 kbp, respectively.

Trp⁺, Leu⁺ or Met⁺ prototrophic convertants were obtained by inoculating 3 ml of enriched medium with a single colony of the JG303 diploid strain. Cultures were grown overnight before harvesting, washing in sterile water and plating both on nonselective completely supplemented medium, and on tryptophan, leucine, or methionine omission media.

Chromogenic screen for sectored colonies: Diploid strain JG303 is mutant at *ADE2* (chromosome XV) and heterozygous at *ADE5* (chromosome VII). The absence of a functional *ADE2* gene product results in the accumulation of a red intermediate generated by the *ADE5* gene product. Crossing over between the centromere and the distal *ADE5* locus will yield daughter cells which are homozygous at the *ADE5* locus through mitotic segregation (in one-half of the cases). One daughter is rendered *ADE5/ADE5*, the other *ade5/ade5*. The latter cell lacks a functional *ADE5* gene; consequently, it cannot synthesize the red intermediate and is white in color. Daughter cells from crossovers (or gene conversions) involving the *ADE5* locus which occur at the time of plating or thereafter give rise to two-toned (red-white) sectored colonies.

Genotypic analysis of sectored colonies: Red-white-sectored colonies were streaked onto synthetic complete medium (GOLIN and ESPOSITO 1981) to obtain pure red and pure white subclones. Six subclones of each color were picked to complete medium and incubated at 30° for 24–48 hr. They were then replica-plated to cycloheximide, sporulation and various omission media. The genotype at *CYH2* was determined as follows. A *cyh2/cyh2* (resistant) diploid shows growth on cycloheximide medium within 24 hr. *CYH2/CYH2* diploids fail to grow even at 96 hr. *CYH2/cyh2* diploids show no growth at 24 hr, but resistant papillae appear by 48 hr. The *LEU1*, *TRP5* and *MET13* sites were scored, on the appropriate omission media, for the presence of prototrophic papillae at 48 hr. Papillae indicate that the diploid contains a pair of heteroalleles at the locus in question since prototrophs arise as the result of heteroallelic gene conversion rather than back mutation (GOLIN and ESPOSITO 1977). If no prototrophs were observed, the locus was assumed to be homoallelic for one of the two alleles. In the case of *MET13*, the alleles are readily distinguished in the homozygous state because the *met13-c* mutation is leaky and shows slight growth after 48–72 hr, whereas the *met13-d* allele shows no growth. An *ADE5/ADE5* diploid is distinguished from a heterozygote in two ways. Homozygotes are a deeper hue of red, and heterozygotes segregate *ade5* spores during meiosis which give rise to white colonies on YPD medium.

Allelism testing: To determine which of two mutant alleles segregated into a given spore, test crosses were made to strains of known genotype and analyzed as described previously (GOLIN and ESPOSITO 1981).

Investigation of chromosome nondisjunction: Nonreciprocal Trp⁺ red-white-sectored colonies, determined by genetic analysis to harbor the genotype represented in Figure 1B, were tested for nondisjunction of chromosome VII by ascus dissection of tetrads. To yield this apparent genotype, an instance of nondisjunction would segregate two *ADE5* homologs and one *ade5* homolog to one daughter (the red segregant). The white segregant consequently would be monosomic,

receiving only an *ade5* homolog. The most direct investigation of chromosome VII nondisjunction, therefore, would be to quantitate spore viability following meiosis of white subclones. However, chromosome VII is suspected of being copy-sensitive since, in a diploid background, monosomes are virtually undetectable (S. KLAPHOTZ, personal communication). To avoid the mitigating effect of any low copy number-prompted chromosome duplications, red subclones were analyzed for trisomy at chromosome VII. Trisomic nondisjunctants produce haploid and disomic spores with equal frequency. A red subclone of a sectored colony generated by nondisjunction (as described above) will cosegregate *ADE5* and *ade5* chromatids in one-third of the meiotic products. These disomic spores will be heteroallelic at *LEU1* and will be capable of producing *Leu*⁺ papillae by gene conversion when replicated to leucine omission medium.

Red subclones from five *Trp*⁺-sectoried colonies were sporulated, and at least 10 tetrads from each were dissected. The resulting colonies were replica-plated to leucine omission medium. After 48 hr incubation at 30°, segregants were scored for the presence of prototrophic papillae. In addition, mating analysis to haploid *leu1-c* and *leu1-12* tester strains was performed to ensure that all meiotic products were capable of generating leucine prototrophic papillae via gene conversion with one or the other of the *LEU1* alleles.

Trisomic analysis

Construction of trisomic strain JG419: Trisomic strain JG419 was made by crossing disomic strain JG417-3A to haploid strain JG418-6A containing the double mutant allele *leu1-c, 12*. To construct the former, the appropriate markers were crossed into the original disomic strain X337-1B (obtained from the Yeast Genetics Stock Center, Berkeley, Ca.) as outlined in Table 1. The final disomic strain (JG417-3A) is heteroallelic at *LEU1* (*leu1-c/leu1-12*), heterozygous at *TRP5*, but homozygous at *CYH2* (drug-sensitive) and *MET13*. The double-mutant stock was constructed as follows. Previous work (GOLIN and ESPOSITO 1984) showed that *Leu*⁺, *Trp*⁺ coincident conversions that arise in a *leu1-c/leu1-12*, *trp5-c/trp5-d* heteroallelic diploid also generate a *leu1-c, 12* homolog at fairly high frequency. Therefore, *Leu*⁺, *Trp*⁺ coincident conversions were selected by plating overnight cultures of the JG384 diploid on leucine, tryptophan double-omission medium. Coincident convertants that were cycloheximide-resistant and homozygous for the *met13-c* allele were initially recovered. These diploids were sporulated, and meiotic segregants were tested for their allelic constitution at *LEU1* and *TRP5*. A recombinant yielding a *leu1-c, 12* homolog was identified (JG443). This strain fails to give *Leu*⁺ recombinants when backcrossed to either a *leu1-12* or *leu1-c* tester stock. The *trp5-c* allele was introduced into the resulting strain JG382-7A by crossing JG443 to JG253 as indicated. An additional cross was made (Z506-13A) to obtain the correct mating type, thus yielding the strain JG418-6A. The final trisomic strain (JG417-3A × JG418-6A) was checked by sporulation and ascus dissection. Good spore viability (85% germination) and the expected aberrant segregation of chromosome VII markers were observed. For example, since the strain is *MET13/MET13/met13-c*, most 4-spored tetrads showed either a 4 *Met*⁺:0 *Met*⁻ or 3 *Met*⁺:1 *Met*⁻ pattern of inheritance instead of the typical 2:2 ratio encountered in a *MET13/met13-c* heterozygote. Similarly, *cyh2* (resistant), *CYH2/CYH2* (sensitive) and *CYH2/cyh2* ascospore clones were recovered as well as disomic clones that were heteroallelic for the *leu1-12* and *leu1-c* alleles. The latter yielded *Leu*⁺ papillae when replica-plated to leucine omission medium. During the analysis of the trisomic strain, the *trp5-20* and *trp5-c* alleles were found to be inseparable by genetic recombination and are likely either same-site mutations or very close to one another.

Selection of independent *Leu*⁺ recombinants: Independent *Leu*⁺ recombinants were selected from the trisomic strain for further genetic analysis. Colonies that were plated on synthetic complete media were picked to YPD master plates. These plates were incubated at 30° for 48 hr before replica plating to leucine omission medium. Several *Leu*⁺ papillae arose from each patch after 48 hr incubation. A single *Leu*⁺ papilla (recombinant) was then picked from each of the original colonies for analysis as described in RESULTS.

Tetrad analysis: Ascus dissection and tetrad analysis was carried out by conventional means.

Allelism testing: To determine whether a spore was *leu1-c*, *leu1-12* or *leu1-c, 12*, crosses were made to haploid *leu1-12* and *leu1-c* tester strains. The resulting diploids were sporulated and replica-plated to leucine omission media to detect *Leu*⁺ papillae.

Subcloning of *Leu*⁺ recombinants: Subcloning of recombinants was performed by streaking cells on YPD medium and picking 10–12 single colonies to YPD master plates. After incubation for 24 hr at 30°, the plates were replica-plated to diagnostic medium for determination of allelic composition at the pertinent sites.

RESULTS

Experimental design: The homothallic diploid strain JG303, which contains the marked chromosome illustrated in Figure 2, was used to detect both reciprocal and nonreciprocal recombination events. It carries heteroallelic sites at *LEU1*, *TRP5* and *MET13*. Gene conversion at these loci, therefore, can be monitored by the selection of prototrophs on various omission media. In addition, the diploid is heterozygous at *CYH2* and *ADE5*. The latter is used to assess crossing over by means of a simple chromogenic assay (ROMAN 1956) described in MATERIALS AND METHODS. In short, exchanges which lead to homozygosity of the *ade5* allele give rise to a white daughter from a red parent. Red-white-sectoried colonies reflect exchanges occurring at the time of plating and comprise both mitotic segregants of the recombination event. Sectoried colonies, therefore, enable one to recover all of the chromosomes involved in an act of recombination. Finally, strain JG303 is hemizygous for the plasmid insertion pKSH (GOLIN and FALCO 1988). As a result of its integration during transformation, chromosomal material is replaced and a *URA3* gene is introduced between *LEU1* and *TRP5*. The strain, consequently, is *Ura*⁺. Loss of pKSH results in a *Ura*⁻ phenotype. In addition, the insertion of *URA3* at this location provides a marker close to the selected sites (*LEU1*, *TRP5*) of conversion in these studies.

Genetic analysis of *Trp*⁺ red-white-sectoried colonies: Prototrophic gene conversion at *TRP5* in strain JG303 was observed at a median frequency of 6.9×10^{-6} . This value is similar to those reported previously for related, but nonisogenic, diploids (GOLIN and ESPOSITO 1981, 1984; GOLIN and FALCO 1988; GOLIN *et al.* 1986). White and red-white sectoried colonies were recovered at median values of 1.9 and 1.1% of total *Trp*⁺ convertants, respectively.

TABLE 2
Genotypes of markers distal to *TRP5* among nonmosaic *Trp*⁺-sectoried convertants

Class	Red side	White side	No. recovered
I	<i>CYH2 met13-d ADE5</i>	<i>cyh2 met13-c ade5</i>	116
II	<i>CYH2 met13-d ADE5</i>	<i>cyh2 met13-c ade5</i>	63
III	<i>CYH2 met13-d ADE5</i>	<i>cyh2 met13-c ade5</i>	2
IV	<i>CYH2 met13-d ADE5</i>	<i>CYH2 met13-d ade5</i>	10
V	<i>CYH2 met13-d ADE5</i>	<i>CYH2 met13-d ade5</i>	4
Mosaics	(See Table 5)		32
Totals			227

A total of 227 red-white-sectoried *Trp*⁺ colonies were selected and subcloned to obtain pure red and white colonies for genetic analysis, as described in MATERIALS AND METHODS. The genotypes that were recovered can be grouped into six general classes (Table 2). One hundred and sixteen (51.1%) appear to be the result of conversion-associated reciprocal exchange (class I). In contrast, 63 (27.8%) sectoried recombinants exhibited a 3:1 segregation of distal markers (class II) and, thus, are analogous to those originally described by ESPOSITO (1978). In these cases, the white subclones were homozygous for all markers distal to *TRP5*, while red subclones remained heterozygous and heteroallelic for the same markers. Of the remaining sectoried colonies that were not included in class I or class II, sixteen (7.0%) yielded red and white subclones which were heterozygous at *CYH2* and sometimes at *MET13* (classes III–V). Finally, thirty-two (14.1%) were mosaic, yielding subclones with three rather than two genotypes, and therefore were similar to those reported previously (GOLIN and TAMPE 1988; ROMAN and RUZINSKI 1990). All of the mosaic colonies contained subclones with genotypes indicative of a nonreciprocal exchange. However, in addition, subclones with genotypes representative of a reciprocal exchange were often recovered from the same colony (Table 3). The relative importance of this mosaic class of sectoried recombinants will be discussed in detail later.

Nonreciprocal genotypes are not attributable to chromosome nondisjunction: The observed class II nonreciprocal (3:1) marker segregations could result from a variety of gene conversion events or chromosome nondisjunction. Conversion events are statistically associated with nondisjunction of chromosomes, particularly those involving centromere-linked markers (CAMPBELL and FOGEL 1977). Ascus dissection and analysis of tetrads from heterozygous red subclones of nonreciprocal *Trp*⁺ red-white-sectoried colonies was performed as described in MATERIALS AND METHODS. There was no evidence for nondisjunction of chromosomes.

Evidence for conversion in G1: ESPOSITO (1978) demonstrated that class I (reciprocal)-sectoried colonies arise before G2 in the cell cycle. To determine whether class II recombinants also arise before DNA replication, the complete genotype at *TRP5* for 31 class II-sectoried colonies was determined as previously described. The results, found in Table 4, indicate that most (29/31) of the *Trp*⁺ conversion events occurred before DNA replication. Had they converted during G2, the red subclones would have been genotypically *TRP5/trp5-d*, and the white subclones *TRP5/trp5-c* [see ESPOSITO (1978) and GOLIN and ESPOSITO (1981) for discussion]. Only two sectoried colonies produced this genotype (class C, Table 3).

Analysis of conversion at *LEU1* and *MET13*: Gene conversion was analyzed at the *LEU1* and *MET13* loci in a manner analogous to that described for *TRP5*. Sectoried recombinants were selected on appropriate omission medium. The genotypes of *Leu*⁺ and *Met*⁺ convertants are very much like those *Trp*⁺ recombinants described above. Of the 189 *Leu*⁺-sectoried colonies analyzed, 108 (57.1%) were reciprocal for distal sites, 60 (31.8%) were nonreciprocal and 21 (11.1%) were mosaic (see below). These frequencies of sector classes are quite similar to those observed among the *Trp*⁺ convertants analyzed.

Ninety-nine *Met*⁺-sectoried convertants were also analyzed. Twenty-eight were found to be mosaic for at least one marker. To determine whether *Met*⁺-sectoried colonies showed nonreciprocal segregation, we examined the genotype of the *ADE5* locus (see MATERIALS AND METHODS) in 72 colonies. Of these, 39 (54.2%) showed a reciprocal segregation, 18 (25%) showed a nonreciprocal recovery, and 15 (20.8%) were mosaic. Tables 5 and 6 compare mosaic frequencies for the three types of selected convertants: *Leu*⁺, *Trp*⁺ and *Met*⁺. The frequency of recombinants exhibiting mosaicism among each of the three selected classes of convertants is presented in Table 5. Among *Met*⁺ convertants, the

TABLE 3
Allelic configuration of Trp⁺-mosaic sectors

Class	Red side					White side					Not recovered
	<i>LEU1</i>	<i>URA3</i>	<i>TRP5</i> ^a	<i>CYH2</i>	<i>MET13</i>	<i>LEU1</i>	<i>URA3</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	
1 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	12
b	12/c	+	+	S	d						
2 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	3
b	12/c	+	+	S/R	d						
3 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	12/c	+	—	S/R	d/c						
4 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	12/c	+	+	R	d						
5 a	12/c	+	+	S/R	d/c	12/c	+	+	S/R	c	1
b	12/c	+	—	S/R	d/c						
6 a	12/c	+	+	S/R	d/c	12/c	+	+	S/R	c	1
b	12/c	+	—	S	d						
7 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	12/c	+	d/c	S	d						
8 a	12/c	+	+	S/R	d/c	12/c	+	+	S	d/c	1
b	12/c	+	+	S	d/c						
9 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	12/c	+	+	S	c						
10 a	+	+	+	S/R	d/c	+	+	+	R	c	1
b	+	+	+	S/R	d						
11 a	12/c	+	+	S/R	d/c	+	+	+	R	c	1
b	+	—	—	S	d						
12 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	h	+	+	S/R	d/c						
13 a	12/c	+	+	S/R	d/c	h	—	+	R	c	1
b	12/c	+	+	S	d						
14 a	12/c	+	+	S/R	d/c	h	+	+	R	c	1
b	12/c	+	+	S	d/c						
15 a	12/c	+	+	S/R	d/c	12/c	+	+	S/R	c	1
b						12/c	+	+	S	c	
16 a	12/c	+	+	S/R	d/c	12/c	+	+	S/R	d/c	1
b						12/c	+	+	S/R	d	
17 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b						h	—	+	R	c	
18 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	h	—	+	S	d	h	+	+	R	c	
19 a	12/c	+	+	S/R	d/c	12/c	+	+	R	c	1
b	12/c	+	+	S	d						
c	12/c	+	—	R	d/c						

Designations are as follows: +, prototrophic; —, auxotrophic; h, homoallelic (alleles not determined). For *CYH2*: S, sensitive (*CYH2*); R, resistant (*cyh2*). The *ADE5* marker was not scored for mosaicism in these colonies.

^a In seven cases Trp⁺ subclones were recovered from Trp⁺-sectored colonies (see text for discussion).

frequency of mosaicism is substantially higher than among Leu⁺ or Trp⁺ recombinants. Table 6 specifies the number of times a given locus exhibits mosaicism for each of the classes of convertants.

To determine whether the location of a given marker relative to a site of selected conversion influences whether the former exhibits mosaicism, a comparison was made between the frequency of mosaicism at *TRP5* among Leu⁺ vs. Met⁺ convertants (see Table 6). The *TRP5* locus is distal and tightly linked to *LEU1*. Among Leu⁺-sectored colonies exhibiting mosaicism, 12/21 involved *TRP5*. In contrast, *TRP5* is proximal and relatively distant (*ca.* 94 Cm) from *MET13*. Only 2/28 Met⁺-mosaic-sectored colonies involved *TRP5*. A Chi square test was employed to determine whether these ratios are significantly different. The probability that no difference exists between the ratios of *TRP5* mosaicism

among Leu⁺- or Met⁺-sectored convertants is less than 0.01.

A more direct representation of the disparity between the frequencies of mosaicism for markers distal to the selected site of conversion and those proximal is that Leu⁺ convertants are often mosaic at the distal *TRP5* site (12/21), but Trp⁺ convertants are rarely mosaic at the proximal *LEU1* site (3/32). The difference between these values is statistically significant at a *P* < 0.005.

Genetic analysis of sectored colonies arising on complete (nonselective) medium: The selection of prototrophic gene convertants could be intrinsically biased and might not reflect spontaneous mitotic recombination in general. For this reason, we also analyzed the genotypes of red-white-sectored colonies arising on medium containing all nutritional requirements (complete medium, GOLIN and ESPOSITO 1977). A total of 29 sectored colo-

TABLE 4

TRP5 genotypes of thirty-one *Trp*⁺ class II-sector colonies

Class	Red subclones	White subclones	No. recovered
A	<i>TRP5</i>	<i>TRP5</i>	14
	<i>trp5-d</i>	<i>trp5-d</i>	
B	<i>TRP5</i>	<i>TRP5</i>	10
	<i>trp5-c</i>	<i>trp5-c</i>	
C	<i>TRP5</i>	<i>TRP5</i>	2
	<i>trp5-d</i>	<i>trp5-c</i>	
D	<i>TRP5</i>	<i>TRP5</i>	2
	<i>trp5-c,d</i>	<i>trp5-c,d</i>	
E	<i>TRP5</i>	<i>TRP5</i>	1
	<i>trp5-d</i>	<i>trp5-c,d</i>	
F	<i>TRP5</i>	<i>TRP5</i>	1
	<i>trp5-d</i>	<i>TRP5</i>	
G	<i>TRP5</i>	<i>TRP5</i>	1
	<i>TRP5</i>	<i>TRP5</i>	
Total			31

TABLE 5

Frequency of mosaics among sector colonies

Conversion class	No. of sectors	No. of mosaics (%)
<i>LEU1</i>	189	21 (11)
<i>TRP5</i>	227	32 (14)
<i>MET13</i>	99	28 (28)

nies (their frequency is extremely low in an unselected population) were collected under these nonselective conditions. The genotypes recovered from subclones of these sector colonies are listed in Table 7 and represent the same general classes seen among sector colonies, but at somewhat different relative proportions. Only one of the colonies appears to have resulted from a reciprocal exchange. Twenty-three of twenty-nine, however, were nonreciprocal with the red side remaining heterozygous or heteroallelic for the sites rendered homozygous in the white subclones. This proportion, which is much higher than that observed among prototrophic convertants, is misleading since, in twelve of the cases, only the distal *ADE5* marker is rendered homozygous in white subclones. The observed genotype, therefore, could simply represent a gene conversion at *ADE5*. The 11 others, however, result from recombination events localized to various genetic intervals proximal to *ADE5* with at least one additional marker homozygous among white subclones. Mosaicism was also detected in five instances. It is important to note that none of the red subclones isolated from the mosaic colonies demonstrated a genotype reciprocal to that observed for its white counterpart. This observation demonstrates that these colonies did not arise from reciprocal exchanges occurring subsequent to cell division on the plate, which would yield parental and reciprocal

TABLE 6

Involvement of various sites among mosaic convertants

Locus or loci	No. of mosaic/convertant class		
	Leu ⁺	Trp ⁺	Met ⁺
Total no. mosaics/site ^a			
<i>LEU1</i>	1	3	0
<i>URA3</i>	1	3	0
<i>TRP5</i>	12	8	2
<i>CYH2</i>	11	25	10
<i>MET13</i>	17	25	18
<i>TRP5</i> involvement	52%	25%	0.07%
Single-site mosaicism			
<i>LEU1</i>	0	1	0
<i>URA3</i>	1	0	0
<i>TRP5</i>	3	2	2
<i>CYH2</i>	1	2	6
<i>MET13</i>	5	2	16
Coincident mosaicism ^b			
<i>LEU1-MET13</i>	0	1	0
<i>URA3-MET13</i>	0	1	0
<i>TRP5-MET13</i>	6	5	0
<i>CYH2-MET13</i>	4	17	4
<i>TRP5, MET13</i>	2	0	0
<i>LEU1, MET13</i>	1	0	0
<i>LEU1, URA3</i>	0	1	0

^a Since a given mosaic can involve multiple sites, the total number of sites involved is greater than the total number of mosaic sectors.

^b Markers separated by a hyphen (e.g., *LEU1-MET13*) indicate those markers and all intervening markers.

genotypes in the product colony. Rather, they are true counterparts to those mosaic colonies recovered among prototrophic convertants, where parental (auxotrophic) genotypes are selected against.

The proportion of mosaics in this sample (5/29), albeit small, appears similar to that found among prototrophic convertants. These results suggest that mosaic colonies represent a significant fraction of spontaneous mitotic recombinants. It also shows, as did previous work by BRUSCHI and ESPOSITO (1983), that spontaneous genetic recombination resulting in red-white-sector colonies on complete medium is largely nonreciprocal.

The nonreciprocal transfer of genetic information centromere-distal to the selected site of conversion, and preferential mosaicism of distal sites, is suggestive of a chromosome break-and-repair mechanism. Repair of broken chromosomes is well documented in yeast (McCUSKER and HABER 1981; HABER and THORNBURN 1984; FALCO *et al.* 1982; DUNN *et al.* 1984) and has been shown to be homolog-dependent (HABER and THORNBURN 1984). We propose, as have others (HASTINGS 1984; BORTS and HABER 1987), that the gene conversion process may be prone to the generation of specific strand lesions (e.g., nicks or gaps) in participating DNA duplexes. Lesions might result from inefficient ligation after endonucleolytic resolution of Holliday intermediates, or might be a direct consequence of excision repair of heteroduplexed DNA (HASTINGS 1984). Strand lesions of this type are processed to double-strand breaks via DNA replication. Nonreciprocal genotypes could then

TABLE 7
Genotypes of sector colonies arising on complete medium

Class	Red subclones						White subclones						No. recovered
	<i>LEU1</i>	<i>URA3</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	<i>ADE5</i>	<i>LEU1</i>	<i>URA3</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	<i>ADE5</i>	
A	12/c	+	d/c	S/R	d	A/A	12/c	+	d/c	R	c	a/a	1
B	12/c	+	d/c	S/R	d/c	A/a	12/c	+	d/c	S/R	d/c	a/a	12
C	12/c	+	d/c	S/R	d/c	A/a	12/c	+	d/c	R	c	a/a	4
D	12/c	+	d/c	S/R	d/c	A/a	12/c	+	—	R	c	a/a	2
E	12/c	+	d/c	S/R	d/c	A/a	h	—	—	R	c	a/a	3
F	12/c	+	d/c	S/R	d/c	A/a	12/c	+	d/c	S/R	c	a/a	2
G	12/c	+	d/c	S/R	d/c	A/a	12/c	—	—	R	c	a/a	1
H	h	+	—	S	d	A/a							
	12C	+	d/c	S/R	d/c	A/a	12/c	+	d/c	S/R	d/c	a/a	1
I	12/c	+	d/c	S/R	d/c	A/a	12/c	+	d/c	R	c	a/a	1
	12/c	+	d/c	S	d	A/a							
J	12/c	+	d/c	S/R	d/c	A/a	12/c	+	d/c	R	c	a/a	1
	12/c	+	d/c	S/R	d	A/a							
K	12/c	+	d/c	S/R	d/c	A/a	12/c	—	—	R	c	a/a	1
	h	+	d/c	S	d/c	A/a							

Designations are as follows: +, prototrophic; —, auxotrophic; S, sensitive (*CYH2*); R, resistant (*cyh2*); A, *ADE5*; a, *ade5*; h, homoallelic.

result from repair processes that use nonsister homologs as templates.

Trisomic analysis

RAY *et al.* (1989) documented triparental recombination processes in yeast, showing them to be stimulated by double-strand breaks. Their analysis utilized an ectopic site as the third contributor. A region of greater homology, however, should be a more effective substrate. To test the chromosome break-repair model, we constructed a yeast strain that was trisomic for markers on chromosome VII as shown in Figure 3. This strain permits selection of a *Leu*⁺-gene-conversion event between homologs I and II. Homolog III contains both mutant alleles and, as shown below, is virtually incapable of contributing *Leu*⁺ revertants. It is, however, a suitable template for repair of a broken homolog. Repair events that utilize this homolog (III) alter the ratio of the uniquely marked *CYH2* and *MET13* alleles. The genetic predictions for the model are shown in Figure 4. A case in which the trisomic strain contains a single broken chromatid in G2 is illustrated. Five complete helices capable of serving as templates for repair synthesis are present. When the *CYH2* locus is considered, three (1, 3 and 4) of these are genetically identical (*CYH2*), while the remaining two contain a different (*cyh2*) allele. If the broken duplex (chromatid 3) is repaired using one of the three *CYH2* chromatids, the original trisomic genotype is restored. Alternatively, if either chromatid 5 or 6 is used, a novel genotype arises. Cells are *CYH2/cyh2/cyh2* rather than *CYH2/CYH2/cyh2*. Similarly, they could also be *MET13/met13/met13* rather than *MET13/MET13/met13*. Quantitative predictions are difficult to make with respect to how frequently the new genotype would appear among *Leu*⁺ convertants, because the frequency of broken chromosomes resulting

FIGURE 3.—Trisomic strain JG419. The final trisomic strain is marked at four loci on the left arm of chromosome VII. Each homolog carries a distinct *LEU1* allele. The *leu1-c,12* homolog is also uniquely marked at *CYH2* and *MET13*.

from recombination-induced lesions is unknown. In the JG303 diploid, the frequency of sectored *Trp*⁺ or *Leu*⁺ colonies exhibiting a 3:1 segregation of markers was about 1.0% of the total convertants recovered. Assuming that these arose from the repair of broken chromosomes, one would expect to see a similar frequency of *CYH2/cyh2/cyh2* recombinants among *Leu*⁺ convertants that are selected in the trisomic strain.

The frequency of *Leu*⁺ conversion in trisomic strains: The frequency of *Leu*⁺ convertants was measured both in the disomic strain JG417-3A and in the trisomic construction JG419. We also attempted to measure the reversion frequency of the *leu1-c,12* double mutant allele present in JG418-6A. The median frequency of conversion in the trisomic strain (1.4×10^{-5}) is about one-third the value obtained with the disome (4.1×10^{-5}). This difference is not unexpected since *leu1-c,12/leu1-c* and *leu1-c,12/leu1-12* combinations, which represent two-thirds of the pairing possibilities, fail to generate prototrophs in the trisomic strain. The frequency observed in the disomic strain is in fact very similar to previously reported values (GOLIN and ESPOSITO 1981, 1984; GOLIN and FALCO 1988; GOLIN *et al.* 1986). No revertants were observed among eight cultures of the double-mutant strain (representing a total of 5×10^8 cells) plated on

FIGURE 4.—Genetic predictions of a break/healing mechanism for recombination events taking place in trisomic strain, JG419. Illustrated here are the salient steps in the hypothesized process whereby, during conversion of *LEU1* (a selected event), lesions in the form of nicks or gaps in the converting duplex are subsequently processed to a double-strand break by replication. At the top of the figure, asymmetric G1 heteroduplex spanning the *LEU1* locus (generated by *leu1-c* strand invasion of the *leu1-12* duplex) has by isomerization produced a Holliday structure. A DNA strand lesion (not specifically indicated) in the converted duplex, resulting from mismatch repair and/or partial endonucleolytic processing, is converted (A) to a double strand break via DNA replication. The broken chromosome can subsequently be healed (B) by using one of the remaining five helices as a repair template. The uniquely marked *CYH2* locus serves as an indicator of template selection and, hence, template-based repair. If helix 1, 3 or 4 is used, the original trisomic genotype (*CYH2*/*CYH2*/*cyh2*) is restored. If helix 5 or 6 is used, a new genotype (*CYH2*/*cyh2*/*cyh2*) emerges in the cell segregating chromatid 2 during mitosis.

leucine-omission medium, even after five days incubation. Furthermore, it is also known that the single *leu1-c* and *leu1-12* mutants revert at very low frequencies (*ca.* 10^{-7} ; GOLIN and ESPOSITO 1977). We also measured the frequency of *Leu*⁺ revertants in *leu1-c/leu1-c,12* and *leu1-12/leu1-c,12* diploids. The reversion frequencies were on the order of 10^{-7} (data not shown). Therefore, most, if not all, of the *Leu*⁺ colonies recovered from the trisomic strain arise via gene conversion between the two alleles rather than by back mutation.

Selection and analysis of *Leu*⁺ convertants: As described in MATERIALS AND METHODS, 943 independent *Leu*⁺ recombinants were selected for analysis. They were

replica-plated to diagnostic media (leucine, tryptophan and methionine omission media as well as cycloheximide-containing medium) and scored at 24 and 48 hr. The observed phenotypes are found in Table 8. Of the 943 recombinants, 34 exhibited a new cycloheximide-resistance phenotype (Figure 5). Instead of few or no resistant papillae, these *Leu*⁺ colonies yielded numerous resistant segregants. Consequently, these clones were termed "*Hcr*⁺" (high-number cycloheximide-resistant recombinants). Since this phenotype was not encountered in 1008 nonrecombinant clones, we conclude that *Hcr*⁺ colonies are a conversion-associated phenomenon.

TABLE 8

Frequency of various recombination events

Phenotype ^{a, b}	Parental (1008)	Leu ⁺ (943)
Hcr ⁺ , Trp ⁻ , Met ⁺	0	18 (0.019)
Hcr ⁺ , Trp ⁺ , Met ⁺	0	15 (0.016)
Hcr ⁺ , Trp ⁺ , Met ⁻	0	1 (0.001)
sen, Trp ⁻ , Met ⁺	0	53 (0.056)

^a Hcr⁺, high number cycloheximide resistant recombinants; sen, sensitive (*CYH2/CYH2/cyh2*).

^b These phenotypes were derived before subcloning (see text) and searching for mosaic colonies. As listed in Table 10, three *cyh2/cyh2/cyh2* and two Met⁻ recombinant subclones were recovered among the mosaic colonies.

FIGURE 5.—The Hcr⁺ phenotype. Of 943 independent Leu⁺ recombinants isolated in this study, 34 exhibited the unique phenotype shown, when replica-plated to cycloheximide medium (1 mg/liter). By 48 hr incubation at 30°, many resistant papillae are easily observed. In contrast, the parental clones exhibit little or no papillation.

About half of the Hcr⁺ recombinants (18/34) were also Trp⁻. There were an additional 53 recombinants that were Trp⁻ but exhibited the usual cycloheximide phenotype (few or no resistant papillae). Perhaps the simplest explanation for this latter class of Trp⁻ colonies is that they represent gene conversion and associated crossing over between homologs I and II. Alternatively, some may also represent coincident conversion at *LEU1* and *TRP5* (see GOLIN and ESPOSITO 1984).

Further genetic analysis was performed on 20 of the 34 Hcr⁺ Leu⁺ convertants. The complete genotype for the markers on the left arm of chromosome VII was determined by tetrad analysis. Approximately 10–15 tetrads were dissected for each original recombinant. The resulting ascospore clones were replica-plated to appropriate omission media for scoring. In addition, allelism

TABLE 9

Genotypes of twenty Leu⁺ convertants showing the Hcr⁺ phenotype

Class	Genotype				No. recovered
1	<i>LEU1</i>	<i>trp5-c</i> ^a	<i>CYH2</i>	<i>MET13</i>	10
	<i>leu1-c</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
	<i>leu1-c, 12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
2	<i>LEU1</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	3
	<i>leu1-12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
	<i>leu1-c, 12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
3	<i>LEU1</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	1
	<i>leu1-c</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
	<i>leu1-c, 12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
4	<i>LEU1</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	2
	<i>leu1-c</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
	<i>leu1-12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
5	<i>LEU1</i>	<i>TRP5</i>	<i>CYH2</i>	<i>MET13</i>	1
	<i>leu1-c, 12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13</i>	
6	<i>LEU1</i>	<i>trp5-c</i>	<i>CYH2</i>	<i>MET13</i>	2
	<i>leu1-c, 12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
7	<i>LEU1</i>	<i>trp5-c</i>	<i>CYH2</i>	<i>MET13</i>	1
	<i>leu1-c, 12</i>	<i>trp5-c</i>	<i>cyh2</i>	<i>met13-c</i>	
	<i>leu1-12</i>				

^a The *trp5-c* designation is used universally since the *trp5-20* allele was genetically indistinguishable.

testing was carried out to determine the complete *LEU1* genotype as described in MATERIALS AND METHODS. The genotypes of the recombinants are found in Table 9. Several points bear mentioning. First, 3 of the 20 Leu⁺ recombinants were no longer trisomic. Rather, their marker segregation indicated that these were heterozygous for the markers and thus contained only two copies of chromosome VII. This result is consistent with the observation of CAMPBELL and FOGEL (1977), who showed an association of conversion and chromosome loss in disomic strains of yeast. Furthermore, HABER and HEARN (1985) showed that *RAD52* independent mitotic recombination results in the loss of chromosomes. Interestingly, one recombinant from our analysis (class 7) retained three different *LEU1* alleles, but was Trp⁻ and showed no evidence of trisomy at *CYH2*. A chromosome rearrangement may have occurred in this recombinant.

Of the 20 clones, 16 remained trisomic for all of the markers. These have several important features. First, each recombinant yielded three different *LEU1* alleles upon ascospore analysis. Most of the recombinants (14/16) carried a Leu⁺ homolog and a *leu1-c, 12* homolog, in addition to one with either the *leu1-c* or *leu1-12* allele. In two cases, however, the *leu1-c, 12* allele was replaced so that in addition to the Leu⁺ homolog, both *leu1-c* and *leu1-12* were recovered. Second, among the Hcr⁺ clones analyzed, three distinct cycloheximide phenotypes were observed. Resistant, sensitive and heterozygous ascospore segregants were found from each of the recombinants (Figure 6). Heterozygous segregants appear sensitive on inhibitor medium at 24 hr, but many resistant papillae arise by 48 hr. The Hcr⁺ recombinants

FIGURE 6.—Phenotypes of ascospore clones from a single tetrad. The $Leu^+ Hcr^+$ recombinants were sporulated and subjected to tetrad analysis. Three distinct phenotypes were found among the ascospore clones from each. Significantly, two resistant segregants (A and B) grow up by 24 hr on cycloheximide medium. At 48 hr, heterozygous clones (C) yield numerous resistant papillae, but sensitive ones (D) fail to grow. From this analysis, we infer that the Hcr^+ recombinant phenotype often is indicative of a $CYH2/cyh2/cyh2$ genotype.

generally yielded four-spored tetrads in which two segregants were resistant (data not shown). This pattern was not observed in the initial, $CYH2/CYH2/cyh2$ trisomic strain and is not expected to occur frequently. This result strongly suggests that the Hcr^+ recombinants are genotypically $CYH2/cyh2/cyh2$. Finally, all Hcr^+ , Met^+ recombinants were $MET13/met13-c/met13-c$, which was determined as follows. When the original nonrecombinant trisomic strain was subjected to tetrad analysis, most tetrads yielded $Met^+ : Met^-$ ratios of 4:0 or 3:1 since the original strain was $MET13/MET13/met13-c$. In contrast, among tetrads analyzed from the 16 Leu^+ recombinants in question, primarily 2 $Met^+ : 2 Met^-$ segregations were found, although 1 $Met^+ : 3 Met^-$ patterns were also observed in a minority of the four-spored cases examined. To verify that the inferred genotype was correct, we crossed Met^+ spores that were heterozygous at $CYH2$ (and hence, disomic) from five of the Leu^+ recombinants to a standard Met^+ strain (JG200). The resulting diploids were sporulated and subjected to tetrad analysis. In each of the cases, tetrad analysis revealed the presence of Met^- segregants (data not shown).

The Hcr^+ Met^- and $cyh2/cyh2/cyh2$ recombinants arise as sectored or mosaic colonies: Before the ascus dissection described above, each of the original Hcr^+ recombinants was subcloned by restreaking and picking as described in MATERIALS AND METHODS. Three sectored (two different genotypes) and four mosaic colonies were recovered. Their phenotypes are listed in Table 10. The observed frequency of mosaics (4/34 or 11.7%) is very similar to the value reported from nontrisomic strains (ROMAN and RUZINSKI 1990). Of note (see DISCUSSION) is the recovery of two additional Met^- and three $cyh2/cyh2/cyh2$ -resistant subclones from the mosaics. The genotypes of the recombinants in question were verified

TABLE 10

Phenotypes of sectored and mosaic colonies of JG419

Recombinant	Sub-clone	<i>LEU1</i>	<i>TRP5</i>	<i>CYH2</i> ^a	<i>MET13</i>
Leu^+ 17 (mosaic)	a	+	+	sen	+
	b	+	+	res	+
	c	+	+	Hcr	+
Leu^+ 45 (mosaic)	a	+	+	Hcr	+
	b	+	—	Hcr	+
	c	+	+	sen	+
Leu^+ 436 (sector)	a	+	—	Hcr	+
	b	+	+	sen	+
Leu^+ 690 (sector)	a	+	+	Hcr	+
	b	+	—	Hcr	+
Leu^+ 786 (sector)	a	+	—	Hcr	+
	b	+	+	Hcr	+
Leu^+ 883 (Mosaic)	a	+	—	Hcr	+
	b	—	+	res	—
	c	+	+	Hcr	+
Leu^+ 921 (Mosaic)	a	+	—	res	+
	b	+	—	sen	+
	c	+	—	Hcr	—

^a For the *CYH2* locus: sen, sensitive; res, resistant; Hcr, high number of resistant papillae.

using tetrad analysis. The two Met^- clones exhibiting the Hcr^+ phenotype (Tables 7 and 9) were found to be $CYH2/cyh2/cyh2$ using the criteria described above. Both clones also segregated three *LEU1* alleles, as did the $cyh2/cyh2/cyh2$ recombinants.

DISCUSSION

In diploid strain JG303, prototrophic gene conversion events at *LEU1*, *TRP5* or *MET13* produced red-white-sector colonies at a frequency of about 1% of the total convertants at each locus. Among the sectored convertants, three distinct genotypic classes were represented. The majority (up to 60%) appear to result from conversion-associated reciprocal exchange since markers distal to the conversion site are homozygous in subclones of both the red and white sides. These recombinants are well documented in the literature and have been attributed to replicational resolution of G1 conversion site heteroduplexes (ESPOSITO 1978; GOLIN and ESPOSITO 1981). A second class of sector colonies, comprising 30–40% of the total, is nonreciprocal, yielding red subclones that are heterozygous for all distal markers and white subclones that are homozygous for the same markers. The recovery of distal alleles from these colonies is 3:1, as opposed to the 2:2 seen with reciprocal events. Aberrant segregation of proximal markers is also observed, but at a much lower frequency. The third class of sector colonies, representing about 12–15% of the total, also contains a nonreciprocal component; however, these colonies are considered mosaic because three or more (rather than two) different genotypes are recovered among the subclones. This

is particularly interesting since the prototrophic convertants were determined to have arisen from G1 intermediates.

Models of recombination

Models previously proposed to account for nonreciprocal segregation of multiple markers have focused on either the formation of long heteroduplexes in G1 (BRUSCHI and ESPOSITO 1983) or multiple intermediates (GOLIN and ESPOSITO 1984; GOLIN and FALCO 1988; RAY *et al.* 1989). The observations reported here are not entirely consistent with the expectations of either of these mechanisms. Although some mitotic recombination events are no doubt due to multiple intermediates, it is unlikely that our nonreciprocal recombinants have arisen from them because in each independent sector all of the distal markers (rather than a subset) display 3:1 segregations. This observation is, however, consistent with the formation of long heteroduplexes during G1. Intermediates of this type cannot account for all of our observations either, for two reasons. First, heteroduplexes arising in G1 cannot generate mosaics. (A more detailed discussion of this recombinant class is presented below.) Second, to explain the observations of this study, one would have to assume that all intermediates originated at, or close to, the selected site of conversion. It is otherwise expected that long heteroduplex intermediates initiate at any site along the chromosome arm and give rise to prototrophic convertants by mismatch repair at the selected site. Closely linked proximal markers, therefore, would segregate nonreciprocally in many instances. This was not found. Among 63 nonmosaic Trp^+ -sectored colonies exhibiting nonreciprocal segregation for distal sites (class II), there were only four cases of aberrant segregation for pKSH:URA3 , a proximal marker that is tightly linked (10 cM). A potential objection to this line of argument is that the pKSH insertion creates a large (11.6 kb) region of nonhomology proximal to TRP5 which might impede heteroduplex movement. This is unlikely for two reasons. First, GOLIN and ESPOSITO (1981) and BRUSCHI and ESPOSITO (1983) used diploids that are closely related to ours but contained no plasmid insertion. Reexamination of their data reveals that the proximal LEU1 alleles segregated normally in all 24 Trp^+ -sectored colonies showing nonreciprocal recovery for the distal markers. Second, we also examined conversion at LEU1 , which is proximal to the insertion. When red-white-sectored Leu^+ colonies were examined, 60/168 of the nonmosaic convertants showed nonreciprocal recovery for all distal sites, including the distal plasmid. This frequency was similar to that observed for TRP5 (57/175). Thus, the pKSH heterozygosity does not appear to impede formation of the nonreciprocal genotype.

A break healing model of recombination: The nonreciprocal transfer of long tracts of genetic information

and the differential behavior of proximal and distal markers are most directly explained by a chromosome break-and-repair process. Although the initial conversion is a G1 event, chromosome breakage and repair would have to occur concurrent with or subsequent to DNA replication. Several investigators have suggested G2 resolution of G1-initiated recombination processes (KLAR and STRATHERN 1984; ROMAN and FABRE 1983) and broken chromosomes have been implicated as G1 conversion intermediates with G2 repair in *rad52* strains (HABER and HEARN 1985). The break-and-repair model, as we propose it, posits that strand transfer, mismatch repair, and Holliday junction resolution of G1 conversion events create nicks or gaps in strands of participating chromatids which sometimes remain unrepaired before the initiation of S-phase. DNA replication processes single-strand nicks to double-strand breaks. Thus, conversion of the selected locus occurs before or during DNA synthesis, and repair (with the concordant conversion of distal markers) takes place after synthesis. If a single chromatid is broken, repair processes, depending on the template used, either restore the original marker configuration or generate a 3:1 ratio of alleles distal to the break point (Figure 7, panel A). Strand lesions leading to breakage of both chromatids involved in the act of conversion (Figure 7, panel B) can result in repair processes which produce 2:2, 3:1 or mosaic allelic segregations as shown in Figure 8.

The mechanism proposed here is analogous to the "break-and-replicate" recombination processes documented in bacteriophage T4 (MOSIG 1987). In yeast, the healing of broken duplexes by using intact homologs as templates has been suggested by several investigators (RESNICK 1976; MCCUSKER and HABER 1981; FALCO *et al.* 1982; HABER and THORNBURN 1984; DUNN *et al.* 1984). Although repair of double-strand breaks in yeast is not a rapid process and requires at least 1 hr for completion (NICKOLOFF *et al.* 1989), broken chromosomes are healed with high efficiency (HABER and THORNBURN 1984). The break-and-repair model explains why proximal markers that are not near the site of conversion are seldom involved, and tightly linked proximal markers sometimes yield mosaicism (compare TRP5 mosaicism among Met^+ convertants *versus* LEU1 mosaicism among Trp^+ convertants). If the centromere-containing chromosomal fragment is partially degraded by exonuclease action before strand invasion and repair synthesis, as proposed by ORR-WEAVER and SZOSTACK (1983), this could be observed.

Evidence for gene conversion-associated chromosome breakage and repair is provided by triparental events in the trisomic strain JG419. Among the Leu^+ , Hcr^+ recombinants, information from the *leu1-c* or *leu1-12* homolog was replaced with information from the *leu1-c*, 12 donor chromosome spanning the distance between LEU1 and MET13 (113 cM) in almost every

FIGURE 7.—Chromosome breakage-healing model. Asymmetric heteroduplex spanning the *TRP5* locus during G1 is shown at the top of each panel. The initial nicks which permit strand displacement and invasion are subsequently healed by DNA ligase. Either excision repair of mismatched basepairs or resolutive cleavage of the Holliday junction leaves a single (panel A) or both (panel B) strands unligated. Replication processes the nicks to double-strand breaks. Chromosome fragments representing information distal to the break point are acentromeric and cannot segregate properly, leaving them susceptible to degradation. Retained centromeric fragments are healed in G2 by invasion of intact homologs for template-based repair synthesis. In panel A, an *ADE5* chromatid has been broken. If repair ensues using an *ade5* chromatid template, a 3:1 ratio of alleles distal to the break point results. Repair off an *ADE5* chromatid would restore the original configuration of distal markers. In theory, either chromatid participating in the conversion event has the same probability of being broken. However, breakage and repair of an *ade5* chromatid would not result in red-white-sectored colonies and, therefore, is not detected by our assay. When two chromatids are broken (panel B), repair processes can produce 2:2, 3:1 and mosaic segregation patterns, as illustrated in Figure 8.

case examined. Half of the *Leu*⁺, *Hcr*⁺ recombinants were *TRP5/trp5/trp5*, and the remainder (18/34) were *trp5/trp5/trp5*, suggesting that each of the homologs involved in the act of conversion has an equal probability of being broken. This could not be tested in the diploid strain JG303 where conversion events leading to breakage and subsequent loss of genetic information from an *ade5* chromatid do not result in a red and a white daughter, even when a genetically different homolog is used as a repair template. Thus, these events were virtually undetectable without the trisomic strain.

Mosaic colony formation

Mosaic colonies are a striking feature of both spontaneous and X ray-induced mitotic recombination (GOLIN and TAMPE 1988; ROMAN and RUZINSKI 1990). Their origin remains poorly understood and may be due to more than one mechanism. Heteroduplex DNA intermediates manifested during G1 cannot give rise to mosaic colonies unless the generation of G1 heteroduplexes is repeated in subsequent cell division cycles. Even then only the major class (class 1), comprising less than half of the mosaic-sectored colonies, can be accounted for. Lengthy G2 heteroduplex intermediates

could account, as a direct consequence of mismatch repair possibilities, for all of the observed classes of mosaic-sectored colonies. This type of long-tract heteroduplex intermediate could be generated at the four-strand stage as represented in Figure 8. The free 5' and 3' termini of a broken chromatid could invade independent homologs for template-based repair. Each of the restored strands, consequently, would be allelically distinct, and a hybrid chromosome arm would be created. DNA replication in the subsequent division cycle would resolve the heteroduplex. While this is an interesting proposal, and split-end models of double-strand break repair have been suggested previously for lambda recombinants (ROSENBERG and HASTINGS 1991), there is no evidence to suggest how the free 5' end could be primed for repair synthesis. Therefore, other possibilities must be entertained. Multiple events along the chromosome arm could produce many, if not all, of the observed classes. It is possible that the original breakage event serves as a marking mechanism for subsequent recombinatorial processes along that chromosome arm. This is reminiscent of the explanation given by ROMAN and RUZINSKI (1990); however, the molecular nature of such a marking mechanism remains unclear. In addition, the dis-

FIGURE 8.—Repair possibilities for two broken chromatids. Events which generate two broken chromatids (as depicted in Figure 7, panel B) can account, based on the nature of the repair process, for each of the observed classes of recombinants. Invasion of sister chromatids by free ends of each of the damaged duplexes will restore the parental genotype. However, invasion of nonsister chromatid by each of the broken chromatids can produce reciprocal recovery of distal alleles (A). Nonreciprocal genotypes are generated by invasion of a nonsister chromatid by one of the two damaged duplexes (B). Mosaic colonies are most easily accounted for by the generation of long regions of hybrid DNA during G_2 . This could occur if the free 3' end of each broken duplex invaded one intact homolog for template-based repair, and the 5' end invaded a genetically distinct homolog. The repair of free 5' ends might be performed by the extension of telomere-based primers. Excision repair at various mismatched loci within the hybrid chromosome arms would give rise to mosaic colonies following replicational resolution of the heteroduplexes and subsequent segregation of the altered chromosomes.

joined chromosome fragment, corresponding to the material distal to the break point, can be considered highly recombinagenic. The free ends might invade an intact duplex resulting in single- or multiple-site conversions.

A final point regarding the mosaic colonies is that in seven cases Trp^- red subclones were recovered. This could be accounted for if the original conversion occurred in G_2 and resulted in one wild-type strand on each of the participating chromatids. Subsequent replication and segregation could produce a Trp^- red cell which was cross-fed by the Trp^+ sister cells.

Interpretations for the generation of mosaic colonies and other important recombinants: We also observed additional classes of recombinants that may provide important clues about the mechanism of mitotic recombination. These include not only mosaic colonies, but trisomic subclones that are *cyh2/cyh2/cyh2* and/or Met^- . With regard to these, several tentative conclusions can be reached. First, the presence of such homozygous recombinants suggests that sometimes two homologs

are broken and repaired using the *leu1-c,12* chromosome as a template. Second, as noted by ROMAN and RUZINSKI (1990), the existence of mosaics argues for the presence of hybrid DNA during G_2 phase at least in some cases. Each of these observations is open to alternative explanations. For instance, both the homozygotes and the mosaics could be produced by multiple rounds of recombination. Indeed, this explanation was proposed by GOLIN and TAMPE (1988) in the first description of mosaic recombinants. There was, however, a fundamental difference in the basic observation made in that study. GOLIN and TAMPE (1988) looked amid coincident Leu^+ - Trp^+ convertants for mosaicism. Under these conditions, the mosaicism was not limited to three genotypes. Rather, 6/11 mosaics yielded four or more distinct genotypes. It is not clear, therefore, why a multiple round model should be limited to three clonal types. Thus considered, there may be more than one mechanism for mosaic colony production.

The detection of nonreciprocal recombinants among unselected populations of cells on complete medium

clearly demonstrates that the phenomenon is not restricted to a specific subset of gene convertants. The exact frequency of gene conversion-associated chromosome breakage cannot be determined precisely because the use of some repair templates restores the original marker configuration, and it is not known whether a bias exists in repair template selection. Additionally, a significant fraction of chromosomes broken by this process may be healed via double-strand-gap repair, and, therefore, may not result in a loss of distal genetic information. Nevertheless, the results presented here suggest a nonrandom association between mitotic gene conversion and chromosome breakage.

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