Coincident Recombination During Mitosis in Saccharomyces: Distance-Dependent and -Independent Components

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ABSTRACT

In mitosis, coincident recombination events between widely separated markers occur more frequently than expected for two independent acts. Several different mechanisms have been proposed to account for this phenomenon. It has been argued that coincident recombination could be due to either an extensive region of heteroduplex DNA or some other distance-dependent mechanism. Alternately, it has been suggested that at least some is due to subpopulations of cells which undergo recombination at very high frequencies. The purpose of these experiments is to evaluate the possible contribution of distance-dependent and distance-independent components. By comparing the coincident recombination frequencies for markers on the same homolog as well as pairs of unlinked sites, we show that there is a strong distance-dependent component for at least 8.8–35-kbp, depending on the type of recombination event (conversion or intrachromosomal exchange). For larger distances separating sites, a distance-independent mechanism(s) results in higher than expected frequencies.

DURING mitosis, coincident gene conversion events involving the linked *LEU1* and *TRP5* loci occur far more frequently than expected for two independent events (GOLIN and ESPOSITO 1981, 1984) even though these sites are separated by about 35 kbp (BALZI *et al.* 1987). Two mechanisms have been proposed to account for these observations (GOLIN and ESPOSITO 1984; ORR-WEAVER and SZOSTAK 1985). First, coincident recombinants could be due to either an extensive region of heteroduplex DNA or to some other distance-dependent mechanism such as concerted formation of multiple recombination intermediates. (GOLIN, FALCO and MARGOLSKEE 1986). Alternatively, it has been suggested that at least some coincident conversion is due to subpopulations of cells which undergo recombination at very high levels. In this paper, we provide evidence that both distance-dependent and -independent mechanisms operate to produce some of the multiple recombination events. The argument for a distance-dependent component comes from two observations. First, in an extension of work reported previously (GOLIN and ESPOSITO 1984), we show that the enhanced frequency of double conversions drops with increasing genetic distance. Second, we have used our observation that a plasmid insertion flanked by a direct repeat and located 8.8 kbp from *LEU1* is lost by recombination at high efficiency (about 30%) among convertants selected at this locus. When convertants are selected at sites that are unlinked to the insertion, the frequency of plasmid loss drops 4–5-fold. Nevertheless, plasmid instability is still greater among recombinants than it is among nonrecombinants. This latter observation argued for an additional distance-independent component such as a subpopulation of cells with enhanced recombination. Two additional lines of evidence suggest that this interpretation is plausible. When coincident convertants at *LEU1* and *TRP5* are selected and subcloned, 4% of the original coincident recombinants yield subcolonies containing mixtures of at least three genotypes for some (or all) of the genetic markers on chromosome *VII*. The simplest explanation for these results is to posit that they represent two acts of recombination separated by a cell division. In addition to these mosaic colonies, we also find a high frequency (4%) of recombination events on two different homologs once the initial Leu^+ Trp^+ convertant is selected.

MATERIALS AND METHODS

Strains and media: The genotypes of the JG300 and JG303 diploids used in this study are shown in Table 1. A fine structure map is given in Figure 1. The construction and insertion of the pJM53 and pKSH plasmid was recently described (GOLIN, FALCO and MARGOLSKEE 1986; GOLIN and FALCO 1988). These plasmid insertions contain a functional *URA3* gene. Loss of plasmid results in uracil auxotrophy. The recipes for YPD, cycloheximide and synthetic media are described elsewhere (GOLIN and ESPOSITO 1977, 1981).

Isolation of Leu^+Trp^+ coincident gene convertants: To isolate Leu^+Trp^+ coincident convertants, single colonies of the JG303 diploid were suspended in 2 ml of YPD broth and incubated at 30° for 24 hr. Cells were harvested, washed one time with sterile, distilled water, and plated on leucine-tryptophan omission media. Plates were incubated

TABLE 1

Yeast strains

Designation	Genotype	Comments
JG 33-18B	*HO a/α leu1-c trp5-c cyh2 met13-c ade5 ade2 lys2-2 tyr1-2 his7-2 ura3-1*	GOLIN and ESPOSITO (1977)
JG 34-38A	*HO a/α leu1-12 trp5-d CYH2 met13-d ADE5 ade2 lys2-1 tyr1-1 his1 ura3-313*	GOLIN and ESPOSITO (1977)
JG44	JG33-18B × JG34-38A	
JG 231	JG34-38A with pJM53 (*URA3*) integrated between *leu1-12* and *trp5-d*	GOLIN, FALCO and MARGOLSKEE (1986)
JG 300	JG 231 × JG 33-18B	
JG 267	JG 34-38A with pKSH (URA3) integrated between *leu1-12* and *trp5-d*	
JG 303	JG 267 × JG 33-18B	

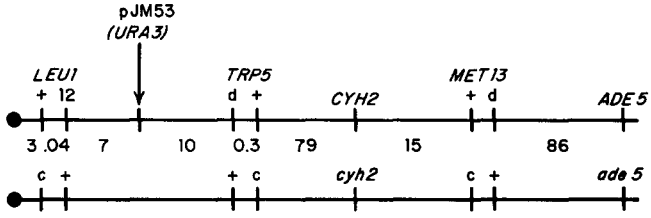

FIGURE 1.—Fine structure of the *LEU1-TRP5* region. The diploids employed in this study carry a series of markers on chromosome *VII* used to monitor recombination. The site of pJM53 (and pKSH) integration is indicated by an arrow. In addition, there are three pairs of noncomplementing alleles *LEU1*, *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).

at 30° for 4 days. The resulting convertants were analyzed further as described below.

Measurement of recombination frequencies: Recombination frequencies were determined as previously described (MALONE, GOLIN and ESPOSITO 1980; GOLIN and ESPOSITO 1981).

Selection of independent convertants: Selection of independently derived convertants has been described (GOLIN and ESPOSITO 1981, 1984).

Analysis of genotypes: The complete genotype for each recombinant was determined by replica plating as previously described (GOLIN and ESPOSITO 1977, 1981).

RESULTS

Evaluating the fraction of distance-dependent coincident recombination events: To determine whether coincident recombination has a distance-dependent component, we took advantage of our observation that a plasmid insertion, pJM53, located 8.8 kbp from the *LEU1* locus and 27 kbp from the *TRP5* locus, is lost with high efficiency (70%) among Leu^+Trp^+ coincident convertants (GOLIN, FALCO and MARGOLSKEE 1986). As a result, cells are rendered auxotrophic for uracil. This is most likely due to intrachromosomal exchange between a set of 6.1 kbp direct repeats which are generated by plasmid insertion (GOLIN, FALCO and MARGOLSKEE 1986). We evaluated the loss of pJM53 as a function of distance. Independently derived convertants were selected at the heteroallelic *LEU1*, *TRP5* or *MET13* loci. These are located, 7, 13 and 90 cM, respectively, from the pJM53 insertion. In addition, convertants were selected at the unlinked *TYR1* site. The recombinants were tested to determine whether they were Ura^-. The results are found in Table 2. Roughly 27% of all Leu^+ convertants (which also remained heterozygous for distal markers) lost the pJM53 insertion. The Trp^+ and Met^+ recombinants showed significantly less loss of the plasmid. In fact, the loss at these latter two loci was probably no greater than from the unlinked Tyr^+ convertants. Still, the value (7%) is considerably higher than the nonrecombinant control (0 of 500).

Coincident gene conversion is distance dependent: The evidence presented above argues for a strong distance-dependent component of coincident recombination operating in the *LEU1*-pJM53 interval. The assay measured heteroallelic conversion at *LEU1* and most probably intrachromosomal exchange at pJM53 (GOLIN, FALCO and MARGOLSKEE 1986). Previously, we showed that coincident, heteroallelic gene conversion at *LEU1* and *TRP5* had a distance-dependent component because the enhancement observed at these sites was six times greater than observed for coincident conversion at the *LEU1* and the distal (90 cM) *MET13* loci (GOLIN and ESPOSITO 1984). The analysis was extended using the same strain (JG44) to evaluate coincident conversion events at two unlinked sites: *LEU1* and *TYR1*. Cultures were grown and plated as previously described and the frequency of Leu^+Tyr^+ convertants measured. The results are found in Table 3. The enhancement for unlinked markers is, at most, four to eight times higher than expected for two independent events. In contrast, the Leu^+Trp^+ recombinants show a 60-fold enhancement.

Detection of Leu^+Trp^+ recombinants with more than one genotype: The plasmid loss experiment and coincident conversion experiments suggest that both distance-dependent and -independent coincident recombination exist. Thus, plasmid loss among selected convertants is higher than nonrecombinant controls, but such loss also declines with genetic distance. This indicated that there might be a subpopulation of cells with enhanced capacity for recombination. We reasoned that if this was the case, the effect might persist

TABLE 2

Loss of pJM53 as a function of distance[a]

Locus analyzed	Distance from pJM53	No. convertants tested	No. Ura$^-$	Percent Ura$^-$	95% limits
LEU1	7 cM, 8.8 kbp[b]	303	81	27	22–32
TRP5	13 cM, 27 kb[c]	215	16	8	4–14
MET13	120 cM	127	1	<1	0–5
TYR1	Unlinked	200	14	7	2–13
Nonrecombinant		500	0	<.2	

[a] Some data for the *LEU1* and *TYR1* loci appeared previously (ESPOSITO *et al.* 1985).
[b] JEANNE MARGOLSKEE, personal communication.
[c] BALZI *et al.* 1987.

TABLE 3

Enhancement of various coincident conversion events

Genetic loci	Frequency $(A)/10^5$	Frequency $(B)/10^5$	Frequency $(/10^{10})$ $(A)(B)$ observed	Frequency $(/10^{10})$ $(A)(b)$ expected	Enhancement observed/expected
LEU1, TRP5[a,b] (A) (B)	2.6	1.9	300	4.9	61
TRP5, MET13 (A) (B)	2.6	1.2	140	3.1	44
LEU1, MET13[a] (A) (B)	2.1	1.3	16	2.7	6
LEU1, TYR1[c] (A) (B)	1.9	.25	<2	0.47	<4

[a] Data are from GOLIN and ESPOSITO (1984). Comparable results for the *LEU1, TRP5* convertants were obtained recently (GOLIN, FALCO and MARGOLSKEE 1986; GOLIN and FALCO 1988).
[b] At least nine cultures of the diploids were plated to obtain median frequencies.
[c] Among 47×10^8 cells plated on leucine, tyrosine omission media, no instances of Leu Tyr coincident conversion were observed. If a single coincident convertant was recovered, the frequency would be approximately $0.02/10^8$. Had there been a 60-fold enhancement, *ca.* 18 colonies would have been recovered.

for several cell divisions. Two recombination events separated by a cell division would yield subcolonies with more than two genotypes as shown in Figure 2. To determine whether coincident convertants segregated colonies of mixed genotypes, we collected 267 independently derived Leu$^+$Trp$^+$ recombinants. Each of these was streaked on nonselective (YEPD) media and 12 of 20 of the resulting subcloned colonies were picked. The complete genotype of each was determined. The results are found in Table 4.

Approximately 11% (31 of 267) of the Leu$^+$Trp$^+$ convertants segregated colonies with two or more distinct genotypes for the markers on chromosome 7. Many of the colonies containing just two genotypes are best explained as having resulted from a coincident conversion event at the *LEU1, TRP5* sites which is coupled to an intergenic exchange. If a recombinant is plated before mitotic segregation occurs, a colony with cells of two genotypes can be produced [see GOLIN and ESPOSITO (1981) for discussion].

Of particular interest are the 11 coincident convertants which segregated three or more genotypes when the subclones were tested. Such a mosaic colony could be indicative of two or more recombination events. A summary of these recombinants is found in Table 5. Some of the genotypes are produced in relatively few steps. The genotypes of colony M-2, for example, can be accounted for by two acts of recombination. In the first step, an act of gene conversion at *CYH2* produces a *CYH2/CYH2* daughter cell that is nevertheless heterozygous at *MET13* and *ADE5*. Cell division and a second recombination event results in a *Ura/Ura*-genotype among some of the subclones.

Several mosaic colonies which segregated numerous genotypes were produced by many acts of conversion and/or exchange.

Appearance of Leu$^-$ and Trp$^-$ coincident convertants: Some Leu$^+$Trp$^+$ coincident convertants yielded subcolonies that were Leu$^-$ or Trp$^-$. Since selection initially occurred on leucine-tryptophan omission media, this observation was unexpected. One possible explanation is that these auxotrophs were cross-fed by the other Leu$^+$Trp$^+$ cells.

Recombination events on two chromosomes: Roughly 5% (14 of 267) of the Leu$^+$Trp$^+$ coincident conversions were also recombinant for markers on other chromosomes. These genotypes are summarized in Table 6. There were instances of gene conversion as well as intergenic exchange. For ex-

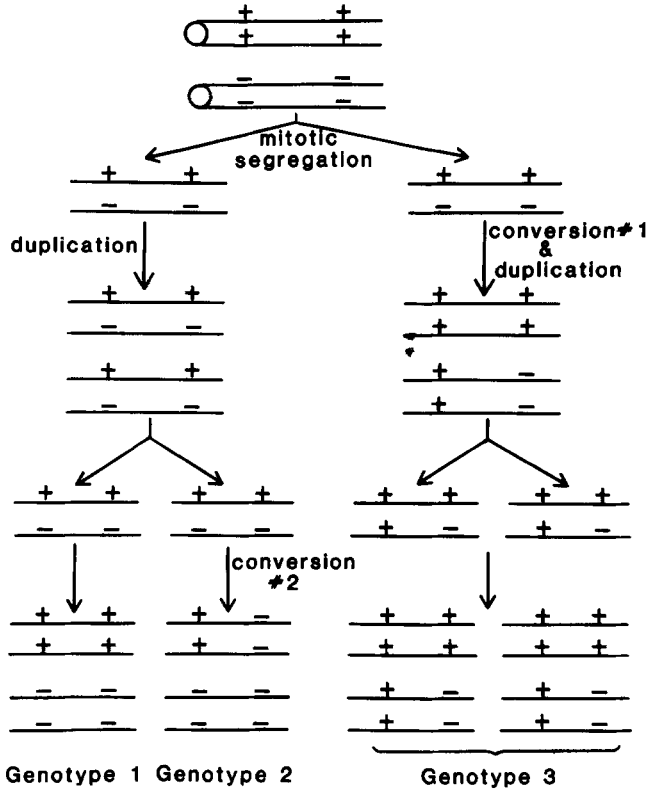

FIGURE 2.—Generation of a mosaic colony. A mosaic colony is one that yields three or more genotypes when subcolonies are picked and tested. The explanation favored for mosaicism is that such colonies represent two acts of recombination separated by cell division.

TABLE 4

Summary of recombination events

Total Leu$^+$Trp$^+$ analyzed	No. of colonies with: Two genotypes	Three or more genotypes (mosaics)	Multiple chromosome involvement
267	20 (0.07)	11 (0.04)	14 (0.05)

ample, colony 13 is Tyr$^+$ and had therefore undergone gene conversion at the *TYR2* locus. It remained heteroallelic at *LYS2* and heterozygous at *HIS7*. In contrast, colony 8 was homozygous (homoallelic) for all three genes on chromosome *II* and probably resulted from intergenic exchange.

Analysis of recombination in cells that did not undergo coincident gene conversion: Two controls were performed to determine whether mosaic colonies and multichromosome recombinants arise selectively in coincident, Leu$^+$Trp$^+$ convertants.

In the first experiment, the JG303 strain was plated out on nonselective (YPD) media. Of the resulting colonies, 103 were streaked on YPD for single subcolony isolation. Twelve subcolonies were picked and tested in a fashion identical to the Leu$^+$Trp$^+$ coincident convertants. Virtually all colonies retained the original JG303 genotype at all loci on chromosome *VII*, *V* and *II*. Among such colonies, there were no instances of mosaicism for the markers on chromosome *VII*. Rare, single recombination events were found for the *CYH2* and *ADE5* loci on chromosome *VII*. There were three separate cases of recombina-

TABLE 5

Mosaic colonies

Colony		No. subclones recovered	Phenotypes at:[a] L	U	T	C	M	A
M-1	a	6	+	+	+	H	H	H
	b	5	+	+	+	S	H	H
	c	2	+	−	+	S	H	H
M-2	a	13	+	+	+	H	ND	H
	b	5	+	+	+	S	ND	H
	c	4	+	−	+	H	ND	H
M-3	a	9	+	+	+	H	ND	−
	b	2	+	+	−	H	ND	H
	c	1	−	+	+	H	ND	−
	d	1	+	+	+	H	ND	H
	e	1	−	+	+	H	ND	H
M-4	a	14	+	+	+	R	ND	−
	b	1	+	+	−	R	ND	H
	c	1	+	+	+	H	ND	−
	d	1	+	+	+	H	ND	H
M-5	a	9	+	−	+	S	H	H
	b	1	+	−	+	H	H	H
	c	1	+	+	−	H	H	H
M-6	a	5	+	+	+	R	c	−
	b	5	+	+	+	H	c	+
	c	1	+	−	+	H	c	+
	d	1	+	−	+	R	c	−
M-7	a	6	+	−	+	H	d	+
	b	4	+	−	−	R	d	−
	c	2	+	−	+	R	c	−
M-8	a	9	+	−	+	S	d	+
	b	4	+	−	+	H	d	+
	c	3	+	−	+	R	c	−
	d	1	+	−	−	R	d	+
	e	1	+	−	−	R	c	+
	f	1	+	−	−	H	c	−
M-9	a	4	+	+	+	S	H	H
	b	4	+	−	−	H	H	H
	c	3	+	+	+	H	H	H
	d	1	+	−	+	H	H	H
M-10	a	6	+	+	+	H	ND	H
	b	5	+	+	+	S	ND	+
	c	2	+	−	−	H	ND	+
M-11	a	6	+	+	+	H	ND	H
	b	2	−	+	+	H	ND	H
	c	2	+	+	+	H	ND	−
	d	2	−	+	+	H	ND	−

[a] The abbreviations are as follows: (1) L: *LEU1*, U: pKSH, T: *TRP5*, C: *CYH2*, M: *MET13*, A: *ADE5*. (2) For L, U, T, + means the cell is a prototroph, − means the cell is an auxotroph, (3) For *CYH2*: H means heterozygous, S means sensitive (*CYH2/CYH2*) and R means resistant (*cyh2/cyh2*). (4) For *ADE5*: H means heterozygous, − means *ade5/ade5* (white) and + means *ADE5/ADE5*. ND = not determined.

TABLE 6

Multiple chromosome recombinants

Recombinant No.	Description
1	· All subclones *his7/his7*
2	· All subclones *his1/his1, his7/his7*
3	Mixture of *his1/his1, HIS1/his1* subclones
4	· All subclones *his1/his1, his7/his7*
5	· All subclones *his7/his7*
6	· All subclones homoallelic at *lys2*
7	· All subclones *LYS2* (Lys$^+$), *TYR1* (Tyr$^+$), *his7/his7*
8	· All subclones homoallelic at *lys2, tyr1, his7/his7*
9	· All subclones *his7/his7*
10	· All subclones homoallelic at *lys2, tyr1*
11	· All subclones homoallelic at *lys2*
12	One subclone is *LYS2* (Lys$^+$)
13	· All subclones *TYR1* (Tyr$^+$)
14	Two subclones *TYR1* (Tyr$^+$)

TABLE 7

Summary of multichromosome recombinants

Description	No. analyzed	No. recombinants	Percent
JG303 coincident convertants	420	19	4.5
JG303 control (nonrecombinant)	2175	2	0.09

tion at the *ADE5* locus to produce a white *ade5/ade5* colony. In each case, the recombinants made up a small (*ca.* 1/100) proportion of the otherwise red subcolonies (these recombinants were deliberately picked and would likely have been missed if 12 subcolonies were selected at random). There was also one *CYH2/CYH2* recombinant among 12 subcolonies from control C-62. This single subclone was homoallelic for the chromosome *II* loci as well. In addition, C-27 contained a single subcolony that was *HIS1/HIS1*.

In the second experiment, we made further use of material from the leucine-tryptophan omission plates. Generally, when a culture of JG303 is plated on such media, a few Leu$^+$Trp$^+$ convertants arise as single colonies. The remaining cells do not grow. They can, however, be recovered by restreaking a patch of the material on a YPD plate. For about one-third of the Leu$^+$Trp$^+$ convertants analyzed, this corresponding control was performed. In each case, 12 subcolonies were picked and tested. This control determined whether a given mosaic colony was due to an infrequent, jackpot of recombinants unrelated to the act of coincident gene conversion. There were no detectable cases of spontaneous recombination among 88 of these controls. A summary of all the data for the chromosome *II* markers is found in Table 7. It includes an analysis of *ca.* 150 additional Leu$^+$Trp$^+$ convertants which were picked and tested for their genotype at the chromosome *II* loci, but were not tested for a mosaic genotype by the subcloning procedure. The expected frequency of coincident convertants which are recombinant for chromosome *II* markers can be estimated as: (frequency of coincident Leu$^+$Trp$^+$ convertants) (frequency of spontaneous recombination events on chromosome *II* among controls C1-C103). This is about (3×10^8) (10^3) or 3×10^{11}. The observed frequency is (3×10^8) (0.05) or 1.5×10^9. A 10–70-fold enhancement is found (when the 95% confidence limits are used in the calculation). The data underestimate the enhancement since the three Leu$^+$Trp$^+$ recombinants (3, 12 and 14—see Table 6), which were genotypically mixed for the chromosome *II* loci, were not included in this calculation.

TABLE 8

Recombination at the *CYH2* locus in JG303 and M-9

Strain	Culture No.	Total *cyh2/cyh2* $\times 10^4$
M-9	1	2.0
	2	9.1
	3	2.0
	4	1.8
	5	2.0
	6	3.7
	7	2.3
Median frequency		2.3
JG303	1	7.0
	2	3.0
	3	2.5
	4	1.8
	5	1.9
	6	3.3
	7	6.5
Median frequency		3.0

Mosaic colonies do not represent the action of hyper-recombination mutations: It could be argued that, since Leu$^+$Trp$^+$ coincident convertants arise at low frequency, some mosaic colonies represent the action of newly selected dominant or semi-dominant hyper-recombination mutations (GOLIN and ESPOSITO 1977). To determine whether this was the case, we measured the frequency of mitotic recombination leading to cycloheximide resistance in *CYH2/cyh2* subclones of the mosaic colony M-9. The frequencies were compared to those obtained from the JG303 diploid. The results, shown in Table 8, demonstrate that there is no significant difference between the strains. Thus, the M-9 colony does not carry a dominant hyper-recombinogenic mutation.

DISCUSSION

Distance dependency of some coincident recombination: The results presented in this paper argue that both distance-dependent and distance-indepen-

dent mechanisms contribute to the phenomenon of coincident recombination. Our analysis of pJM53 loss as a function of distance from a site of gene conversion argues that the concomitant events have a strong distance component for perhaps 8–10 kbp. Thereafter, coincident recombination becomes largely distance independent. For example, loss of pJM53 from Trp^+ convertants is no greater than loss among unlinked Tyr^+ convertants. These results are similar but not identical to those found for coincident heteroallelic conversions (GOLIN and ESPOSITO 1984; this study). In these studies coincident gene conversion rates at the *LEU1* and *TRP5*, *MET13*, or *TYR1* sites were determined. *LEU1 TRP5* coincident convertants occurred about ten times more frequently than the *LEU1 MET13* or *LEU1 TYR1* counterpart.

Plasmid loss *vs.* gene conversion experiments: Both the plasmid loss and coincident heteroallelic recombination experiments suggest distance-dependent components; however, they differ in the extent to which such a mechanism operates. The plasmid loss experiment suggests that distance dependence extends to the plasmid insertion, but does not include *TRP5*. The coincident conversion experiment indicates that the distance-dependence mechanism(s) extends to that site. The discrepancy may reflect the fact that each study assays a different feature of recombination. The plasmid loss experiment measured conversion at *LEU1* and coincident intrachromosomal exchange while the other study examined at acts of gene conversion at two loci. Perhaps the dependency on distance is different for each. This could, for example, reflect different recombination pathways. It is known that, while the *rad52* mutation causes a profound depression in gene conversion, its effect on intergenic exchange is less pronounced (see MALONE and ESPOSITO 1980; PRAKASH *et al.* 1980).

Subpopulation of cells with increased levels of mitotic recombination: The results presented in this study also argue that, although vegetative recombination is rare, additional acts of conversion and/or exchange may occur more readily in a subpopulation of cells. Similar findings have been reported by others as well for both spontaneous and X-ray-induced mitotic recombination (FOGEL and HURST 1963; MINET, GROSSENBACHER-GUNDER and THURIAUX 1980; MONTELONE, PRAKASH and PRAKASH 1981). Mosaic colonies suggest that the factor(s) required for recombination persist over at least several cell divisions once they are synthesized or assembled. The difference between a normal mitotic cell and one with increased capacity for recombination could be relatively small. NICKOLOFF, CHEU and HEFFRON (1986) have shown that the action of the HO (mating type) nuclease near a site where recombination is assayed is sufficient for the efficient induction of conversion.

Frequency of mosaic clones is probably an underestimate: In this paper, we show that coincident multiple events separated in space and time are common among selected Leu^+Trp^+ convertants. It is likely that this frequency is an underestimation. Some Leu^+Trp^+ recombinants that *did not* yield mixed subclones could have been the product of events that took place in earlier generations and segregated to purity prior to plating. In addition, the abundance of clones that display only one member of a particular genotype suggests that the screening method is missing additional mixed clones.

Other explanations for mosaic colonies: Two additional explanations for mosaic colonies bear discussion. Since it is known that some diploid strains undergo meiosis and ascospore formation at low levels even in nitrogen-rich media, it could be argued that the mosaic colonies arise from a small subset of cells engaged in meiosis. The mosaic colonies that we recovered did not have a meiotic exchange or segregation of chromosomes, since, in all 11 instances, they retained heteroallelic or heterozygous configurations for all the markers on the other linkage groups. We cannot rule out the interesting possibility that a subset of cells were induced for some products that increase recombination and are generally meiotic specific. A second explanation for mosaic colonies is that they are the result of long heteroduplexes formed in G-2 which are incompletely repaired (ROTHSTEIN 1984). Following chromosome segregation, more than three genotypes could be recovered. This scenario also remains a possibility.

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