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Gene conversion and associated reciprocal recombination are separable events in vegetative cells of *Saccharomyces cerevisiae*

(mitotic exchange/heteroduplex/half-chiasma/cleavage/strand isomerization)

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ABSTRACT **Mitotic gene conversion occurs in both the G1 and G2 phases of cell growth. We postulate that the DNA strand or strands that connect the duplexes in G1 as a consequence of strand transfer are cleaved by an endonuclease. When this takes place in G1, crossing-over occurs in G2 at a frequency that is higher than would be expected from independent events. Conversion and associated reciprocal recombination are therefore separable with respect to the stage in the cell cycle when each occurs and possibly with respect to mechanism.**

Gene conversion and reciprocal recombination (crossing-over) have been demonstrated both in vegetative cells and in cells undergoing meiosis in the yeast *Saccharomyces cerevisiae*. In meiotic cells, it is generally thought that gene conversion results from the following series of events: One of the strands of a DNA duplex breaks away from its complementary strand and invades the homologous duplex by replacing a strand of the same polarity there. Gene conversion is detected if the newly formed heteroduplex is noncomplementary for one or more base pairs. Noncomplementarity in the heteroduplex is corrected by excision of either one of the two strands and by repair synthesis using the other strand as a template. Heteroduplexes can be formed simultaneously in the two homologous duplexes, involving the same stretch of DNA (symmetrical hybrid DNA). In this case, repair can occur in both heteroduplexes or in one or the other. Another parameter is the resolution of the half-chiasma that ties the two duplexes together when symmetry obtains. Resolution can be by cleavage of the two strands involved in the half-chiasma, with the consequence that nearby markers are not recombined, or by cleavage of the uninvolved strands, in which case the outside markers recombine with reference to the locus undergoing gene conversion. A theoretical model of hybrid DNA was suggested by Holliday (1) to account for gene conversion and was later incorporated in a general theory of recombination to include both symmetrical and asymmetrical hybridization by Meselson and Radding (2). The alternative cleavage possibilities were suggested as consequences of the geometric isomerization of the two DNA duplexes (3). Thus, a physical basis was established for the well-known correlation between conversion and the reciprocal recombination of nearby markers.

Mitotic gene conversion and associated reciprocal recombination seemed to follow similar rules. However, Esposito (4) and Fabre (5) found that gene conversion could occur in the G1 phase of cell growth, a result anticipated by Wildenberg (6). It had also been assumed (7) that the cell was at the four-chromatid stage, corresponding to pachytene in meiosis, when recombination occurred. Fabre made use of *cdc4*, a cell division cycle mutant that is temperature sensitive and becomes arrested in G1 at 35°C. A diploid heteroallelic for *cdc4* produced colonies at 35°C at a rate suggestive of gene conversion. Golin and Esposito (8) analyzed sectored colonies in which conversion had occurred and concluded (*i*) that conversion was a G1 event exclusively or nearly so and (*ii*) that sectoring was the result of replication without cleavage of the half-chiasma (Fig. 2).

It is the purpose of this paper to offer an alternative explanation for the sectored colonies. We intend to show that (*i*) gene conversion can occur in G2 as well as in G1, albeit with lower frequency, and (*ii*) that cleavage of the strands forming the half-chiasma occurs in G1. Cleavage in G1 by itself will not result in a sectored colony after DNA replication. A crossover in G2 is required to achieve sectoring.

MATERIALS AND METHODS

Strain. The diploid strain C821 was used in all experiments. The genotype of this strain is *trp5 LEU1 ade6-21 CLY8 SUC1 mal1/TRP5 leu1 ade6-1 cly8 suc1 MAL1* in the chromosome VII linkage group (9). The strain is heteroallelic for *ade6* and requires adenine for growth. The symbols *trp5* and *leu1* stand for mutant genes effecting tryptophan dependence and leucine dependence, respectively; *cly8* is responsible for temperature sensitivity in a strain that was isolated by L. H. Hartwell and that stops growth at 35°C. *SUC1* and *MAL1* are codominants that cause the fermentation of sucrose and maltose, respectively. The average distances in centimorgans between successive intervals are: *trp5*-18-*leu1*-2-centromere-30-*ade6*-20-*cly8*. *SUC1* and *MAL1* are distal to *cly8* but show no linkage with it. *SUC1* and *MAL1* segregate as if they were alleles and their placement with respect to each other is arbitrary. We infer from recombination studies that *ade6-21* is nearer the centromere than is *ade6-1* (unpublished data).

Media. Cells were grown to logarithmic or stationary phase in YEP, consisting of, per liter, 10 g of yeast extract, 20 g of peptone, and 20 g of glucose, supplemented with 40 mg of adenine; 20 g of agar was added for solid medium. Diagnostic plates for sucrose fermentation and maltose fermentation were made by substituting sucrose (20 g/liter) or maltose (40 g/liter) for glucose. Before pouring, a 1.5% solution of bromthymol blue was added to the sucrose medium at 5 ml/liter and the pH was adjusted to 7.6 or a 0.4% solution of bromcresol purple was added to the maltose medium at 9 ml/liter and the pH was adjusted to 6.

Methylbenzimidol-2-yl Carbamate (MBC) Stock Solution. This stock solution was prepared by dissolving MBC in dimethyl sulfoxide at 20 mg/ml. Various amounts of the stock solution were added to cells growing in YEP (see *Experimental Procedures*).

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Abbreviation: MBC, methylbenzimidol-2-yl carbamate.

Source of Radiation. A Machlett OEG-60 x-ray tube, operated at 50 kV and 20 mA, was used. The dose rate was 110 rad/sec (1 rad = 0.01 gray).

Experimental Procedures. We obtained sectored colonies by following a procedure similar to that of Esposito (4). In our experiments, 10^6 cells of the genotype and disposition diagrammed in Fig. 2 were x-rayed and plated on each plate of adenineless medium, to select for convertants at the *ade6* locus. The colonies were then replicated to indicator media to identify colonies that were sectored for the *SUC1* gene on one side and *MAL1* on the other. The number of sectored colonies picked up from the platings of G1 cells in three experiments were 9/1,857 cells plated, 13/2,601, and 10/3,993; the numbers in four MBC experiments were 5/1,892, 12/2,532, 13/6,091 and 15/2,576. There were less than 100 colonies per adenineless plate, except for one of the MBC experiments, in which there was an average of 260 colonies per plate. This experiment also gave a relatively low yield of sectored colonies, 13/6,091, probably due to crowding that made the picking of sectored colonies uncertain. The colonies that showed sectoring were spread again on adenineless medium and retested on indicator plates to verify that they were SUC^+ MAL^- and SUC^- MAL^+ as expected. The two sides of each sectored colony therefore were subjected to two preconditions: both sides of the sector had to be ADE^+ and one side had to be SUC^+ MAL^- and the other SUC^- MAL^+. The cells of each side of the sectored colony were sporulated and a sufficient number of asci were dissected to identify the disposition of the markers on chromosome VII of the daughter cells from which the colony arose.

In three of the four experiments, stationary-phase cells (G1) were exposed to MBC for 7 hr at a concentration of 10 μg per 10^8 cells per ml of fresh YEP; in one experiment (80-6-24, Fig. 1) logarithmic-phase cells were similarly exposed for $3^1/_2$ hr in 50 μg of MBC per 4×10^6 cells per ml. MBC has been shown to interact with fungal tubulin and thus to affect spindle formation (10); yeast cells are arrested in mitosis after DNA replication (11). In our hands, using lower concentrations of MBC and shorter exposures to avoid loss of viability, the block was not complete. Fig. 1 shows the pattern of budding of cells held under the two different conditions of growth. The cells were stained by the 4,6-diamidino-2-phenylindole technique (12) to reveal their nuclei. The most numerous type in each case was a doublet in which one cell had a single nucleus and the other had none. We assume that the nucleus of this doublet was in G2. Confirmation of DNA replication was obtained by the diaminobenzoic acid fluorometric method (13), which showed that there was approximately a doubling in DNA content in cells blocked by MBC compared with the amount present in G1 cells.

The frequency of induced convertants (x-ray dose = 3,300 rad) in G1 cells is higher than in those blocked by MBC. In four experiments the induced frequencies were 501, 540, 588, and 532 per 10^6 cells in G1. The corresponding values at the MBC block were 156, 114, 167, and 109 per 10^6 cells, counting each doublet as two cells. The frequencies of preexisting prototrophs averaged 4% and 11%, respectively, of the number of prototrophs in each set of experiments.

Expt.	Budding Pattern				Total P.U.
	⊙	⊙⊙	⊙⊙	⊙⊙	
80-1-24	52(10)	288(57)	79(16)	88(17)	507
80-6-24	0(0)	133(80)	20(12)	13(8)	166

FIG. 1. Cell types after exposure to MBC. Stationary-phase cells of 80-1-24 were held in MBC for 7 hr, then they were stained with 4,6-diamidino-2-phenylindole for cytological observation and x-irradiated for sectoring. Logarithmic-phase cells of 80-6-24 were held in MBC for $3^1/_2$ hr and were then handled like 80-1-24. Percent of each type is given in parentheses. P.U., plating unit.

RESULTS AND DISCUSSION

The data obtained from sectored colonies induced by x-ray treatment of cells in stationary phase or cells blocked by MBC are shown in Table 1. There are 77 sectored colonies, 32 from cells treated in stationary phase and 45 from cells treated at the MBC block. The colonies fall into 25 distinguishable categories. Two of these, 6 and 23, do not have *cly8* in either sector. We assume, particularly because there is no sign of gene conversion at the *cly8* locus in any of the other cases, that these represent an event, such as gene conversion, that occurred some time prior to the time of irradiation, to produce a cell homozygous for the *CLY8* allele.

The experimental procedure required an *ADE6* gene in both sectors of a colony. In addition, there were two instances that could be due to conversion at other loci. In category *18*, the single colony of this genotype exhibited a 3:1 segregation at the *leu1* locus; in category *4*, one of the colonies segregated 1:3 for *MAL1*. All other loci segregated 2:2. Thus there was no evidence for simultaneous conversions of distal markers as was reported earlier for spontaneous conversion (4, 8).

Evidence that Gene Conversion Occurs in G2 as Well as in G1. If gene conversion occurred exclusively in G2, only one type of sectored colony would be observed, that in which both *ade6* alleles are found, one in each sector of a colony. These are of the type shown in categories *1*, *2*, and *3* (Table 1). There are, however, only two cases of this type from stationary-phase cells, whereas there are 12 from MBC blockage. This difference is significant at $P = 0.025$. Thus we conclude that gene conversion can take place in G2 as well as G1. Using other techniques, L. H. Johnston (personal communication) has reached the same conclusion.

A number of assumptions underlie the foregoing conclusion. To obtain sectored colonies in which each side carries a different *ade6* allele from an event occurring in G1, there would have to be symmetrical hybrid DNA including the *ade6* locus and a repair process that would leave one *ade6-21* (− +) and one *ade6-1* (+ −) unrepaired (4). The other way of obtaining the same result is to have gene conversion take place in G2 between two homologous chromatids, the other two chromatids being uninvolved and going each to a different one of the two daughter cells that are to produce the sectored colony (4, 7). 4,6-Diamidino-2-phenylindole staining of the cells in MBC showed that the majority of cells at the block were candidates for being in G2, having one nucleus and a bud of approximately the same size as the mother cell (Fig. 1). The others either had a single nucleus in each cell and were probably in G1 or had a nucleus stretched between two cells and were probably at a later stage than G2. Since conversion occurs with a higher frequency in G1, the finding that some 33 of 45 sectored colonies from MBC-blocked cells showed evidence of G1 gene conversion is consistent with the staining results.

That MBC has no marked effect on DNA repair can be seen by comparing the frequency of conversion in G1 cells exposed to MBC and those that were not exposed (categories *4–25*, Table 1). The cells are known to be in G1 if the sectored colonies that they produce have a different input allele in each sector, as in categories *1*, *2*, and *3*. Thus, of 63 colonies that fall into this category, 37 have *ade6-21* in each sector, 21 of which were from MBC-treated cells and 16 were not. The relative proportions are 21/33, vs. 16/30. Of those that had *ade6-1* in each sector, 8 were from MBC-treated cells and 9 were not. Finally, among those that had 3 of the 4 chromatids converted to new

Table 1. Categories of sectored colonies obtained from x-irradiation of cells in G1 (stationary phase) and after exposure to MBC

Category	Mutant *ade6* in each sector	*TRP5*	*LEU1*	*ADE6-21*	*ADE6-1*	*CLY8*	*SUC1*	*MAL1*	MBC	G1
1	21/1	−	+	−	+	+	+	−	8	1
		+	−	+	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	+	−	−	−	+		
2	21/1	−	+	−	+	+	+	−	1	1
		+	−	+	+	+	+	−		
		−	+	+	−	−	−	+		
		+	−	+	+	−	−	+		
3	21/1	−	+	+	+	+	+	−	3	0
		+	−	−	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	+	−	−	−	+		
4	21/21	−	+	−	+	+	+	−	15	10*
		+	−	+	+	+	+	−		
		−	+	−	+	−	−	+		
		+	−	+	+	−	−	+		
5	21/21	−	+	−	+	−	+	−	0	1
		+	−	+	+	−	+	−		
		−	+	−	+	+	−	+		
		+	−	+	+	+	−	+		
6	21/21	−	+	−	+	+	+	−	1	0
		+	−	+	+	+	+	−		
		−	+	−	+	+	−	+		
		+	−	+	+	+	−	+		
7	21/21	−	+	−	+	+	+	−	2	2
		+	−	+	+	−	+	−		
		−	+	−	+	+	−	+		
		+	−	+	+	−	−	+		
8	21/21	−	+	−	+	−	+	−	1	0
		+	−	+	+	+	+	−		
		−	+	−	+	−	−	+		
		+	−	+	+	+	−	+		
9	21/21	−	+	+	+	+	+	−	1	0
		+	−	−	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	−	+	−	−	+		
10	21/21	−	+	+	+	+	+	−	0	2
		+	−	−	+	+	+	−		
		−	+	−	+	−	−	+		
		+	−	+	+	−	−	+		
11	21/21	−	+	−	+	+	+	−	1	1
		+	−	+	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	−	+	−	−	+		
12	1/1	−	+	+	+	+	+	−	3	3
		+	−	+	−	+	+	−		
		−	+	+	+	−	−	+		
		+	−	+	−	−	−	+		
13	1/1	−	+	+	+	+	+	−	3	3
		+	−	+	−	−	+	−		
		−	+	+	+	+	−	+		
		+	−	+	−	−	−	+		
14	1/1	−	+	+	+	−	+	−	1	0
		+	−	+	−	+	+	−		
		−	+	+	+	+	−	+		
		+	−	+	−	−	−	+		
15	1/1	−	+	+	−	−	+	−	1	0
		+	−	+	+	+	+	−		
		−	+	+	−	−	−	+		
		+	−	+	+	+	−	+		
16	1/1	−	+	+	−	+	+	−	0	1
		+	−	+	+	−	+	−		
		−	+	+	−	+	−	+		
		+	−	+	+	−	−	+		
17	1/1	−	+	+	−	+	+	−	0	1
		+	−	+	+	+	+	−		
		−	+	+	−	−	−	+		
		+	−	+	+	−	−	+		
18	1/1	−	+	+	−	+	+	−	0	1
		+	+	+	+	+	+	−		
		−	+	+	−	−	−	+		
		+	−	+	+	−	−	+		
19	21/21,1	−	+	−	+	+	+	−	1	2
		+	−	+	+	+	+	−		
		−	+	−	−	−	−	+		
		+	−	+	+	−	−	+		
20	21,1/1	−	+	−	−	+	+	−	1	0
		+	−	+	+	+	+	−		
		−	+	+	−	−	−	+		
		+	−	+	+	−	−	+		
21	21,1/1	−	+	−	−	+	+	−	1	0
		+	−	+	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	+	−	−	−	+		
22	+/21	−	+	+	+	+	+	−	0	1
		+	−	−	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	+	+	−	−	+		
23	+/21	−	+	+	+	+	+	−	0	1
		+	−	−	+	+	+	−		
		−	+	+	+	+	−	+		
		+	−	+	+	+	−	+		
24	+/1	−	+	+	+	+	+	−	1	0
		+	−	+	+	+	+	−		
		−	+	+	−	−	−	+		
		+	−	+	+	−	−	+		
25	+/1	−	+	+	+	+	+	−	0	1
		+	−	+	+	+	+	−		
		−	+	+	+	−	−	+		
		+	−	+	−	−	−	+		

The genotypes of the two sectors are shown above and below the horizontal line for each category. *LEU1* and *TRP5* are on one side of the centromere; *ADE6* and the fermentation markers *SUC1* and *MAL1* are on the other side (see text). With MBC there were 45 cases from 13,091 cells plated; with G1 cells there were 32 cases from 8,451 cells.

*One was $\begin{matrix} - & + & - & + & - \\ + & - & + & + & - \end{matrix}\begin{pmatrix} - & + \\ - & - \end{pmatrix}$ on one side; brackets indicate ambiguity of placement.

ade6 products (*19–25*), and must have arisen from symmetrical heteroduplexes in G1, 4 originated from MBC-treated cells and 5 did not. It therefore appears that MBC does not by itself have a noticeable effect on repair or some other parameter of the conversion process.

Evidence that Gene Conversion in G1 is Followed by a Crossover in G2. Of the 63 cases of G1 conversion (excluding the 14 cases of categories *1–3*), 43 can be accounted for by assuming either (*i*) that DNA replication follows heteroduplex formation and thereby resolves the half-chiasma or (*ii*) that heteroduplex formation is followed by endonucleolytic cleavage of the two DNA strands of the half-chiasma (an example is shown in Fig. 2) or of the two uninvolved strands. In the case of cleavage, crossing-over is required in G2 to produce chromatids that will sort out at anaphase to produce the sectored colony. These 43 sectored colonies are therefore indeterminate in supplying information on the origin of the sectored colony.

However, the 20 remaining cases are informative because each of these requires at least one crossover in G2 (Table 2), if the *cly8* locus is not included in the heteroduplex. The table summarizes the exchanges in G2 that would be required if the half-chiasma is resolved (*a*) by DNA replication in G1 or (*b*) by endonucleolytic cleavage in G1. The number of exchanges is further subdivided in the table according to whether the *cly8* locus is not or is included in the heteroduplex that would then stretch from the *ade6* locus to the *cly8* locus. We have no evidence that the *cly8* locus is ever included in the heteroduplex, in prototrophs from irradiated or untreated (unpublished data) cultures, and we discuss this as a possibility because it has been proposed that in mitotic conversion there can be exceedingly long heteroduplexes (4, 8).

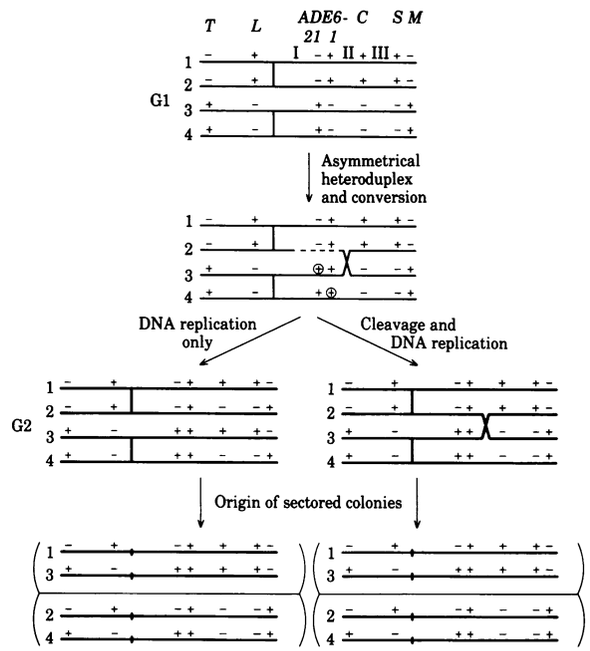

FIG. 2. Diagram to show alternative ways of obtaining category *4* (Table 1) from an asymmetrical heteroduplex and the replication mode of resolution of the half-chiasma in G1 (left side of diagram) or from endonucleolytic cleavage of the two strands of the half-chiasma and a crossover in region II in G2 (right side). If symmetrical heteroduplexes are formed and if the two *ADE*$^+$ conversions are not in the same heteroduplex, the same result would be obtained from events in region I rather than region II. Strand isomerization and cleavage of the other two strands would have no observable consequences. (In the case of cleavage, note that scission of the single strand involved in strand transfer is sufficient and the half-chiasma need not be formed; in this case strand isomerization would not apply.) Vertical lines represent centromeres. The thinner horizontal lines represent DNA strands in G1; the broken line represents repair synthesis after asymmetrical strand transfer. Chromatids are shown in G2 by thicker lines. Conversions to *ADE6* are indicated by ⊕. Sectoring is obtained when the chromatids are distributed to daughter cells as shown. *T, L, C, S,* and *M* represent *TRP5, LEU1, CLY8, SUC1,* and *MAL1,* respectively.

Table 2. Categories that do or do not require G2 recombination if the half-chiasma in G1 is resolved by DNA replication (*a*) or by cleavage (*b*)

Category	No. of cases	*CLY8* locus not included in heteroduplexes		*CLY8* locus included in heteroduplexes	
		a	b	a	b
10	2	1	1	—	—
11	2	1	1	—	—
14	1	1	2	1	1
21	1	1	2	—	—
7	4	2	1	0	1
8	1	2	1	2	1
13	6	2	1	0	1
15	1	2	1	2	1
16	1	2	1	2	1
5	1	2	3	2	1

Expectations are shown if *CLY8* locus is or is not included in the heteroduplex. If it is included, we assume that mismatch repair has not occurred at this locus. Dashes imply that the locus is not included in the heteroduplex.

The cases in Table 2 are of two types with respect to the disposition of the *cly8* alleles. Six of these, belonging to categories *5, 10, 11,* and *21,* are homozygous in each sector of the colony. All of the others, 14 cases, are heterozygous at this locus. Diagrammatic explanations of categories *10, 11, 14,* and *21* are given in Fig. 3. It is necessary to postulate at least one G2 crossover event in these four categories because each involves a three-strand exchange that would be impossible if resolution of the half-chiasma is by replication alone. Category *5* requires a triple crossover if the cleavage mode of resolution is assumed. If conversion had been preceded by recombination in region III or if both had occurred simultaneously, only a single crossover in G2 would be needed. Categories *7, 8, 13, 15,* and *16* can be

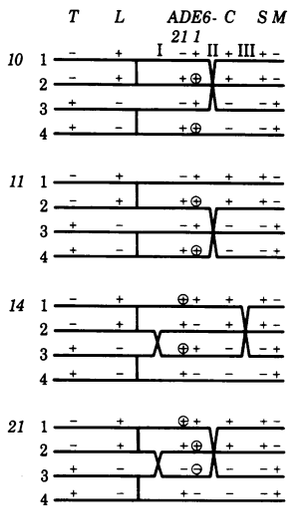

FIG. 3. G2 distribution of crossovers between homologous chromatids, assuming gene conversion in G1 (as a result of symmetrical heteroduplexes in DNA strands 2 and 3) and the cleavage mode of resolution. Symbols are as in Fig. 2. Conversions at the *ADE6* locus are indicated by ⊕ and ⊖. Sectoring follows from 1–4, 2–3 chromatid distributions in categories *10, 11, 14,* and *21.*

accounted for by a single crossover, in region III, if cleavage has occurred in G1 or by a double crossover, one in region III, if resolution has been achieved by DNA replication (Table 2). In two categories (*8* and *15*), composing two cases, strand isomerization (2, 3) is required. The other parameters that enter into the reconstructions of what might have happened to produce the sectored colony are (*i*) the repair or nonrepair in the region of the heteroduplex where there is noncomplementarity, (*ii*) whether the DNA is hybrid in one or both of the duplexes (asymmetry or symmetry), and (*iii*) the region in which strand transfer has occurred in G1 or chromatid exchange in G2. Explanations other than those given are therefore possible, but the results and conclusions would remain the same.

If DNA replication is the cause of sectoring in the 20 colonies of Table 2, there must have been 6 single exchanges in G2 and 14 double exchanges. If, on the other hand, cleavage in G1, which cannot by itself produce a sectored colony, is followed by exchange in G2 to produce sectoring, there would need to be 17 single exchanges, 2 double exchanges, and 1 triple exchange. If economy of exchange is the rule in these cases, as it is in meiotic crossing-over, then resolution by cleavage would be favored over resolution by replication. However, since we do not know the rules that govern the mitotic events, resolution by replication cannot be eliminated on this basis as a hypothesis.

Linkage Relations of Centromere-Linked Markers and CLY8. In 14 colonies (categories *7*, *8*, *13*, *14*, *15*, and *16*) *cly8* is heterozygous in each sector. The centromere-linked *leu1* marker is on the same chromosome as the + allele of *cly8*, and *cly8* is on the same chromosome as the + allele of *leu1*. These linkages would not be disturbed if the *cly8* locus were included in the heteroduplex and if the half-chiasma in G1 is resolved by DNA replication. Thus, analyzing the four chromatids of the sectored colony, this type of segregation would result in a parental ditype (− +/+ −) for these two loci. The data show, however, that there are three types of *cly8* heterozygotes. Eleven (categories *7*, *12*, and *16*) are parental ditypes expected from either the cleavage or replication mode of resolution of the half-chiasma. Three others are exceptions to the replication mode of resolution. Two (*8* and *15*) are nonparental ditypes (i.e., −−/++) and can be explained by assuming strand isomerization and cleavage of the strands not involved in the original half-chiasma. One (*14*) is a tetratype, −−/++ in one sector and −+/+− in the other, and requires a crossover in G2 between chromatids from one involved and one noninvolved strand in the half-chiasma in G1. Thus, while the parental ditypes are indeterminate as to which mechanism is operative, the three cases that are exceptions cannot be explained by DNA replication alone but require instead cleavage in G1 followed, in the case of the tetratype, by a three-strand double crossover as shown in Fig. 3.

The sectored colonies in which *cly8* is heterozygous in both sectors offer additional evidence that the *cly8* locus is not included in the heteroduplex. The second point of interest is that, assuming the cleavage explanation for all of these cases, an estimate of the relative frequency of strand isomerization can be made. Thus, in the 14 cases in which an estimate is possible, only in 2 is it necessary to invoke strand isomerization. This ratio (2/14) compares favorably with the finding of about 20% recombination associated with induced mitotic gene conversion (14).

Concluding Remarks. We have shown that 20 of 63 cases of sectored colonies in which conversion had occurred in G1 require in addition at least one crossover in G2. The 43 other cases can be explained either as the consequence of DNA replication after G1 conversion or by assuming cleavage in G1 and crossing-over in G2. Possibly both explanations are valid in specific cases. If one alone is responsible for sectoring, then there is no choice but to assume that G1 conversion is accompanied by cleavage of the half-chiasma created by heteroduplexing. Recombination associated with conversion in *unselected* mitotic tetrads (6), in which parental ditypes predominate, also is at variance with resolution by replication, which predicts mainly tetratypes. We do not mean to imply that crossing-over in G2 is the result of a conversional intermediate. It may well be that crossing-over in G2 in mitosis is the consequence of quite another mechanism, such as double-strand breakage (15).

The coming together of two duplexes in G1 to allow for strand transfer can also be regarded as providing for the nearness of the four chromatids that are formed after DNA replication, especially in the region of strand transfer in G1. Thus one of the conditions for crossing-over has been met, the proximity of the interacting chromatids. That associated recombination in vegetative cells is not an experimental artifact incidental to x-ray induction is seen from the fact that the frequency of recombination associated with induced conversion at the *ADE6* locus is about 20%, whereas recombination among nonconvertants treated the same way is less than 1% (ref. 14; unpublished data).

Mitotic gene conversion and crossing-over may or may not be different from meiotic recombination with respect to underlying mechanisms (see ref. 16 for a discussion of the problem in meiosis and for other references on the subject). If it is not different, then the issue of the relationship between conversion and crossing-over in both needs reassessment.

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