Mechanisms of Gene Conversion in *Saccharomyces cerevisiae*

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Manuscript received May 2, 1989
Accepted for publication August 26, 1989

ABSTRACT

In red-white sectored colonies of *Saccharomyces cerevisiae*, derived from mitotic cells grown to stationary phase and irradiated with a light dose of x-rays, all of the segregational products of gene conversion and crossing over can be ascertained. Approximately 80% of convertants are induced in G_1, the remaining 20% in G_2. Crossing over, in the amount of 20%, is found among G_1 convertants but most of the crossovers are delayed until G_2. About 20% of all sectored colonies had more than one genotype in one or the other sector, thus confirming the hypothesis that conversion also occurs in G_2. The principal primary event in G_2 conversion is a single DNA heteroduplex. It is suggested that the close contact that this implies carries over to G_2 when crossing over and a second round of conversion occurs.

THE term gene conversion was originally meant to apply only to irregular segregations, i.e. departures from the 2+: 2 ratio expected in tetrads from the Mendelian segregation of genes in heterozygous (+/−) condition. It has since been applied to sister-strand repeats and to heterologous recombination between homologous genes on nonhomologous chromosomes. It has also been applied to the incorporation of plasmid DNA into the homologous site on the chromosome.

A variety of mechanisms have been proposed to account for gene conversion. The first of these that was generally accepted (HOLLIDAY 1964) called for the cutting of single homologous strands of DNA of the same polarity and the reciprocal transfer of segments of the broken strands to the opposite duplexes, thus forming two heteroduplexes. If the region that was transferred was heterozygous for a gene before transfer, the transfer itself would create a condition in both duplexes of noncomplementarity. The mismatches, as they are frequently referred to, can either be repaired by excision-repair or they can go unrepaired in either of the two duplexes. In the latter case, segregation would achieve complementarity in the ensuing post-meiotic division. The half-chiasma created by the reciprocal transfer of the DNA segments could then be resolved either by cutting and rejoining the strands that were involved in the transfer or by cutting and rejoining the two other strands of the duplexes. In the latter case, the result would be a conversion-associated reciprocal recombination that has been observed to accompany gene conversion at a much higher than expected frequency.

A refinement of the Holliday hypothesis was proposed by MESELSON and RADDING (1975) based on the accumulating evidence (reviewed in RADDING, 1982) that single strand invasion of DNA of one duplex can displace an homologous segment in the other duplex. The deletion in the duplex from which the segment was transferred is filled in by new DNA synthesis and the point of transfer can move to increase the length of the heteroduplex while at the same time more DNA synthesis occurs on the other strand to keep up with the advancing heteroduplex. At some indefinite time the newly synthesized strand attaches to the homologous strand in the other duplex and from then on further advance creates two heteroduplexes just as in the Holliday model. The Meselson and Radding hypothesis has the property of being dynamic and of allowing for single strand transfer and subsequent reciprocal strand exchange. Thus gene conversion events can in principle come from a single heteroduplex or reciprocal heteroduplexes.

Another explanation for gene conversion derives from the observation in yeast by ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) that transformation occurred much more frequently when the transforming circular plasmid was cut to make it linear. Moreover, if a segment was removed from the circular plasmid by cuts in two places, the "gapped" linear plasmid also transformed with high efficiency and the gap was repaired during the process. These data suggested to SZOSTAK *et al.* (1983) an alternative hypothesis to account for gene conversion. According to this Hypothesis a cut in one of the two homologous DNA duplexes severed both strands of the duplex. The cut

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was expanded to create a gap in the duplex. Repair of the gap was accomplished by single-strand transfer of DNA from the other duplex and new DNA synthesis in both duplexes to replace the transferred material in one and the gap in the other was postulated. Finally, loose ends from both duplexes were joined to complete the process. The gap-repair hypothesis differs from the Holliday and Meselson-Radding models in two respects: There are two half-chiasmata in the gap-repair model compared with one in the others and there are two ways of achieving irregular segregation, one involving the formation of a heteroduplex, the other simply resulting from the repair of the gap.

Gene conversion can also occur in mitotic cells albeit with a much lower frequency. In fact, meiotic and mitotic gene conversion are generally dealt with as separate subjects. Most recent reviews of gene conversion either treat them in separate chapters or in separate sections (see for example ESPOSITO and WAGSTAFF 1981; FOGEL, MORTIMER and LUSNAK 1981; ORR-WEAVER and SZOSTAK 1985). The following observations have been interpreted as denoting differences between the two types of conversion: (1) The frequency of mitotic conversion is two or three orders of magnitude less than that of meiotic conversion; (2) the frequencies within a gene are polarized, *i.e.*, the frequency of conversion is higher at one end of the gene than at the other in meiosis but the same gradient does not appear to be as extreme if it exists at all in mitosis; (3) mutations affecting the frequency of conversion in meiosis do not seem to alter the frequency in mitosis, and vice versa; (4) the heteroduplex, which is generally regarded as the basic mechanism underlying gene conversion, seems on the average to be longer in mitosis than in meiosis; (5) there has been some discussion about whether associated crossing over, in regions adjacent to the gene undergoing conversion, occurs more frequently in meiosis than in mitosis but the evidence is too scanty to permit a firm conclusion.

It would seem unlikely that the underlying mechanism of gene conversion is different in mitosis and meiosis. Most of the criteria mentioned above can be taken to indicate a difference between the two types of cell division rather than a difference in the fundamental process of gene conversion. Both require to the best of our information the transfer of a region of DNA from one DNA duplex to another to form a heteroduplex as a prior condition for gene conversion. The next event that is generally assumed is repair of noncomplementarity in the heteroduplex by excision-repair or by replication of the DNA strands to restore complementarity. (We are mindful of the exception posed by the gap-repair hypothesis.)

In *Saccharomyces cerevisiae*, gene conversion can be analyzed in both mitosis and meiosis because this yeast has a stable diplophase in which cells divide mitotically and the heteroallelic or heterozygous condition can be established. Furthermore, the opportunity to study segregation like that in tetrad analysis is available also in mitosis by making use of sectored colonies (ROMAN and FABRE 1983), discussed in more detail in this report. We will present evidence that confirms the relatively high incidence of gene conversion in G_1 of mitosis as compared with G_2 and we will also discuss the relationship between gene conversion and crossing over.

WILDENBERG (1970) was the first to show that gene conversion can take place in G_1 of mitosis. This finding was confirmed by ESPOSITO (1978) and by FABRE (1978). Esposito defined the genetic consequences that distinguish between conversion in G_1 from that in G_2. ROMAN and FABRE (1983) then showed that gene conversion could occur in both G_1 and G_2; conversion in G_1 was followed quite frequently by crossing over in G_2. This provides a third mechanism for the relationship between conversion and crossing over that is further elaborated in this paper.

Our interpretation of the evidence was contrary to existing hypotheses designed to explain the highly correlated relationship between gene conversion and crossing over in a nearby region (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). Because our interpretation was regarded even by us as quite unorthodox and because our data were based on a severe selection regimen, we thought it possible that we had selected a special sample of cells that was not representative of the majority of cells in which gene conversion could occur. The experiments reported here have been designed to test the temporal separation hypothesis under less stringent selection conditions. In fact, the only condition that was imposed was that of red-white sectoring in a colony originating from a cell of appropriate genotype.

MATERIALS AND METHODS

Genotypes of strains: The genotypes of the diploid strains used in these experiments are given in Table 1 for the markers on chromosome VII. The strains are also homozygous for *ade2* (chromosome *XV*, not shown in Table 1). Strains which are homozygous for *ade2* and either heterozygous or homozygous for *ADE6* produce colonies that are red; if they are homozygous *ade6* or are heteroallelic at this locus (for example, *ade6*-1/*ade6*-21), the colonies are white.

The recessives *trp5* and *leu1* represent requirements for tryptophane and leucine in the growth medium; *cly8* is a temperature-sensitive mutation that allows normal growth at room temperature (*ca.* 23°) but the cells do not grow at 35°; *SUC1* and *MAL1*, which are distal to and show no linkage to *cly8* are codominants for sucrose and maltose fermentation. The fermentation genes are in fact near the end of the chromosome arm (CELENZA and CARLSON 1985). We infer from recombination studies that the order of the *ADE6* alleles are: centromere–*ade6*-21–*ade6*-1–*ade6*-123 (JONES 1964; ROMAN 1980). The intergenic distances in centimorgans between markers are shown in Table 1.

Media: The basic media, in grams per liter, were 10 g of yeast extract, 20 g of peptone, and 20 g of dextrose (YEPD), supplemented with 100 mg of adenine, 20 mg of tryptophane, and 20 mg of uracil. The synthetic complete medium contained 6.7 g of Difco Yeast Nitrogen Base, to which was added 10 mg of adenine, 60 mg of leucine, and 40 mg of tryptophane. These two media were modified for diagnostic purposes as described below. For solid media, 20 g of Difco Noble agar was added.

Diagnostic plates for sucrose fermentation and maltose fermentation were made by substituting sucrose (20 g/liter) or maltose (40 g/liter) for dextrose in YEPD medium, modified to contain 10 g/liter peptone for sucrose plates and 20 g/liter for maltose plates. Before pouring, a 1.5% solution of bromthymol blue was added to the sucrose medium at 5 ml/liter, and the pH 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 adjusted to 6.0. Tryptophane, leucine, and adenine requirements were diagnosed on synthetic complete plates lacking the appropriate amino acid or purine. As a precautionary measure, glycerol (30 ml/liter) replaced dextrose in YEPD for the purpose of detecting petites, which do not sporulate and were therefore avoided.

The sporulation medium consisted of potassium acetate (10 g/liter), yeast extract (2.5 g/liter), dextrose (1 g/liter), adenine (100 mg/liter), uracil (20 mg/liter) and agar (20 g/liter).

Source of radiation: A Machlett OEG-60 x-ray tube, operated at 50 kV and 20 mA, was used. The dose rate was approximately 110 rad/sec.

To obtain convertants: Cells were grown to stationary phase, sonicated, diluted and plated for approximately 200 colonies per plate on synthetic complete medium and were x-rayed for 30 sec, approximately 3000 rad. In making the cell counts for the dilutions, cells with a discernible bud were counted as two cells; the unbudded cells accounted for 93% to 99% of the total depending on the strain used in the experiment. When the *ADE6* heterozygotes were irradiated and plated, both red-white sectored colonies and whole white colonies were found among the predominantly red colonies. When heteroallelic strains were used, red-white and whole red colonies were found among the predominantly white colonies. The red-white colonies were picked up and plated, after appropriate dilution, for the isolation of whole reds and whole whites from the same colony. Six of each of these were tested for the other markers on chromosome *VII*. The exceptional whole white and whole red colonies were handled the same way.

Distinction between conversion in G_1 and conversion in G_2; the homoallelic/heteroallelic test: To paraphrase ESPOSITO (1978) in our context, the white sectors of the red-white colonies from the heteroallelic strains should be homoallelic or heteroallelic for the *ade6* input alleles if conversion of *ade6* occurred in G_1; only heteroallelism would be expected if conversion occurred in G_2. The white isolates were sporulated and if red colonies were obtained the white isolates were scored as heteroallelic. If only white colonies were obtained, the genotype was recorded as homoallelic. We did not attempt to determine which allele was present in the homoallelic isolate.

Tests for *ADE6 ade6 vs. ADE6 ADE6:* The red isolates from the sectored and whole red colonies were tested for the presence of the *ade6* allele. The isolates were sporulated, checked for the presence of asci, and spread on synthetic complete plates, approximately 500 cells per plate. The presence of white colonies or red-white sectors indicated that the isolate was *ADE6/ade6*. If only red colonies resulted, the genotype was recorded as *ADE6/ADE6*.

Tests for heterozygosity or homozygosity at the *CLY8* locus: Determination of the *CLY8* genotype was accomplished by sporulating those red and white isolates which grew at 35° and spreading 500 cells on synthetic complete plates. YEPD replica plates were incubated at 23° and 35°. Genotypes were scored as *CLY8/cly8* if there was evidence of failure to grow at 35° of whole colonies or of sectors within colonies, and as *CLY8/CLY8* if there was no such evidence.

Tests for the disposition of markers between the chromosome *VII* homologs: The red and white isolates that proved to be *CLY8/cly8* and *SUC1/MAL1* were plated for 200–400 colonies per plate and irradiated for 60 seconds. The *cly8* sectors or whole colonies that were the consequence of a crossover between the centromere and the *CLY8* locus were tested for the fermentation marker that was on the same chromosome as *cly8*. In this way the alignments *CLY8 SUC1/cly8 MAL1* and CLY8 MAL1/*cly8 SUC1* could be distinguished.

In a similar way tests were made for the alignment of markers with respect to the *ADE6* locus. The red isolates that were *ADE6/ade6* were plated for 200–400 colonies and irradiated for 60 sec. The white sectors or whole whites resulting from the irradiation were tested for *cly8*, if the colony from which they came was *CLY8/cly8*, and for the fermentation marker that was on the same chromosome as *ade6*.

EXPERIMENTAL RESULTS

The role of *TRP5* and *LEU1* heterozygotes in these experiments: All of the cultures were *TRP5 leu1/trp5 LEU1* (Table 1). These are on the other side of the centromere from the markers that were analyzed for gene conversion and its consequences. Since both *TRP5* and *LEU1* are linked to the centromere, they serve to indicate whether the cells are in mitosis or meiosis. If mitosis, both markers should remain heterozygous in both sectors of the sectored colonies save for occasional crossovers between the centromere and either of the two loci or conversions at either or both of the loci. Among 416 sectored colonies from strains heterozygous for *ADE6* (+/*ade6*) or heteroallelic for *ade6*, all were heterozygous for *TRP5* and four showed the recessive phenotype of *leu1* among the six samples chosen from each side of the sectored colony. Further, these four were in the white sector and were found only among the treated cells of the *ADE6* heterozygotes. These can be explained by conversion that would give 3*leu1*: 1*LEU1* segregation and, consequently, *leu1/leu1* in the white sector or the red sector. It is evident therefore that the cells in these experiments were in mitosis.

The origin of red-white sectored and whole white colonies in +/ade6 heterozygotes: Table 2 shows the frequencies of aberrant colonies from various heterozygous strains. The frequencies of sectored colonies average 1% and those of whole whites average 0.26%. Among the sectored colonies, those with ½ white sectors were most frequent, followed by those with either ½ or ¾ white sectors. Those that were classified

TABLE 1

Disposition of markers on chromosome *VII*

Expt. No.	Diploid strains	Regions: *ADE6* alleles	*TRP5* (17)	*LEU1* (5)	I *CEN7* (35)	II *ADE6* (37)	*CLY8*	III *SUC1* (1)	*MAL1*
85-11-15	C971	21	−	+		−	+	+	−
		+	+	−		+	−	−	+
86-09-08									
84-10-17	C955	1	−	+		−	−	−	+
-11-13		+	+	−		+	+	+	−
-11-28									
85-09-06									
86-05-20	C267	123	+	−		−	+	+	−
-08-29		+	−	+		+	+	−	+
85-04-15	C965	21 +	−	+		− +	+	+	−
		+ 1	+	−		+ −	−	−	+
85-04-16	C967	21 +	−	+		− +	+	−	+
		+ 1	+	−		+ −	−	+	−
85-04-17	C968	21 +	−	+		− +	+	+	−
-05-02		+ 1	+	−		+ −	−	−	+
-11-06									
86-06-03	C973	1 +	+	−		− +	+	+	−
		+ 123	−	+		+ −	−	+	−
86-04-29	C972	21 +	+	−		− +	+	−	+
-05-21		+ 123	−	+		+ −	−	+	−

TRP5 and *LEU1* are to the left of the centromere (C) and *ADE6*, *CLY8* and *SUC1 MAL1* are to the right. The distances between loci are given in centimorgans (MORTIMER *et. al.* 1989). See MATERIALS AND METHODS for details.

TABLE 2

Frequency of aberrant colonies from diploids heterozygous for different *ade6* alleles

Expt. No.	*ade6* allele	X-ray dose (sec)	Sectored colonies ≤1/8	1/4	1/2	3/4	≥7/8	O.C. colonies	Whole whites	Total colonies	*S*	Percent Sectors	Percent Whites
85-11-15	21	30	2	20	35	4	1	1	23	6,896	96.8	0.90	0.33
		0	0	0	0	0	0	0	0	1,789		0.00	0.00
86-9-8	21	30	3	18	67	22	13	7	40	15,652	92.4	0.79	0.26
		0	0	0	0	0	0	0	1	2,259		0.00	0.04
84-10-17	1	30	0	8	32	10	1	0	8	5,146	94.0	0.99	0.16
		0	0	0	0	0	0	0	0	2,738		0.00	0.00
84-11-13	1	30	0	7	13	3	1	0	12	3,343	98.8	0.72	0.36
		0	0	0	0	0	0	0	0	1,692		0.00	0.00
84-11-28	1	30	5	9	50	12	2	0	16	7,253	97.1	1.08	0.22
		0	0	1	0	0	0	0	0	1,867		0.05	0.00
85-9-6	1	30	7	11	35	9	1	12	13	5,883	95.2	1.07	0.22
		0	0	1	0	0	0	0	1	2,060		0.05	0.05
86-5-20	123	30	3	24	67	18	7	7	35	9,972	101.5	1.19	0.35
		0	0	0	0	0	0	0	1	1,637		0.00	0.06
86-8-29	123	30	5	38	116	36	35	3	51	20,602	108.7	1.12	0.25
		0	0	0	2	0	0	0	1	2,526		0.08	0.04

The experiments are numbered according to the year, month and day when each was started. The fractions indicate colonies that have white sectors of that size beginning about or near the center of the colony. The whole-white colonies are in a separate column. The O.C. colonies are those that have small sectors that are distinctly off-center. They were few in number and were not counted as sectored colonies. *S* represents the percent survival relative to the untreated control. The results are summarized in Table 3.

as having ⅛ or less or ⅞ or more white sectors were few in number. Sectored colonies among the controls were rare. Reconstruction experiments have been done in which white cells and red cells were placed side by side with a micromanipulator and a similar range and frequency distribution of sector size among colonies obtained from the pairings have been observed (ROMAN 1973). We therefore conclude that sector size is variable because of uncontrolled factors in colony formation, such as a lag in the start of growth of one of the two daughter cells, and therefore that in treated cells the segregation of the recombination products takes place mainly in the first division of the treated cell. In fact, the size of the sector seem to be of little or no importance in determining the genotypes of the sectored colony. Among the 145 colonies

TABLE 3

Summary of data for each *ade6* allele in Table 2

ade6 allele	X-ray dose (sec)	Sectored colonies					Whole whites	Total colonies	Percent	
		≤1/8	1/4	1/2	3/4	≥7/8			Sectors	Whites
21	30	5	38	102	26	14	63	22,548	0.82	0.28
	0	0	0	0	0	0	1	4,048	0.00	0.02
1	30	12	35	130	34	5	49	21,625	1.00	0.23
	0	0	1	1	0	0	1	8,357	0.02	0.01
123	30	8	62	183	54	42	86	30,574	1.14	0.28
	0	0	0	2	0	0	2	4,163	0.05	0.05
Totals	30	25	135	415	114	61	198	74,747	1.00	0.26
	0	0	1	3	0	0	4	16,568	0.02	0.02

analyzed in the next section, five were classified as ≤⅛, 18 were ¼, 96 were ½, 22 were ¾, and four were ≥⅞.

There were in addition small sectors that were confined to the margin of the colony. These were in colonies from the treated cells and thus were the consequence of the treatment. We presume that they were delayed effects due to induced recombinational ability (FABRE and ROMAN 1977; F. FABRE, personal communication). There was also a sizable number of whole white colonies from the treated cells. The significance of these will be discussed in a later section. The pertinent data in Table 2 are summarized in Table 3.

Origin of sectored colonies obtained from +/*ade6-1* heterozygotes: In Table 4, column 5, are listed the genotypes of the sectored colonies of +/*ade6-1*. We chose to present the data from +/*ade6-1* because more cases of this heterozygote were completely analyzed than were the heterozygotes +/*ade6-21* or +/*ade6-123* although the smaller amount of data from the latter two heterozygotes agreed well with those in Table 4. Of the 145 cases analyzed, belonging to 28 different classes, 96 did not require gene conversion at the *ADE6* locus to achieve a white sector. In reconstructing the events that lead to sectoring, we start with a pair of homologous DNA duplexes in column 2 (G_1), the two innermost strands being of the same polarity, opposite to that of the two outermost strands. The centromere of chromosome *VII* (not shown) is to the left. The four loci shown in the table are from left to right, *ADE6, CLY8, SUC1, MAL1*, in that order. The assignments of *SUC1* and *MAL1* are arbitrary; they do not exhibit crossing over between them and their order could very well be reversed. The region between the centromere and the *ADE6* locus is marked by I, between *ADE6* and *CLY8* by II, and between *CLY8* and the *SUC1 MAL1* complex by III.

Class 1, by far the most numerous, requires no gene conversion in G_1 and only a crossover between two of the four chromatids in G_2 in region I; our preference for the event occurring in G_2 rather than in G_1 was first expressed in ROMAN and FABRE (1983) and will become clearer in later sections. The next column shows the results of the crossover and finally segregation of the chromatids gives us the sectored colony, with a genotype as shown in column 5. Classes 6, 10, 11, 13, 16, 17, 19, and 21 also do not require gene conversion at the *ADE6* locus but either exhibit additional crossovers in G_2 or conversion at other loci in G_1. The remaining 49 (classes 2, 3, 4, 5, 7, 8, 9, 12, 14, 15, 18, 20, 22, 23, 24, 25, 26, 27, and 28) do require conversion at the *ADE6* locus, to give 1*ADE6*:3*ade6* with, in some cases, a crossover in the ensuing G_2. The heading of Table 4 explains the symbolism of the dotted lines and the parentheses surrounding certain loci.

It should be emphasized, however, that there is more than one explanation for the location of the crossovers in G_2. There is in addition to the placement of the crossovers shown in Table 4, alternative placements that involve chromatids of identical composition. This applies as well to multiple crossovers as to single crossovers. For example, in Classes 6, 24 and 28, the double crossovers could just as well be three-strand doubles, to give a sectored colony having the same genotype. Similarly, the single crossovers in 1, 8, 9, 14–17, 19, 23, 26 and 27 could involve the other like chromatid.

The 49 sectored colonies are interpreted as being due to gene conversion at the *ADE6* locus in G_1 after the transfer in region I of a segment of DNA carrying *ade6* that replaces *ADE6* in the homologous DNA strand of the same polarity. We assume that the strand that now connects the two duplexes is cut by an endonuclease and that the space left open by the transfer of material is now filled in by new DNA synthesis (dotted line). However, in thirty-four of these cases (classes 2, 3, 4, 5, 7, 18, 20, 22, 25), no crossover is required in G_2 to produce the corresponding sectored colony. Nine of the thirty-four cases have additional transfers in regions II or III or both and eight of these (classes 4, 22, and 25) are assumed to have an excision-repair replacement of the segment

TABLE 4

Genotypes of sectored colonies obtained from +/*ade6-1* diploids

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	No. of cases
	I II III	I II III			
1	+ + + − + + + − − − − + − − − +	+ + + − + + + − − − − + − − − +	+ + + − − − − + + + + − − − − +	+ + + − + + + − − − − + − − − +	82
2	+ + + − − + + − − + − + − − − +	None	+ + + − − + + − − + − + − − − +	+ + + − − + − + − + + − − − − +	7
3	+ + + − − + + − − − − + − − − +	None	+ + + − − + + − − − − + − − − +	+ + + − − − − + − + + − − − − +	7
4	+ + + − − + + − − + − + − (+) − +	None	+ + + − − + + − − + − + − + − +	+ + + − − + − + − + + − − + − +	6
5	+ + + − − + + − − + − + − − − +	None	+ + + − − + − + − + + − − − − +	+ + + − − − − + − + + − − + − +	6
6	+ + + − + + + − − − − + − − − +	+ + + − + + + − − − − + − − − +	+ + + − − − + − + + − + − − − +	+ + + − + + − + − − + − − − − +	4
7	+ + + − − − − + − − − + − − − +	None	+ + + − − − − + − − − + − − − +	+ + + − − − − + − − − + − − − +	4
8	+ + + − − + + − − − − + − − − +	+ + + − − + + − − − − + − − − +	+ + + − − − − + − + + − − − − +	+ + + − − + + − − − − + − − − +	3
9	+ + + − − + − + − − − + − − − +	+ + + − − + − + − − − + − − − +	+ + + − − − − + − + − + − − − +	+ + + − − + − + − − − + − − − +	3

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	No. of cases
10	+ + + − + + + − − − − + − − − +	+ + + − + + + − − − − + − − − +	+ + − + − − + − + + − + − − + −	+ + − + + + − + − − + − − − + −	2
11	+ + + − + + + − − + + − − − (+ −)	+ + + − + + + − − + + − − − + −	+ + + − − + + − + − + − − + + −	+ + + − + − + − − + + − − + + −	2
12	+ + + − − + + − − − + − − − − +	+ + + − − + + − − − + − − − − +	+ + + − − − + − − − + − − + − +	+ + + − − + − + − − + − − − + −	2
13	+ + + − + + + − − − − + − − − +	+ + + − + + + − − − − + − − − +	+ − − + − + + − + − − + − + + −	+ − − + + − − + − + + − − + + −	2
14	+ (−) + − − − − + − − − + − − − +	+ − + − − − − + − − − + − − − +	+ − − + − − − + − − − + − − + −	+ − − + − − − + − − − + − − + −	1
15	+ + (− +) − + − + − − − + − − − +	+ + − + − + − + − − − + − − − +	+ + − + − − − + − + − + − − − +	+ + − + − + − + − − − + − − − +	1
16	+ + + − + + − + − − − + − − − +	+ + + − + + − + − − − + − − − +	+ + + − − − − + + + − + − − − +	+ + + − + + − + − − − + − − − +	1
17	+ + (−) − + + − − − − − + − − − +	+ + − − + + − − − − − + − − − +	+ + − − − − − + + + − − − − − +	+ + − − + + − − − − − + − − − +	1
18	+ + + − − − + − − − − + − − − +	None	+ + + − − − + − − − − + − − − +	+ + + − − − − + − − + − − − − +	1

TABLE 4—Continued

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	No. of cases
19	+ + + − + − + − − − − + − − − +	+ + + − + − + − − − − + − − − +	+ + + − − − − + + − + − − − − +	+ + + − + − + − − − − + − − − +	1
20	+ (−) (− +) − − − + − − − + − − − +	None	+ − − + − − − + − − − + − − − +	+ − − + − − − + − − − + − − − +	1
21	+ + + − + + + − − + − + − − − +	+ + + − + + + − − + − + − − − +	+ + + − − + − + + − − + − + + −	+ + + − + − − + − + + − − + − +	1
22	+ + + − − − + − − − + − − − (+ −)	None	+ + + − − − + − − − + − − − + −	+ + + − − − + − − − + − − − + −	1
23	+ + + − − + + − − − − + − − − +	+ + + − − + + − − − − + − − − +	+ − − + − + + − − + + − − − − +	+ − − + − − − + − + + − − + + −	1
24	+ + + − − − − + − − − + − − − +	+ + + − − − − + − − − + − − − +	+ − + − − + − + − − − + − − − +	+ − + − − − − + − + − + − − − +	1
25	+ + (− +) − − − + − − − + − − − +	None	+ + − + − − − + − − − + − − − +	+ + − + − − − + − − − + − − − +	1
26	+ + + − − − + − − − − + − − − +	+ + + − − − + − − − − + − − − +	+ + + − − − − + − − + − − − − +	+ + + − − − + − − − − + − − − +	1
27	+ + + − − + + − − − − + − − − +	+ + + − − + + − − − − + − − − +	+ − − + − + + − − + + − − − − +	+ − − + − + + − − − − + − + + −	1

TABLE 4—Continued

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	No. of cases
28	+ + + − − + + − − − − + − − − +	+ + + − − + + − − | − | − + − − − +	+ + + − − − + − − + − + − − − +	+ + + − − + − + − − + − − − − +	1
					145

The genotypes of the sectored colonies are shown in the fifth column. The red sector genotype is above the line, the white sector genotype below the line. Column two represents the two DNA duplexes of G_1 with the markers as shown on the right arm of chromosome *VII* in Table 1. The dotted lines represent the material that was transferred from one DNA strand to the homologous strand of the same polarity and also the resynthesis of DNA to replace the material that is transferred. See text for other details. The symbols in parentheses represent mismatch-repair to restore complementarity. In column 3 the vertical lines represent reciprocal recombination between homologous chromatids (see text for details). The fourth column shows the resolution of the crossovers before segregation of the chromatids to sister cells (fifth column). The number of each type of sectored colony is given in the sixth column.

in one strand by a copy of a segment in the homologous strand. An additional case of excision-repair involving *CLY8* and *SUC1 MAL1* (class 20) was also found. *SUC1* and *MAL1* are generally excised simultaneously as if they were the same locus or closely neighboring loci. It should be noted that the dotted line spanning more than one locus (representing new DNA synthesis) may or may not be continuous (ESPOSITO 1978; JUDD and PETES 1988; BORTS and HABER 1987; GOLIN and FALCO 1988; GOLIN and TAMPE 1988). New synthesis can occur on the same strand or on the homologous strand (class 2 for example).

Of the 42 sectored colonies that exhibited gene conversion at loci other than *ADE6* (conversion at this locus is required to obtain the sectored colony), 14 required excision-repair (there were two instances in class 20 but since is not clear that they are unrelated we are counting them as one). We are however missing half of the cases of mismatch-repair if parity prevails in these cases. Moreover, some, perhaps all, of the remaining classes that are indicated by dotted lines as being the result of DNA resynthesis might actually be cases of mismatch-repair. Thus repair may occur as frequently in mitosis as in meiosis. One other item of interest is the relationship of crossing over to gene conversion among the convertants of Table 4 compared with the frequency of crossing over among the nonconvertants. Of the 90 cases of sectored colonies in which conversion cannot be demonstrated, eight had one or two crossovers, for a total of 13.3% crossing over, not including the crossover in region I that was responsible for sectoring in the first place. Among the 55 cases in which gene conversion had occurred, there were also 12 crossovers or 21.8%. We interpret these data to mean that when the homologous duplexes are paired, even though the pairing might not be complete, the condition of close contact that this implies is carried over to G_2 and is appropriate for crossing over; the idea of a strict dependence of crossing over on conversion, at least in mitosis, is therefore not justified.

Gene conversion in heteroallelic diploids: Table 5 shows the distribution of aberrant colonies induced in different heteroallelic strains. The frequency of sectored colonies averages about 0.28% and the frequency of whole reds is not significantly different from the control. The heteroallelic colonies that are white and red sectors occur when one of the recessive alleles is converted to *ADE6*. More rarely, an exchange between the two alleles can produce a strand carrying *ADE6*, the other strand carrying both *ade6* mutations. The data are summarized in Table 6.

We cannot account for the asymmetry of the sector sizes in five of the six experiments. The relative absence of sectors larger than one-half red is not a consequence of selection on the synthetic complete medium. Whereas the white sector has a selective advantage on YEPD medium, there is no selection for either sector on synthetic complete medium. Also, dissections showed that each gene on chromosome *VII* segregated as expected of a heterozygote. In the 21/123 diploid used in experiment 86-4-29 the half-sectored colony is again predominant and there is no evidence of asymmetry when this diploid is treated and plated for sectored colonies.

Table 7 shows one of the possible reconstructions of the events that could have given rise to the observed sectored colonies (column 5). The symbols are the same as in Table 4 except that the two adenine heteroalleles are shown together at the left. All of the 74 sectored colonies were derived from C972 which is heteroallelic *ade6*-21/*ade6*-123. The DNA duplexes in G_1 have dotted lines and parentheses with the same meaning as in Table 4. Also, as in Table 4, the exchanges shown under the heading G_2 Exchange, can be between the other like chromatid without altering the result. Thus in Cx (column 3) the exchange shown between the two inner chromatids

TABLE 5

Frequency of sectored colonies and whole red colonies induced by x-rays in heteroallelic strains listed in Table 1

Expt. No.	*ade6* alleles	X-ray dose (sec)	Sectored colonies					Whole red	Total colonies	S^a	Percent	
			≤1/8	1/4	1/2	3/4	≥7/8				Sectored colonies	Whole reds
85-4-15	21/1	30	7	6	6	0	0	2	8216	96.8	0.23	0.02
		0	0	0	0	0	0	0	2122		0.00	0.00
85-4-16	21/1	30	2	6	5	2	0	0	7012	94.8	0.21	0.00
		0	0	0	0	0	0	0	1849		0.00	0.00
85-4-17	21/1	30	5	9	8	4	0	2	7764	94.9	0.34	0.026
		0	0	0	0	0	0	0	2045		0.00	0.00
85-5-2	21/1	30	2	8	14	1	1	1	8405	91.5	0.31	0.01
		0	1	0	0	0	0	0	1837		0.04	0.00
86-6-3	1/123	30	3	11	10	2	1	1	9792	94.3	0.25	0.01
		0	1	0	0	0	0	0	1731		0.06	0.00
86-4-29	21/123	30	4	2	20	5	3	2	9095	100.0	0.37	0.02
		0	0	0	0	0	0	0	1802		0.00	0.00
86-5-21	21/123	30	7	13	17	4	2	1	6940	98.4	0.62	0.01
		0	0	0	0	0	0	0	1410		0.00	0.00

The fractions indicate colonies with red sectors of that size. The data are summarized in Table 6.
[a] Percent survival.

TABLE 6

Summary of data in Table 5

ade6 alleles	X-ray dose (sec)	Sectored colonies					Whole reds	Total colonies	Percent	
		≤1/8	1/4	1/2	3/4	≥7/8			Sectors	Reds
21/1	30	16	29	33	7	1	5	31,397	0.27	0.02
	0	1	0	0	0	0	0	7,859	0.01	0.00
1/123	30	3	11	10	2	1	1	9,792	0.28	0.01
	0	1	0	0	0	0	0	1,731	0.06	0.00
21/123	30	4	2	20	5	3	3	9,095	0.37	0.03
	0	0	0	0	0	0	0	1,802	0.00	0.00

could very well be between the second and fourth chromatid. The x as in Ax is a symbol for homoallelism in the white sector; Ay is a class that is heteroallelic in the white sector. It should be noted that the two A classes are significantly larger than the two B classes. This suggests that the transfer of a segment of DNA is more likely to go in one direction than the other or that mismatch-repair occurs more frequently in the one direction.

Of the 74 examples of sectored colonies, there were 11 cases of single exchanges in one or the other of the distal regions, three double exchanges and one triple exchange, the proximal-most exchange being between the adenine locus and the centromere. Thus there were a total of 11 exchanges among the 74 cases, or 21.6%, in good agreement with the values obtained in the analysis of the data in Table 4. It should also be noted that the number of homoallelic cases is equivalent to the number of heteroallelic cases. This is further evidence that the events are initiated in G_1.

It may seem extraordinary that gene conversion should occur in G_1 and associated crossing over should be delayed until G_2. This subject was discussed in a previous paper (ROMAN and FABRE 1983). The transfer of a segment of DNA from one duplex to its homologue in G_1 requires the close contact of the two duplexes, at least in the region of transfer. We suppose that the nearness that is implied is maintained after DNA replication has taken place to form the chromatids in G_2. Thus one of the conditions for both crossing over and gene conversion has been met. Further evidence of crossing over and gene conversion in G_2 is provided in a later section that deals with mosaic sectors in sectored colonies.

Explanation of whole white and whole red colonies in Tables 3 and 6: As noted earlier, there was a sizable number of the whole white colonies from the treated cells, summarized in Table 3. These were striking against a background of red and sectored colonies from the +/*ade6* heterozygotes. The whole whites would be expected if the *ADE6* allele were

TABLE 7

Explanation of sectored colonies (column 5) obtained from heteroallelic diploid C972 (Table 1)

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	Homo-allelic	Hetero-allelic
Ax	(−) + + − + + − + − + + − − + − + − − + −	None	+ + + − + + − + − + + − − + − + − − + −	+ + − + − − + − − + − + − − + −	18	0
Ay	− + + − + + (−) + − + + − − + − + − − + −	None	− + + − + + + + − + + − − + − + − − + −	"	0	19
Bx	− + + − + − + + − + − + − + − + (−) − + −	None	− + + − + − + + − + − + − + − + + − + −	+ − + − − + − + − − + − − + − +	6	0
By	− + + − + − + + − + (−) + − + − + − − + −	None	− + + − + − + + − + + + − + − + − − + −	" 0	9	
Cx	(−) + + − + + − + − + + − − + − + − − + −	+ + + − + + − + − + + − | − + − + − − + −	+ + + − + + − − + − + − + − + + − − + −	+ + − + − + − + − − + − − − + −	3	0
Cy	− + + − + − + + − + (−) + − + − + − − + −	− + + − + − + + − + + + | − + − + − − + −	− + + − + − + − + − + + + − + + − − + −	" 0	1	
Dx	− + + − + − + + − + − + − + − + (−) − + −	− + + − + − + + − + − + | − + − + + − + −	− + + − + − + − + − − + + − + + + − + −	+ − + − − − + − − + − + − + − +	3	0
Dy	− + + − + + (−) + − + + − − + − + − − + −	− + + − + + + + − + + − | − + − + − − + −	− + + − + + + − + − + − + − + + − − + −	" 0	1	
Ey	− + + − + + (−) − + − + − − + − + − − + −	None	− + + − + + + − + − + − − + − + − − + −	+ − + − − − + − − + − + − − + −	0	1

TABLE 7—Continued

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	Homo-allelic	Hetero-allelic
Fy	− + + − + − + + − + (−) + + + − + − − + −	None	− + + − + − + + − + + + + + − + − − + −	+ + + − − + − + − − + − − + − +	0	1
Gy	− + + − + + (−) + − + + − − − + + − − (+ −)	None	− + + − + + + + − + + − − − + + − − − +	+ + − + − − − + − − − + − − − +	0	1
Hx	(−) + + − + + − + − + + − − − + + − − (+ −)	+ + + − + + − + − + + − | − − + + − − − +	+ + + − + + − − − + + − + − + + − − − +	+ + − + − + − + − − − + − − − +	1	0
Ix	− + + − + − + + − + − + + − + + (−) (−) (+ −)	None	− + + − + − + + − + − + + − + + + + − +	+ + − + − + − + − + − + − + − +	2	0
Jy	− + + − + − + + − + (−) + + − + + − − + −	None	− + + − + − + + − + + + + − + + − − + −	+ + − + − + − + − + − + − − + −	0	1
Kx	− + + − + − + + − + − + − + − + (−) − + −	− + + − + − + + − + − + | − | + − + + − + −	− + + − + − + − − + − + + + − + + − + −	+ − + − − + − + − + + − − − − +	1	0
Ky	− + + − + − + + − + (−) + − + − + − − + −	− + + − + − + | + | − + + + | − | + − + − | − | + −	− + + − + − + − − + + + − + − + − + + −	”	0	1
Lx	− + + − + − + + − + (−) + + + − + (−) − + −	− + + − + − + + | − + | + + | + + − + + | − | + −	− + + + − + + + + − − + − − + + + + − +	+ + + − + + − + − + + − − − − +	1	0
My	− + (+) (− +) + − − + − + − − + − + − − + −	None	− + − + − + + − + − + − − + − + − − + −	+ − + − − − + − − − + − − − + −	0	1

TABLE 7—Continued

Class	G_1	G_2 exchange	Results of exchange	Sectored colony	Homo-allelic	Hetero-allelic
Nx	(−) + + − +	+ + + − +	+ + + − +	+ + − +		
	+ − + − +	+ − + − +	+ − + + −	− − + −		
	+ − − + −	+ − − | + −	+ − − + −	− − − +	1	0
	+ − − + −	+ − − + −	+ − − − +	− + + −		
Ny	− + + − +	− + + − +	− + + + −			
	+ (−) + − +	+ + + | − +	+ + + − +			
	+ − − + −	+ − − | + −	+ − − − +	"	0	1
	+ − − + −	+ − − | + −	+ − − + −			
Oy	(−) + + − +	+ + + − +	+ + + + −	+ + + −		
	+ − + − +	+ − + | − +	+ − − − +	− + − +		
	+ − − + −	+ − | − | + −	+ − + − +	− − + −	1	0
	+ − − + −	+ − − | + −	+ − − + −	− − − +		
					$\overline{37}$	$\overline{37}$

The symbols x and y represent whether the white side of the sectored colony was homoallelic (x) or heteroallelic (y). The two heteroalleles are shown in the second through the fourth columns at the left as − + or + −; otherwise the symbolism is the same as that in Table 4. This designation does not appear in column 5 because mismatch-repair in the red sector has resulted in the replacement of the heteroallele by a + representing *ADE6*. The heteroalleles were not tested for genotype and are therefore represented by a −. The numbers of each class are given in columns 6 and 7.

deleted by excision-repair. If this were the explanation, most of the white colonies should be a mosaic of two genotypes. An analysis of 46 colonies from three experiments (Table 8) showed that all but one had single genotypes. Thirty-six of the 45 single-genotype colonies had a genotype which was like that of the white parent in the original diploid, and could be accounted for as the result of a crossover between the *ADE6* locus and the centromere (see Table 4). The other nine could have been derived from crossovers in the region distal to *ade6*. At any rate, it seems likely that excision-repair plays only a small role in producing the whole white colonies.

Similarly eight whole reds from the heteroallelic cultures were analyzed for genotype. These were easily seen against the background of white and sectored colonies (Table 8). None of these had more than one genotype, although, as in the case of the whole whites, two genotypes would be expected in each colony if they were simply the consequence of excision-repair. Five of the eight are of the parental genotype, assuming that excision-repair produced the *ADE6* gene. A combination of crossing over between the adenine locus and the centromere, and crossovers distal to the adenine locus, would account for these colonies.

Thus it is clear that the whole whites and whole reds are the consequence mainly of the loss of the sister cell in the first division of the colony.

Mosaic sectors in sectored colonies of heteroallelic and heterozygous diploids; evidence of conversion in G_2: In addition to the 74 colonies that were obtained after x-ray treatment of the heteroallelic diploid C972, there were 16 sectored colonies that showed more than one genotype in one or the other sector of the colony. Of the 33 genotypes in these mosaic sectors, 31 were 1+: 3− at the adenine locus. The two others were 2+: 2− at this locus presumably because of a conversion to *ADE6* in each of the two duplexes in G_1. There were 22 homoallelics and 13 heteroallelics among the 35 genotypes. The homoallelic-heteroallelic test applies only to the adenine locus since this locus is the only one that was heteroallelic originally. Thus it is only at the adenine locus that we can monitor for the stage (G_1 or G_2) in which conversion had occurred. Further, only one genotype per sector would be the consequence of a recombinational or conversional event in G_1.

The very fact that sectors can be mosaic proves that mosaicism is the consequence of events that occurred in G_2. On this premise, the explanation of mosaic sectors becomes quite simple. Cases of mismatch repair or nonrepair at *CLY8* and *SUC1 MAL1* that are characteristic of G_2 conversion can be found among the 16 mosaics (Table 9). An explanation for four of these 1, 2, 3 and 16, is given in Table 10. Case 1 is heteroallelic in both white sectors, case 2 is homoal-

TABLE 8

Genotypes of the whole white and whole red colonies, sampled from the diploids at the head of each column

Diploid:	C955		C971		C965		C968		C972	
Genotype:	+ + + − − − − +		+ − − + − + + −		− + + + − + − − − +		− + + + − + − − − +		− + + − + + − − + −	
	Genotypes of white colonies:						Genotypes of red colonies:			
	− − − + − − − +	18	− + + − − + + −	17	+ + + − − + + −	1	+ − − + − − − +	1	+ + − + − − + −	2
	− − + − − − − +	3	− + + − − − − +	1	+ + + − − − − +	1	+ − − + − + + −	2	+ + − + − + − +	1
	− + + − − + − +	2	− + − + − − + −	1				$\overline{2}$		$\overline{3}$
	− + + − − − − +	1	− + + − − + + −	1						
	− + + − − + + −	1	− − − + − − − +							
	− + + − − − − +	$\frac{1}{26}$		$\overline{20}$						

The mosaic white colony is designated by the vertical line. The symbols in parentheses indicate that the orientation of the two markers with reference to the others was not tested.

lelic in both white sectors, case 3 is heteroallelic in one white sector and homoallelic in the other, and case 16 is the only colony that is mosaic for three genotypes and is heteroallelic in all three. Since only six samples from each sector were analyzed for genotype, it is quite possible that we missed finding all of the cases of mosaicism, especially among the multiple mosaics. Thus the mosaic colonies are about 18% of the total number of sectored colonies. As was concluded elsewhere (ROMAN and FABRE 1983; FABRE, BOULET and ROMAN 1984) conversion occurs at an appreciable rate in G_2 but not as frequently as in G_1. Another interpretation of mosaicism is favored by GOLIN and TAMPE (1988).

In C965, C967 and C968, all of which are heteroallelic *ade6*-21/1, there were 63 sectored colonies with no detectable mosaicism in either sector. There were also, in this group, 16 mosaic sectors, of which two showed three genotypes. Thus, of 79 sectored colonies, 16, or 20%, showed genotypic mosaicism in either the red or white sector. These results supplement those given for C972 and show that gene conversion does indeed occur in G_2.

We turn now to the evidence for mosaic sectors in colonies obtained from the heterozygotes. In experiment 85-9-6, utilizing the diploid strain C955 that is heterozygous for *ade6*-1, there were 41 colonies that had one genotype in each sector. Two others were mosaic for two genotypes in the red sector, one had three genotypes in the red sector and also had two genotypes in the white sector. There were thus four mosaics among 44 sectored colonies. In experiment 84-10-17, also with C955, there were 28 colonies with a single genotype in each sector, three colonies with two genotypes in the red sector and one colony with three genotypes in the white sector. Summarizing the results of two other experiments with C955 (84-11-13 and 84-11-28), 76 sectored colonies had one genotype in each sector, 10 were mosaic for 2 genotypes, 8 on the red side and two on the white side. One other had three genotypes in the red sector. Thus there were 19 mosaic colonies among a total of 163 sectored colonies, or 11.7%. In another diploid, C971, there were 51 sectored colonies with no detectable mosaics and four that were mosaic for two genotypes on the red side; one was similarly mosaic on the white side and one was mosaic for three genotypes, two on the red side and one on the white side. Of 57 colonies, therefore, 7 sectors were mosaic in one or the other sector. Thus, in summary, 26 mosaic sectors were obtained in 220 sectored colonies or 11.8%. The difference between this figure and the larger percentage of mosaic sectors from the heteroallelic crosses can be attributed to the difference in the origin of the sectored colonies in the heterozygotes versus the heteroallelics. As the data in Table 4 show, 82 of the 145 sectored colonies sampled from the heterozygotes were due to crossovers proximal to the *ADE6* locus and presumably had no or very little effect on mosaicism. If we reduce the number to those that involve

TABLE 9

Mosaic colonies found among the sectored colonies of C972, in addition to those in Table 7; x and y represent homoallelism and heteroallelism, respectively

	+	+	−	+			+	+	−	+				+	−	+	−			+	−	+	−	
Ay 1	−	−	+	−	y		−	−	−	+	y	11	+	−	+	−	x		−	+	+	−	x	
	−	+	−	+			−	+	−	+			−	−	+	−			−	−	+	−		
	−	−	+	−			−	−	+	−			−	−	+	−			−	−	+	−		
	+	−	+	−			+	−	+	−			+	+	−	+			+	−	+	−		
Bx 2	−	+	−	+	x		−	+	−	+	x	12	−	+	+	−	x		−	−	−	+	y	
	−	+	+	−			−	−	+	−			−	+	−	+			−	−	+	−		
	−	−	−	+			−	+	−	+			−	+	+	−			−	−	−	+		
												or												
	+	−	+	−			+	−	+	−			+	+	−	+			+	−	+	−		
By 3	−	+	−	+	y		−	+	+	−	x		−	+	+	−	y		−	−	−	+	x	
	−	−	+	−			−	−	+	−			−	−	−	+			−	+	−	+		
	−	+	−	+			−	+	−	+			−	−	+	−			−	+	+	−		
	+	+	−	+			+	+	−	+			+	+	−	+			+	−	−	+		
Cx 4	−	+	−	+	y		−	+	−	+	x	13	−	−	−	+	x		−	+	−	+	y	
	−	+	−	+			−	−	+	−			−	+	−	+			−	+	+	−		
	−	−	+	−			−	−	+	−			−	−	−	+			−	−	−	+		
												or												
	+	−	+	−			+	−	+	−			+	+	−	+			+	−	−	+		
Dx 5	−	−	+	−	x		−	−	+	−	x		−	−	−	+	y		−	+	−	+	x	
	−	+	−	+			−	−	+	−			−	+	+	−			−	+	−	+		
	−	+	−	+			−	−	+	−			−	−	−	+			−	−	−	+		
	+	−	+	−			+	−	+	−			+	+	+	−			+	+	+	−		
Dx 6	−	−	+	−	x		−	−	+	−	y	14	−	−	+	−	x		−	−	+	−	x	
	−	+	−	+			−	−	+	−			−	+	−	+			−	+	+	−		
	−	+	−	+			−	−	+	−			−	−	−	+			−	−	+	−		
	+	−	+	−			+	−	+	−			+	+	+	−			+	+	+	−		
Dx 7	+	−	+	−	y		−	−	+	−	x	15	−	−	+	−	x		−	−	+	−	y	
	−	+	−	+			−	+	−	+			−	+	−	+			−	+	+	−		
	−	−	+	−			−	+	−	+			−	−	−	+			−	−	+	−		
or																								
	+	−	+	−			+	−	+	−			+	+	−	+			+	−	+	−		+	+	+	−	
Ey	−	−	+	−	y		+	−	+	−	x	16	−	+	−	+	y		−	−	+	−	y	−	+	+	−	y
	−	+	−	+			−	+	−	+			−	+	+	−			−	+	+	−		−	+	+	−	
	−	−	+	−			−	+	−	+			−	−	+	−			−	−	+	−		−	−	+	−	
	+	+	−	+			+	+	−	+																
Ix 8	−	+	−	+	x		−	+	−	+	x															
	−	+	−	+			−	+	+	−																
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	+	+	−	+			+	+	−	+																
Jy 9	−	+	−	+	x		−	+	−	+	y															
	−	+	−	+			−	+	+	−																
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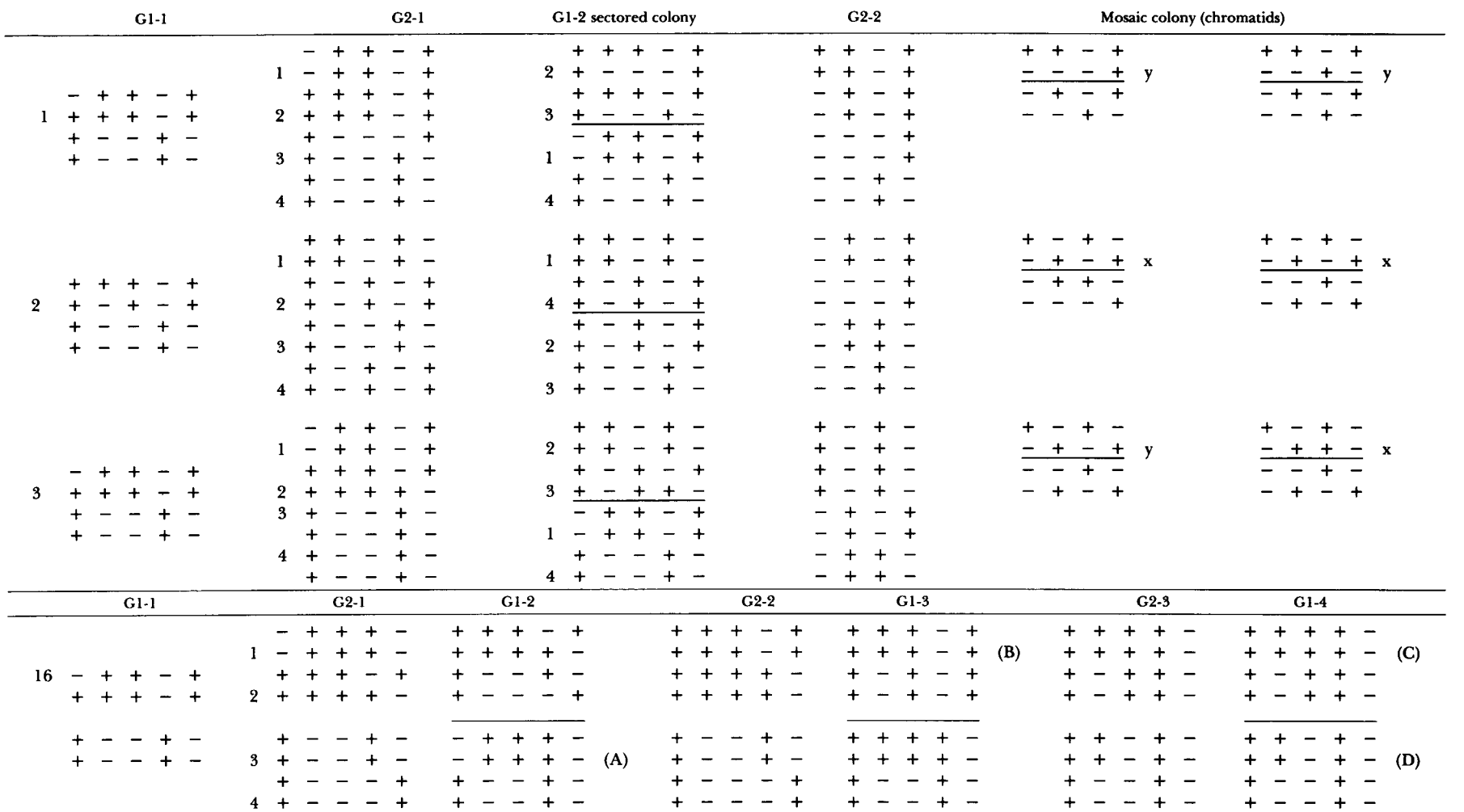
	+	+	+	−			+	+	+	−																
Oy 10	−	+	−	+	x		−	+	−	+	x															
	−	−	+	−			−	+	+	−																
	−	−	−	+			−	+	−	+																

There were 15 sectors with two genotypes and one with three genotypes (see text for details and for additional information). Colonies 7, 12 and 13 are indeterminate as to which genotype in the red sector is associated with one or the other genotype in the white sector. Therefore both possibilities are shown. The designation in the first column indicates that one of the genotypes corresponds to one that is shown in Table 7.

TABLE 10

Explanation of four of the cases in Table 9



The first 4 columns represent DNA duplexes in G1 and G2. The *ade6* heteroalleles are shown at the left, *Cly8/cly* in the middle and *SUC1/MAL1* at the right. All have transfer of DNA strand in G1 to produce + + (wild-type) at *ADE6* locus as shown. Case 1 has a − + displacement of + − in duplex 3 of G2-1. G2-2 represents replication of duplexes of red sector and displacement of + − by − + in duplex 3. Also + = + + and − = − + and + − at *ADE6* locus. Case 2 has crossover in G2-1 between duplexes 1 and 4 in region between *ADE6* and *CLY8* loci. G2-2 represents replication of duplexes of white sector with double crossover between duplexes 2 and 3, in region between *ADE6* and *CLY8* loci and between *CLY8* and *SUC1* loci. Case 3 has crossover in G2-1 between 2 and 3 and displacement of − + by + − in duplex 2. G2-2 represents replication of duplexes of red sector and segregation gives red sectors of correct genotypes. To obtain homoallelic from heteroallelic white sectors conversion and mismatch repair in G1-3 (not shown) is required. Case 16 has a more extensive format. G2-1 has crossover between duplex 1 and 4 and displacement of − + by + − in duplex 2. A is the genotype of the white sector which is the same in all three mosaic genotypes. The G2-2 of the red sector segregates to produce genotype B. The other product of G2-2 (the lower part of G1-3) undergoes a two-strand double crossover in the regions on both sides of the *CLY8* locus, shown in G2-3. All genotypes in case 16 are shown as duplexes. The 1-3, 2-4 segregation produces genotypes C and D. B, C and D are the mosaic genotypes in the red sector of case 16.

the marker region, the proportionate decrease is 43% (63/145). Applying this correction to the percentage of mosaics from the heterozygotes 11.8/.43 gives a value of 27%, not significantly different from that of the mosaics from the heteroallelics ($P = 0.20$).

The frequency of mosaic colonies is higher than one might expect on the basis of the incidence of sectored colonies that do not show mosaicism. However, it should be recalled that x-rays induce recombinational competence (FABRE and ROMAN 1977). The strength of this effect is likely to be more pronounced in the earliest cell divisions of the colony.

DISCUSSION AND CONCLUSIONS

The hypothesis to fit the facts in mitotic gene conversion and crossing over can be summarized as follows. (1) Gene conversion, as defined by irregular segregation can take place, and does so predominantly, in G_1. (2) A single heteroduplex is sufficient to account for most of the evidence, though not all, of gene conversion in *ade6* heterozygotes. (3) Mitotic crossing over, associated with gene conversion, occurs in the following G_2, although it may occur infrequently in G_1. (4) In heteroallelic diploids, a single heteroduplex followed by mismatch correction of one or the other heteroalleles is sufficient to account for most of the instances of conversion. The associated crossovers again occur mostly in G_2, although it is possible, here again, that some take place in G_1 (5). The evidence from mosaic sectors of sectored colonies provides evidence for gene conversion in G_2 in as many as about 20% of the sectored colonies. This is true for both the heteroallelic and heterozygous diploids. In the mosaic sectors, the evidence is compelling that conversion at the *ADE6* locus occurs in G_1 and is followed by conversion and associated crossing over in G_2. In the case of nonrepair in G_2, homozygosity for the marker in question is accomplished by DNA replication in the next division.

It was proposed in a previous paper (ROMAN and FABRE 1983) that gene conversion in G_1 was followed by crossing over in G_2 in mitosis. The reason for this proposal was that sectored colonies were obtained which could not be explained by concomitant conversion and crossing over in G_1. In Tables 4 and 7 of this paper, classes 10, 11, 12, 13, 21, 23, 24, and 27 in Table 4, and classes Ky, Lx, Ny, and Oy in Table 7 fall into the category that cannot be explained by the simultaneous occurrence in G_1 of gene conversion and crossing over. Not included are the ambiguous cases which could have had a different placement of each crossover as explained earlier. These could be accounted for as simultaneous events in G_1 or as conversion in G_1 followed by crossing over in G_2. Further, the existence of mosaic sectored colonies is incompatible with the concept of the simultaneous occurrence of the two events in G_1.

We have not been able to test unequivocally in *Saccharomyces cerevisiae* for the frequency of gene conversion in the G_1 immediately preceding the onset of meiosis. SHERMAN and ROMAN (1963) may have provided evidence of this kind by placing stationary phase cells in sporulation medium and removing a known number of cells at intervals for platings on drop-out media. The prototrophs thus obtained from heteroallelic cells increased in frequency with time in the sporulation medium. It was observed that there were two waves of prototrophs, the two being about equal in size. We can now interpret this finding as possibly indicating the premeiotic (prior to DNA replication) and meiotic contribution to the final conversion frequency. ESPOSITO and ESPOSITO (1974) investigated gene conversion in *S. cerevisiae* by placing cells in sporulation medium and removing aliquots to YEPD at various time intervals. They found that these cells reverted to the mitotic type of division until a time was reached in sporulation medium after which they were committed to meiosis. During the time when they remained uncommitted, these cells showed increasing amounts of gene conversion, significantly more than the spontaneous rate in mitosis. The only inducing agent in their experiments was starvation of the cells in the sporulation medium. The converting heteroalleles were *lys2* (chromosome *II*) and *leu1* (chromosome *VII*). We cannot be sure that their cells were in G_1 when gene conversion was increasing so dramatically. These experiments should be repeated using sectored colonies and applying the homoallelic-heteroallelic test for the transition from G_1 to the G_2 of meiosis.

Some of the other organisms which have been used extensively for studies of conversion in meiosis do not lend themselves to studies in mitosis. *Neurospora crassa* (IYENGAR *et al.* 1977), *Sordaria fimicola* (BELL and THERRIEN 1977) and *Ascobolus immersus* (J.-L. ROSSIGNOL, personal communication) do not have a sustained diplophase in which the heterozygous or heteroallelic condition can be established and, in fact, undergo karyogamy after DNA replication. A diplophase that is durable can be selected for in *Schizosaccharomyces pombe* (EGEL *et al.* 1980; GROSSENBACHER-GRUNDER 1985). Moreover, mutants are available in this organism that arrest premeiotic cells in G_1 (EGEL 1973). Thus *S. pombe* is well suited to studies of mitotic recombination as reported here and offers also the possibility of a direct measurement of the contribution of the premeiotic G_1 to the meiotic frequency of gene conversion.

Another reason for investigating the frequency of premeiotic gene conversion is the occurrence of heterologous conversion, *i.e.*, conversion between non-

homologous chromosomes, found in both *S. pombe* and *S. cerevisiae* (SZANKASI *et al.* 1986; LICTEN, BORTS and HABER 1987). How homologous sequences in different chromosomes find each other is not clear. We suggest that pairing starts in premeiotic G_1 when the DNA duplexes search for homologous partners. In the search for homology, mistakes are sometimes made and homologous sequences on nonhomologous duplexes pair momentarily but long enough to engage in conversion and sometimes even in a crossover that leads to a translocation. Our suggestion can be tested since *Neurospora, Sordaria* and *Ascobolus*, not having a premieotic G_1, should therefore not exhibit heterologous conversion.

We have postulated a third mechanism for gene conversion to account for the results that we have obtained. The most important event in all three mechanisms is the formation of a single heteroduplex. The Meselson-Radding and Szostak *et al.* hypotheses include, in addition, an obligatory double heteroduplex following the single (SZOSTAK *et al.* actually require two double heteroduplexes). SZOSTAK *et al.* propose an additional source of gene conversion, without the intervention of a heteroduplex, in their gap-closure hypothesis. In our results, the double heteroduplex is considerably more rare than the single but it does occur as our evidence of mosaic sectors shows. We prejudice our results against double heteroduplexes in the procedure of obtaining sectored colonies so that not all are detected when they occur. The relatively low frequency of mosaic sectors in G_2 shows double heteroduplexes are not predominant in our material.

We wish to thank URS LEUPOLD, Bern (Switzerland) RICHARD EGEL, Copenhagen (Denmark) and JEAN-LUC ROSSIGNOL, Orsay (France) for prompt replies to our questions; and to FRANCIS FABRE, Orsay (France) for valuable comments regarding the manuscript. This research was funded by U. S. Public Health Service grant GM 27949.

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Communicating editor: D. BOTSTEIN