Meiotic Mismatch Repair Quantified on the Basis of Segregation Patterns in *Schizosaccharomyces pombe*

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ABSTRACT

Hybrid DNA with mismatched base pairs is a central intermediate of meiotic recombination. Mismatch repair leads either to restoration or conversion, while failure of repair results in post-meiotic segregation (PMS). The behavior of three G to C transversions in one-factor crosses with the wild-type alleles is studied in *Schizosaccharomyces pombe*. They lead to C/C and G/G mismatches and are compared with closely linked mutations yielding other mismatches. A method is presented for the detection of PMS in random spores. The procedure yields accurate PMS frequencies as shown by comparison with tetrad data. A scheme is presented for the calculation of the frequency of hybrid DNA formation and the efficiency of mismatch repair. The efficiency of C/C repair in *S. pombe* is calculated to be about 70%. Other mismatches are repaired with close to 100% efficiency. These results are compared with data published on mutations in *Saccharomyces cerevisiae* and *Ascobolus immersus*. This study forms the basis for the detailed analysis of the marker effects caused by G to C transversions in two-factor crosses.

ASCOMYCETOUS fungi amenable to tetrad analysis have provided a wealth of information on meiotic recombination, because all four chromatids resulting from individual meiocytes can be analyzed. Two types of aberrant (non-Mendelian) segregation are observed. One is the nonreciprocal transfer of information from both DNA strands of a chromatid to one of the chromatids of the other homologous chromosome ($6^+:2^-$ and $2^+:6^-$; the numbers refer to the DNA single-strands present in a tetrad). Segregation of this type will be called whole chromatid conversion (WCC). The second type of aberrant segregation is post-meiotic segregation (PMS). The information of only one strand of DNA is transferred between homologous chromatids and the most frequent segregation patterns are $5^+:3^-$, $3^+:5^-$ and aberrant $4^+:4^-$. PMS tetrads contain one ($5^+:3^-$ and $3^+:5^-$) or two (aberrant $4^+:4^-$) haploid spores that segregate both parental alleles of an involved marker in the first mitotic division after meiosis.

The occurrence of PMS tetrads is evidence for the formation of heteroduplex DNA. $5^+:3^-$ and $3^+:5^-$ segregations are more frequent than tetrads with two PMS spores. They probably result from transfer of one DNA single-strand. Tetrads with two PMS spores (aberrant $4^+:4^-$) may be the result of symmetric exchange of two single strands between chromatids. The rare aberrant $6^+:2^-$ and aberrant $2^+:6^-$ tetrads must be explained by independent asymmetric single-strand transfer which can also lead to aberrant $4^+:4^-$ tetrads [see FOGEL, MORTIMER and LUSNAK (1981) for definition of these segregation types]. There is evidence for the formation of symmetric hybrid DNA from the *b2* locus of *Ascobolus immersus* (PAQUETTE and ROSSIGNOL 1978) and from the *grey* locus of *Sordaria fimicola* (KITANI and WHITEHOUSE 1974). In *Saccharomyces cerevisiae* the segregations characteristic for symmetric heteroduplex DNA (aberrant 4:4, aberrant 5:3 and 4:4 apparent 2 strand double crossover) are quite rare compared to the frequencies predicted from the appearance of 6:2 and 5:3 segregations (FOGEL *et al.* 1979; DETLOFF, SIEBER and PETES 1991). These criteria imply that heteroduplex formation is asymmetric rather than symmetric in budding yeast. The tetrad data from *Schizosaccharomyces pombe* (GUTZ 1971; THURIAUX *et al.* 1980) allow no distinction between symmetric and asymmetric hybrid DNA formation.

Two hypotheses have been put forward for the explanation of WCC. The first one assumes hybrid DNA formation followed by mismatch repair. Repair is either toward recipient chromatid information (restoration, non detectable $4^+:4^-$) or toward donor chromatid information (conversion, detectable as $6^+:2^-$, $2^+:6^-$) (HOLLIDAY 1964; MESELSON and RADDING 1975; RADDING 1982). The alternative is conversion by repair of double-strand gaps with sequences copied from the homologous chromatid (SZOSTAK *et al.* 1983). There are several lines of evidence favoring hybrid DNA formation for the major part of genes. First, the relative frequency of PMS for a given marker is not correlated with its position in genes showing a gradient of frequencies of total aberrant tetrads. The segregation pattern for a mutation depends on the type of mismatches rather than the position in the gene (PAQUETTE and ROSSIGNOL 1978; FOGEL, MORTIMER and LUSNAK 1981; WHITE, LUS-

NAK and FOGEL 1985; LICHTEN *et al.* 1990; DETLOFF, SIEBER and PETES 1991). This is incompatible with the proposition that double-strand breaks at initiation sites are extended to gaps reaching far into the high conversion side of genes. Second, the frequencies of PMS and WCC appear to be interconnected. Mutant alleles have been identified that display increased PMS at the expense of WCC relative to nearby alleles (NAG, WHITE and PETES 1989; FOGEL *et al.* 1979; this study). This is best interpreted as mutation or mismatch specific processing of hybrid DNA. Third, yeast strains defective in mismatch repair enzymes show an increased frequency of PMS and a decreased frequency of WCC for most of the alleles analyzed (WILLIAMSON, GAME and FOGEL 1985; BISHOP, ANDERSEN and KOLODNER 1989; KRAMER *et al.* 1989).

If heteroduplex DNA is the major intermediate, the segregation pattern of any mutation is directly related to the repair properties of the mismatches. Two types of mismatches result from each heterology. Base substitutions yield four possible pairs of mismatches: C/C-G/G; A/A-T/T; G/T-A/C and G/A-T/C. Conventional tetrad analysis does not identify the interacting DNA single-strands and thus the specific mismatch created in a chromatid. Therefore PMS frequencies are only a measure for the compound repair efficiencies of a mismatch pair. In *S. pombe* and *S. cerevisiae* PMS generally constitutes a minor fraction of the total aberrant segregations. The reported PMS frequencies in crosses in which the three mismatch pairs A/A-T/T, G/T-A/C and G/A-T/C may occur are below 3% in *S. pombe* (GUTZ 1971; THURIAUX *et al.* 1980) and vary between 3% and 8% in *S. cerevisiae* (FOGEL, MORTIMER and LUSNAK 1981). All base substitutions showing high frequency of PMS are G to C transversions: 26%-28% in fission yeast (this study) and 33%-48% in budding yeast (FOGEL, MORTIMER and LUSNAK 1981). Thus, repair of either the C/C or the G/G mismatch, or both, is inefficient. LICHTEN *et al.* (1990) demonstrated by a physical analysis of spore DNA that the C/C mismatch at *arg4-nsp* is repaired at least fivefold less efficiently than the G/G mismatch. DETLOFF, SIEBER and PETES (1991) used a genetic approach for the identification of persisting mismatches. In agreement with LICHTEN *et al.* (1990) they concluded that C/C mismatches resulting from a G to C transversion in the *HIS4* initiation codon are corrected at least threefold less efficiently than all other mismatches.

Data on mismatch repair in mitotic cells have been obtained by transformation of a mutant with heteroduplexes constructed *in vitro*. The mutation affects colony color and transformed cells develop either into uniformly colored colonies (repair) or colonies of mixed colors (failure of repair). KRAMER *et al.* (1989) found that C/C mismatches escaped repair to a much greater extent than all other mismatches. In a similar study BISHOP, ANDERSEN and KOLODNER (1989) concluded that C/C, A/A and T/T mismatches are less efficiently repaired than A/C, G/T, G/G and C/T. In addition studies of mismatch repair efficiencies in prokaryotes also revealed poor repair of C/C relative to other mismatches [reviewed by MODRICH (1987)].

This work is related to a detailed characterization of the marker effects of G to C transversions observed in two-factor crosses in *S. pombe* (SCHÄR and KOHLI 1993). In order to provide the necessary background for the explanation of the marker effects, the meiotic repair of C/C and G/G mismatches is analyzed. Three G to C transversions and the corresponding control mutations in the genes *sup3* and *ade6* are examined. The low frequency of aberrant segregation at the *ade6* locus forced us to establish a method for PMS rate determination from random spores. The higher aberrant segregation frequencies at *sup3* provides the opportunity for evaluation of the new method by comparison with PMS frequencies obtained from tetrads. A scheme for quantitative treatment of tetrad and random spore data is presented. It allows the estimation of the frequency of hybrid DNA formation as well as of repair efficiencies for mismatch pairs for any particular site. We apply the scheme for calculation also to data from *S. cerevisiae* and *A. immersus* and compare our estimates with the results of a different approach to quantitation (PAQUETTE and ROSSIGNOL 1978). Finally, we discuss the underlying assumptions on mechanisms of recombination.

MATERIALS AND METHODS

Strains, media and general genetic methods: All the *S. pombe* strains used in this study are derived from the original wild-type strain of Leupold (GUTZ *et al.* 1974). The specific mutational changes are described in the listed publications.

972 h^- (GUTZ *et al.* 1974).
ade6-M387 h^+; *ade6-51* h^+; *ade6-52* h^+ (SCHÄR and KOHLI 1093).
ade6-M387 leu2-120 h^-; *ade6-51 leu2-120* h^- (this study).
ura2-10 h^+; *leu2-120* h^-(strain collection, Bern).
ade6-M216 h^+(SZANKASI *et al.* 1988).
ade6-704 sup3-UGA h^+/h^-(KOHLI *et al.* 1984).
ade6-704 sup3-UGA,CA52 h^+ (KOHLI *et al.* 1984).
ade6-704 sup3-UGA,r36 h^-(KOHLI *et al.* 1984).

The strains *ade6-M387 leu2-120* h^- and *ade6-51 leu2-120* h^- were constructed by crossing *ade6-M387* h^+ and *ade6-51* h^+ with *leu2-120* h^-. The standard media, yeast extract agar (YEA), malt extract agar (MEA) and the general genetic methods are described by GUTZ *et al.* (1974). The minimal medium (MMA) consists of 0.67% Difco yeast nitrogen base without amino acids, 1% glucose and 1.8% agar.

Determination of conversion and PMS frequencies by tetrad analysis: Conversion and PMS at the *sup3-UGA* suppressor-inactivating second-site mutations *CA52* and *r36* were investigated by tetrad analysis in crosses homozygous for *ade6-704* and the *sup3-UGA* anticodon. Conversion of *ade6-M387* was studied in tetrads from a one-factor cross with the 972 h^- wild-type strain (Table 2). In these crosses, Mendelian segregation will result in two white and two red colonies on a medium with limiting adenine concentration

(YEA). The red colony phenotype is caused by the unsuppressed *ade6-704* mutation. Aberrant segregations are readily detected by non 2:2 segregation of colony color. PMS tetrads contain one red and white-sectored colony. It may be noted that such sectors never appeared as clean-cut red and white halves. Red and white cell material was always interspersed. This is also seen in reconstruction experiments. If a red and a white cell are placed adjacent to each other on a YEA plate or a MMA plate supplemented with 10 mg/liter adenine, no clear-cut sector was observed among more than 200 cell pairs (data not shown). If cells are placed more than seven cell lengths apart from each other, sharply defined red and white sectors appeared.

All spore colonies from aberrant tetrads (and in the case of PMS colonies both sectors) were checked for prototrophy and auxotrophy. This excludes mutations in early genes of the adenine pathway producing white auxotrophs. Furthermore all were checked for ploidy on the basis of cell size (microscopical inspection) and for regular mating-type segregation.

PMS detection in random spores: Since aberrant segregation rates of *ade6* alleles are low (0.2–0.4%), determination of PMS rates by tetrad analysis is impractical. To circumvent this problem, we established a random spore analysis system for the analysis of PMS at *ade6-M387* and *ade6-51*. As a control PMS rates were determined for *sup3-UGA,CA52* and *sup3-UGA,r36* by tetrad analysis and, in parallel, from random spores. The principle of the method is described in RESULTS AND DISCUSSION. Spore material from the crosses illustrated in Table 2 was treated with snail enzyme to eliminate vegetative cells (MUNZ and LEUPOLD 1979). A sample of 0.1 ml of appropriate dilutions of the spore suspensions was plated on MMA supplemented with limiting amounts of adenine (10 mg/liter). The spore titer was adjusted to yield 30 colonies per plate. In case of the crosses with *ade6-M387* and *ade6-51* the selection for *leu2*$^+$ *ura2*$^+$ recombinants had to be considered. The *leu2*$^+$ *ura2*$^+$ recombinants amount to 1.3% of total spores (2.6 cM genetic distance). The titer was adjusted to 2.3×10^4 spores/ml corresponding to about 30 growing units per 0.1 ml spore suspension. There was no selection in the crosses involving the *sup3* second site mutations; thus, titers were adjusted to 300 spores/ml. The plates were incubated 5 days at 30° and another 2 days at 22° to enhance red color intensity of *ade6* auxotrophs. Each individual colony was then visually inspected for the presence of interspersed red and white sectors. The red and the white parts of colonies unambiguously showing such sectors were reisolated and submitted to the checking procedure already described for the aberrant tetrads. The number of genuine PMS colonies was calculated as described in RESULTS AND DISCUSSION. In addition the content of diploid spores was determined by replicating a representative number of colonies on MMA containing the dye phloxin B (KOHLI *et al.* 1977). The frequency of diploids (colonies staining dark red) was used to obtain the correct number of haploid colonies that were screened for PMS.

RESULTS AND DISCUSSION

Observations on post meiotic segregation in *S. pombe* and other fungi: For the calculation of the frequency of hybrid DNA formation and the efficiency of mismatch repair we are relying on sequenced mutations as far as possible. Figure 1 shows the nature of the mutations in the genes *ade6* and *sup3* we used in this study. C/C-G/G is the first of four possible pairs of conjugate mismatches. They are formed by G to C transversions. In *S. pombe* all mutations of this type show high PMS in tetrad or random spore analysis (Table 1). Among them are *ade6-M387*, *sup3-UGA* and *sup3-UGA,CA52* (Figure 1). High PMS for this type of mutation has also been described in *S. cerevisiae* (WHITE, LUSNAK and FOGEL 1985; LICHTEN *et al.* 1990; DETLOFF, SIEBER and PETES 1991). Furthermore, in both yeasts these are the only base substitutions that confer a strong marker effect on intragenic recombinant frequencies (THURIAUX *et al.* 1980; MOORE *et al.* 1988). This phenomenon is studied in detail in SCHÄR and KOHLI (1993). The second pair of mismatches G/T-A/C is represented by a large number of mutations in *S. pombe*. None of them have shown high PMS. Representatives of this group are *ade6-M216*, a G to A transition at the 5′ end of the gene, and *ade6-52* (T to C transition) and *ade6-51* (C to T transition) separated by 312 nucleotides at the 3′ end of the gene (Figure 1). *ade6-51* is separated by only four nucleotides from the G to C transversion *ade6-M387*. In the tRNA gene *sup3-UAA* and *sup3-UGA,r36* are G to A transitions affecting exactly the same base pairs as the G to C transversions mentioned above (KOHLI *et al.* 1984). Mutations yielding this second type of mismatch pairs do not show high PMS in *S. cerevisiae* either (DETLOFF, SIEBER and PETES 1991). Four mutations in *S. pombe* are known to form the third pair of mismatches A/A-T/T but so far the PMS frequencies are missing. Yet, in *S. cerevisiae* mismatch pairs of this as well as of the fourth type (A/G-T/C) show low PMS (DETLOFF, SIEBER and PETES 1991). The *S. pombe* G to T transversions *ade6-M375*

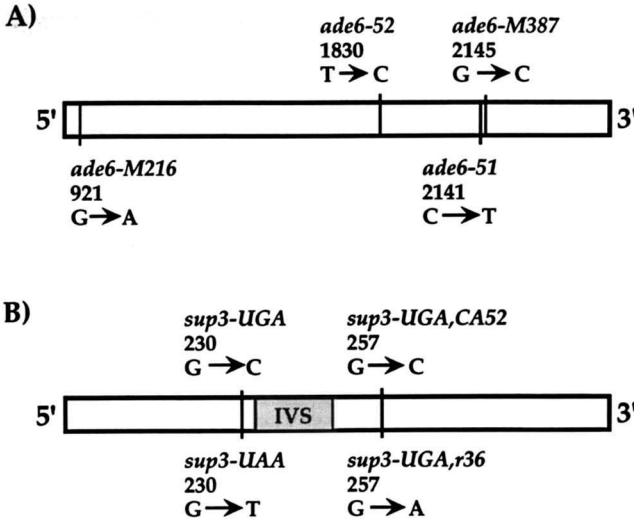

FIGURE 1.—Survey of the *ade6* and *sup3* mutants used in this study. The given 5′ to 3′ polarities of the genes and the mutational changes refer to the nontranscribed strands. (A) The open bar is the *ade6* open reading frame from bp 875 to 2533. The names of the alleles, the position of the mutations (nucleotide number), and the base substitutions are shown. The nucleotide numbering is from SZANKASI *et al.* (1988). (B) The part of the *sup3* gene coding for the precursor of $tRNA^{Ser}$ is shown. It extends from bp196 to 292. The shaded box is the intron (IVS) of 15 bp (HOTTINGER *et al.* 1982).

TABLE 1

Conversion and PMS frequencies determined by tetrad analysis

Allele	Mutation	Total no. of tetrads	WCC			PMS		
			6:2	2:6	%	5:3	3:5	%
ade6-M216[a]	G to A	2353	6	4	0.42	0	0	0
ade6-52[b]	T to C	1032	2	1	0.29	0	0	0
ade6-M387	G to C	2365	4	1	0.21	1	0	0.04 (<0.14)
sup3-UGA[c]	G to C	3999	37	25	1.6	14	9	0.58 (0.33–0.83)
sup3-UAA[a]	G to T	654	7	5	1.8	0	0	0
sup3-UGA,CA52	G to C	1085	9	5	1.3	4	1	0.46 (0.01–0.91)
sup3-UGA,r36	G to A	1105	7	9	1.5	0	1	0.09 (<0.31)

Percentages of PMS tetrads are given with their 95% confidence limits. The values are calculated according to the formula: $L_{95\%} = p \pm (1.96 \sqrt{p(100 - p)/n + 50/n})$, with p = proportion of PMS (%) and n = total number of tetrads analyzed (SNEDECOR and COCHRAN 1967).

[a] Pooled with data from THURIAUX *et al.* (1980).

[b] Data from GUTZ (1971).

[c] Data from MUNZ and LEUPOLD (1979).

and *ade6-M26* produce A/G-T/C mismatch pairs. Both mutations show low PMS in tetrads (GUTZ 1971). None of the mutations of *A. immersus* treated in this work have been sequenced.

Determination of WCC and PMS frequencies in *S. pombe*: In this section we combine data from GUTZ (1971) and THURIAUX *et al.* (1980) with our own results on segregation patterns in tetrads (Table 1) and PMS in random spores (Table 2).

Tetrads were dissected from the one-factor crosses *ade6-704 sup3-UGA* h^- × *ade6-704 sup3-UGA,CA52* h^+ and *ade6-704 sup3-UGA* h^+ × *ade6-704 sup3-UGA,r36* h^-. The G to C transversion *CA52* shows 0.46% PMS. The control mutation *r36* (G to A transition) alters the same nucleotide, but shows only one PMS in 1105 tetrads. The resulting frequency is 0.09% (Table 1). A similar result was obtained earlier for the G to C transversion *sup3-UGA* (0.58% PMS) and its control *sup3-UAA* (G to A transition, no PMS in 654 tetrads). All four mutations yield WCC events. Both G to C transversions show almost identical PMS/WCC ratios (0.35 and 0.36). No gradient of aberrant segregation frequencies is discernible for this short tRNA gene and the frequency of total aberrant segregation is approximately 2% for any mutation at the *sup3* locus (Table 1) (THURIAUX *et al.* 1980).

Aberrant segregation of *ade6* mutations is found with decreasing frequency from *ade6-M216* (0.42% WCC) at the "high" conversion end to the distal alleles *ade6-52* (0.29% WCC) and *ade6-M387* (0.21% WCC + 0.08% PMS). The latter values were obtained from the cross *ade6-M387* h^+ × 972 h^- (Table 1). Under these circumstances a determination of the PMS/WCC ratio for the G to C transversion *ade6-M387* is not feasible by tetrad analysis (PMS < 0.1%). For this reason and for future application to other mutations we established a method for determination of PMS frequencies from random spores. To have a control for the validity of this new method, we analyzed the *sup3* alleles *CA52* (G to C) and *r36* (G to A) along with the *ade6* allele *M387* (G to C) and its control *51* (C to T). Before the results are discussed the experimental procedure is described.

Spores from the four crosses illustrated in Table 2 are plated on minimal medium supplemented with limiting adenine, on which *ade6* mutants form red and *ade*$^+$ cells white colonies. After incubation colonies are counted and inspected for the presence of red/white sectors. Besides genuine PMS colonies sectored colonies of three different origins are expected.

I: Two or more spores from a tetrad expressing different *ade6* phenotypes may not be separated during the plating procedure. The frequency of such sticky spores can be reduced experimentally to about 0.9×10^{-3} spores or 3.6×10^{-3} tetrads by extensive vortexing. But it still interferes with the determination of PMS frequencies below 0.36% as expected for *ade6-M387*. The inclusion of the two auxotrophic markers *leu2-120* and *ura2-10* in the *ade6* crosses allowed a further reduction of these events by selection for *leu2*$^+$ *ura2*$^+$ recombinants. The two loci are closely linked (2.6 cM) and reside on chromosome *I* assuring independent segregation from the *ade6* heteroalleles on chromosome *III*. A single exchange event within the short interval is required to produce one *ura2*$^+$ *leu2*$^+$ spore in a tetrad (tetratype) and only four-strand double-crossover events give rise to two prototrophic spores in one tetrad (nonparental ditype; NPD). Relying on the equations published by SNOW (1979) tetratypes are expected to occur with a frequency of 5×10^{-2} whereas NPDs appear with a probability of only 3.2×10^{-4}. Thus the overall likelihood of NPD tetrads (3.2×10^{-4}) harboring two sticky spores (3.6×10^{-3}) that are prototrophic (1/6) is: $p(\text{NPD}_{(++)}) = (3.2 \times 10^{-4})\ (3.6 \times 10^{-3})\ (1/6) = 1.9 \times 10^{-7}$. The relevant estimate is $p(\text{NPD}_{(++)})/p(\text{tetratypes}) + 2p(\text{NPD}) = 3.8 \times 10^{-6}$. Hence one $\text{NPD}_{(++)}$ per 2.7×10^5 prototrophic colonies no longer interferes with

TABLE 2

PMS frequencies determined by random spore analysis

Allele	Mutation	No. of random spores	Sectored spore colonies	Different mating type	Estimated no. of PMS	PMS per 400 spores[a]
ade6-M387	G to C	54964	23	6	11	0.08 (0.03–0.13)
ade6-51	C to T	55494	6	3	0	0
sup3-UGA,CA52	G to C	8643	21	6	9	0.42 (0.12–0.72)
sup3-UGA,r36	G to A	9305	17	13	0	0

Spores from the following crosses were examined. For a detailed description of the PMS selection system see RESULTS AND DISCUSSION:

ade6-M387 leu2-120 h⁻ × ura2-10 h⁺
ade6-51 leu2-120 h⁻ × ura2-10 h⁺
ade6-704 sup3-UGA h⁻ × ade6-704 sup3-UGA,CA52 h⁺
ade6-704 sup3-UGA h⁺ × ade6-704 sup3-UGA,r36 h⁻

ade6-704 is nonsense suppressible by *sup3-UGA*. *sup3-UGA,CA52* and *sup3-UGA,r36* are second-site suppressor-inactivating base substitutions (Figure 1).

[a] The values PMA/400 spores, which correspond to percentages of PMS tetrads, are given with their 95% confidence limits (for description see legend of Table 1).

the expected PMS frequency of one per 1250 units.

II: A second problem is represented by spores of different *ade6* phenotype that are plated next to each other by chance and grow up to an apparent PMS colony. The frequency of such events is decreased by spreading only a limited number of *leu2⁺ura2⁺* spores per plate (<35). In addition the red and white parts of each sectored colony are tested for mating-type identity. Half of the *ade6⁻* and *ade6⁺* spore-pairs plated in close proximity are expected to express different mating-types. Hence to obtain the true number of PMS events, twice the number of sectored colonies of different mating-type was subtracted from the total number of sectors.

III: Another problem consists of diploid spores heterozygous for the *ade6* alleles. Spores from such diploids grow as white colonies until a mitotic crossover between the centromere and *ade6* provides the chance for homozygous segregation of the two *ade6* alleles leading to red and white sectors. This source of false PMS events is eliminated by checking each sectored colony for ploidy and excluding all with one or two diploid parts. The frequency of diploids is also determined for correction of the total number of haploid spores analyzed in each cross.

The results of PMS analysis in random spores are shown in Table 2. Among 54,964 spores from the cross with *ade6-M387* 11 PMS colonies were inferred, yielding a reliable estimate of a PMS frequency of 0.08%. No PMS was present in a comparable number of spores from the *ade6-51* cross. The reliability of random-spore PMS analysis was tested by determination of the PMS frequency of *sup3-UGA,CA52* in tetrads (Table 1). The observed PMS frequency of 0.46% is not significantly different from that from the random spore data (0.42%, Table 2). This holds also for *ade6-M387* (0.04% in tetrads *vs.* 0.08% in random spores), though only one PMS event was observed in 2365 tetrads which allows no safe estimate of the PMS frequency (Tables 1 and 2). These comparisons show that the described procedure for PMS frequency determination yields reliable results and thus can be applied to mutations in other genes with low frequency of aberrant segregation.

The PMS/WCC ratio resulting for *ade6-M387* is 0.38 (PMS = 0.08%). It is remarkably similar to the values determined for *sup3-UGA* (0.36) and *sup3-UGA,CA52* (0.35). The near identity of these values suggests that there is virtually no effect of sequence context on PMS frequency as the surrounding DNA sequences are different for the three G to C transversions (KOHLI *et al.* 1984; SZANKASI *et al.* 1988; Figure 1).

Quantitations on the basis of a mismatch repair model

For the quantitative interpretation of our data we have developed a scheme for calculation of the frequency of hybrid DNA formation and the efficiency of mismatch repair. These calculations are based on a mismatch repair model that makes specific assumptions about several parameters from the currently favored models for meiotic recombination. In the following sections we introduce our calculation model and state the assumptions. We also discuss experimental data from the literature that support or contradict them. Basically we have developed the model for mismatch repair in *S. pombe*, but we also discuss its application to data from *S. cerevisiae* and *A. immersus*.

The gradient of frequencies of aberrant tetrads for different mutations across a gene is generally taken as evidence for the existence of a preferred initiation site of recombination at the high conversion end and distance-dependent formation of heteroduplex DNA starting from this initiation site. The presence of double-strand (ds) breaks and the extension of ds gaps from the initiation site is controversial. While there

are data demonstrating the role of ds breaks in the initiation of homologous recombination in budding yeast (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; SUN *et al.* 1989; SUN, TRECO and SZOSTAK 1991; LIANG, ALANI and KLECKNER 1990), there is strong evidence for the absence of substantial conversion by gap repair. In *S. cerevisiae* and *A. immersus* no correlation is discernible between the frequency of PMS and the position of a mutation within genes showing strong gradients of aberrant segregation (FOGEL, MORTIMER and LUSNAK 1981; LICHTEN *et al.* 1990; DETLOFF, SIEBER and PETES 1991; PAQUETTE and ROSSIGNOL 1978). This is incompatible with double-strand gaps extending frequently into the gene from the high conversion end. Furthermore, in *S. cerevisiae* the PMS frequencies of various mutations significantly increase in crosses with a *pms1* background (WILLIAMSON, GAME and FOGEL 1985). Though conversion is still observed and could be due to gap repair, we prefer to explain the majority of these events as the result of an alternative system for mismatch repair for which we present evidence in SCHÄR and KOHLI (1993). Our model and calculations apply only to the region of genes that are not subject to double-strand gap formation, where hybrid DNA is assumed to be the exclusive intermediate for recombination. We believe, the assumption is safe for mutations that are distant from fixed recombination initiation sites.

Frequency of hybrid DNA formation: The frequency of hybrid DNA is [hDNA] = [WCC] + [restoration] + [PMS] = 2 [WCC] + [PMS], given three assumptions.

I: The rate of hybrid DNA formation is constant for any given site. It is independent of the type of mutations that may be involved at this site and their patterns of aberrant segregation. We restrict the argument to base substitutions and single base pair deletions and additions. There is no independent experimental evidence for this assumption. But our calculation of hybrid DNA frequencies based on this and the other assumptions leads to closely similar values for different mutations at the same site, even when they show widely different aberrant segregation patterns. This is demonstrated by the data in Tables 3 and 4 and by further calculations (not shown) on the results published by PAQUETTE and ROSSIGNOL (1978).

II: Hybrid DNA formation is asymmetric. The detailed analysis of the *ARG4* locus and of segregation patterns of mutations in many other genes led FOGEL, MORTIMER and LUSNAK (1981) to the conclusion that there is no evidence for symmetric hybrid DNA formation in *S. cerevisiae*. Also the analysis of the extensive segregation data on the very frequently recombining *HIS4* gene (DETLOFF, SIEBER and PETES 1991) yields no evidence for symmetric hybrid DNA formation. In contrast, *A. immersus* genes show asymmetric hybrid DNA formation that can be extended to symmetric hybrid DNA in the direction to the low conversion end (PAQUETTE and ROSSIGNOL 1978). There are not sufficient data for any locus in *S. pombe* to support the assumption. One reason for the lack of data is the generally low frequency of aberrant tetrads observed in fission yeast [<2% for most loci; THURIAUX *et al.* (1980)].

III: Hybrid DNA is repaired yielding restoration and conversion events with equal frequency. The ratio of conversion to restoration is not known for any mutation in fungi, but the question has been addressed by several authors. HASTINGS (1984) has reported genetic approaches for the simultaneous detection of conversion and restoration in genes of *A. immersus* and *S. cerevisiae*. For the *b2* locus he finds equal frequencies of conversion and restoration. For the *HIS1* locus he reports an excess of conversion over restoration. However, he draws this conclusion from a selected class of conversion events that are associated with crossing over. In addition, the conclusion is based on the assumption that crossovers occur only at the low conversion end of the gene, which is contradicted by the results of FOGEL, MORTIMER and LUSNAK (1981). A bias of conversion over restoration is thus not demonstrated convincingly by the experiments of HASTINGS (1984). DETLOFF, SIEBER and PETES (1991) raise an argument for nonrandom repair at the 5′ end of the *HIS4* gene. They assume equal frequencies of inclusion of different, closely linked mutations in hybrid DNA. Then they compare frequencies of total aberrant segregation (PMS + WCC) of a high PMS palindromic insertion mutation (*his4-B102*, 24% PMS + 15% WCC = 39%) with the average of low PMS base substitutions 50bp downstream at the *HIS4* initiation codon (5% PMS + 35% WCC = 40%). From these data the authors conclude that *all* mismatch corrections at the initiation codon are conversions and that no restoration occurs at these well-repaired mismatches. This situation might be specific for the *HIS4* locus, though the data can be explained alternatively by assumption of only weak biases toward restoration in the correction of the poorly repaired heterologies that derive from palindrome insertions. No evidence for biased repair can be derived from aberrant segregation frequencies in the *ARG4* locus as clearly documented by the increase of total aberrant segregations for *arg4-nsp*, when crosses homozygous for *pms1* are compared with controls homozygous for *PMS1*. The higher increase of PMS than decrease of WCC indicates approximate equality of conversion and restoration (Table 4). Clearly further work is needed for the assessment of conversion *vs.* restoration frequencies of the different types of heterologies in hybrid DNA.

The frequency of hybrid DNA formation at a given site is reliably reflected by aberrant segrega-

TABLE 3

Hybrid DNA frequencies and mismatch repair efficiencies in *S. pombe*

Locus	Mutation	Total no. of tetrads	WCC tetrads (%)	PMS tetrads (%)	WCC + PMS (%)	hDNA tetrads (%)[a]	Repair efficiency[b] R	Repair efficiency[b] $r_{(C/C)}$
sup3-UGA[c]	G to C	3999	1.6	0.58	2.2	3.8	84	73
sup3-UAA[d]	G to T	654	1.8	0	1.8	3.6	>96	
sup3-UGA,CA52	G to C	1085	1.3	0.46	1.8	3.1	84	73
sup3-UGA,r36	G to A	1105	1.5	<0.09	1.6	3.1	>97	
ade6-M216[d]	G to A	2353	0.42	0	0.42	0.84	>95	
ade6-52[e]	T to C	1032	0.29	0	0.29	0.58	>86	
ade6-51	C to T		0.25[f]	0[g]	0.25	0.50[f]	>98[g]	
ade6-M387	G to C	2365	0.21	0.08[g]	0.29	0.50	84[g]	73

[a] The percentage of hDNA tetrads = 2 WCC (%) + PMS (%); for description see text.
[b] The overall repair efficiency in percent (R) = 100(2WCC/hDNA); The repair efficiency of the C/C mismatch $r_{(C/C)} = 2R - r_{(G/G)} = 2R - 95$; for description see text.
[c] Data from MUNZ and LEUPOLD (1979).
[d] Pooled with data from THURIAUX *et al.* (1980).
[e] Data from GUTZ (1971).
[f] The tetrad values for *ade6-M387* are used for this estimation (Table 1).
[g] The PMS frequency determined with random spores (Table 2) is used for calculation.

TABLE 4

Hybrid DNA frequencies and mismatch repair efficiencies in *S. cerevisiae* and *A. immersus*

Organism, locus	Mutation[a]	Total no. of tetrads	WCC tetrads (%)	PMS tetrads (%)	WCC + PMS (%)	hDNA[b] tetrads (%)	Repair efficiency[b] R	Repair efficiency[b] $r_{(C/C)}$
S. cerevisiae:								
arg4-17[c]	A to T	12358	7.7	0.44	8.1	16	96	
arg4-16[c]	G to C	5586	4.6	4.2	8.8	13	71	47
arg4-nsp[d]	G to C	1913	9.4	1.5	11	20	94	93
arg4-nsp Δpms1[d]	G to C	157	6.4	7.6	14	20	64	
A. immersus:[e]								
b2-8E	Subst.	33802	13	11	24	37	70	(64)
b2-F1	Add/Del	21320	7.3	19	26	34	43	(42)
b2-G1	?	6239	2.7	19	22	24	23	(27)
b2-24	?	14240	9.0	4.4	13	22	82	(83)
b2-26	?	32619	1.1	18	19	20	11	(28)
b2-A4	Add/Del	18958	0.98	17	18	19	10	(25)

Rare independent double events in the present tetrad samples are scores as follows. 8:0 and 0:8 segregations are considered as two WCC events; 7:1 and 1:7 segregations as one WCC, and one PMS event; and aberrant 6:2, aberrant 2:6 and aberrant 4:4 as two PMS events. To account for the undetected WCC events in 4:4 (2 strand doubles or WCC + WCC), aberrant 5:3 and aberrant 3:5 (WCC + PMS) tetrads, the numbers of the corresponding detectable segregations 8:0 + 0:8 and 7:1 + 1:7, respectively, are multiplied by two.
[a] The alterations of the mutant sense strands are indicated. The *A. immersus* mutations have not been sequences.
[b] For explanation of these calculations see Table 3 and RESULTS AND DISCUSSION.
[c] Data from FOGEL *et al.* (1979).
[d] Data from LICHTEN *et al.* (1990).
[e] Data from PAQUETTE and ROSSIGNOL (1978). The order of the alleles from the top to the bottom of the list reflects their intragenic arrangement from the high to the low conversion end. In parentheses are the repair efficiencies calculated by PAQUETTE and ROSSIGNOL (1978).

tion patterns: Our results demonstrate that the equation [hDNA] = 2[WCC] + [PMS] yields very similar values for closely located alleles with different aberrant segregation patterns (low *vs.* high PMS alleles). This is the case for the data from all three fungi that have been considered (Table 4). In *S. pombe* the mutation pairs *sup3-UGA/sup3-UAA* and *sup3-UGA,CA52/sup3-UGA,r36* represent different substitutions of the same nucleotides (Figure 1). Very similar hybrid DNA frequencies result for the members of each pair (3.8%/3.6% and 3.1%/3.1%, Table 3) although they show different segregation patterns. The same is observed with the *S. cerevisiae* mutation *arg4-nsp* in presence (20%) or absence (20%) of the PMS1 activity and for the pairs of closely linked *b2* mutations of *A. immersus*.

The gradient of aberrant segregation frequencies for mutations across a gene is generally explained by initiation of heteroduplex formation at a fixed point and termination of hybrid DNA formation at random sites in a distance-dependent manner. These gradients are not completely smooth, when the total aberrant

segregation frequencies (WCC + PMS, Tables 3 and 4) are taken as a measure for the inclusion of a mutation in hybrid DNA. Some mutations show comparable or even higher aberrant segregation frequencies in comparison with others that are positioned closer to the presumed initiation site. Such irregularities disappear when the frequency of hybrid DNA is calculated and substituted for total aberrant segregation frequencies. For example in *S. pombe* the *ade6* gene shows a weak polarity of aberrant segregation with the 5′ end as the high conversion end (GUTZ 1971). The calculated hDNA frequencies fall from *ade6-M216* at the 5′ end to *ade6-52* to *ade6-M387* toward the 3′ end (0.84%–0.58%–0.50%). This polarity is not apparent for *52* and *M387* (315 base pairs (bp) separated), when frequencies of total aberrant segregations are compared (WCC + PMS = 0.29% for both sites). The same is true for the *S. cerevisiae* mutations *arg4-17* and *arg4-16*. *Arg4-17* is located 214 bp closer to the high conversion end of the gene than *arg4-16* but its frequency of total aberrant segregation is smaller (WCC + PMS = 8.1% for *arg4-17 vs.* 8.8% for *arg4-16*) while the calculated hybrid DNA frequency reflects the expected gradient (16% *vs.* 13%). The same is observed for the *b2* alleles of *A. immersus* (Table 4).

Calculation of the efficiency of mismatch repair: Based on the assumptions I to III made for hybrid DNA formation, the overall repair efficiency R for a given pair of mismatches in hybrid DNA is:

$$R = \text{corrected events/total events}$$
$$= ([\text{WCC}] + [\text{restoration}])/[\text{hDNA}]$$
$$= 2[\text{WCC}]/(2[\text{WCC}] + [\text{PMS}]).$$

In *S. pombe* the G to T transversion *sup3-UAA*, the G to A transitions *sup3-UGA,r36* and *ade6-M216*, and the T to C transition *ade6-52* show almost no PMS in the samples of tetrads given in Table 1. For the calculation we assume that the next tetrad analyzed would show PMS in those crosses that have not shown PMS yet. Thus the efficiency of repair is >86% for *ade6-52* and > 95% for the other mutations. For *ade6-51* *R* was estimated to be >98% based on the random spore data in Table 2. These mutations form the mismatch pairs T/C-G/A, A/C-G/T and C/A-T/G. The three G to C transversion alleles show PMS frequencies varying from 0.08 to 0.58% (Tables 1 and 2). Yet, when overall repair efficiencies (*R*) are calculated (Table 3), remarkably similar values around 84% are obtained due to the correction for different hybrid DNA frequencies in *sup3* and *ade6*.

In *S. cerevisiae* mismatch repair is a little less efficient. PMS tetrads for G to C transversions may approach 50% of aberrant tetrads and other mutations show values up to 5% or more (Table 4) (FOGEL *et al.* 1979; LICHTEN *et al.* 1990). In *A. immersus* the repair efficiencies are generally lower and scatter over a larger range than in the yeasts. Many mutations show more PMS than WCC tetrads (Table 4) (PAQUETTE and ROSSIGNOL 1978). The calculated repair efficiencies *R* in Table 4 confirm these data with values from 70% to 97% for budding yeast and 10% to 82% for Ascobolus.

Our calculations assume asymmetric hybrid DNA formation but symmetric hybrid DNA occurs within the *A. immersus b2* gene with an increasing frequency from the high to the low conversion end. For the analysis of the aberrant segregation spectra of *b2* alleles PAQUETTE and ROSSIGNOL (1978) developed a calculation model that includes symmetric hybrid DNA formation and the efficiency of mismatch repair as parameters. The rare formation of symmetric hybrid DNA in the proximal part of the gene may explain why our calculations of repair efficiencies for the proximal *b2* sites (Table 4) are remarkably close to the values published by PAQUETTE and ROSSIGNOL (1978). In contrast, rather different values are obtained for the distal mutations in *b2* (*26*; *A4*, Table 4). When symmetric hybrid DNA occurs, some WCC events will be obscured in apparent 4:4 (2 strand doubles), aberrant 5:3 and aberrant 3:5 tetrads, if flanking marker configuration is not considered, while all events without correction (PMS) will be scored (see FOGEL, MORTIMER and LUSNAK 1981 for definition of the segregation types). This leads to the observed underestimate of the repair efficiency, when our formula is used on mutations frequently covered by symmetric hybrid DNA. A further discussion of mismatch repair efficiencies in *A. immersus* has to await the determination of the molecular nature of the mutations.

Two further assumptions (IV and V) are made to obtain a basis for the estimation of the C/C repair efficiency.

IV: The two heteroduplex types are formed with equal frequency for any cross between a mutant and the corresponding wild-type allele. Thus, in case of a G to C transversion the conjugate mispairs C/C and G/G occur with equal frequencies. The assumption can be tested by inspection of the aberrant segregation patterns for any mutation. When two chromatids interact, four possibilities for strand displacement exist. Randomness may be restricted on either the chromatid level or the DNA strand level. Preference of one chromatid for strand invasion (cp) is detected as disparity of aberrant segregation, whereas preferential transfer of one of the two DNA strands of a chromatid (sp) is difficult to monitor [but see NAG and PETES (1990)]. Four combinations of the two preferences are possible: (A) cp−/sp−; (B) cp+/sp−; (C) cp−/sp+ and (D) cp+/sp+ with (−) indicating randomness and (+) preference. Among these four possibilities only the coincidence of a chromatid preference and a strand preference (D) produces an excess of one mismatch over the other. This will be revealed by mutation-

independent disparity of aberrant segregation. Thus, parity can be taken as evidence for equal frequencies of the two mismatches. It is observed for the great majority of point mutations in *S. pombe* (GUTZ 1971), *S. cerevisiae* (FOGEL, MORTIMER and LUSNAK 1981; DETLOFF, SIEBER and PETES 1991) and *A. immersus* (PAQUETTE and ROSSIGNOL 1978). The few exceptions are excluded from consideration, because they are thought to be near to fixed recombination initiation sites (for example *ade6-M26*: GUTZ 1971; SCHUCHERT *et al.* 1991). Near parity is also observed for all mutations studied in this work (*S. pombe*, Table 1; *S. cerevisiae*, Table 4).

V: Assumption V states that the G/G mismatch is repaired as efficiently as all other non-C/C mismatches and PMS of G to C transversions then is largely due to failure of C/C repair. Several lines of evidence support this assumption. A strong correlation of correction rates exists for given mismatches in *Escherichia coli*, *Streptococcus pneumoniae*, *S. cerevisiae*. In *E. coli* and *S. pneumoniae* C/C mispairs are generally inefficiently corrected relative to all other point mismatches (reviewed by MODRICH 1987). For vegetatively growing *S. cerevisiae* cells KRAMER *et al.* (1989) have reported inefficient correction of C/C mismatches only, while BISHOP, ANDERSEN and KOLODNER (1989) have published that C/C, A/A and T/T mismatches are poor repair substrates. In addition, two meiotic studies support efficient repair of G/G and poor repair of C/C mismatches. LICHTEN *et al.* (1990) have physically investigated the presence of heteroduplex DNA in spores formed by a diploid heterozygous for the G to C transversion *arg4-nsp*. Only C/C is detected and the authors conclude that there is at least a fivefold excess of unrepaired C/C over G/G mismatches. DETLOFF, SIEBER and PETES (1991) have used G to C mutations at the *HIS4* initiation codon in a special tetrad analysis system that allows the identification of the transferred strand and, thus of the mismatches present in spores producing PMS colonies. They report that repair of C/C mismatches is about threefold less efficient than repair of G/G mismatches. Furthermore, PMS colonies resulting from unrepaired G/G mismatches are detected at roughly the same frequency as for all other non-C/C mismatches. Besides the finding that the behavior of G to C transversions in one- (high PMS) and two-factor crosses (marker effects) is identical in fission and budding yeast (SCHÄR and KOHLI 1993), there is no direct evidence on efficiency of C/C *vs.* G/G repair in *S. pombe*. The segregation data from *S. pombe* are compatible with the general observation that C/C mismatches, in contrast to the other base substitution mismatches represent poor repair substrates.

Given the additional assumption IV, the overall correction efficiency (R) of the two conjugate mismatches of a mismatch pair can be expressed as:

$$R = (r_1 + r_2)/2,$$

where r_1 and r_2 are the efficiencies of repair of the individual mismatches. The repair efficiency of an individual mismatch then is:

$$r_1 = 2R - r_2,$$

according to assumption V we set the efficiency for G/G (r_2) repair at 95%. In consequence the three values obtained for C/C repair are around 73% (Table 3). For *S. cerevisiae* we also assume 95% repair for G/G; then the corresponding efficiencies for C/C repair are 47% and 93% for the two examples in Table 4. The larger difference between the two mutations may be due to different genetical backgrounds of the budding yeast strains. The high C/C repair efficiency for *arg4-nsp* could be due to a silent mutation nearby that yields well repaired mismatches. Frequent cocorrection of *arg4-nsp* C/C mismatches would then result. Such efficient cocorrection of G to C transversion mismatches has been observed before (FOGEL, MORTIMER and LUSNAK 1981; THURIAUX *et al.* 1980) and would also explain the unusual strong increase of PMS at *arg4-nsp* in strains with a *PMS1* deletion (Table 4).

The method for determination of PMS frequencies with random spores presented in this study yields results that are consistent with tetrad data. Together with the quantitation of hybrid DNA formation and mismatch repair efficiencies this forms the basis for further investigation on the relationship between heteroduplex DNA formation, mismatch repair and gene conversion in *S. pombe* and especially for the quantitative analysis of the formation of prototrophic recombinants in two-factor crosses. In particular we are now able to tackle the marker effects observed in two-factor crosses involving G to C transversions (HOFER *et al.* 1979; THURIAUX *et al.* 1980; MOORE *et al.* 1988). This further analysis is presented in SCHÄR and KOHLI (1993).

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LITERATURE CITED

BISHOP, D. K., J. ANDERSEN and R. D. KOLODNER, 1989 Specificity of mismatch repair following transformation of *Saccharomyces cerevisiae* with heteroduplex plasmid DNA. Proc. Natl. Acad. Sci. USA **86:** 3713–3717.

DETLOFF, P., J. SIEBER and T. D. PETES, 1991 Repair of specific base pair mismatches formed during meiotic recombination in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 737–745.

FOGEL, S., R. K. MORTIMER and K. LUSNAK, 1981 Mechanisms of meiotic gene conversion, or "wandering on a foreign strand," pp. 289–339 in *The Molecular Biology of the Yeast Saccharomyces*, Vol. 1, edited by J. N. STRATHERN, E. W. JONES and J. W.

BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

FOGEL, S., R. MORTIMER, K. LUSNAK and T. TAVARES, 1979 Meiotic gene conversion: A signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

GUTZ, H., 1971 Site-specific induction of gene conversion in *Schizosaccharomyces pombe*. Genetics **69:** 317–337.

GUTZ, H., H. HESLOT, U. LEUPOLD and N. LOPRIENO, 1974 *Schizosaccharomyces pombe*, pp.395–446 in *Handbook of Genetics*, Vol. 1, edited by R. C. KING. Plenum Press, New York.

HASTINGS, P. J., 1984 Measurement of restoration and conversion: its meaning for the mismatch repair hypothesis of conversion. Cold Spring Harbor Symp. Quant. Biol. **49:** 49–53.

HOFER, F., H. HOLLENSTEIN, F. JANNER, M. MINET, P. THURIAUX and U. LEUPOLD, 1979 The genetic fine structure of nonsense suppressors in *Schizosaccharomyces pombe*. Curr. Genet. **1:** 45–61.

HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. **5:** 282–304.

HOTTINGER, H., D. PEARSON, F. YAMAO, V. GAMULIN, L. COOLEY, T. COOPER and D. SOELL, 1982 Nonsense suppression in *Schizosaccharomyces pombe*. The *S. pombe sup3-e* transfer RNA Ser UGA gene is active in *S. cerevisiae*. Mol. Gen. Genet. **188:** 219–224.

KITANI, Y., and H. WHITEHOUSE, 1974 Aberrant ascus genotypes from crosses involving mutants at the *g* locus in *Sordaria fimicola*. Genet. Res. **24:** 229–250.

KOHLI, J., H. HOTTINGER, P. MUNZ, A. STRAUSS and P. THURIAUX, 1977 Genetic mapping in *Schizosaccharomyces pombe* by mitotic and meiotic analysis and induced haploidization. Genetics **87:** 471–489.

KOHLI, J., P. MUNZ, R. AEBI, H. AMSTUTZ, C. GYSLER, W. D. HEYER, L. LEHMANN, P. SCHUCHERT, P. SZANKASI, P. THURIAUX, U. LEUPOLD, J. BELL, V. GAMULIN, H. HOTTINGER, D. PEARSON and D. SOELL, 1984 Interallelic and intergenic conversion in three serine tRNA genes of *Schizosaccharomyces pombe*. Cold Spring Harbor Symp. Quant. Biol. **49:** 31–40.

KRAMER, B., W. KRAMER, M. S. WILLIAMSON and S. FOGEL, 1989 Heteroduplex DNA correction in *Saccharomyces cerevisiae* is mismatch specific and requires functional PMS genes. Mol. Cell. Biol. **9:** 4432–4440.

LIANG, C., E. ALANI and N. KLECKNER, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:** 1089–1101.

LICHTEN, M., C. GOYON, N. P. SCHULTES, D. TRECO, J. W. SZOSTAK, J. E. HABER and A. NICOLAS, 1990 Detection of heteroduplex DNA molecules among the products of Saccharomyces cerevisiae meiosis. Proc. Natl. Acad. Sci. USA **87:** 7653–7657.

MESELSON, M. S., and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:** 358–361.

MODRICH, P., 1987 DNA mismatch correction. Annu. Rev. Biochem. **56:** 435–466.

MOORE, C. W., D. M. HAMPSEY, J. F. ERNST and F. SHERMAN, 1988 Differential mismatch repair can explain the disproportionalities between physical distances and recombination frequencies of *cyc1* mutations in yeast. Genetics **119:** 21–34.

MUNZ, P., and U. LEUPOLD, 1979 Gene conversion in nonsense suppressors of *Schizosaccharomyces pombe*. I. The influence of the genetic background and of three mutant genes (*rad2*, *mut1* and *mut2*) on the frequency of postmeiotic segregation. Mol. Gen. Genet. **170:** 145–148.

NAG, D. K., and T. D. PETES, 1990 Genetic evidence for preferential strand transfer during meiotic recombination in yeast. Genetics **125:** 753–761.

NAG, D. K., M. A. WHITE and T. D. PETES, 1989 Palindromic sequences in heteroduplex DNA inhibit mismatch repair in yeast. Nature **340:** 318–320.

ORR-WEAVER, T., J. SZOSTAK and R. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

PAQUETTE, N., and J. L. ROSSIGNOL, 1978 Gene conversion spectrum of 15 mutants giving post-meiotic segregation in the *b2* locus of *Ascobolus immersus*. Mol. Gen. Genet. **163:** 313–326.

RADDING, C., 1982 Homologous pairing and strand exchange in genetic recombination. Annu. Rev. Genet. **16:** 405–437.

SCHÄR, P., and J. KOHLI, 1993 Marker effects of G to C transversions on intragenic recombination and mismatch repair in *Schizosaccharomyces pombe*. Genetics **133:** 825–835.

SCHUCHERT, P., M. LANGSFORD, E. KAESLIN and J. KOHLI, 1991 A specific DNA sequence is required for high frequency of recombination in the *ade6* gene of fission yeast. EMBO J. **10:** 2157–2163.

SNEDECOR, G. W., and W. G. COCHRAN, 1967 *Statistical Methods*, Ed. 6. The Iowa State University Press, Ames, Iowa.

SNOW, R., 1979 Maximum likelihood estimation of linkage and interference from tetrad data. Genetics **92:** 231–245.

SUN, H., D. TRECO and J. W. SZOSTAK, 1991 Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell **64:** 1155–1161.

SUN, H., D. TRECO, N. SCHULTES and J. W. SZOSTAK, 1989 Double-strand breaks at an initiation site for meiotic gene conversion. Nature **338:** 87–90.

SZANKASI, P., W. D. HEYER, P. SCHUCHERT and J. KOHLI, 1988 DNA sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*. Wild-type and mutant alleles including the recombination hot spot *ade6-M26*. J. Mol. Biol. **204:** 917–925.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand-beak repair model for recombination. Cell **33:** 25–35.

THURIAUX, P., M. MINET, P. MUNZ, A. AHMAD, D. ZBAEREN and U. LEUPOLD, 1980 Gene conversion in nonsense suppressors of *Schizosaccharomyces pombe*. II. Specific marker effects. Curr. Genet. **1:** 89–95.

WHITE, J. H., K. LUSNAK and S. FOGEL, 1985 Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate. Nature **315:** 350–353.

WILLIAMSON, M. S., J. C. GAME and S. FOGEL, 1985 Meiotic gene conversion mutants in *Saccharomyces cerevisiae*. I. Isolation and characterization of *PMS1-1* and *PMS2-2*. Genetics **110:** 609–646.

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