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Specificity of mismatch repair following transformation of *Saccharomyces cerevisiae* with heteroduplex plasmid DNA

(gene conversion/mismatch correction/*PMS1* function/genetic recombination)

DOUGLAS K. BISHOP*†, JANET ANDERSEN†, AND RICHARD D. KOLODNER†‡§

*Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02135; †Dana–Farber Cancer Institute, 44 Binney Street, Boston, MA 02115; and ‡Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

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ABSTRACT **A method is described for genetic detection of mismatch repair products following transformation of *Saccharomyces cerevisiae*. The method is based on the detection of β-galactosidase activity in clonal derivatives of cells transformed with heteroduplex plasmid DNA. Heteroduplex plasmid substrates were constructed by insertion of an oligonucleotide heteroduplex into the coding sequence of the *Escherichia coli lacZ* gene. The plasmid and oligonucleotides were designed so that one strand of the construct would code for a functional β-galactosidase and the other strand would contain an in-frame nonsense codon. The frequencies of transformed clones containing only Lac$^+$ cells, only Lac$^-$ cells, or a mixture of the two Lac phenotypes provided information on the efficiency of the repair reaction. With this method, plasmids carrying single-base substitution mismatches, a single-base frameshift mismatch (T/Δ), or a 3-base-pair substitution mismatch (TGA/GAA) were tested. A/C, G/T, G/A, G/G, and T/Δ mismatches were repaired with significantly greater efficiencies than C/C, A/A, T/T, and TGA/GAA. T/C was repaired with an intermediate efficiency. The frequencies of products obtained with G/G, G/A, and T/Δ mismatches suggested modest inequality of repair in the two possible directions. Strains carrying the repair-deficient *pms1-1* mutation were also tested. The efficiencies of repair of A/C, G/T, G/G, and A/A mismatches were reduced in *pms1-1* cells compared with wild-type cells. In addition, a change in repair inequality was detected when transformation of the two strains with an A/C mismatch was compared.**

The ability of cells to recognize and eliminate mismatched nucleotide base pairs has been well documented (for reviews see refs. 1–3). Mismatch repair can promote genetic stability by correcting errors in DNA synthesis (3–5) or by correcting G/T mispairs resulting from deamination of 5-methylcytosine (6). In addition, mismatch repair plays a role in homologous recombination by acting on mismatches formed within heteroduplex recombination intermediates (7–11). To study mismatch repair in a genetically tractable, eukaryotic organism, we developed a method for transforming *Saccharomyces cerevisiae* with purified heteroduplex plasmid DNA and analyzing the resulting repair products (11, 12). Using that method, we previously showed that two different insertion/deletion heterologies, as well as A/C and C/T mismatches, were repaired following transformation (12). In those studies we also showed that a strain carrying a *pms1-1* mutation (10) was partially deficient in repair following transformation (11).

Here we present an improved heteroduplex transformation method that allows phenotypic detection of heteroduplex-derived plasmid genotypes. Plasmid substrates containing base-substitution or frameshift mismatches in the same (or in nearly the same) sequence context were analyzed. The data provide information on the specificity of mismatch repair in yeast and the dependence of repair specificity on *PMS1*.

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METHODS AND MATERIALS

Materials. Bacteriophage T4 DNA ligase was prepared essentially as described by Tait *et al.* (13). T4 polynucleotide kinase was prepared by the method of Panet *et al.* (14). Restriction endonucleases were purchased from New England Biolabs and used as recommended by the supplier. Oligonucleotides were synthesized on an Applied Biosystems model 380A DNA synthesizer using protocols and reagents provided by the manufacturer. Oligonucleotides were purified, annealed to form duplexes, and phosphorylated at their 5′ ends by previously described methods (15). The structures of oligonucleotide duplexes used in heteroduplex plasmid construction are shown in Fig. 1*B*.

Strains and Media. *Escherichia coli* strain RDK1007 (GM48: *dam-3, dcm-6, thr-1, thi-1, leuB6, lacY1, galK2, galT22, ara-14, tonA31, tsx-78, supE44* obtained from M. Marinus, University of Massachusetts Medical School) was used for plasmid maintenance. Identical results were obtained when plasmid DNA was prepared from a *dam*$^+$*dcm*$^+$ strain (23). *S. cerevisiae* strains MW3022-9D (*MATα, trp1Δ, ura3-52, leu2, his7-2, ade2-1*) and MW3022-4B (*MATα, trp1Δ, ura3-52, leu2, his7-2, ade2-1, pms1-1*)¶ obtained from S. Fogel (University of California, Berkeley) were used as recipient strains in heteroduplex transformation experiments. Media used for growing yeast and *E. coli* have been described (24, 25).

Plasmids. The construction of pRDK94, which was used as vector in all heteroduplex transformation experiments, has been described (23). The plasmid (Fig. 1*A*) was designed to have unique *Bam*HI and *Xho* I sites to facilitate its use in the construction of substrates containing mismatched oligonucleotide insertions. In addition pRDK94 was designed to allow genetic identification of mismatch-repair products following transformation with heteroduplex derivatives of the plasmid. The plasmid carries the *TRP1* gene, which serves as a selectable marker in yeast transformation and during growth of the transformed clones. Yeast *ARS1* and *CEN3* elements promote autonomous and stable maintenance of the plasmid at ≈1 copy per cell (26). pRDK94 also carries the coding region of the *E. coli* gene *lacZ* and a 5′ portion of the yeast histone gene *HTB1*, including the promoter and 60 nucleotides of the coding sequence (16). A 0.94-kb fragment of pBR322 DNA, flanked by unique *Bam*HI and *Xho* I sites, lies between the *HTB1* and *lacZ* sequences.

Abbreviation: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

§To whom reprint requests should be addressed at the Dana–Farber Cancer Institute.

¶As a result of a bookkeeping error, the strain MW3022-4B was previously identified (10) as carrying the *pms1-2* allele instead of *pms1-1* (M. Williamson, personal communication).

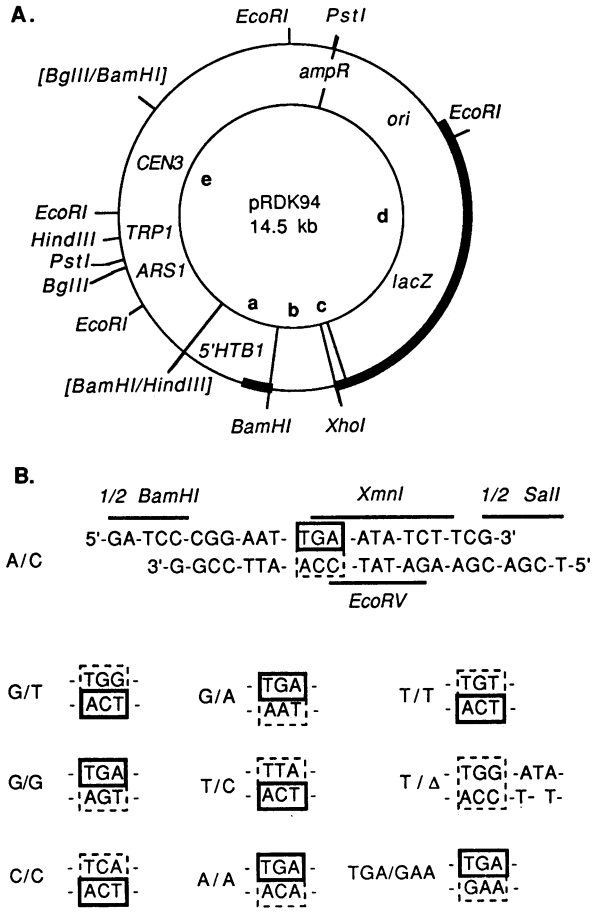

FIG. 1. (*A*) Structure of pRDK94. Fragment lengths are not to scale. Restriction sites that were destroyed by blunt-end ligation during plasmid construction are indicated in brackets. The locations of *HTB1* and *lacZ* coding sequences are defined by bold lines. The sources of DNA fragments defined by lowercase boldface letters are as follows: **a**, 1.1-kilobase (kb) *Hin*dIII–*Bam*HI fragment of pFB1-20a (16) containing the 5′ end of the yeast *HTB1* gene including its promoter and 60 nucleotides of coding sequence; **b**, 1-kb *Bam*HI–*Xho* I fragment of pRDK39 (17); **c**, an oligonucleotide adapter designed to convert the *Bam*HI site of pFB1-20a to an *Xho* I site; **d**, 5.4-kb *Bam*HI–*Pst* I fragment of pFB1-20a containing the "*lacI-z*" gene (18) and pBR322 sequences including the ColE1 origin and the 3′ portion of the ampicillin-resistance (*ampR*) gene; and **e**, 7.0-kb *Pst* I–*Bam*HI fragment from A106p3 (Andrew Murray, unpublished work) containing pBR322 sequence from nucleotide position 3609 through 4361 (19), bacteriophage λ sequence from nucleotide position 46,348 through 415 (20), a 2.6-kb *Eco*RI–*Hin*dIII fragment containing the yeast *CEN3* element (21), a 1.4-kb *Eco*RI fragment containing the yeast *TRP1* and *ARS1* elements (22), and pBR322 sequence from nucleotide position 1 to 375 (19). (*B*) Structure of oligonucleotide heteroduplexes. The entire sequence of the A/C heteroduplex is shown including recognition sites for restriction endonucleases. Only mismatched codons are shown for the remainder of the heteroduplexes; flanking sequences are identical to the sequence shown for A/C. "1/2 *Sal* I" and "1/2 *Bam*HI" refer to 5′ overhanging ends identical to those generated by the respective restriction endonucleases. Solid lines enclose nonsense codons.

Heteroduplex Construction. Heteroduplex plasmid DNA was constructed by replacing the 0.94-kb *Bam*HI–*Xho* I pRDK39 fragment with a 23-base-pair oligonucleotide heteroduplex having compatible ends (Fig. 1*B*). Plasmid DNA was typically purified from 6 liters of stationary-phase bacterial cells by a scaled-up version of a published method (27). pRDK94 plasmid DNA (200–400 μg) was digested with a 2-fold excess of *Xho* I. The linear DNA was extracted with phenol, precipitated with ethanol, and dissolved in 1 ml of 10 mM Tris/1 mM Na_2EDTA, pH 8. A 10-fold molar excess of oligonucleotide was added to *Xho* I-digested pRDK94 DNA (at a final concentration of 100 μg/ml) in a ligation mixture containing 50 mM Tris (pH 7.8), 20 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM ATP, and 200 μg of bovine serum albumin per ml. The reaction mixture was incubated at 12.5°C for 3–5 hr with T4 DNA ligase (100 PP_i units/ml). The DNA was then purified by phenol extraction and ethanol precipitation.

The purified DNA was digested with a 2- to 4-fold excess of *Bam*HI, a step predicted to yield linear pRDK94 molecules with a single oligonucleotide heteroduplex joined to the *Xho* I "sticky end." *Bam*HI-digested DNA was phenol-extracted and purified from oligonucleotides by gel filtration on 5-ml agarose A5m columns (Bio-Rad) run in 100 mM NaCl/10 mM Tris, pH 8/1 mM EDTA. Fractions containing high molecular weight DNA were pooled and incubated with *Bam*HI as before and reprecipitated with ethanol. The DNA was then incubated with T4 ligase at low DNA concentration (1 μg/ml) under conditions that have been described for intramolecular ligation of DNA (28). Ligation was often inefficient: <10% of the DNA was converted to covalently closed circles as judged by agarose gel electrophoresis. The DNA was then concentrated by ethanol precipitation in the presence of *E. coli* B tRNA (Schwarz/Mann) at 10 μg/ml followed by centrifugation at 20,000 rpm for 30 min in a Beckman SW27 rotor at 2°C. The yield of DNA after concentration was observed to be 20–50%. The DNA was then digested with a 5-fold excess of *Sal* I to linearize plasmid circles that contained multiple oligonucleotide insertions (intermolecular ligation of oligonucleotide duplexes creates a *Sal* I site). Covalently closed circular DNA was then purified on CsCl/ethidium bromide density gradients (28).

The purity of the heteroduplex preparations was assayed by electrophoresis in agarose gels (data not shown). Heteroduplex preparations were free of plasmid dimers (<5%), were resistant to digestion with *Xho* I and *Sal* I (<95%), and yielded a single 14-kb linear fragment following digestion with *Bam*HI. Between 70% and 95% of the heteroduplex DNA was in the covalently closed circular form and the remaining DNA was in the open circular form. A previous experiment, in which closed and open circular heteroduplex plasmids were compared, revealed no effect of random single-strand interruptions on the distribution of transformant genotypes (12).

Transformation. Yeast spheroplasts were prepared for transformation by published methods (12, 24). Two-tenths of a microgram of pRDK94 heteroduplex DNA was used, and 0.05 μg of pRDK76 DNA was usually added as an internal cotransformation control (the 4-fold difference in the amounts of plasmid DNA compensated for the relative transformation efficiencies of the two plasmids). Twenty micrograms of salmon sperm DNA was added to the transforming DNA to eliminate cotransformation. After transformation, spheroplasts were plated on three types of media: lacking tryptophan, lacking uracil, or lacking both tryptophan and uracil. The efficiency of transformation to tryptophan prototrophy (pRDK94 transformants) was between 300 and 1000 transformants per μg of pRDK94 DNA. The frequency of cotransformation was calculated as 2 times the average number of transformants on plates lacking uracil and tryptophan, divided by the average number of transformants on single-omission plates. In most cases no cotransformation occurred (<1%), and the highest calculated value of cotransformation was 5%.

Identification of Plasmid-Derived Phenotypes. The Lac phenotype of transformed clones was determined by streaking individual clones on tryptophan-omission medium, allowing single colonies to form for 3 days at 30°C, replica-plating the tryptophan-omission plates to tryptophan-omission plates containing 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal; Bethesda Research Laboratories; ref. 29) at 100

μg/ml, and incubating the X-Gal plates for an additional 3 days at 30°C. Three different phenotypes could be distinguished: streaks containing only blue colonies, streaks containing only white colonies, and streaks containing both blue and white colonies. Streaks with fewer than 10 single colonies were not scored.

Physical Identification of Plasmid Genotypes. Methods for the preparation of yeast DNA from transformed clones and for the characterization of plasmid DNA by restriction mapping and Southern blot analysis have been described (12). M13 phage mp17 was used as the probe for Southern blots and was labeled with ^{32}P by the random-primer method (30).

Statistical Analysis. Differences between proportions were evaluated with a χ^2 test (31) or, when sample sizes were small, with a Fisher exact test (32). Significant inequalities in the frequencies Lac^+ plus Lac^- transformants were detected by evaluating the difference of the $Lac^+/(Lac^+ + Lac^-)$ proportion from 0.5 with a continuity-corrected normal approximation to test a single proportion (31).

Wild-type cells were transformed with the same heteroduplex substrate (the A/C derivative of pRDK94) on five different days. There was no evidence of day-to-day variation. The A/C data were collapsed as were the data on the other occasions when an experiment was repeated on more than one day.

If 0.05 had been used as the P value for significance, the large number of probabilities being calculated would ensure that, by chance, some outcomes would have probabilities of <0.05 even in the absence of a true underlying difference or association. A conservative way to control for a high false-positive rate is a Bonferroni adjustment (33), which divides the nominal P value for significance by the estimated number of analyses being performed on the same data. Based on this adjustment, $P \leq 0.0005$ was considered significant and $P > 0.0005$ but < 0.05 was viewed as suggestive but not conclusive.

RESULTS

To determine whether *S. cerevisiae* repairs different mismatched nucleotide base pairs with different efficiencies, a series of heteroduplex plasmid DNAs was constructed and used to transform mitotic yeast cells. Heteroduplex DNA was constructed by inserting oligonucleotide duplexes containing a single mismatch into a yeast centromere plasmid vector, pRDK94 (Fig. 1). The vector was designed so that, after ligation of the oligonucleotide heteroduplexes into the plasmid, the two DNA strands differ in their potential for directing synthesis of β-galactosidase in yeast cells; one codes for a functional gene, the other a nonsense mutation. Three plasmid-derived phenotypes are predicted to result following transformation: repair favoring one strand would yield a plasmid that codes for functional histone–β-galactosidase fusion protein (Lac^+), repair favoring the other single strand would yield a nonsense-mutant plasmid (Lac^-), and failure of repair would yield one Lac^+ and one Lac^- plasmid after the first round of replication. Transformants were assigned to one of the three predicted categories by first streaking transformed clones for single colonies and then growing the single colonies on X-Gal indicator plates. Ten different substrates were constructed, each of which carried a single mismatched site. The mismatches tested were A/C, G/T, G/G, C/C, G/A, T/C, A/A, T/T, a single-base frameshift mispair designated T/Δ, and a 3-base-pair substitution (TGA/GAA) (see Fig. 1). The frequencies of transformants in the three possible classes for each of the 10 substrates are given in Table 1.

Different Substrates Yield Different Frequencies of Mixed-Phenotype Transformants. Wild-type and *pms1-1* mutant strains of yeast were transformed (Table 1). In wild-type cells, the relative frequency of mixed-phenotype transformants (which are interpreted as resulting from failure of mismatch repair) varied from 0.10 to 0.57. Pairwise evaluation of the difference in the proportion of mixed-phenotype transformants (see Statistical Analysis under *Materials and Methods*) allowed the substrates to be divided into two statistically distinguishable categories; transformation by the A/C, G/T, G/A, G/G, and T/Δ substrates yielded low frequencies of mixed-phenotype transformants (10–20%), whereas C/C, A/A, T/T, and TGA/GAA substrates yielded significantly higher frequencies ($P < 0.0005$) of mixed-phenotype transformants (45–57%). The frequency of mixed transformants obtained with the T/C substrate fell between the two categories defined by the repair efficiencies of the other substrates. The same analysis of the data from transformation of *pms1-1* cells allowed the substrates to be divided into three categories. The frequency of transformants with a mixed Lac phenotype was as follows: 9% for T/Δ; 26–40% for A/C, G/T, G/A, T/C, G/G, and C/C; and 67–73% for A/A, T/T, and TGA/GAA.

***pms1-1* Increases the Yield of Mixed-Phenotype Transformants.** A/C, G/T, G/G, and A/A substrates yielded a significantly higher proportion of mixed-phenotype transformants after transformation of *pms1-1* cells than after transformation of wild-type cells. While the effect of the *pms1-1* mutation on the yields of mixed-phenotype transformants was modest (<3-fold), statistically significant differences were reproducible (data not shown). In no case did a substrate yield a significantly higher frequency of mixed phenotypes in wild-type compared with *pms1-1* transformation.

Table 1. Frequencies of heteroduplex plasmid-derived phenotypes

	Wild type					*pms1-1*					
	Frequency				Repair inequality	Frequency				Repair inequality	*pms1-1*-dependence
Substrate	Lac^+	Lac^-	Mixed	Total	P value*	Lac^+	Lac^-	Mixed	Total	P value*	P value†
A/C	0.47	0.35	0.18	403	(0.0066)	0.25	0.43	0.32	157	(0.006)	0.0004
G/T	0.40	0.40	0.20	171		0.32	0.28	0.40	112		0.0002
G/A	0.28	0.53	0.19	143	(0.0006)	0.21	0.50	0.29	141	0.0003	
T/C	0.31	0.40	0.29	151		0.35	0.27	0.38	158		
C/C	0.25	0.30	0.45	186		0.24	0.38	0.38	141	(0.02)	
G/G	0.57	0.33	0.10	173	(0.0009)	0.55	0.19	0.26	166	<0.0001	0.0001
A/A	0.21	0.29	0.50	87		0.16	0.10	0.73	89		<0.0001
T/T	0.25	0.18	0.57	61		0.18	0.10	0.73	62	(0.03)	
T/Δ	0.60	0.30	0.10	113	0.0005	0.64	0.27	0.09	126	<0.0001	
TGA/GAA	0.20	0.24	0.56	127		0.09	0.24	0.67	116		(0.003)

Significant P values are given without parentheses, "suggestive" P values are given in parentheses (see Statistical Analysis).
*Differences of the $Lac^+/(Lac^+ + Lac^-)$ proportion from 0.5.
†Differences in the proportion of mixed-phenotype transformants over the total when *pms1-1* and wild-type data are compared.

Different Plasmids Yield Slightly Different Ratios of Lac$^+$ to Lac$^-$ Transformants. If mismatch repair favors one of the two single strands of the mismatched duplex, then the frequencies of pure Lac$^+$ and Lac$^-$ transformants are expected to be unequal. In wild-type cells, T/Δ displayed significant repair inequality in favor of conservation of information on the coding strand of the fusion gene (T/A > Δ/Δ; see Table 1). Analysis of the data obtained by transformation of the G/G and G/A substrates revealed nearly significant repair inequalities. Transformation of the *pms1-1* mutant revealed significant inequalities of similar magnitude for both G/G (C/G > G/C) and G/A (G/C > T/A) (Table 1). The data obtained with A/C were suggestive of repair inequality (A/T > G/C) in wild-type cells.

Transformation of *pms1-1* cells also yielded significant inequality between the frequencies of pure Lac$^+$ and Lac$^-$ transformants. The repair inequality of the T/Δ and G/A mismatches was roughly the same as that calculated from the respective data obtained by transformation of wild-type strains. The inequality of G/G repair, favoring conversion of G/G to C/G rather than to G/C, was somewhat more pronounced in *pms1-1* cells than in wild-type cells ($P = 0.05$). A significant difference in the inequality of repair of A/C was detected by a comparison of wild-type (A/T > G/C) and *pms1-1* (G/C > A/T) transformation data ($P = 0.0004$). In both wild-type and *pms1-1*, inequalities in the yields of the two single-phenotype transformants were ≤3-fold.

Physical Analysis of Plasmid DNA from Heteroduplex Transformants. To confirm that plasmid-derived phenotypes correspond to predicted plasmid genotypes, the A/C substrate was designed with restriction endonuclease cleavage-site heterologies as well as heterologies in protein-coding potential (Fig. 1*B*). Plasmid DNAs from 34 A/C transformants were analyzed by digestion with *Eco*RV and *Xmn* I. Thirty of the 34 transformants gave cleavage patterns predicted by the plasmid-derived phenotype using a single-strand excision resynthesis model for mismatch repair, including 11 Lac$^+$ transformants (cleaved at the marked site with *Eco*RV but not *Xmn* I), 12 Lac$^-$ transformants (cleaved with *Xmn* I but not *Eco*RV), and 7 mixed-phenotype transformants (partially cleaved with both *Eco*RV and *Xmn* I). These results show that about 90% of the observed phenotypes accurately reflect the genetic information at the mismatched site.

Three of the four transformants that yielded unexpected restriction endonuclease cleavage patterns included a Lac$^-$ transformant with plasmid DNA that failed to be cleaved with either *Xmn* I or *Eco*RV and two Lac$^-$ transformants with plasmid DNA that was cleaved to completion with both *Eco*RV and *Xmn* I. While the method of heteroduplex construction and the assay for heteroduplex purity eliminate the possibility that a large proportion of the heteroduplex plasmids lack oligonucleotide insertions or contain multiple insertions (see *Methods and Materials*), these three exceptional transformants can be explained if the purified preparations contain a small fraction of aberrant molecules. Alternatively, mismatches may occasionally be resolved by mechanisms other than single-strand excision resynthesis. One possibility is that mismatch recognition leads to the formation of double-strand breaks in the DNA (34, 35). In the absence of homologous sequences, repair of the breaks by intramolecular ligation may produce the exceptional classes recovered. Intramolecular ligation of linear plasmid DNA has been shown to occur during transformation of yeast (36).

The fourth exceptional transformant was Lac$^+$ and contained plasmid DNA that was partially cleaved by both *Eco*RV and *Xmn* I. This transformant can be explained if the plasmid-borne *CEN3* element failed to promote proper segregation during the first division following transformation. The detection of a mixed-genotype transformant with a Lac$^+$ phenotype suggested that a proportion of unrepaired substrate molecules were scored as having been repaired to *lac*$^+$. However, seven of eight transformants with mixed genotypes based on restriction analysis did display a mixed phenotype, and therefore the method appears to be reasonably sensitive in detecting failure of repair.

In a previous study (12), we measured the repair of an A/C and a C/T substrate by using a restriction mapping method. While the sample sizes in those experiments were small, the results obtained are in agreement with the results of the genetic experiments presented here and provide further support for the validity of the method.

DISCUSSION

Plasmids containing genetically marked mismatched nucleotides were used to transform mitotic yeast cells. The distribution of plasmid-derived phenotypes among transformants, and the results of restriction mapping of the products obtained in some experiments, suggests that yeast cells can recognize and remove mismatched bases from the DNA. In the present study, evidence for specific recognition of mismatches includes statistically significant differences in the yields of mixed-phenotype transformants when different substrates were transformed into either wild-type or *pms1-1* cells and the dependence of the yield of mixed-phenotype transformants on the *pms1* genotype. Previous analysis of the plasmid heteroduplex system has demonstrated that these results cannot be explained by loss or failure to detect repair products (11, 12, 23). As discussed below, there are a number of similarities between the results obtained here and the results of previous studies on recombination and repair.

A/C or G/T mismatches were repaired with relatively high efficiency following transformation. Efficient repair of A/C and G/T relative to most transversion mispairs has also been inferred from transformation studies in *Pneumococcus* (37–39), and *E. coli* (40–43). Previous evidence concerning efficient repair of transition relative to transversion mispairs also included *in vitro* studies in *E. coli* (44, 45) and yeast (15). Repair of A/C has been reported to be somewhat less efficient than repair of G/T in African green monkey cells (46, 47). Finally, marker effects observed during recombination in *Schizosaccharomyces pombe* (48) and *S. cerevisiae* (49) are consistent with efficient correction of transition mismatches formed in heteroduplex recombination intermediates.

Another similarity between the data reported here and data reported previously is the difference in the efficiency of repair of G/G compared with C/C. Evidence that G/G mismatches were repaired more efficiently than C/C mismatches has been reported for *Sch. pombe* (48), *Pneumococcus* (39), *E. coli* (40), and monkey cells (47). The observation that *pms1-1* reduces the efficiency of repair of a G/G mismatch without affecting repair of a C/C mismatch is similar to observations on the effects of *hex* mutations in *Pneumococcus* (37, 39) and *mutS* and *mutL* mutations in *E. coli* (40). Inefficient repair of C/C following wild-type yeast transformation is also consistent with the observation that the yeast *arg4-16* (a G/C → C/G transversion mutation) exhibits relatively high frequencies of postmeiotic segregation compared with other markers (49). In contrast to the results obtained by transformation, repair was not detected in a yeast cell-free system with either G/G or C/C substrates (15). The difference could be explained if the nucleotide sequences surrounding the mismatch play a role in promoting recognition of G/G mispairs in yeast or if the *in vitro* system was defective in a component required for G/G recognition.

A/A and T/T mismatches were repaired with similar efficiency to that of C/C mismatches. Previous studies in *E. coli* and *Pneumococcus* suggested that A/A and T/T mismatches were corrected more efficiently than C/C mis-

matches (37, 39–41). Furthermore, tetrad analysis of a yeast strain heterozygous for the *arg4-17* mutation suggested that A/A and T/T were efficiently repaired during meiosis (49). It is possible that the efficiency of repair of A/A and T/T in yeast may be influenced by sequence environment, since repair efficiency of A/A and T/T varies in *Pneumococcus* and *E. coli*, depending on the location of the marker (37, 39, 43). Alternatively, repair of mismatched nucleotides following transformation of mitotic cells may not completely reflect meiotic gene conversion.

Inequalities in the frequencies of Lac^+ and Lac^- transformants were detected with substrates containing G/G, T/Δ, and G/A mismatches. Since the repair inequality obtained with G/G is more easily accounted for by a sequence context effect than by simple mismatch recognition, it is difficult to assess whether the T/Δ and G/A inequalities resulted from an asymmetry during mismatch recognition, an effect determined by the sequence environment, or both. The change in the repair inequality observed with the A/C substrate when wild-type and *pms1* data are compared suggests that mismatch recognition plays a role in repair inequality. However, further experiments are required to distinguish the effects of mismatch structure on repair inequality from those of mismatch location.

While the mechanisms of mismatch repair in mitotic and meiotic cells may differ, the results presented here are consistent with certain predictions made on the basis of a heteroduplex correction model for meiotic gene conversion (9, 10, 48). For example, modest (3-fold or less) disparity in the frequencies of 6:2 and 2:6 segregations of markers observed after meiosis are consistent with modest inequalities in the directionality of repair as reflected by the numbers of Lac^+ and Lac^- transformants (7, 9, 10). In addition, significant changes in postmeiotic segregation frequency and conversion disparity detected by comparison of wild-type and *pms1-1* tetrad data (10) predicted that at least some mismatched substrates would yield more mixed-phenotype transformants following transformation of *pms1-1* cells than following transformation of the wild-type strain, and the present studies confirm this prediction. Finally, relative repair efficiencies of the various mismatches display some similarity to those predicted on the basis of postmeiotic segregation frequencies (49).

The results presented above confirm our earlier conclusions that mismatch repair can be quite efficient (11, 12), that A/C and T/C mispairs are repaired following transformation (12), and that the *PMS1* gene is involved in mismatch repair (11). The range of repair efficiencies observed in the *pms1* strain reported here (as well as the range of postmeiotic segregation frequencies previously reported for *pms1-1* homozygotes) suggests either that the *pms1-1* mutation does not completely eliminate *PMS1* gene function or that a *PMS1*-independent repair pathway(s) can function in mismatch repair and recombination. Further genetic and biochemical analysis will be required to resolve this point.

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