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Cloning and Nucleotide Sequence of DNA Mismatch Repair Gene *PMS1* from *Saccharomyces cerevisiae*: Homology of PMS1 to Procaryotic MutL and HexB

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The *PMS1* gene from *Saccharomyces cerevisiae*, implicated in DNA mismatch repair in yeast cells (M. S. Williamson, J. C. Game, and S. Fogel, Genetics 110:609–646, 1985), was cloned, and the nucleotide sequence was determined. The nucleotide sequence showed a 2,712-base-pair open reading frame; the predicted molecular mass of the deduced protein is 103 kilodaltons. Deletion mutants of the open reading frame were constructed and genetically characterized. The deduced amino acid sequence of the *PMS1* gene exhibited homology to those of the *mutL* gene from *Salmonella typhimurium* and the *hexB* gene from *Streptococcus pneumoniae*, genes required for DNA mismatch repair in these organisms. The homology suggests an evolutionary relationship of DNA mismatch repair in procaryotes and eucaryotes.

The finding of different repair efficiencies for different base-base mismatches (6, 31) verified the existence of DNA mismatch repair in eucaryotes. Currently, however, little is known about the genes involved and their possible functions. In the yeast *Saccharomyces cerevisiae*, mutants carrying mutations in the *PMS1* locus were isolated as meiotic hyperrecombination mutants for closely linked markers (44). These mutants, carrying *pms1-1* and *pms1-2*, are characterized by increased postmeiotic segregation frequencies in unselected tetrads. A rationale proposed for this mutant phenotype was that mismatches in heteroduplex DNA intermediates formed during meiotic recombination (22, 27) were corrected with diminished efficiency. Similarly, the mitotic mutator phenotype of the *pms1* mutants could also be accounted for by a defect in DNA mismatch repair if replication errors were not efficiently removed by postreplicative mismatch correction. It was demonstrated by transformation of yeast cells with in vitro-constructed heteroduplex DNAs containing self-complementary loop structures that a *pms1* mutation affects the repair of such structures (4).

In contrast, DNA mismatch repair in procaryotes and the genes involved have been studied extensively (for reviews, see references 11, 28, and 34). In *Escherichia coli* (and its close relative *Salmonella typhimurium*), the *mutS*, *mutL*, *mutH*, and *uvrD* (*mutU*) genes and in *Streptococcus pneumoniae* the *hexA* and *hexB* genes were identified as DNA mismatch repair genes. A specific function has already been assigned to several of the *E. coli* gene products participating in the mismatch correction process: the *mutH* protein is a sequence-specific endonuclease that cleaves unmethylated GATC sequences (43), the *uvrD* protein is DNA helicase II (20), and the *mutS* protein recognizes and binds to base-base mismatches (40, 41).

Comparison of the deduced amino acid sequences of the *mutS* gene from *S. typhimurium* (19) and the *hexA* gene from *S. pneumoniae* (32) revealed significant homology (36% identity) between the two gene products. This observation argues for an evolutionary relationship of DNA mismatch repair in these two organisms and suggests that the functions of the two proteins are very similar.

This paper reports the cloning and nucleotide sequence of the yeast DNA mismatch repair gene *PMS1*, the characterization of null mutations of this gene, and a comparison of its predicted amino acid sequence with those of the *hexB* gene from *S. pneumoniae* and the *mutL* gene from *S. typhimurium*.

MATERIALS AND METHODS

Yeast strains. The following haploid yeast strains were used: MW3069-5A (*trp1 ura3-52 hom3-10 his3-Kpn*I *leu2-3, 112 MAT***a** *pms1-1*), MW3069-15A (*trp1 ura3-52 ade2-1 hom3-10 lys2-20 his3-Kpn*I *MAT*α), MW3071-8A (Δ*trp1 ura3-52 ade2-1 ade8-14 hom3-10 leu2-3,112 MAT***a** *pms1-1*), MW3157-63C (*ura3-52 ade2-1 hom3-10 his4-519 met4 MAT*α *pms1-1*), MW3070-8B (*trp1 ura3-52 ade2-1 ade8-14 hom3-10 his3-Hin*dIII *leu2-3,112 MAT***a**), MW3070-8Bpms1Δ (same as MW3070-8B but Δ*pms1*), MW3271-10C (*ura3-52 ade2* Δ*ade8 hom3-10 lys1-1 lys2-20 his4-519 leu2-3,112, thr1, MAT***a**). MW3271-10Cpms1Δ (same as MW3271-10C but Δ*pms1*), MW3317-21A (Δ*trp1 ura3-52 ade2* Δ*ade8 hom3-10 his3-Kpn*I *met4 met13 MAT*α), and MW3317-21Apms1Δ (same as MW3317-21A but Δ*pms1*). The alleles *his3-Hin*dIII and *his3-Kpn*I were constructed in vitro by cleavage with the respective enzymes and fill-in (*his3-Hin*dIII) or removal (*his3-Kpn*I) of the protruding ends with subsequent religation (unpublished data). Haploids MW 3271-10C and MW3317-21A are the parents of diploid WC149, and haploids MW3271-10Cpms1Δ and MW3317-21Apms1Δ are the parents of diploid WC150.

Media. Yeast media were as previously described (7). Regeneration agar was a modified synthetic medium lacking uracil and containing 1 M sorbitol and 3% agar.

Genetic techniques. Mass mating, isolation of zygotes by micromanipulation, and plate dissection of unseleted tetrads were carried out as described previously (14, 29). Postmeiotic segregations were scored as sectored prints on the appropriate selective medium. For some markers, e.g., *his3* versus *his4*, postmeiotic segregations were scored by complementation as described elsewhere (44). The *leu2-3,112*

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allele was treated as a single mutation. Map distances were calculated as described by Mortimer and Schild (30).

The mitotic mutator phenotype of *pms1* mutants was monitored by scoring the frequency of canavanine-resistant papillae on colony prints as described previously (44) and by determining the number of *hom3-10* revertants as outgrowths on colony prints on synthetic medium lacking threonine or, for strains carrying an additional threonine auxotrophic marker, methionine. On replica prints from ascosporal colonies (diameter, ca. 5 mm) of *pms1* segregants, two to eight threonine prototrophic papillae were typically observed. In contrast, on prints of *PMS1* wild-type segregants, even a single papilla occurred only rarely.

Mutation rates were determined in a fluctuation test (24). Eleven cultures in rich medium (10 ml each) were inoculated at 20 cells per ml and grown to stationary phase. The viable titer and the number of mutants were determined by plating different dilutions (or 1:10 concentrations) of the cultures onto the appropriate media. Rates were calculated from the median frequency as described previously (44).

Plasmids. For constructing the centromere vector pWBK1, the 972-base-pair (bp) *Eco*RI-*Nru*I fragment of the centromere vector YCp50 (36), which comprises most of the tetracycline resistance gene, was replaced by the 144-bp *Eco*RI-*Pvu*II fragment from bacteriophage M13mp18 (45), which carries the polylinker region.

An f1 origin (12), isolated as a 518-bp *Dpn*I fragment from the plasmid pMac254 (38a), was cloned into the filled-in *Xho*I site of pWBK1 to construct plasmid pWBK2 (23a). A filled-in 237-bp *Mae*I-*Fnu*DII fragment of plasmid pMac254, which carried the f1 transcription terminator (35; nucleotides 1500 to 1604 in reference 3) in duplicate, was cloned into the filled-in *Eco*RI site of pWBK2 to construct plasmid pWBK3. The terminators in pWBK3 were oriented such that transcription starting in the vector DNA into the polylinker region would be terminated to prevent fortuitous cotranscription of DNA cloned into the polylinker.

The integration vector pWBK4 was constructed by replacing the 377-bp *Eco*RI-*Bam*HI fragment of YIp5 (39), which carries part of the tetracycline resistance gene, with the 21-bp *Eco*RI-*Bam*HI fragment from the polylinker region of phage M13mp18 (45).

Preparation of chromosomal yeast DNA. A 1-liter culture of strain MW3069-15A, which was known to complement the mutator phenotype of the *pms1-1* mutation in diploids, was grown at 30°C to a cell density of 10^8/ml in rich medium. Cells were harvested by centrifugation, washed with 50 mM EDTA (pH 8), and incubated in 30 ml of 1 M sorbitol–50 mM EDTA (pH 8)–70 mM dithiothreitol for 20 min at room temperature. After centrifugation, the cells were suspended in 14 ml of 1 M sorbitol–100 mM EDTA (pH 7.5), ca. 10 mg of zymolyase (100,000 U/g; Miles Laboratories, Inc.) was added, and the cell suspension was incubated for 1 h at 37°C. Then 20 ml of 150 mM NaCl–100 mM EDTA (pH 8) containing 4 mg of proteinase K was added, and the cells were lysed by addition of 4.5 ml of 10% sodium dodecyl sulfate. The mixture was incubated at 60°C for 30 min and extracted with buffer-saturated phenol. The aqueous phase was reextracted with chloroform and overlayed with 2 volumes of ethanol. The nucleic acids were spooled onto a glass pipette at the phase border. The collected precipitate was redissolved in 10 ml of 10 mM Tris hydrochloride–1 mM EDTA (pH 8) containing 50 μg of RNase A per ml at 37°C, extracted with phenol-chloroform (1:1, vol/vol), and precipitated from 0.3 M sodium acetate with 1 volume of ethanol. The pellet was dried under vacuum and redissolved in 5 ml of 10 mM Tris hydrochloride–1 mM EDTA (pH 8). The isolated chromosomal DNA ranged in size from ca. 60 to ca. 300 kb, as determined by orthogonal-field-alternation gel electrophoresis (8).

Construction of the nonamplified library. The chromosomal DNA was partially digested with the restriction enzyme *Sau*3A as described previously (25). The digest was fractionated by two successive sedimentation centrifugations through sucrose gradients. The fractions containing DNA of 10 to 25 kb in size were pooled. Construction of the library was carried out as described by Siede and Eckhardt-Schupp (37). A 7.5-μg sample of partially digested chromosomal DNA was mixed with 7.5 μg of *Bam*HI-linearized, dephosphorylated plasmid DNA of pWBK1 and incubated in 250 μl of 66 mM Tris hydrochloride–6.6 mM $MgCl_2$–10 mM dithiothreitol–0.4 mM ATP–0.3 mM spermidine with 5 U of T4 DNA ligase (Boehringer GmbH) overnight at room temperature. The ligase reaction mixture was extracted with phenol-chloroform (1:1, vol/vol); the DNA was precipitated with ethanol, washed with 70% ethanol, and redissolved in 50 μl of 10 mM Tris hydrochloride–0.1 mM EDTA (pH 8).

Transformation and screening of the library. The nonamplified library, constructed as described above, was used to transform the *pms1-1* strain MW3069-5A by a modified spheroplast transformation procedure (21). A 100-ml culture of strain MW3069-5A was grown in rich medium to a cell density of 10^7/ml. The cells were washed with water, suspended in 10 ml of 1 M sorbitol–25 mM EDTA (pH 8)–50 mM dithiothreitol, and incubated for 10 min at 30°C. The cells were washed with 1 M sorbitol, suspended in 10 ml of 1 M sorbitol–100 mM sodium citrate–10 mM EDTA (pH 5.8), ca. 0.5 mg of powdered zymolyase (100,000 U/g; Miles) was added, and the mixture was incubated at 30°C. The extent of spheroplasting was estimated at ca. 5-min intervals. After spheroplasting was greater than 95%, cells were gently washed twice with 1 M sorbitol and resuspended in 10 ml of 1 M sorbitol, and 2-ml samples were dispensed into sterile 14-ml polypropylene screw-cap tubes. The samples were centrifuged, and the pellets were suspended in 1 M sorbitol–6.5 mM $MgCl_2$–50% rich medium and incubated with very gentle agitation for 1 h at 30°C. The spheroplasts were collected by centrifugation and suspended for each transformation in 120 μl of 1 M sorbitol–10 mM $CaCl_2$–10 mM Tris hydrochloride (pH 7.4). The solution of the nonamplified library (50 μl) was adjusted to 1 M sorbitol and 10 mM $CaCl_2$ and added to the spheroplast suspension. The mixture was incubated for 10 min at room temperature, 1.2 ml of 20% polyethylene glycol 3500–10 mM $CaCl_2$–10 mM Tris hydrochloride (pH 7.5) was added, and the mixture was dispensed into four sterile plastic tubes. After incubation for 10 min at room temperature, ca. 5 to 10 ml of molten regeneration agar, cooled to 50°C, was added to each tube, mixed briefly, and plated immediately onto synthetic medium lacking uracil. The plates were incubated at 30°C for about 4 days. A total of ca. 5,000 primary transformants was obtained from two transformations.

The yeast cells were eluted from the top agar by passing the agar several times through a hypodermic needle. The suspension of cells resulting after addition of water to the agar was diluted and plated to synthetic medium lacking uracil. After incubation for several days at 30°C, the colonies were replica plated to synthetic medium lacking threonine to screen for *hom3-10* revertants. Among 18,000 colonies screened, two clones that displayed a markedly decreased frequency of *hom3-10* revertants were identified. These two clones were not necessarily independent. By crossing each

to a *ura3-52 pms1* haploid, with subsequent sporulation and tetrad analysis, both haploids were shown to contain (at least) two different plasmids. However, only one plasmid complemented the mutator phenotype of a *pms1-1* mutant. One ascosporal segregant of these crosses, WC13-2-6C, containing only the complementing plasmid was used for further studies.

Subcloning and integration. Plasmid DNA was isolated from WC13-2-6C and used to transform *E. coli* HB101 (*hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44*). From the ca. 23-kbp insert DNA of this plasmid, several subclones were constructed in pWBK3 or YCp50 and tested for the ability to complement the mutator phenotype of yeast strain MW3071-8A. The subclone used for further analysis was a 7.9-kbp *Kpn*I fragment cloned into the *Kpn*I site of YCp50.

For constructing integrants, the 7.9-kbp *Kpn*I fragment was cloned into the integration vector pWBK4. Strain MW3071-8A was transformed to uracil prototrophy with circular DNA of the resulting plasmid by a modified lithium acetate procedure (23, 23a). To map the integration site, seven transformants were crossed with strain MW3157-63C, and the resulting diploids were sporulated and subjected to tetrad analysis with 8 to 12 tetrads per cross. All integrations had occurred at the *PMS1* locus, as indicated by the linkage to *met4* (44). Additional tetrads were analyzed from a single hybrid to obtain a more reliable estimate of the map distance.

Nucleotide sequence analysis. Nucleotide sequence analysis was carried out as described by Bankier et al. (2) (shotgun sequencing). Sequence analysis of the *PMS1* gene was achieved in two steps. First, a partial sequence of the 7.9-kbp *Kpn*I fragment was obtained. On the basis of the information of this sequence analysis, further subcloning into pWBK3 yielded a 4-kbp *Bgl*II-*Sal*I subclone, which also complemented the mutator phenotype of a *pms1* mutant. This fragment was sequenced as described above. Combining the information from the two sequence analyses yielded almost the complete double-stranded nucleotide sequence of the *PMS1* gene. To close a few gaps that were read from only one strand, existing subclones were resequenced for extended readings or as double-stranded DNA (10), using the reverse sequencing primer dCAGGAAACAGCTATGAC (Amersham Corp.). The individual sequences were assembled into the complete sequence with the GEL program available in the BIONET package (IntelliGenetics Inc.). The SEQ and PEP programs from the same package were used for various analyses of this sequence.

Construction of the *pms1* deletion mutants. The plasmid carrying the 7.9-kbp *Kpn*I fragment with the *PMS1* gene cloned into YCp50 was cut with *Mlu*I and *Sst*I, and the large fragment was isolated from a low-melting-point agarose gel (25). The isolated fragment was treated with DNA ligase in the presence of the oligonucleotide 5′-pCGCGAGCT-3′ (Synthetic Genetics) to allow the joining of the otherwise incompatible *Mlu*I and *Sst*I ends. In the resulting plasmid, almost the entire open reading frame (ORF) of the *PMS1* gene was deleted (Fig. 1 and 2). The *Kpn*I fragment, which was now shortened to 5.2 kbp, was cloned into the integration vector pWBK4.

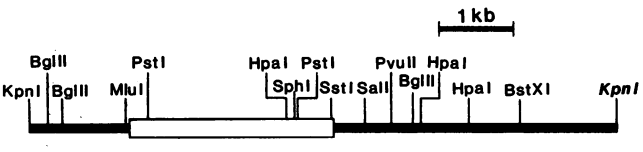

FIG. 1. Restriction map of the 7.9-kbp *Kpn*I fragment carrying the *PMS1* gene. The open box marks the ORF of the *PMS1* gene. The *Kpn*I site on the right is derived from the vector sequence.

This plasmid DNA was linearized with *Bst*XI and used for transformation of several yeast strains to uracil prototrophy by a modified lithium acetate protocol (23, 23a). Intrachromosomal Ura$^-$ recombinants (snapouts) were selected on plates containing 5-fluoroorotic acid (5). The selected snapouts were then tested for mutator phenotype. The presence or absence of the deletion was verified by Southern blot analysis (38).

RESULTS

Cloning and nucleotide sequence analysis of the *PMS1* gene. The mitotic mutator phenotype of *pms1* mutants can be monitored by a dramatically increased reversion frequency of the *hom3-10* marker (Table 1). This phenotypic response was exploited to clone the *PMS1* gene by complementation as described in Materials and Methods. A nonamplified library was used for cloning, since it was not certain a priori whether the *PMS1* gene could be propagated in *E. coli* (37).

A 7.9-kbp *Kpn*I subclone in the centromere plasmid YCp50 (36) was obtained from the original isolate, which carried an insert of ca. 23 kbp. A restriction map of the 7.9-kbp *Kpn*I fragment is shown in Fig. 1. (Note that the rightmost *Kpn*I site is derived from the vector sequence.) The 7.9-kbp *Kpn*I fragment was cloned into the integration vector pWBK4. The resulting plasmid was integrated into the genome of a *ura3-52 pms1-1* strain (MW3071-8A). The genetic map distance between the plasmid-borne *URA3* gene and the *MET4* gene in an integrant that had lost the mutator phenotype was determined to be 12 centimorgans (33 parental ditypes:0 nonparental ditypes:10 tetratypes), a value in close agreement with the map distance estimated for the original *pms1* mutation relative to *met4* (44).

A 4-kbp *Bgl*II-*Sal*I subclone that complemented the *pms1* mutator phenotype was obtained from the 7.9-kbp *Kpn*I subclone. The nucleotide sequence of this fragment was determined by the shotgun sequencing method (2) as described in Materials and Methods. The sequence showed two ORFs. The first of these two ORFs was probably incomplete; i.e., its start seemed to lie outside of the *Bgl*II-*Sal*I fragment (data not shown). Therefore, the *PMS1* gene was tentatively assigned to the second ORF. The nucleotide sequence of the second ORF with some flanking DNA is shown in Fig. 2. The ORF is 2,712 bp long (if the first available ATG codon is taken as the start) and can encode a protein of 904 amino acids with a molecular mass of 103 kilodaltons.

Construction and characterization of deletion mutants. To confirm the assignment of the *PMS1* gene to the second

TABLE 1. Spontaneous mitotic mutation rates

Strain	Mutations/cell division	
	Forward mutation to *can* resistance	Reversion and/or suppression of *hom3-10*
MW3070-8B	1.1×10^{-7}	2.1×10^{-9}
MW3070-8Bpms1Δ	2.2×10^{-6}	3.0×10^{-6}
MW3271-10C	1.1×10^{-7}	ND[a]
MW3271-10Cpms1Δ	2.9×10^{-6}	5.8×10^{-6}
MW3317-21A	2.2×10^{-7}	2.4×10^{-9}
MW3317-21Apms1Δ	6.8×10^{-6}	9.6×10^{-6}

[a] ND, Not determined.

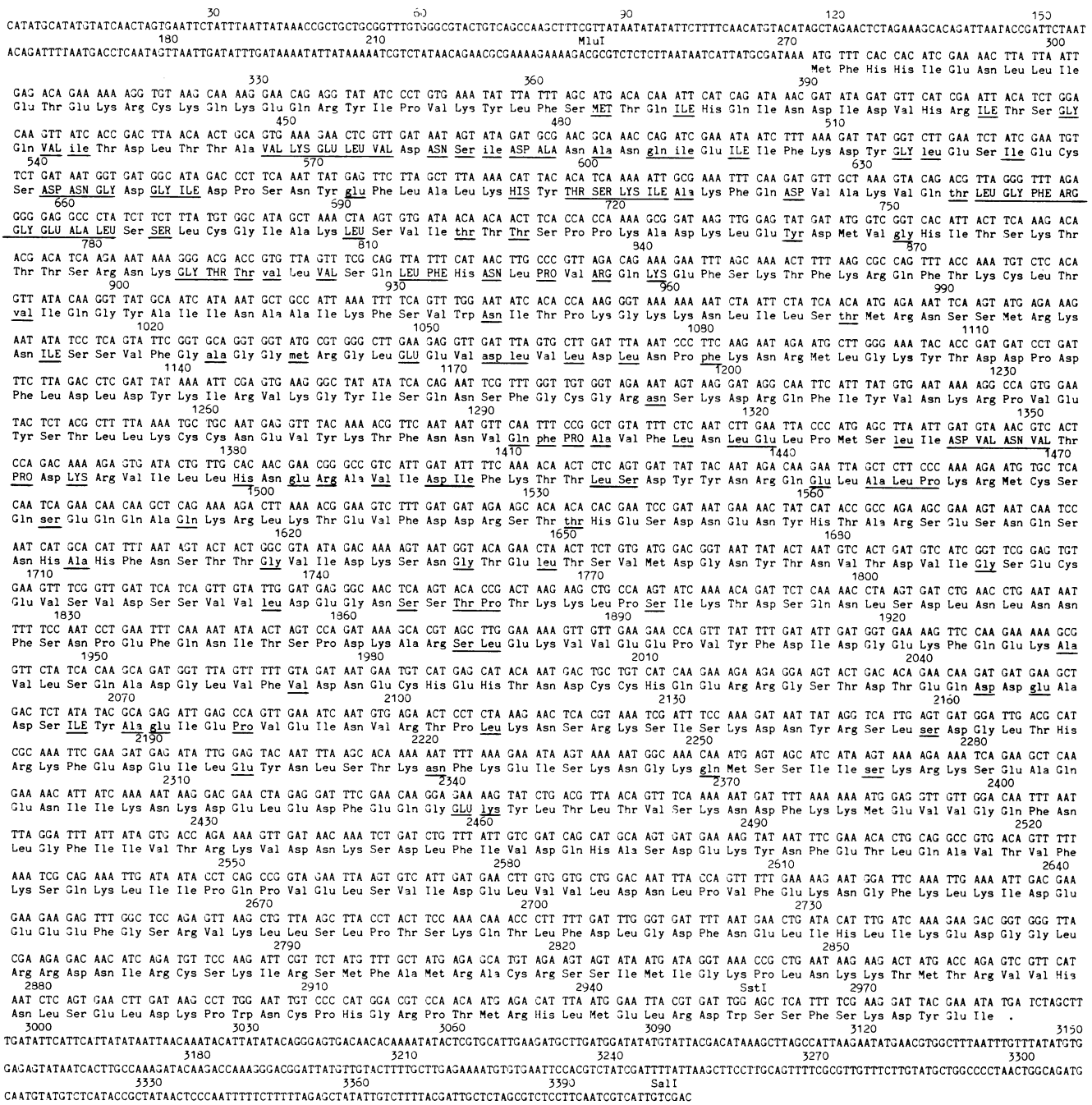

FIG. 2. Nucleotide sequence of the *PMS1* gene. Identical amino acids shared between the PMS1 protein and the MutL or HexB protein after optimized alignment are underlined. The underlined amino acids are categorized further as follows: if the symbol for the amino acid is in uppercase, the amino acid is identical in all three proteins; if the symbol is in lowercase, the amino acid is identical only between the PMS1 and HexB proteins; if the first letter is capitalized, the amino acid is identical between the PMS1 and MutL proteins.

ORF, chromosomal deletion mutants were constructed. A deletion of almost the entire ORF was constructed on a plasmid by removing the 2.7-kbp DNA fragment between the *Mlu*I site (position 241) and the *Sst*I site (position 2958). Having demonstrated that deleting this ORF is not lethal by introducing a similar deletion into diploid cells, with subsequent sporulation and tetrad analysis (data not shown), we introduced the *Mlu*I-*Sst*I deletion construction into haploid yeast cells by using the two-step gene replacement technique (5). This protocol yields not only the desired deletion mutants but also completely isogenic wild-type strains, which are very important for genetic characterizations.

The mutation rates for the forward mutation to canavanine resistance and for the reversion of the *hom3-10* mutation to prototrophy were determined for several *pms1* deletion strains and the corresponding isogenic wild-type strains (Table 1). Whereas the increase in the forward mutation rate to canavanine resistance resulting from the deletion of the *PMS1* gene was moderate (ca. 20- to 30-fold), the increase for the reversion rate of the *hom3-10* mutation in the same

TABLE 2. Aberrant segregations in unselected tetrads from diploids WC149 (*PMS1/PMS1*) and WC150 (Δ*pms1*/Δ*pms1*)

Heterozygous marker	No. of events				%BCF[a]	%PMS[b]
	Gene conversion		Postmeiotic segregation			
	6:2[c]	2:6[c]	5:3[c]	3:5[c]		
WC149 (175 tetrads)						
leu2-3,112	3	2	0	0	2.8	
Δ*trp1*	0	0	0	0		
thr1	3	1	0	0	2.3	
lys1-1	3	3	0	0	3.4	
his4-519	4	9	0	0	7.4	
met13	8	1	0	0	5.1	
*his3-Kpn*I	1	3	0	0	2.3	
met4	0	4	0	0	2.3	
lys2-20	0	1	0	0	0.6	
WC150 (97 tetrads)						
leu2-3,112	1	4	2	1	8.2	38
Δ*trp1*	0	0	0	0		
thr1	2	1	1	1	5.2	40
lys1-1	2	0	3	1	6.2	67
his4-519	1	1	3	5	10.3	80
met13	1	1	6	6	14.4	86
*his3-Kpn*I	0	1	0	0	1.0	
met4	0	1	0	1	2.1	50
lys2-20	1	0	0	1	2.1	50

[a] %BCF, Basic conversion frequency, defined as (total aberrant segregations/total tetrads) × 100.

[b] %PMS, (Total postmeiotic segregation events/total aberrant segregations) × 100.

[c] The first integer refers to the wild-type allele; the second refers to the mutant allele.

strains was dramatic (over 1,000-fold). The increase observed for the forward mutation rates was comparable to that found with the *pms1-1* and *psm1-2* mutants, for which a ca. 50-fold increase was observed (44).

The influence of the *PMS1* gene on meiotic recombination was investigated in two diploids that were isogenic except for the *PMS1* locus; one (WC150) was homozygous for the *pms1* deletion, and the other (WC149) was homozygous for the *PMS1*$^+$ allele. Segregation patterns among unselected tetrads were monitored at several loci (Table 2). The frequency of postmeiotic segregation in tetrads from the Δ*pms1* diploid was greatly increased in comparison with results for tetrads from the *PMS1*$^+$ diploid. In tetrads from the *PMS1*$^+$ diploid, all 46 aberrant segregations were gene conversions; none represented a postmeiotic segregation. In contrast, the homozygous Δ*pms1* diploid yielded 31 postmeiotic segregations among 48 aberrant segregations. The remaining 17 aberrant segregations were gene conversions. These results are in accord with findings for the *pms1-1* and *pms1-2* mutations (44). However, although the frequency of postmeiotic segregations was dramatically increased in tetrads from the Δ*pms1* diploid, gene conversions continued to occur.

It was previously reported that survival among spores from homozygous *pms1* diploids is greatly reduced (44). This finding was attributed to an increase in haploid-lethal mutations during mitotic growth of the diploid cells. To test this hypothesis, survival rates of spores from differently treated diploids, homozygous for the *pms1* deletion, were compared. In the first experiment, isolated zygotes were grown into colonies on rich medium before sporulation. The second set of data was derived from diploids that were transferred to sporulation medium shortly after zygote formation; i.e., the haploids were mixed on rich medium and incubated for 4 h at 30°C before the mating mixture was transferred to sporulation medium. The survival of spores from diploids that experienced only few cell divisions between zygote formation and sporulation was 76% (243 surviving spores among 320 from 80 dissected tetrads). This percentage is considerably better than that for spores from diploids that underwent many divisions between zygote formation and sporulation (59%; 709 surviving spores among 1,200 from 300 dissected tetrads). This finding demonstrates that the accumulation of haploid-lethal mutations was at least partially responsible for the low spore survival observed previously (44). However, spore survival from Δ*pms1*/Δ*pms1* diploids that were immediately sporulated still fell short of that observed in the isogenic *PMS1*$^+$ diploids (97% [771 surviving spores of 796 dissected from isolated zygotes] and 96% [154 surviving spores of 160 dissected from the mating mix]). Therefore, it cannot be excluded that the *PMS1* gene has a function in addition to DNA mismatch repair that improves spore viability.

Homology between the PMS1 and MutL and HexB proteins. The deduced amino acid sequence of the PMS1 protein was compared with those of the MutL protein from *S. typhimurium* (26) and the HexB protein of *S. pneumoniae* (33). Overall homology after optimized alignment of the PMS1 protein to the MutL protein was 15% (99 identical amino acids of 658) and to the HexB protein 13% (87 identical amino acids of 677). The region of highest homology, which extended from amino acid 32 of the PMS1 protein to amino acid 190 (the sequence for nucleotides 367 to 843 in Fig. 2), lay in the N-terminal part of the protein (Fig. 3). In this region, overall homologies to the MutL and HexB proteins were 32 and 33%, respectively. The homologous amino acids were not distributed randomly but rather were clustered in several regions of up to eight adjacent amino acids that were identical in all three proteins. Another region with slightly weaker homology was found further downstream (amino acid sequence for nucleotides 1290 to 1410 in Fig. 2). The remaining C-terminal part of the PMS1 protein showed much weaker homology to either of the two procaryotic proteins

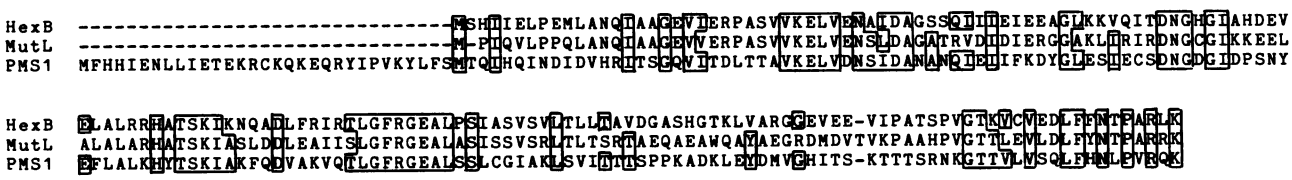

FIG. 3. Comparison between the N-terminal parts of the deduced amino acid sequences of the HexB, MutL, and PMS1 proteins. Identical amino acids shared between the PMS1 protein and the HexB and MutL proteins after optimized alignment are boxed. Amino acids identical between the HexB and MutL proteins but not found in the PMS1 protein are not marked. Hyphens indicate gaps that were introduced to optimize the alignment.

(Fig. 2). The PMS1 protein as deduced from the nucleotide sequence (with the first ATG taken as the start) was found to be considerably larger than the two procaryotic proteins. As determined from the optimized alignment, it carries 31 additional amino acids at the N terminus and ca. 215 additional amino acids at the C terminus.

DISCUSSION

The following results of this study demonstrate that the cloned DNA fragment contains the *PMS1* gene: (i) plasmids carrying the ORF assigned to the *PMS1* gene are able to suppress the mitotic mutator phenotype of the *pms1-1* allele; (ii) when plasmids carrying the cloned DNA fragment are integrated into the chromosome, the integration site exhibits about the same linkage to the *met4* locus as does the locus assigned to the original *pms1* mutations; and (iii) deletion of the ORF assigned to the *PMS1* gene on the chromosome results in mutants with a phenotype essentially identical to that of strains with the original mutant alleles *pms1-1* or *pms1-2* (44).

Some of the earlier genetic characterizations performed with the original *pms1* mutants were repeated in this study by using *pms1* deletion mutants. Such analyses bear on formulating a definitive interpretation of the previous data (44) because the use of a deletion mutant excludes the possibility that the phenotypes of the original mutants were caused by leaky alleles. Furthermore, construction of deletion mutants via integration and subsequent intrachromosomal recombination provides wild-type strains that are completely isogenic to the deletion mutants, allowing comparisons in which the residual genetic background is held constant.

One characterization concerned the determination of mutation rates in *PMS1* wild-type and *pms1* deletion strains. It was found that the increase in mutation rates was much higher for the reversion of the *hom3-10* mutation (over 1,000-fold) than for forward mutations to canavanine resistance (ca. 20- to 30-fold). If the sole difference between the $PMS1^+$ and the *pms1* deletion strains is the lack of DNA mismatch repair, the increased mutation frequencies can be attributed to the fraction of mutagenic events that are (repairable) DNA polymerase errors. For the reversion of the *hom3-10* mutation, these are most likely the only important events. However, mutations arising from mismatches not corrected by the *PMS1*-dependent system and several other mutagenic events, such as insertions of transposable elements, deletions, duplications, or UV lesions (9, 18), should be expected to contribute equally frequently to the inactivation of the *CAN1* gene in both mutant and wild type regardless of the presence or absence of the *PMS1* gene. If these events constitute a substantial fraction of total mutations, the lack of postreplicative DNA mismatch repair will elicit only a comparably moderate increase in the observed mutation rates.

The frequency of postmeiotic segregations is dramatically higher in tetrads from the *Δpms1/Δpms1* diploid than in those from the *PMS1/PMS1* diploid. A postmeiotic segregation event arises from an unrepaired heteroduplex DNA in that particular ascospore. Unless the *PMS1* gene has a function in recombination in addition to DNA mismatch repair, it is conceivable that an equivalent quantity of heteroduplex DNA is also present in the *PMS1* wild-type strains. The conspicuous paucity of postmeiotic segregations in wild-type strains implies that such presumptive heteroduplex DNAs are repaired. Depending on the strand that serves as template for the mismatch correction, either a gene conversion or a restoration to the parental genotype will result. From the experiment described here, we cannot determine what percentage of gene conversions in DNA mismatch repair-proficient strains actually represent mismatch-specific correction of heteroduplex DNA. It is possible that repair occurs with a strong bias against the invading strand, as found in transformation of *S. pneumoniae*. Here, only markers that lead to a heteroduplex DNA with poorly repairable mismatches show high transformation efficiencies (1, 11).

Apart from heteroduplex DNA repair, gene conversion might originate from recombinogenic repair of double-strand gaps (42). Initiation of a substantial amount of all gene conversions via a double break would explain the occurrence of gene conversions in the *pms1* deletion strain, for which residual *PMS1*-dependent repair caused by leakiness of the mutation can be ruled out. There are, however, other possible explanations for the observed gene conversions in the *pms1* deletion strains. It cannot be excluded that these events scored as gene conversion are in fact postmeiotic segregations that went undetected either because one of the sectors was too small or because one of the cells died at the first mitotic division. Another possibility is that residual repair, which is independent of the *PMS1* gene, is acting on the heteroduplex DNA recombination intermediate. This residual repair need not be specific for DNA mismatches. Rather, it could represent fortuitous corepair of the mismatch, such as by nick translation or repair of other DNA lesions such as chemically modified bases, apurinic-apyrimidinic sites, or pyrimidine dimers (16, 17). Such residual repair might also account for the hyperrecombination phenotype of *pms1* mutants at very closely linked alleles (44), which seems to exist also in *pms1* deletion mutants (data not shown).

Another previous observation characterizing the *pms1-1* and *pms1-2* mutants is the low ascosporal viability encountered in homozygous mutant diploids. Because in the experiments described above the *pms1* deletion mutant was compared with a completely isogenic wild-type diploid, the low spore viability is not attributable to genetic background differences between mutant homozygotes and wild type. It could be shown that at least part of the low spore viability resulted from the accumulation of haploid-lethal mutations during vegetative growth of the diploid cells. This result clearly demonstrates that although the *PMS1* gene is not essential for growth under laboratory conditions, DNA mismatch repair is of great importance for yeast cells, particularly in the diploid state, when numerous detrimental mutations can be accumulated without immediately affecting the fitness of an individual yeast cell.

The genetic data presented here suggest a function of the *PMS1* gene in DNA mismatch repair. This implication is strongly supported by the finding that the correction efficiencies of heteroduplex DNAs with defined loop structures (4) or defined base-base mismatches (23a) are greatly reduced in *pms1* mutants.

Another argument for a function of the *PMS1* gene in DNA mismatch repair is the finding that the deduced amino acid sequence is homologous to that of the procaryotic genes *mutL* and *hexB*, loci known to be involved in DNA mismatch repair (11, 28, 34). The homology is found primarily in the N-terminal region of the proteins, suggesting a similar function of this region in all three organisms. Whereas the homology between the MutL and HexB protein starts at the very N terminus of both proteins, the PMS1 protein carries some 30 additional N-terminal amino acids before the start of

the homologous region. However, the homologous region starts also in the PMS1 protein with a methionine. Thus, the PMS1 protein may have acquired an additional 5′ exon during evolution. A possible function of the additional amino acids may be to provide a nuclear localization signal (13). However, such sequences might as well be located in the C-terminal segment, which is markedly larger than in the procaryotic proteins. Another possibility for explaining the apparently longer N terminus of the PMS1 protein is that translation actually starts at the second ATG codon of the ORF.

The homology between the PMS1, MutL, and HexB proteins indicates that DNA mismatch repair has emerged early in evolution, at least before the separation of procaryotes and eucaryotes. This view predicts also that the MutS and HexA proteins have counterparts in yeast cells. Potential candidates for such proteins are the gene products of the other mismatch repair genes *PMS2* and *PMS3* (23a), which were identified together with the *PMS1* gene (44; described as *cor2*, *cor3*, and *cor4* in reference 15).

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