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Dual Requirement in Yeast DNA Mismatch Repair for *MLH1* and *PMS1*, Two Homologs of the Bacterial *mutL* Gene

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We have identified a new *Saccharomyces cerevisiae* gene, *MLH1* (*mutL* homolog), that encodes a predicted protein product with sequence similarity to DNA mismatch repair proteins of bacteria (MutL and HexB) and *S. cerevisiae* yeast (PMS1). Disruption of the *MLH1* gene results in elevated spontaneous mutation rates during vegetative growth as measured by forward mutation to canavanine resistance and reversion of the *hom3-10* allele. Additionally, the *mlh1*Δ mutant displays a dramatic increase in the instability of simple sequence repeats, i.e., $(GT)_n$ (M. Strand, T. A. Prolla, R. M. Liskay, and T. D. Petes, Nature [London] 365:274–276, 1993). Meiotic studies indicate that disruption of the *MLH1* gene in diploid strains causes increased spore lethality, presumably due to the accumulation of recessive lethal mutations, and increased postmeiotic segregation at each of four loci, the latter being indicative of inefficient repair of heteroduplex DNA generated during genetic recombination. *mlh1*Δ mutants, which should represent the null phenotype, show the same mutator and meiotic phenotypes as isogenic *pms1*Δ mutants. Interestingly, mutator and meiotic phenotypes of the *mlh1*Δ *pms1*Δ double mutant are indistinguishable from those of the *mlh1*Δ and *pms1*Δ single mutants. On the basis of our data, we suggest that in contrast to *Escherichia coli*, there are two MutL/HexB-like proteins in *S. cerevisiae* and that each is a required component of the same DNA mismatch repair pathway.

In both procaryotes and eucaryotes, DNA mismatch repair plays a prominent role in the correction of errors made during DNA replication and genetic recombination (17, 38). In *Escherichia coli*, methyl-directed (or long patch) mismatch repair involves the products of the mutator genes *mutS*, *mutL*, *mutH*, and *uvrD* (17). Isolation of the respective proteins has allowed characterization of their biochemical functions: in vitro, MutS is a DNA mismatch-binding protein (54, 55), UvrD is DNA helicase II (20), and MutH is a latent endonuclease that incises at the transiently unmethylated strands of hemimethylated GATC sequences (60). Although no biochemical activity for MutL has been identified, the protein appears to couple mismatch recognition by MutS to MutH incision at d(GATC) sequences in an ATP-dependent manner. In the process, MutL enhances the endonuclease activity of the MutH protein. Furthermore, DNase I protection experiments have shown that the MutL protein interacts with a MutS-heteroduplex DNA complex in the presence of ATP to alter the nature of the MutS footprint (18). Sancar and Hearst have proposed that MutL acts as a "molecular matchmaker," a protein that promotes the formation of a stable complex between two or more DNA-binding proteins in an ATP-dependent manner without itself being part of a final effector complex (47). Single-stranded DNA-binding protein, DNA polymerase III holoenzyme, exonuclease I, and DNA ligase are also required for in vitro reconstitution of *E. coli* methyl-directed DNA mismatch repair (28).

Elements of the *E. coli* MutLHS system appear to have been evolutionarily conserved in procaryotes and eucaryotes. Genes encoding structural homologs of MutL and MutS have been identified in *Salmonella typhimurium* (19, 31), *Streptococcus pneumoniae* (35, 37), the budding yeast *Saccharomyces cerevisiae* (27, 34, 41, 58), the fission yeast *Schizosaccharomyces pombe* (13), and mammals (7, 14, 29). The first molecular evidence for the conservation of DNA mismatch repair in yeasts came from the characterization of the *S. cerevisiae PMS1* gene, the protein product of which exhibits homology to MutL (27, 61). Mutations in the *PMS1* gene result in elevated rates of spontaneous mutation and increased the levels of postmeiotic segregation (PMS). PMS is the result of unrepaired heteroduplex DNA generated during the process of meiotic recombination. Recently, two *S. cerevisiae* DNA mismatch repair genes *MSH1* and *MSH2*, have been identified and found to encode proteins displaying homology to procaryotic MutS (40, 41). Disruption of the *MSH2* gene affects nuclear mutation rates and PMS levels in a manner similar to mutation of the *PMS1* gene. The *MSH1*-encoded protein is involved in mitochondrial DNA maintenance. A third *mutS* homolog, *MSH3*, has been identified by the PCR (46) with degenerate oligonucleotides (34) and also as a result of the yeast chromosome III mapping project (58). Disruption of the *MSH3* gene slightly increases PMS for some markers but does not induce a strong mitotic mutator phenotype (34).

Because multiple *mutS* homologs exist in yeasts, and in light of our laboratories' findings of at least two *mutL* homologs in the mouse (7), we used the PCR with degenerate oligonucleotides to search for additional *S. cerevisiae mutL*-like genes. The following report describes the initial structural and functional characterization of one such gene, *MLH1* (*mutL* homolog). In this report, we show that the mutator, PMS, and spore lethality effects of *MLH1* disruption are essentially identical to the phenotypes caused by *PMS1* disruption. Additionally, a recent study has shown that both MLH1 and PMS1 have a major and indistinguishable role in maintaining the genetic stability of simple sequence repeats, i.e., $(GT)_n$ (53). Finally, in this report we show that the *mlh1*Δ *pms1*Δ phenotype is indistinguishable

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TABLE 1. Yeast strains used

Strain	Genotype
NK860	*MATα ho::LYS2 lys2 ura3 leu2::hisG his4X*
NK860-*mlh1Δ*	Same as NK860 but *mlh1Δ::LEU2*
NK860-*pms1Δ*	Same as NK860 but *pms1Δ*
NK860-*pms1Δ mlh1Δ*	Same as NK860 but *mlh1Δ::LEU2 Δpms1*
RKY1101	*MATa ho::LYS2 lys2 ura3 leu2::hisG*
TAP200	Same as NK860 but *can1 trp1-H3*
TAP210	Same as RKY1101 but *cyh*
TAP300	Same as TAP200 but *mlh1Δ::LEU2*
TAP310	Same as TAP210 but *mlh1Δ::LEU2*
TAP400	Same as TAP200 but *pms1Δ*
TAP410	Same as TAP210 but *pms1Δ*
TAP500	Same as TAP200 but *mlh1Δ::LEU2 pms1Δ*
TAP510	Same as TAP210 but *mlh1Δ::LEU2 pms1Δ*
MW3317-21A	*MATa trp1 ura3-52 ade2 ade8 hom3-10 his3-KpnI met4 met13*
MW3317-21A-*pms1Δ*	Same as MW3317-21A but *pms1Δ*
MW3317-21A-*mlh1Δ*	Same as MW3317-21A but *mlh1Δ::URA3*
MW3317-21A-*mlh1Δ pms1Δ*	Same as MW3317-21A but *mlh1Δ::URA3 pms1Δ*

from that of either single mutant and suggest that the *MLH1* and *PMS1* gene products operate in the same DNA mismatch repair pathway.

MATERIALS AND METHODS

Strains and media. *S. cerevisiae* strains used in this study are listed in Table 1. Strains NK860 and RKY1101 are isogenic derivatives of SK1 and were a gift from R. D. Kolodner, Harvard University. Strains MW3317-21A and MW3317-21A-*pms1Δ* were a gift from D. Maloney and S. Fogel, University of California, Berkeley. The TAP strains were derived during this study. Selection of cycloheximide- and canavanine-resistant strains was performed as previously described (51). The *trp1-H3* allele in strain TAP200 was obtained by two-step replacement (49) using plasmid R1285, a gift from G. S. Roeder, Yale University. *pms1* disruption strains NK860-*pms1Δ* and TAP410 were obtained by two-step replacement with plasmid pWBK2 (27). All *pms1Δ* and *mlh1Δ* constructions were verified by Southern blot analysis. Strains TAP400, TAP500, and TAP510 were obtained by mating strains TAP410 and TAP300, sporulating the resulting diploids, and dissecting tetrads. The *his4-X* and *trp1-H3* alleles used in this study are each 4-bp insertion mutations.

Yeast cells were grown nonselectively on YPD (1% Bacto Yeast Extract, 2% Bacto Peptone, 2% glucose, 2% agar). Nutritional markers were scored on synthetic media missing one amino acid (Bio 101). Canavanine plates contained synthetic complete medium lacking arginine, supplemented with 30 mg of canavanine per liter. Cycloheximide plates contained YPD medium supplemented with 10 mg of cycloheximide per liter.

E. coli DH1 was used as the host for the YCp50- and YEp24-based yeast genomic libraries and for all plasmid constructions.

PCR. Two 29-mer deoxyoligonucleotides, 5′-CTTGATTC TAGAGC(T/C)TCNCCNC(T/C)G(A/G)AANCC-3′ and 5′-A GGTCGGAGCTCAA(A/G)GA(A/G)(T/C)TNGTNGAN AA-3′, were synthesized by the oligonucleotide synthesis facility at Yale University School of Medicine. The 5′ oligonucleotide contains an *Xba*I site and the 3′ oligonucleotide contains a *Sac*I site to facilitate cloning of PCR products. PCR (46) was carried out in 100-μl reaction volumes containing 10 ng of yeast genomic DNA, 1.0 mM each oligonucleotide primer, 5 IU of *Taq* polymerase (Cetus), 50 mM KCl, 10 mM Tris, and 1.5 mM $MgCl_2$. Reactions were carried out in a Gene Machine II thermal cycler as follows: 1 min at 94°C, 1 min at 43°C, and 1.5 min at 62°C for 35 cycles. Fragments of the expected size (approximately 210 bp) were cloned into a pUC19 vector.

Genomic Southern blots. Genomic DNAs of yeast strains were digested with restriction enzymes and electrophoresed on a 0.8% agarose gel. DNA was transferred from agarose gels to nylon-reinforced nitrocellulose membranes. Southern blot hybridizations (52) were carried out by the method of Church and Gilbert (9). DNA probes were prepared by the method of Feinberg and Vogelstein (12).

Screening of genomic libraries. YCp50- and YEp24-based *S. cerevisiae* genomic libraries were obtained from Merl Hoekstra, ICOS Corp. For library screening, bacterial colonies were grown overnight on LB plates overlaid with nylon membranes (Magnagraph). Individual membranes were replica plated onto two other filters and regrown for 12 h. Membranes were then treated with lysis buffer (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 5% sodium dodecyl sulfate [SDS]) and then subjected to microwave baking for 4 min. Filters were prehybridized with 2× SSC–1% SDS–0.5% dry milk at 65°C for 12 h. Hybridization reaction mixtures contained 6× SSC, 1% SDS, 1.5% dry milk, and 30 ng of ^{32}P-labeled probe.

Nucleotide sequence analysis. *MLH1* gene restriction fragments were subcloned into a pBluescript vector. Double-stranded plasmid DNA was sequenced on both strands by the dideoxy-chain termination method (United States Biochemical Sequenase kit). Sequences were aligned with the Genetics Computer Group programs (11).

***MLH1* chromosomal mapping.** The *MLH1* gene was mapped to chromosome XIII by Southern hybridization analysis of yeast chromosomal DNA separated electrophoretically in a contour-clamped homogeneous electric field gel (8). The chromosome assignment was confirmed by hybridization with a *RAD52* probe, a known chromosome XIII locus (50).

***MLH1* disruption constructs and yeast transformation.** Two disruption plasmids, *mlh1Δ::URA3* and *mlh1Δ::LEU2* (Fig. 1), were constructed as follows. A 4-kb *Sac*I-*Bam*HI DNA fragment of *MLH1* was subcloned into a Bluescript vector in which the *Hin*dIII site in the polylinker had been destroyed. This subclone, which is truncated at position 1300 in the *MLH1* gene, was then digested with *Hin*dIII and *Sph*I, overhangs were filled in with T4 polymerase, and the fragment was ligated to a YEp13 *Hpa*I fragment containing the *LEU2* gene. Transformation of yeast cells with this construct inserts the *LEU2* gene and deletes 230 bp of upstream and 300 bp of *MLH1* coding region, including the ATG start codon and the highly conserved, PCR-amplified region. Digestion of this plasmid with *Sac*I and *Bam*HI, to release a 4-kb fragment, was performed prior to all transformations. An identical construction strategy was used to make a plasmid for *mlh1Δ::URA3* disruption. In this case, the final

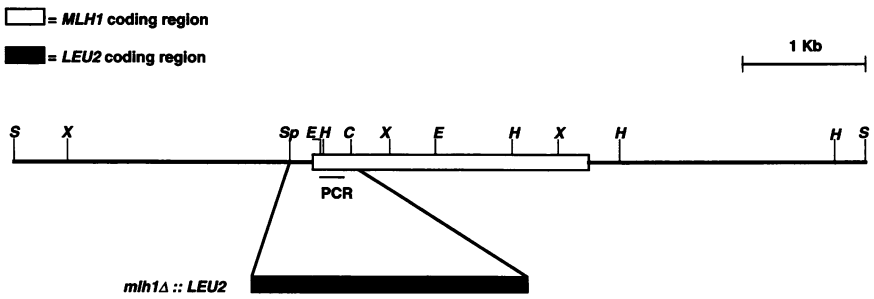

FIG. 1. Restriction map of the wild-type *MLH1* gene and the *mlh1*Δ::*LEU2* deletion created by targeted gene disruption. The approximate location of the 210-bp PCR amplified fragment containing the conserved N-terminal domain is shown. S, *Sac*I; X, *Xba*I; C, *Cla*I; Sp, *Sph*I; H, *Hin*dIII, E, *Eco*RI.

ligation step introduced the *URA3* gene contained in the *Sca*I-*Hin*dIII fragment derived from plasmid YEp24.

Yeast transformation was performed by a lithium acetate transformation method (23), using the BIO 101 yeast transformation kit, followed by selection on appropriate media.

Growth protocols for tetrad analysis. (i) Colony size growth regimen. Haploid parents were grown as patches on YPD plates for 24 h. The strains were then mixed, and the mixture was patched back onto a YPD plate. Mating was allowed to proceed for 5 h. After 5 h, zygotes were isolated by micromanipulation. These diploid cells were allowed to grow into single colonies (3-mm diameter) on YPD plates and then transferred to sporulation plates.

(ii) Zero growth regimen. Haploid strains were patched onto YPD medium and allowed to grow for 24 h. Haploids of opposite mating types were then mixed, and the mixture was patched back onto a YPD plate. After 5 h, the mixture was transferred to sporulation plates. In both regimens, sporulation was evident after 24 h.

Tetrad analysis. Tetrads were dissected onto YPD medium by using a Micro Video Instruments, Inc., tetrad dissection system. Master plates were directly replica plated onto appropriate drop-out plates after 3 days of nonselective growth. One day later, the colony replicas on the various selective media were examined with the microscope (25-fold magnification) to detect sectors.

Mutation rate measurements. Rates were determined by a fluctuation test (30). Ten cultures in rich medium (10 ml each) were inoculated with freshly grown single colonies and grown to saturation phase. The viable titer and the number of mutants were determined by plating different dilutions of the cultures onto appropriate media.

RESULTS

PCR amplification of *MLH* gene fragments. Degenerate oligonucleotide primers based on two highly conserved regions between the *S. typhimurium* MutL, *S. pneumoniae* HexB, and *S. cerevisiae* PMS1 proteins were used to perform PCR amplification with *S. cerevisiae* genomic DNA as the template (16). Primer locations are shown in Fig. 3. As determined by restriction enzyme digestion, four different classes of DNA fragments of the expected size were amplified (data not shown). The PCR-amplified material was cloned into pBluescript via *Sac*I and *Xba*I ends. Sequencing of representative fragments from each of the four classes revealed that one class represented the *PMS1* gene, while one did not encode an open reading frame. The other two fragments encode open reading frames with significant amino acid sequence similarity to PMS1, MutL, and HexB. These represent portions of genes designated *MLH1* and *MLH2* to conform to the nomenclature adopted by Reenan and Kolodner for MutS homologs (41). Northern (RNA) hybridization to total yeast RNA of vegetative cells detected low-abundance transcripts for both *MLH1* and *MLH2* (data not shown). Next, the PCR fragments were used as probes to clone the entire *MLH1* and *MLH2* genes from *S. cerevisiae* genomic libraries. The *MLH2* gene is currently being characterized further and will be the subject of a future publication.

Cloning and nucleotide sequence of the *MLH1* gene. The 210-bp DNA fragment encoding a portion of the *MLH1* gene was used to screen a YCp50-based *S. cerevisiae* genomic DNA library. Only one positive clone was isolated from this screen. Sequencing revealed that it did not contain a complete *MLH1* gene. Next, we screened a YEp24-based library constructed with the same source of genomic *S. cerevisiae* DNA and isolated the entire *MLH1* gene on a 10-kb insert. The restriction map of a 7-kb *Sac*I fragment that contains the entire *MLH1* gene is shown in Fig. 1. Sequencing of both strands of *MLH1* revealed an open reading frame capable of encoding 769 amino acids (Fig. 2). The MLH1 predicted protein displays significant homology to MutL, HexB, and PMS1, although homology is limited mostly to the N-terminal 300 amino acids (Fig. 3). Overall, the *MLH1* gene product displays 24% amino acid sequence identity to the PMS1 protein. Interestingly, sequence identity over the first N-terminal 350 amino acids is stronger between MLH1 and MutL than between PMS1 and MutL (31% versus 25%). The sequence GFRGEAL, at position 130 of MLH1 (Fig. 3), is found in the N-terminal portion of all known MutL homologs, including mouse and human (7, 27, 31, 37). The small region of C-terminal homology shared by PMS1 and HexB is not observed in the C-terminal region of MLH1 (Fig. 3).

***MLH1* disruption markedly increases spontaneous mutation rates.** Plasmid p*mlh1*Δ-*LEU2* was used for one-step disruption (44) of the *MLH1* gene in strain NK860. The spontaneous forward mutation rate to canavanine resistance is approximately 30-fold higher in the *mlh1*Δ mutant than in the isogenic wild-type strain (Table 2). Disruption of the *PMS1* gene in the same strain background results in a similar mutation rate. Furthermore, the mutation rates of the *mlh1*Δ and *pms1*Δ single mutants are the same as that of the *mlh1*Δ *pms1*Δ double mutant.

One noteworthy feature of the *pms1* mutator phenotype is a greater than 1,000-fold increase in reversion to threonine prototrophy of strains carrying the *hom3-10* mutation (27), a

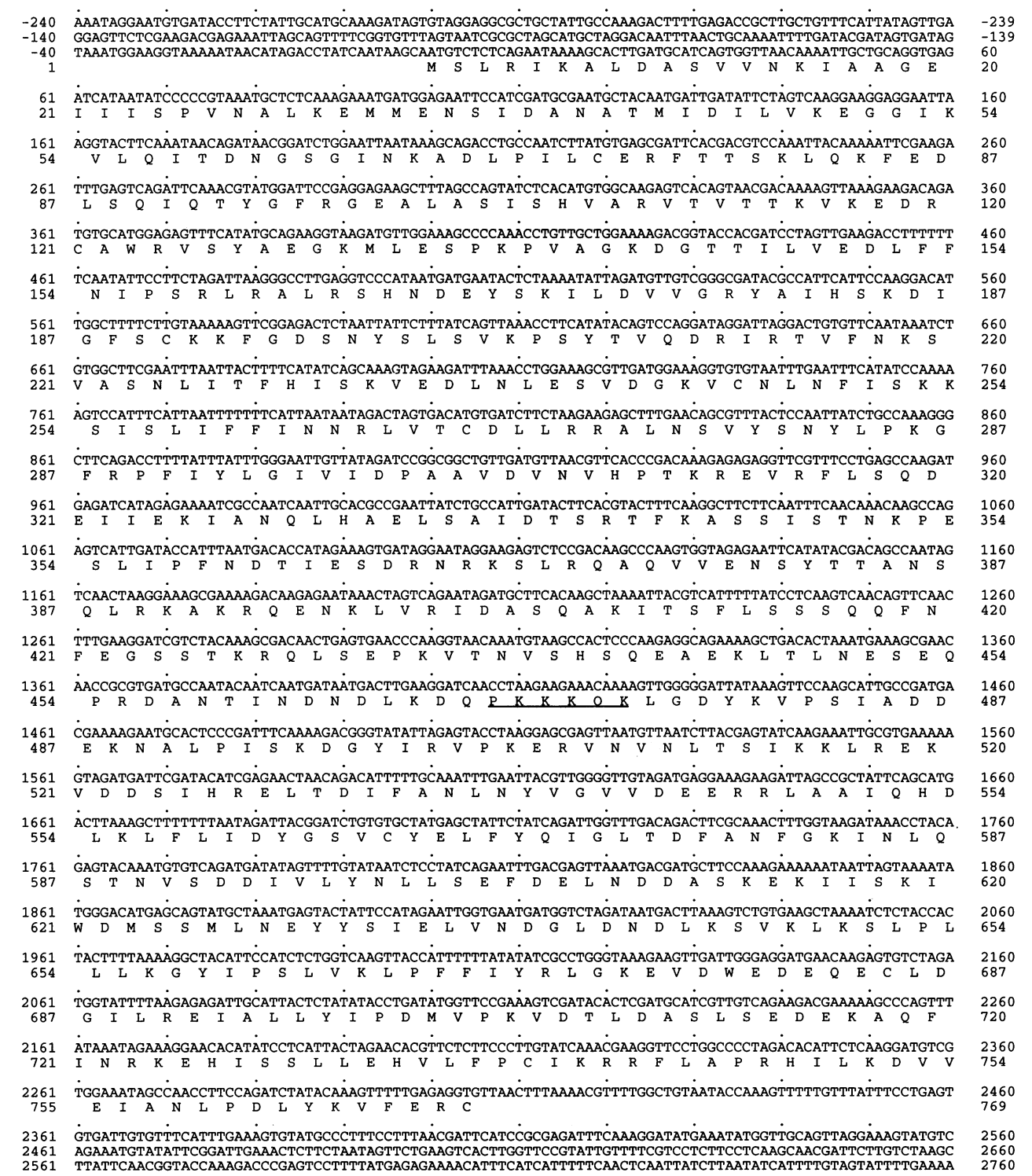

FIG. 2. Complete DNA and protein sequences of the yeast *MLH1* gene. The entire DNA coding sequence and flanking 5′ and 3′ untranslated sequences are shown. The underlined protein sequence PKKQK is similar to a known nuclear localization sequence (43).

G/C base pair insertion (59). We disrupted *MLH1* in a *hom3-10* strain and observed an increase in reversion rate indistinguishable from that observed for the isogenic *pms1*Δ strain (Table 2). Analogous to results in the canavanine resistance assay, rates of reversion measured at the *hom3-10* marker are indistinguishable between *mlh1*Δ, *pms1*Δ, and *mlh1*Δ *pms1*Δ strains (Table 2).

Introduction of the entire *MLH1* gene on a multicopy plasmid, YEp24, to strain NK860-*mlh1*Δ restores forward mutation rate to canavanine resistance back to wild-type

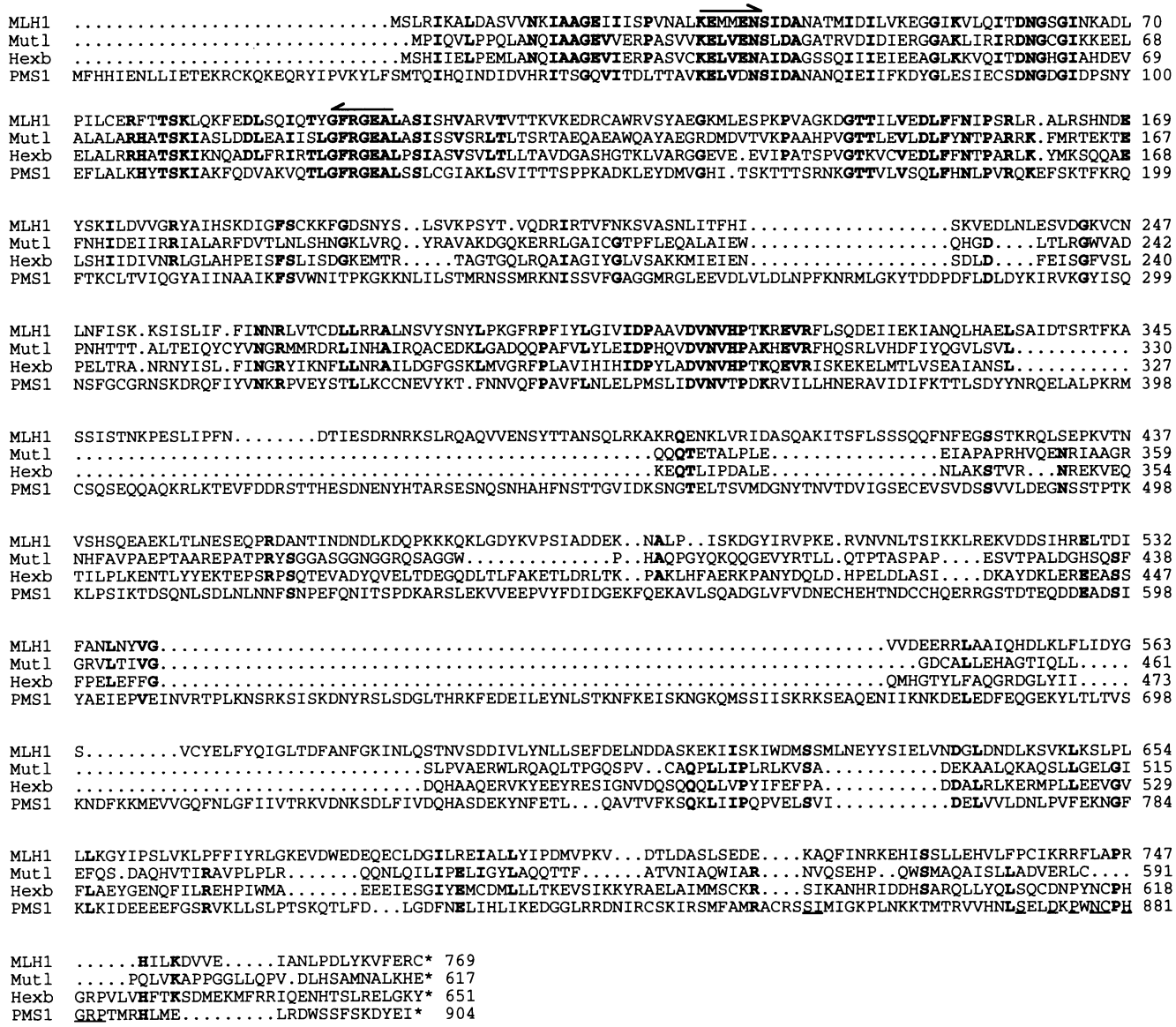

FIG. 3. Comparison of the yeast MLH1 protein sequence with sequences of other MutL homologs. Sources of proteins: MLH1, *S. cerevisiae*; PMS1, *S. cerevisiae*; MutL, *S. typhimurium*; HexB, *S. pneumoniae*. Sequences were aligned with the PILEUP program of the Genetics Computer Group (11). Gap weight = 2.0, and gap weight length = 0.1. Amino acids that are identical for at least three proteins are shown in boldface. The positions of the degenerate PCR primers used to isolate the *MLH1* gene are shown by the arrows. The underlined regions correspond to regions conserved between PMS1 and HexB only.

levels (data not shown). However, introduction of *MLH1*, on YEP24, to strain NK860-*pms1*Δ does not reduce the mutation rate (data not shown). Therefore, *MLH1* expression on a multicopy plasmid cannot overcome *PMS1* deficiency.

***MLH1* disruption reduces spore viability.** The reduced spore viability seen in *pms1* and *msh2* mutants can be improved by limiting premeiotic growth of the diploids (27, 61). We tested spore viability under zero growth and colony size growth conditions (see Materials and Methods). Under both sets of conditions, spore viability is similar for *mlh1*Δ, *pms1*Δ, and *mlh1*Δ *pms1*Δ strains (Table 3).

***MLH1* disruption increases the level of PMS.** Because a defect in the DNA mismatch repair genes *PMS1* and *MSH2* each increases the frequency of PMS, we measured PMS in

TABLE 2. Mitotic mutation rates

Strain	No. of events/cell/generation	
	Forward mutation to canavanine resistance	Reversion of *hom3-10*
NK860	5.2×10^{-7}	NA[a]
NK860-*mlh1*Δ	1.7×10^{-5}	NA
NK860-*pms1*Δ	1.0×10^{-5}	NA
NK860-*pms1*Δ *mlh1*Δ	1.3×10^{-5}	NA
MW3317-21A	ND[b]	4.1×10^{-9}
MW3317-21A-*mlh1*Δ	ND	9.0×10^{-6}
MW3317-21A-*pms1*Δ	ND	9.2×10^{-6}
MW3317-21A-*pms1*Δ *mlh1*Δ	ND	7.7×10^{-6}

[a] NA, not applicable.
[b] ND, not determined.

TABLE 3. Spore viability

Relevant genotype	Spore viability (%)[a]	
	Zero growth	Colony size growth
MLH1 PMS1 *MLH1 PMS1*	98	95
mlh1Δ *mlh1Δ*	78	53
pms1Δ *pms1Δ*	86	61
mlh1Δ pms1Δ *mlh1Δ pms1Δ*	76	56

[a] Determined by dissecting at least 50 tetrads from each diploid. For the colony size growth experiment, at least three independently generated diploids were used for each genotype. Growth conditions are described in Materials and Methods.

mlh1Δ mutants. A pair of haploid strains (TAP200 and TAP210), with markers at four loci (*his4-X*, *trp-H3*, *can1*, and *cyh*), were mated to form multiply heterozygous diploids. The aberrant segregation patterns at these loci in the wild-type, *mlh1Δ*, *pms1Δ*, and *mlh1Δ pms1Δ* strains are shown in Table 4. Overall, these results indicate that like *PMS1* mutation, disruption of *MLH1* increases the number of PMS events for all of the tested loci, which include 4-bp insertion mutations (*his4-X* and *trp1-H3*) and presumed point mutations (*can1* and *cyh*). Furthermore, as determined by a two-tailed Fisher exact test (62), the *mlh1Δ pms1Δ* strain displays PMS/gene conversion ratios for each individual locus that are not significantly different from those for either single mutant (at $P = 0.05$). The increased PMS/gene conversion ratio observed for the *pms1Δ* strain at *HIS4* is marginally significant ($P = 0.056$) compared with that observed for the *mlh1Δ pms1Δ* strain, a result that we attribute to the small data set obtained for the *pms1Δ* mutant.

DISCUSSION

We have identified a gene (*MLH1*) in the yeast *S. cerevisiae*, the deduced amino acid sequence of which is homologous to sequences of DNA mismatch repair proteins of bacteria (MutL and HexB) and of the previously described *S. cerevisiae PMS1* gene product. In this report, we show that disruption of *MLH1* causes increased rates of spontaneous mutation, as measured by forward mutation to canavanine resistance and reversion of the *hom3-10* allele. Additionally, homozygous *mlh1Δ* diploids show an increase in spore lethality. The latter can be reduced by limiting premeiotic growth as a diploid, suggesting that much of this spore lethality results from the accumulation of recessive lethal mutations. *MLH1* gene disruption also results in increased levels of PMS, a reflection of inefficient heteroduplex repair, at the four loci tested. These heterozygous sites scored for PMS comprise two 4-bp insertion mutations (*his4-X* and *trp1-H3*) and two presumed point mutations (*can1* and *cyh*). Our observations are suggestive of a general defect in the correction of mismatched base pairs, or heteroduplex DNA, generated during DNA replication and recombination. Therefore, the *MLH1* gene product appears to play a role in nuclear DNA mismatch repair.

Recent results have demonstrated that *pms1*, *mlh1*, and *msh2* mutants display a 100- to 700-fold increase in $(GT)_n$ tract instability, while mutations that eliminate the proofreading function of the DNA polymerases have little effect (53). Furthermore, the patterns of GT tract length alteration are similar between *mlh1* and *pms1* strains. No additional increases in tract instability or in the spectrum of GT tract alterations were observed in *pms1 mlh1* double mutants compared with either single mutant. The finding that DNA mismatch repair gene mutations greatly destabilize simple sequence repeat tracts (53) suggests a possible underlying basis for one form of familial colorectal cancer (1, 21, 57).

Therefore, the levels of spontaneous mutation as measured by three assays (two reported in this study and one

TABLE 4. Gene conversion and postmeiotic segregation

Relevant genotype	No. with aberrant segregation of:				Total no. of tetrads	% AS[a]	% PMS[b]
	6:2	2:6	5:3	3:5			
Wild type							
his4-X/HIS4	5	8	0	0	355	3.7	0
trp1-H3/TRP1	1	2	0	0	355	0.85	0
can1/CAN1	1	0	0	0	355	0.28	0
cyh/CYH	2	0	0	0	355	0.56	0
mlh1Δ/mlh1Δ							
his4-X/HIS4	4	5	4	2	537	2.7	40
trp1-H3/TRP1	1	0	3	3	537	1.3	86
can1/CAN1	0	2	1	0	537	0.55	33
cyh/CYH	2	0	4	2	537	1.4	75
pms1Δ/pms1Δ							
his4-X/HIS4	1	0	6	2	303	2.9	88
trp1-H3/TRP1	1	2	0	2	303	1.6	40
can1/CAN1	0	1	0	1	303	0.66	50
cyh/CYH	0	2	3	0	303	1.7	60
pms1Δ mlh1Δ/pms1Δ mlh1Δ							
his4-X/HIS4	10	3	10	3	562	4.4	52
trp1-H3/TRP1	1	2	0	4	562	0.9	83
can1/CAN1	2	2	0	0	562	0.7	0
cyh/CYH	1	2	3	1	562	1.4	62

[a] Aberrant segregation (AS) represents non-Mendelian segregation.
[b] Calculated as (5:3 + 3:5)/total number of aberrant segregations.

mentioned above) and PMS at four loci in *mlh1* mutants are all indistinguishable from those levels seen in *pms1* mutants in an isogenic strain background. Furthermore, the mutator and PMS phenotypes for the *mlh1Δ pms1Δ* double mutants are not significantly different from those of either of the single mutants. Also of note is the observation that *MLH1* expression on a high-copy-number plasmid cannot correct *PMS1* deficiency. Taken as a whole, the results lead us to conclude that the *PMS1* and *MLH1* gene products are each required components of the same DNA mismatch repair pathway in *S. cerevisiae*. The requirement for two MutL homologs in the same pathway in yeast cells indicates that despite general similarities between procaryotic and eucaryotic DNA mismatch repair, significant differences do exist. Furthermore, our finding of two MutL homologs in mice that are strongly homologous to MLH1 and PMS1, respectively (7), suggests a similar requirement for two MutL homologs in mammals.

As reported elsewhere, disruption of the *S. cerevisiae MSH2* gene in strains isogenic to those used here increases levels of spontaneous mutation, PMS, and spore death (40) to an extent similar to that found for *MLH1* disruption. The MSH2 protein appears to be responsible for most if not all of the DNA mismatch binding activity in *S. cerevisiae* nuclear extracts, as assayed by gel shift assays of mismatched oligonucleotides (32). Meiotic studies indicate that *msh2* single-mutant and *msh2 pms1* double-mutant strains display similar levels of PMS at the *HIS4* locus (2). We predict that an *mlh1 msh2* double-mutant strain would behave similarly and propose that the *MSH2*, *PMS1*, and *MLH1* gene products are components of the same DNA mismatch repair pathway. This pathway most likely repairs errors generated during DNA replication that have escaped the proofreading function of DNA polymerases, as well as sites of heteroduplex DNA generated during meiotic recombination.

S. cerevisiae gene product pairs displaying high levels of homology include RAS1 and RAS2, the DNA topoisomerases TOP1 and TOP2, the two histone subtype pairs H2B1-H2B2 and H2A1-H2A2, and the two mitogen-activated protein kinase-kinase homologs MKK1 and MKK2. In each case, double mutants display a much more severe phenotype than the respective single mutants (15, 22, 24, 25, 45). In fact, single mutants at H2B1 and H2B2, H2A1 and H2A2, and MKK1 and MKK2 do not display an obvious phenotype, suggesting functional redundancy. That the *mlh1Δ pms1Δ* double-mutant strain phenotype is no more severe than that of either single mutant is therefore unusual. On the basis of in vitro studies with purified *E. coli* mismatch repair proteins, the initiation of methyl-directed mismatch repair at hemimethylated GATC sequences appears to be induced by MutH activation in a reaction that requires MutS, Mg^{2+}, ATP, and MutL (3). Although the stoichiometry of the various proteins required for methyl-directed DNA mismatch repair in *E. coli* is not known, the bacterial MutL protein is known to exist as a dimer in solution (18). Therefore, one possibility is that PMS1 and MLH1 act together as a heteropolymeric complex in the DNA mismatch repair process.

Given that *E. coli* apparently needs only one MutL-like protein, why does *S. cerevisiae* require two MutL homologs? One requisite difference between *S. cerevisiae* and *E. coli* is the mechanism of discriminating between the old strand and the new strand. It is clear that transient undermethylation of GATC sequences in the newly synthesized strand provides a mechanism for strand discrimination in *E. coli* (33). However, also clear is that *S. cerevisiae* has no detectable DNA methylation (36). Therefore, a requirement for two MutL-like proteins in *S. cerevisiae* might be related to strand discrimination. Other, more speculative possibilities for the requirement of two MutL homologs might involve specific interactions between MLH1, PMS1, and DNA replication factors or a differential role in leading- versus lagging-strand DNA replication.

Two independent lines of evidence suggest that the *pms1*, *mlh1*, and *msh2* single mutants and the *mlh1 pms1* double mutant each display residual meiotic gene conversion. In each of the single- and double-mutant strains tested, approximately 50% of aberrant segregations remain as gene conversions. The level of PMS appears to be both allele and mutation specific but never reaches 100%. Moreover, correction of heteroduplex-containing plasmid constructs is reduced, but not eliminated, in *pms1* strains (5, 6, 26). These observations suggest that additional heteroduplex repair pathways might operate during meiotic recombination in *S. cerevisiae*. A recent study has provided genetic evidence that in *S. pombe*, two different mechanisms for mismatch repair operate during meiosis (48). The results suggest that one major system, which involves long excision tracts, recognizes all base mismatches, except C/C, with high efficiency, while a minor system, having short excision tracts, recognizes all mismatches, but with equal, lower efficiency. A relevant *S. cerevisiae* study that examined the effect of all base mismatches on meiotic gene conversion at the *S. cerevisiae HIS4* locus has demonstrated that all mismatches are repaired with equal efficiency except the C/C mismatch, which shows reduced repair (10). Therefore, one explanation for the persistence of gene conversion events in *pms1*, *mlh1*, *mlh1 pms1*, and *msh2* strains (all of which appear to be deficient in the same DNA mismatch repair pathway) is that a second pathway, which perhaps corrects only a small fraction of recombination heteroduplexes in wild-type cells, is responsible for residual gene conversions in *mlh1*, *pms1*, *mlh1 pms1*, and *msh2* strains. Possibly, the small effect of *MSH3* mutants on PMS levels (34) reflects the existence of such a second pathway in *S. cerevisiae*. The study of *mlh1 msh3* double mutants might clarify this possibility. A second and contrasting explanation for the residual gene conversions is that other repair pathways that do not involve heteroduplex correction, e.g., double-strand gap repair (56), are responsible for gene conversion.

In addition to a role in replicative error correction and the processing of heteroduplex DNA formed during homologous recombination, the *E. coli mutHLS* system helps to maintain genomic stability by preventing recombination between related but diverged (homeologous) sequences (39). In *S. cerevisiae*, mutation of the *PMS1* gene results in a small increase in the rate of ectopic recombination between the diverged *SAM1* and *SAM2* genes (4) but has no effect on spontaneous intragenic recombination between the *HIS4* genes of *Saccharomyces carlsbergensis* and *S. cerevisiae*, which are 15 to 20% divergent at the DNA level (42). At present, there is no clear evidence that DNA mismatch repair proteins influence homeologous recombination in *S. cerevisiae*. Possibly, *MLH1* and/or *MLH2* play some role in homeologous recombination.

In summary, we have identified a new *S. cerevisiae* gene, *MLH1*, that encodes a protein displaying homology to previously identified DNA mismatch repair proteins of bacteria (MutL and HexB) and *S. cerevisiae* (PMS1). Genetic characterization of *MLH1* indicates an involvement in the correction of replicative errors, including those occurring in simple sequence repeats (53), and the repair of heteroduplex

sites present in meiotic recombination intermediates. No significant differences were observed between the phenotype of the *mlh1 pms1* double mutant and that of either single mutant, suggesting that in *S. cerevisiae*, unlike bacteria, two MutL-related proteins are required in what is likely to be the major DNA mismatch repair pathway. Furthermore, the results of Alani and Kolodner (2) suggest that the *MSH2* gene product is also a component of this DNA mismatch repair pathway. Finally, we do not yet know why *S. cerevisiae* requires two MutL homologs or whether the PMS1, MLH1, and MSH2 proteins are actually part of the same complex during DNA mismatch repair. Future molecular and biochemical characterization yeast DNA mismatch proteins should help to clarify these and other issues.

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REFERENCES

1. **Aaltonen, L. A., P. Peltomaki, L. F. S., P. Sistonen, L. Pylkkanen, J.-P. Mecklin, H. Jarvinen, S. M. Powell, J. Jen, S. R. Hamilton, G. M. Petersen, K. W. Kinzler, B. Volgestein, and A. de la Chapelle.** 1993. Clues to the pathogenesis of familial colorectal cancer. Science **260**:812–816.
2. **Alani, E., and R. D. Kolodner.** Personal communication.
3. **Au, K. G., K. Welsh, and P. Modrich.** 1992. Initiation of methyl-directed mismatch repair. J. Biol. Chem. **267**:12142–12148.
4. **Bailis, A. M., and R. Rothstein.** 1990. A defect in mismatch repair in Saccharomyces cerevisiae stimulates ectopic recombination between homeologous genes by an excision repair dependent process. Genetics **126**:535–547.
5. **Bishop, D. K., J. Anderson, 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.
6. **Bishop, D. K., M. S. Williamson, S. Fogel, and R. D. Kolodner.** 1987. The role of heteroduplex correction in gene conversion in Saccharomyces cerevisiae. Nature (London) **328**:362–364.
7. **Bronner, C. E., S. M. Baker, R. J. Bollag, A. R. Godwin, D.-M. Christie, and R. M. Liskay.** Unpublished results.
8. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234**:1582–1585.
9. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81**:1991–1995.
10. **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.
11. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **16**:7025–7042.
12. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
13. **Fleck, O., H. Michael, and L. Heim.** 1992. The swi4 gene of Schizosaccharomyces pombe encodes a homologue of mismatch repair enzymes. Nucleic Acids Res. **20**:2271–2278.
14. **Fujii, H., and T. Shimada.** 1989. Isolation and characterization of cDNA clones derived from the divergently transcribed gene in the region upstream from the human dihydrofolate reductase gene. J. Biol. Chem. **264**:10057–10064.
15. **Goto, T., and J. C. Wang.** 1985. Cloning of yeast TOP1, the gene encoding DNA topoisomerase I, and construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II. Proc. Natl. Acad. Sci. USA **82**:7178–7182.
16. **Gould, S. J., S. Subramani, and I. E. Scheffler.** 1989. Use of the DNA polymerase chain reaction for homology probing: isolation of partial cDNA or genomic clones encoding the iron-sulfur protein of succinate dehydrogenase from several species. Proc. Natl. Acad. Sci. USA **86**:1934–1938.
17. **Grilley, M., J. Holmes, B. Yashar, and P. Modrich.** 1990. Mechanisms of DNA-mismatch correction. Mutat. Res. **236**:253–267.
18. **Grilley, M., K. M. Welsh, S.-S. Su, and P. Modrich.** 1989. Isolation and characterization of the Escherichia coli mutL gene product. J. Biol. Chem. **264**:1000–1004.
19. **Haber, L. T., P. P. Pang, D. I. Sobbel, J. A. Mankovich, and G. C. Walker.** 1988. Nucleotide sequence of the *Salmonella typhimurium mutS* gene required for mismatch repair: homology of *mutS* and *hexA* of *Streptococcus pneumoniae*. J. Bacteriol. **170**:197–202.
20. **Hickson, I. D., H. M. Arthur, D. Bramhil, and P. T. Emmerson.** 1983. The E. coli uvrD gene product is DNA helicase II. Mol. Gen. Genet. **190**:265.
21. **Ionov, Y., M. A. Peinado, S. Malkhosyan, D. Shibata, and M. Perucho.** 1993. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature (London) **363**:558–561.
22. **Irie, K., M. Takase, K. S. Lee, D. E. Levin, H. Araki, K. Matsumoto, and Y. Oshima.** 1993. *MKK1* and *MKK2*, which encode *Saccharomyces cerevisiae* mitogen-activated protein kinase homologs, function in the pathway mediated by protein kinase C. Mol. Cell. Biol. **13**:3076–3083.
23. **Ito, H., K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
24. **Kataoka, T., S. Powers, C. McGill, O. Fasano, J. Strathern, J. Broach, and M. Wigler.** 1984. Genetic analysis of yeast RAS1 and RAS2 genes. Cell **37**:437–445.
25. **Kolodrubetz, D., M. C. Rykowski, and M. Grunstein.** 1982. Histone H2A subtypes associate interchangeably in vivo with histone H2B subtypes. Proc. Natl. Acad. Sci. USA **82**:7178–7182.
26. **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.
27. **Kramer, W., B. Kramer, M. S. Williamson, and S. Fogel.** 1989. Cloning and nucleotide sequence of DNA mismatch repair gene *PMS1* from *Saccharomyces cerevisiae*: homology of PMS1 to procaryotic MutL and HexB. J. Bacteriol. **171**:5339–5346.
28. **Lahue, R. S., K. G. Au, and P. Modrich.** 1989. DNA mismatch correction in a defined system. Science **245**:160–164.
29. **Linton, J. P., J.-Y. J. Yen, E. Selby, Z. Chen, J. M. Chinsky, K. Liu, R. E. Kellems, and G. F. Crouse.** 1989. Dual bidirectional promoters at the mouse *dhfr* locus: cloning and characterization of two mRNA classes of the divergently transcribed *Rep-1* gene. Mol. Cell. Biol. **9**:3058–3072.
30. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
31. **Mankovich, J. A., C. A. McIntyre, and G. C. Walker.** 1989. Nucleotide sequence of the *Salmonella typhimurium mutL* gene required for mismatch repair: homology of MutL to HexB of *Streptococcus pneumoniae* and to PMS1 of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **171**:5325–5331.
32. **Miret, J. J., M. Milla, and R. S. Lahue.** 1993. Characterization of a DNA mismatch binding activity in yeast extracts. J. Biol. Chem. **268**:3507–3513.
33. **Modrich, P.** 1987. DNA mismatch correction. Annu. Rev. Biochem. **56**:435–466.
34. **New, L., K. Liu, and G. F. Crouse.** The yeast gene MSH3 defines a new class of eukaryotic Muts homologues. Mol. Gen. Genet., in press.
35. **Priebe, S. D., S. M. Hadi, B. Greenberg, and S. A. Lacks.** 1988. Nucleotide sequence of the *hexA* gene for DNA mismatch repair

in *Streptococcus pneumoniae* and homology of *hexA* to *mutS* of *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **170:**190–196.
36. **Proffitt, J. H., J. R. Davie, D. Swinton, and S. Hattman.** 1984. 5-Methylcytosine is not detectable in *Saccharomyces cerevisiae* DNA. Mol. Cell. Biol. **4:**985–988.
37. **Prudhomme, M., B. Martin, V. Mejean, and J. Claverys.** 1989. Nucleotide sequence of the *Streptococcus pneumoniae hexB* mismatch repair gene: homology of HexB to MutL of *Salmonella typhimurium* and to PMS1 of *Saccharomyces cerevisiae*. J. Bacteriol. **171:**5332–5338.
38. **Radman, M.** 1988. Mismatch repair and genetic recombination, p. 169–192. *In* R. Kucherlapati and G. R. Smith (ed.), Genetic recombination. American Society for Microbiology, Washington, D.C.
39. **Rayssiguier, C., D. S. Thaler, and M. Radman.** 1989. The barrier to recombination between Escherichia coli and Salmonella typhimurium is disrupted in mismatch-repair mutants. Nature (London) **342:**396–401.
40. **Reenan, R. A., and R. D. Kolodner.** 1992. Characterization of insertion mutations in the Saccharomyces cerevisiae MSH1 and MSH2 genes: evidence for separate mitochondrial and nuclear functions. Genetics **132:**975–985.
41. **Reenan, R. A., and R. D. Kolodner.** 1992. Isolation and characterization of two Saccharomyces cerevisiae genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. Genetics **132:**963–973.
42. **Resnick, M., Z. Zgaga, P. Hieter, J. Westmoreland, S. Fogel, and T. Nilsson-Tillgren.** 1992. Recombinational repair of diverged DNAs: a study of homeologous chromosomes and mammalian YACs in yeast. Mol. Gen. Genet. **234:**65–73.
43. **Richardson, W. D., B. L. Roberts, and A. E. Smith.** 1986. Nuclear localization signals in polyoma virus large-T. Cell **44:**77–85.
44. **Rothstein, R.** 1983. One-step disruption in yeast. Methods Enzymol. **101:**202–211.
45. **Rykowski, M. C., J. W. Wallis, J. Choe, and M. Grunstein.** 1981. Histone H2B subtypes are dispensable during the yeast cell cycle. Cell **25:**477–487.
46. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487–491.
47. **Sancar, A., and J. E. Hearst.** 1993. Molecular matchmakers. Science **259:**1415–1420.
48. **Schar, P., P. Munz, and J. Kohli.** 1993. Meiotic mismatch repair quantified on the basis of segregation patterns in Schizosaccharomyces pombe. Genetics **133:**815–824.
49. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed in vitro. Proc. Natl. Acad. Sci. USA **76:**4951.
50. **Schild, D., B. Konforti, C. Perez, W. Gish, and R. Mortimer.** 1983. Isolation and characterization of the yeast DNA repair genes. I. Cloning of the RAD52 gene. Curr. Genet. **7:**85–92.
51. **Sikorski, R. S., and J. D. Boeke.** 1991. In vitro mutagenesis and plasmid shuffling: from cloned gene to mutant yeast, p. 302–318. *In* C. Guthrie and G. Fink (ed.), Guide to yeast genetics and molecular biology, vol. 194. Academic Press, Inc., New York.
52. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.
53. **Strand, M., T. A. Prolla, R. M. Liskay, and T. D. Petes.** 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature (London) **365:**274–276.
54. **Su, S. S., R. S. Lahue, K. G. Au, and P. Modrich.** 1988. Mispair specificity of methyl directed DNA mismatch correction in vitro. J. Biol. Chem. **263:**6829–6835.
55. **Su, S. S., and P. Modrich.** 1986. E. coli mutS encoded protein binds to mismatched DNA base pairs. Proc. Natl. Acad. Sci. USA **83:**5057–5061.
56. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33:**25–35.
57. **Thibodeau, S. N., G. Bren, and D. Schaid.** 1993. Microsatellite instability in cancer of the proximal colon. Science **260:**816–819.
58. **Valle, G. E., G. Bergantino, G. Lanfranchi, and G. Carignani.** 1991. The sequence of a 6.3 kb segment of yeast chromosome III reveals an open reading frame coding for a putative mismatch binding protein. Yeast **7:**981–988.
59. **Wang, Q., U. G. G. Henning, R. G. Ritzel, E. A. Savage, and R. C. von Borstell.** 1990. Double stranded base sequencing confirms the genetic evidence that the hom3-10 allele of Saccharomyces cerevisiae is a frameshift mutant. Yeast **6:**S76.
60. **Welsh, K. M., A.-L. Lu, S. Clark, and P. Modrich.** 1987. Isolation and characterization of the Escherichia coli mutH gene product. J. Biol. Chem. **262:**15624–15629.
61. **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 pms1-2. Genetics **110:**609–646.
62. **Zar, J. H.** 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.