**MPH1, A Yeast Gene Encoding a DEAH Protein, Plays a Role in Protection of the Genome From Spontaneous and Chemically Induced Damage**

Jürgen Scheller, Anke Schürer, Christian Rudolph, Stefan Hettwer and Wilfried Kramer

*Abteilung Molekulare Genetik und Präparative Molekulargenetik, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, 37077 Göttingen, Germany*

Manuscript received January 14, 2000
Accepted for publication April 5, 2000

**ABSTRACT**

We have characterized the MPH1 gene from Saccharomyces cerevisiae. mph1 mutants display a spontaneous mutator phenotype. Homologs were found in archaea and in the EST libraries of Drosophila, mouse, and man. MpH1 carries the signature motifs of the DEAH family of helicases. Selected motifs were shown to be necessary for MPH1 function by introducing missense mutations. Possible indirect effects on translation and splicing were excluded by demonstrating nuclear localization of the protein and splicing proficiency of the mutant. A mutation spectrum did not show any conspicuous deviations from wild type except for an underrepresentation of frameshift mutations. The mutator phenotype was dependent on REV3 and RAD6. The mutant was sensitive to MMS, EMS, 4-NQO, and camptothecin, but not to UV light and X-rays. Epistasis analyses were carried out with representative mutants from various repair pathways (msh6, magl, apn1, rad14, rad52, rad6, mms2, and rad3). No epistatic interactions were found, either for the spontaneous mutator phenotype or for MMS, EMS, and 4-NQO sensitivity. mph1 slightly increased the UV sensitivity of mms2, rad6, and rad14 mutants, but no effect on X-ray sensitivity was observed. These data suggest that MPH1 is not part of a hitherto known repair pathway. Possible functions are discussed.

**CELLS have evolved a broad arsenal of repair mechanisms to defy threats to the integrity of their genetic information** (for overview see Friedberg et al. 1995). Chemical modifications of DNA constituents by environmental and intracellular factors such as radiation or chemical agents may alter the informational content of the DNA or lead to cell death by blocking transcription or replication. A variety of general pathways coping with such lesions have been defined so far. In base excision repair, the damaged base is removed by a specific glycosylase, the resulting apurinic or apyrimidinic site is incised by an AP-lyase activity or an AP-endonuclease, the abasic sugar is removed, and an undamaged nucleotide is inserted using the information of the complementary strand. Nucleotide excision repair, which is mainly involved in the removal of bulky adducts, excises an oligonucleotide containing the damage by cutting several nucleotides to the left and right of the damage on one strand. The oligonucleotide is removed by a helicase and the remaining gap is filled by DNA polymerase. Recombinational repair corrects damage by recombination with a homologous double-stranded DNA molecule. This type of repair is particularly important if both strands of a DNA double helix are damaged in the same region, e.g., by a double-strand break or an interstrand cross-link.

Damage tolerance mechanisms allow the cells to proceed with vital processes such as transcription and replication even in the presence of DNA damage. The price the cells have to pay is an increase in the probability of acquiring a mutation, since these mechanisms involve the mutagenic bypass of lesions that block normal replicative DNA polymerases (Bridges 1999). This does not result in removal of the damage, but allows the cells to continue growth, if repair is not fast enough. In Escherichia coli, lesion bypass synthesis is carried out by the UmuD\(^{-}\)-C complex (pol V), which is formed in response to DNA damage after proteolysis of UmuD by activated RecA (Tang et al. 1999). In the yeast Saccharomyces cerevisiae, several enzymes are involved in translesion synthesis. The REV1 gene product inserts cytosines opposite apurinic/apyrimidinic sites (Nelson et al. 1996a), as one major source of AP sites is the depurination of G. The RAD30 gene product is a DNA polymerase that inserts two As opposite of a cyclobutane thymidine dimer (Johnson et al. 1999). A more general translesion DNA polymerase is probably DNA polymerase \(\xi\), which consists of two subunits encoded by REV3 and REV7 (Nelson et al. 1996b). These mechanisms are subsumed in a pathway that has been coined postreplicative repair, since stalling of DNA synthesis after DNA damage with successive release of the block has been observed (Rupp and Howard-Flanders 1968), and that has an error-
prone and an error-free branch. Involved in this pathway are also ubiquitin-conjugating enzymes such as Rad6 (Jentsch et al. 1987) and Ubc13 (Hofmann and Pickart 1999), whose exact functions are not clear. One plausible role could be the removal of stalled replication or transcription complexes via ubiquitin-mediated proteolysis to allow access of the repair machinery to the damage.

Another source of mutations, which is independent of any chemical modification of DNA, is the misincorporation or insertion/deletion of nucleotides during replication. Mechanisms presently known to counteract such errors are the nucleotide selection step of the DNA polymerases, preferential exonucleolytic removal of misincorporated nucleotides at the replication fork by proofreading (Doublié and Ellenberger 1998; Roberts and Kunkel 1999), and postreplicative correction by DNA mismatch repair (Modrich and Lahue 1996) or by the recently discovered RAD27-dependent pathway (Tishko et al. 1997).

Organisms with more complex genomes require a higher replicational fidelity to maintain their genetic information than those with simpler genomes (Eigen and Schuster 1979), as is revealed, e.g., by the low accuracy of reverse transcriptions from RNA viruses and the absence of DNA mismatch repair in several microorganisms as evidenced by the lack of the respective orthologues in their complete genomic sequences (Eisen 1998). On the other hand, the importance of these mechanisms for higher organisms is demonstrated by severe disorders elicited in humans through defects in certain repair systems. Well-documented examples are xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy, which are caused by defects in the nucleotide excision repair (Chu and Mayne 1996), and hereditary nonpolyposis colon cancer (HNPPC), which is due to inactive alleles of DNA mismatch repair genes (Modrich and Lahue 1996). The correlation between heritable human diseases and DNA repair makes the investigation of pathways with an impact on fidelity control important from a medical standpoint to define possible new determinants of such diseases. Here we describe the characterization of the new mutator gene MPH1 from the yeast S. cerevisiae that also has homologs in mammals.

Mph1 has the seven signature motifs characteristic for the DEAH/DExH family of putative RNA helicases (Gorbalenya and Koonin 1993). Members of this protein family are numerous in both pro- and eucaryotes and fulfill a large variety of different cellular functions (Lükings et al. 1998). They have been shown to act in translation (Pause and Sonenberg 1992), splicing (Hamm and Lamond 1998), mRNA stability (lost and Dreyfus 1994), transcription, UV repair (Deschavanne and Harosh 1993), and other processes involving nucleic acids. The function of several conserved motifs has been delimited by both mutational analyses (Pause and Sonenberg 1992; Pause et al. 1993; Gross and Shuman 1995, 1996) and the structure determination of the hepatitis C virus (HCV) RNA helicase domain (Yao et al. 1997). The Walker motif A (Walker et al. 1982; helicase motif I) is involved in binding of the terminal phosphate of the ATP, and the aspartate and glutamate of motif B (helicase motif II), which are part of the DExH sequence, are required for Mg$^{2+}$ binding and hydrolysis. The histidine residue in this sequence may be involved in an NTP-dependent conformational change. The TAT sequence (helicase motif III) probably serves as a hinge region for rotation of the RNA-binding domain, which may be essential for helicase activity. RNA binding/unwinding requires the QRxG RxG RxG sequence (helicase motif VI), which presumably binds to single-stranded RNA via electrostatic interactions (Pause et al. 1993).

**MATERIALS AND METHODS**

**Yeast strains and disruption plasmids:** The MPH1 disruption plasmid was constructed starting from the pBluescript II KS(-) based plasmid pH12 (source: A. Hinnen, Entian et al. 1999), which contained a URA3 gene flanked by 5′ and 3′ adjacent chromosomal regions of MPH1. The URA3 gene was deleted by cleavage with Smal and Clai, fill-in, and religation. Subsequently, the 3.8-kb BamHI/ BglII fragment from pKN51 containing the URA3 gene flanked by two direct repeats of the Salmonella typhimurium hisG gene (Alani et al. 1987) was inserted into the BamHI site of the pH12 derivative with the deleted URA3 gene. The resulting plasmid, which was named pH12-UH, contains the 5′ flanking region of MPH1 from chromosomal coordinates 360398–361138 and the 3′ flanking region from 357405–357658. For mph1 deletion construction, pH12-UH was cleaved with PvuII and used for transformation of strains CEN.PK2-1Ca (trp1-289 leu2-3,112 ura3-52 his3-D2) and MW3317-21A (Krämer et al. 1989). ura3– deletions were selected on 5-fluoroorotic acid (5-FOA; Sikorski and Boeke 1990), yielding strains CEN.PK2-1Cmhp1Δ and MW3317-21Amhp1Δ, respectively. The structure of the deletion was verified by PCR analysis. The mutant phenotype of CEN.PK2-1Cmhp1Δ could be complemented by introduction of the centromere plasmid pRS133-MPH1, which contained the MPH1 gene as a 4161-bp Sphi-Msal fragment (chromosomal coordinates: 360791–356631) inserted into the EcoRV-Smal sites of pRS313 (Sikorski and Hieter 1989). Strains CEN.PK2-1C and CEN.PK2-1Cmhp1Δ were transformed with a linear URA3 gene fragment, isolated by PstI-HindIII cleavage of plasmid pH12-UH, to uracil prototrophy to yield strains CEN.PK2-1C-URA3Δ and CEN.PK2-1Cmhp1Δ-URA3Δ, respectively. hom3-10 derivatives were constructed as described (Krämer et al. 1996). The presence of the hom3-10 allele was verified by threonyne auxotrophy and the increased reversion rate after additional deletion of MSH2 (Reenan and Kolodner 1992; Lühr et al. 1998). msh6Δ mutants of CEN.PK2-1C were constructed as described (Lühr et al. 1998). The mph1 msh6 double mutant was constructed by introducing the msh6 deletion into CEN.PK2-1Cmhp1Δ. Disruption mutants of CEN.PK2-1C and CEN.PK2-1Cmhp1Δ for mag1, apn1, and mms4 were constructed using the plasmids pJC8901 (mag1::hisG-URA3-hisG; Chen et al. 1990), pSCP19A (apn1::hisG; Ramotar et al. 1991), and pWX1603 (mms4::hisG-URA3-hisG; Xiao et al. 1998), which were obtained from W. Xiao. The disruption strains could be complemented with plasmids containing the respective wild-type genes, which were also obtained from W. Xiao. re3, rad6,
rad14, mm2, and rad52 deletion mutants were constructed by the method of Wach et al. (1994) using the kanMX module of plasmid pFA6 with PCR primers adding 40 nucleotides homology from the regions around the start and the stop codon at the ends of the kanamycin cassette. The PCR products were used to transform CEN.PK2-1C and CEN.PK2-1Cmph1Δ to G418 resistance. The deletion construction was verified by PCR analysis.

**Nucleotide sequence of ura3 mutations:** Strains CEN.PK2-1C-URA3* and CEN.PK2-1C-mp1Δ-URA3*, respectively, were streaked for single cells onto synthetic medium lacking uracil and 55 or 58, respectively, single-cell colonies were transferred individually onto 5-FOA plates (Sikorski and Boeke 1991). Single 5-FOA-resistant papilla were restreaked for single-cell colonies onto rich medium and one single-colony each was tested for ura3 auxotrophy by lack of growth on synthetic medium without uracil and by complementation of the uracil auxotrophy by a plasmid-borne URA3 gene. Chromosomal DNA from each thus characterized mutant clone was prepared (Gram positive bacteria & yeast DNA isolation kit, Puregene) and a 1237-bp fragment (chromosome V: 115917–117153) containing the ura3 gene (116167–116967) was amplified by PCR (2 min denaturation at 95°; 30 cycles: 95° 45 sec, 55° 45 sec, 72° 90 sec; primers: 5′-TAATGTTGGCT TGTTTACGGTGC-3′ and 5′-TCTGGGAGGTATG TAGTTCC-3′) using Tfi-DNA-Polymerase (Promega, Madison, WI). The PCR products were purified with a QiAquick PCR purification kit (QIAGEN, Hilden, Germany) and both strands were sequenced with a cycle sequencing kit (Amersham, Uppsala, Sweden) using RDI 800 labeled primers (MWG Biotech; forward, 5′-CTGAACACCAATGGCATACG-3′; nucleotide position on chromosome V. 116019; reverse, 5′-GAGCT CTAATTTGGAG-3′; nucleotide position on chromosome V. 117024). Sequences were analyzed on a LiCor DNA sequencer model 4000L.

**Construction of the MPH1-GFP fusion gene:** For construction of the MPH1-GFP fusion plasmid pRS316-GAL1-MPH1-GFP, the first GFP fragment (Siemerig et al. 1996) was PCR amplified using the primers gfp Sma: 5′-TCC CCC GGG ATG GCT AGC AAA GGA GAA GAA C-3′ and 5′-ATG CCT GCA GGT CGA CTC TAG AGG-3′, which introduced an additional SmaI site at the 5′ end of the gene. The PCR fragment was digested with SmaI and cloned into the SmaI site of plasmid pRS316-GAL1-MPH1. This plasmid contains the MPH1 gene under control of the GAL1 promoter. The SmaI site was introduced at the very end of the MPH1 gene changing the second last codon from an aspartic acid codon to a glutamic acid codon and introducing an additional lysine codon before the stop codon. (Details of the constructions are available from the authors.) Furthermore, a SmaI/FspI fragment from pRS316-GAL1-MPH1-GFP, carrying the MPH1-GFP fusion gene, was cloned into the Ed136II site of plasmid pYES2 (Invitrogen, Carlsbad, CA) to yield plasmid pYES2-MPH1-GFP. Control plasmids expressing GFP alone were constructed by cloning the SmaI-digested PCR-amplified GFP fragment (see above) into the SmaI site of pRS316-GAL1 or into the Ed136II site of pYES2, yielding the plasmids pRS316-GAL1-GFP and pYES2-GFP, respectively.

For delimiting the nuclear localization signal of Mph1, a HincII fragment of MPH1 coding for amino acids 675–838 was cloned into the Ed136II site of pYES-GFP, located 9 bp upstream of the stop codon of the GFP5 gene. Using the primers gfp-Sma (see above) and 5′-GCT CTA GAT TTT TTT GGC TTC ACC TT or 5′-GCT CTA GAA ACT TTC TTC TTT GT as primers at the 3′ end of the gene, fragments of the fusion gene containing Mph1 segments coding for amino acids 675–696 or 675–704, respectively, were amplified where an XbaI site was introduced via 3′ primers. The PCR fragments were cleaved with MscI/XbaI (MscI is an internal site in the GFP gene) and cloned into the corresponding sites of pYES-GFP.

Transformants of CEN.PK2-1C with these plasmids were grown for 24 hr in synthetic medium lacking uracil with 2% galactose as a carbon source before evaluating the fluorescence with an Axioskop fluorescence microscope (Zeiss, Oberkochen, Germany). For identification of nuclei, cells were stained with 4′,6-diamidino-2-phenylindole (DAPI; 0.5 μg/ml) after brief fixation in 40% ethanol/0.1 m sorbitol. For gfp fluorescence Zeiss filter set 487709 was used and for DAPI fluorescence Zeiss filter set 487702 was used.

**Mutation rates:** Mutation rates were determined according to the method of the median (Lea and Coulson 1948) with 11 parallel cultures unless otherwise indicated. Five-milliliter cultures were inoculated with ~100-1000 cells and grown at 30° for 3 days in YPD or, for plasmid-containing strains, in selective synthetic medium and aliquots were plated onto selective plates. For strains containing plasmids, the plates were also selective for the plasmid marker. The titer in the cultures was counted with a hematocytometer or, for the epistasis analyses with rad6, rad14, mm2, and rad52, the viable titer was determined. Heteroduplex-DNA repair assays were as described (Lühr et al. 1998).

**Sensitivity against chemicals and radiation:** Methyl methane-sulfonate (MMS) and 4-nitroquinoline 1-oxide (4(NOQ)) were purchased from Fluka Chemical (Buchs, Switzerland); 6-azauracil, hydroxyurea, and camptothecin were from Sigma (St. Louis). Sensitivity tests were performed on plates by growing an overnight culture of the respective strain in liquid YPD at 30°, adjusting the cell density, and spotting serial 1:10 dilutions onto SC plates containing 60 μg/ml 6-azauracil or onto YPD plates containing 100 mm hydroxyurea or MMS, EMS, 4(NOQ), or camptothecin at the concentrations indicated. Plates were incubated at 30° for 2–4 days.

For MMS survival curves, overnight cultures of the respective strains were diluted into YPD to a cell density of 1×10⁷ cell/ml and grown with shaking to a density of 1×10⁷ cell/ml. An aliquot was removed for determination of the viable titer and MMS was added to a final concentration of 0.3%. Samples were withdrawn after 20, 40, and 60 min, the cells were washed with YPD, and appropriate dilutions were plated onto YPD plates to determine the viable titer.

UV survival curves were recorded by growing cells as described above for MMS killing curves to a density of 1×10⁷ cells/ml. Cells were washed and resuspended in water at a density of 1.5×10⁷ cell/ml. Cells were irradiated with a noncalibrated UV lamp (UV-C range, burner Osram HNS10W, distance ~30 cm). Aliquots were withdrawn at different times, diluted, and plated onto YPD plates to determine the number of survivors after incubation at 30°. For semiquantitative plating tests, 4×10³ cells of overnight cultures in YPD were streaked across a square YPD plate (12×12 cm) and segments of the streaks were irradiated for various times. Plates were incubated in the dark at 30° for 2 days.

For X-ray survival curves, overnight cultures in YPD were washed and resuspended in 100 mm sodium-potassium phosphate buffer, pH 7.2. Cells were irradiated with agitation with a calibrated X-ray source (200 kV, 20 mA, filter 0.5 Cu; Siemens) for different doses. Aliquots were withdrawn and dilutions were plated on YPD plates to determine the number of survivors after incubation at 30°.

**RESULTS**

In a systematic approach to analyze the function of unknown open reading frames of S. cerevisiae discovered during the yeast genome sequencing project, mutants with a deletion of open reading frame YIR002c were
found to have an increased spontaneous mutation rate in a forward mutation assay to canavanine resistance and in a reversion assay of the amber mutation trpl-289 (CAG → TAG; codon 130 of TRP1 gene). The mutant did not display any pleiotropic phenotype in other tests employed in the functional analysis network such as growth on different carbon sources (glucose, galactose, mannose, fructose, ethanol, acetate, glycerol), formation of rho− cells, distribution of cells in different stages of the cell cycle, sensitivity to various stresses (H2O2, NaCl, KCl, CuSO4, temperature), changes in morphology and cytoskeleton (bud scars, nucleus, mitochondria, vacuole, actin, tubulin), mating, invasive growth, glycosylation and secretion, plasmid loss and sensitivity to thiabendazole, and UV-induced mutations (Entian et al. 1999).

YIR002, which had been renamed MPH1 (mutator phenotype; Entian et al. 1999), is located on the right arm of chromosome IX (position 357415–360393) and could code for a protein of 993 amino acids. According to the classification given by Gorbalenya and Koonin (1993), Mph1 belongs to the DEAH family in the superfamily 2 (SF2) of helicases, since it contains seven characteristic motifs (motif I, PTGMKGT; motif Ia, PTRPLV; motif II, VIDEAH; motif III, ALTAT; motif IV, RVIIFT; motif V, TSIGEGLDI; motif VI, QRMGRTGR).

For the present study, we have determined the transcription of the MPH1 gene and the meiotic behavior of mutants. It was shown by reverse transcription (RT)-PCR (Kramer et al. 1996) that transcription is not cell cycle regulated and is not induced during meiosis (data not shown). Some induction (~3.8-fold) has been reported after treatment with MMS (Jelinsky and Samson 1999). Homozygous mph1 diploids displayed a slight sporulation defect. Starting from isolated zygotes, wild-type cells produced 30% four spore asci (78 asci from a total of 255 cells), whereas the homozygous mph1 mutant yielded only 8% four spore asci (19 asci from a total of 256 cells). Spore survival, however, which is a sensitive indicator for several disturbances during sporulation, was not reduced. After tetrad dissection, 89.6% viable spores were observed in homozygous mph1 diploids (190 survivors/212 spores) and 92.4% in wild type (207 survivors/224 spores; three isolated zygotes each). It can be concluded that mph1 mutants do not have a severe meiotic defect.

Possible indirect effects and nuclear localization: Members of the DEAH family are often associated with RNA metabolism (Lüking et al. 1998). The mutator effect of mph1 mutants could therefore be due to an indirect effect, e.g., that lack of the MPH1 gene product may reduce the translational efficiency of a protein involved in fidelity control or may not allow efficient splicing of an intron containing pre-mRNA. To investigate this in more detail, the subcellular localization of the MPH1 gene product was determined. The gene for green fluorescent protein (gfp) was fused to the 3′ end of the open reading frame of MPH1 and the fusion gene was expressed from a multicopy plasmid under control of the GAL1 promoter. The MPH1-gfp fusion gene (with the natural MPH1 promoter) was able to complement the mutator phenotype of an mph1 mutant (data not shown). As can be seen in Figure 1A, the Mph1-gfp derived fluorescence colocalizes with the DAPI fluorescence indicative for the nucleus. Expressing gfp without the Mph1 moiety resulted in uniform staining of the cell.

Inspection of the mph1 amino acid sequence revealed several clusters of basic amino acids between positions 675 and 838 (Figure 1B), which are typical for nuclear localization signals. To investigate whether sequences in this region are sufficient for directing nuclear localization, several peptides from this region were shown in Figure 1B were produced as C-terminal fusion with gfp. As judged from fluorescence microscopy, all peptides were able to direct localization of gfp to the nucleus (data not shown).

The nuclear localization of Mph1 largely excludes indirect effects on translation. To test for possible defects in splicing, total RNA from wild-type and mph1
mutant strains was prepared and the amounts of spliced and unspliced mRNA for MM S2, UBC13, UBC4, and ACT2 were determined by RT-PCR (Krämer et al. 1996) using PCR primers flanking the intron. M M S2 (Broomfield et al. 1998) and UBC13 (Hofmann and Pickart 1999) were chosen, since the phenotypes of mm s2 and ubc13 mutants have some resemblance to the phenotype of mph1 mutants (see below) and thus a splice defect might result in phenocopies of the respective mutants. No significant increase in the amount of unspliced mRNA was observed in any of the four genes in the mph1-derived RNA preparation compared to wild type (data not shown).

To test whether the conserved motifs in Mph1 are necessary for its function in fidelity control, single-amino-acid substitution mutations were constructed in motif I (K113Q), motif II (D209N, E210Q, H212D), and motif VI (Q603D). The mutant genes were introduced on a centromere plasmid into mph1 mutants and tested for complementation. None of the mutant genes was able to complement the mutator phenotype of the mph1 mutant (data not shown). To exclude the possibility that the mutant proteins produced were unstable, fusions of the mutant proteins with gfp were constructed. As judged from fluorescence microscopy, all fusion proteins were present in similar amounts and were localized to the nucleus (data not shown).

**Mutation spectrum:** To possibly delimitate the function of MPH1, the spectrum of spontaneous mutations in a wild-type strain and an isogenic mph1 Δ mutant was recorded. In case a particular type of mutation would occur predominantly in mph1 mutants, important clues to the possible function could be obtained. First, the ura3-52 alleles in CEN.PK2-1C and CEN.PK2-1Cmph1Δ were replaced by transformation with a functional URA3 gene. To select for spontaneous uracil auxotrophic mutants, 55 or 58 uracil prototrophic single-cell colonies from wild type or mutant, respectively, were streaked on 5-FOA. One mutant colony derived from each single-cell colony was restreaked for single-cell colonies; the ura3 gene from one colony was amplified by PCR and sequenced. This procedure ensured that all mutations were independent events. The results of this analysis are summarized in Table 1.

The ura3 mutations both in wild type and in mph1 mutants were more or less evenly distributed over the entire length of the gene with no obvious hotspots (data not shown). It can thus be concluded that MPH1 does not exert a strong preference for a particular sequence context. Also, no strong preponderance of a particular type of mutation occurring in the mph1 mutants was observed compared to wild type (Table 1). The conspicuous disparity in transition mutations (A → G vs. G → A and C → T vs. T → C) is similar in wild type and mph1 mutants and thus most likely constitutes an intrinsic property of the experimental system, since it has also been observed in other mutation spectra using URA3

| TABLE 1 |
| Summary of the spontaneous URA3 mutation spectrum in a wild-type and a mph1 mutant strain |

<table>
<thead>
<tr>
<th></th>
<th>Wild type</th>
<th>mph1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transversions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A:T→T:A</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>A:T→C:G</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>G:C→T:A</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>G:C→C:G</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Σ</td>
<td>24</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>(44%)</td>
<td>(59%)</td>
</tr>
<tr>
<td>Transitions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A:T→G:C</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>G:C→A:T</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Σ</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(36%)</td>
<td>(36%)</td>
</tr>
<tr>
<td>Deletions/insertions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+GG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td>(15%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>Duplications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+CAAA</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>+46-bp</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Σ</td>
<td>3</td>
<td>'0'</td>
</tr>
<tr>
<td></td>
<td>(5%)</td>
<td>(0%)</td>
</tr>
<tr>
<td>Rearrangements</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>TAAAAACACA→A₁₄</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>CTGATTT→CTAATT</td>
</tr>
<tr>
<td>Σ</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(3%)</td>
</tr>
<tr>
<td>Σ</td>
<td>55</td>
<td>58</td>
</tr>
</tbody>
</table>

Mutation spectra were recorded using the isogenic strains CEN.PK2-1C-URA3⁺ and CEN.PK2-1C-mph1Δ-URA3⁺. For insertions, deletions, and rearrangements, the changes occurring on the coding strand are indicated. For base substitutions, the classes comprise mutations where the first nucleotide of an indicated base pair was on the coding strand, as well as mutations where the first nucleotide was on the noncoding strand.

(Kunz et al. 1998). There is, however, an underrepresentation of one or two nucleotide insertions/ deletions in the mph1 mutant (one in mph1 mutant, eight in wild type).

To gather further evidence for the conjecture derived from the mutation spectrum that frameshift mutations are not increased in mph1 mutants, the reversion rate of the hom3-10 allele was determined. hom3-10 is a T addition in a run of six Ts (Marsischky et al. 1996). The reversion rate of hom3-10 in a mph1 background was 7.5 × 10⁻⁹ vs. 7.8 × 10⁻⁹ in the isogenic wild-type strain and thus not increased in the mutant, consistent with the interpretation of the mutation spectrum. The forward mutation rate to canavanine resistance of the mph1 mutant was increased eightfold compared to wild type in this experiment (3.7 × 10⁻⁷ vs. 4.5 × 10⁻⁶).

The predominance of base substitutions vs. inser-
tion/deletions in mph1 mutants resembles the mutation spectrum of an msh6 mutant, whereas another DNA mismatch repair mutant, msh2, showed a strong predominance of frameshifts over base substitutions (Mar-sischky et al. 1996). To see whether MPH1 acts in a MSH 6-dependent pathway, an epistasis analysis of the mutator phenotypes was carried out. The forward muta-
tion rate to canavanine for the mph1 mutant was increased 12-fold compared to wild type (1.3 × 10⁻⁶ vs. 1.1 × 10⁻⁷) and for the msh6 mutant 25.5-fold (2.8 × 10⁻⁶). The mutation rate of the mph1 msh6 double mutant was elevated 35.5-fold (3.9 × 10⁻⁶) and is thus additive. It can therefore be concluded that MPH1 is not part of the MSH 6-dependent branch of DNA mismatch repair.

MPH1 might nevertheless be involved in DNA mismatch repair having some more general function. In E. coli, the DNA helicase UvrD is required for fully efficient mismatch repair (Modrich and Lahue 1996). To test this in more detail, an mph1 mutant was transformed with heteroduplex DNA with defined mismatches (G/T, G/G, A/Δ, C/C) in an assay as described in Lühr et al. (1998). No obvious difference in the repair of the selected mismatches could be observed between mph1 mutants and wild type (data not shown). Therefore, it can be concluded that mph1 is likely not involved in DNA mismatch repair.

Sensitivity of mph1 mutants against DNA-damaging treatments: A variety of mutants affected in several pathways for repair of premutagenic lesions show a spontaneous mutator phenotype. Therefore, the sensitivity of mph1 mutants against various physical treatments or chemicals damaging DNA or otherwise interfering with nucleic acid metabolism was tested (UV light, X rays, MMS, EMS, 4-NQO, camptothecin, hydroxyurea, 6-azauracil). Far UV light induces mainly bulky lesions such as cyclobutane pyrimidine dimers and 6-4-pho-
toprodugs (Cadet 1994). X rays induce double-strand breaks both via direct absorption and radicals produced by radiolysis, which also cause a whole spectrum of other damages (Hutchinson 1985; Ward 1988). MMS and EMS (ethyl methanesulfonate) are both alkylating agents transferring methyl and ethyl groups, respectively, to nucleophilic centers in DNA thus forming a variety of different adducts. The second most frequent MMS adduct after N'-methylguanine is the toxic N³-alkyladenine (Pegg 1984), which blocks replication. O⁶-alkylguanine is probably more prominent among the EMS adducts (Drake and Baltz 1975; Singer 1975).

After metabolic activation, 4-NQO forms bulky adducts with nucleobases (Tur sky 1994), which are primarily targets for nucleotide excision repair. Therefore, 4-NQO is sometimes referred to as UV mimetic, although it produces a significant amount of oxidative damage as well (Ramoør et al. 1998). Camptothecin is not a DNA-damaging agent by itself, but traps the covalent topoisomerase I/DNA intermediate (Pommier et al. 1998), which could lead to double-strand breaks during replication (Liu et al. 1996). Hydroxyurea reduces the amount of dNTPs thus inhibiting DNA synthesis, and 6-azauracil reduces the UTP and GTP pools thus interfering with transcription (Hampsey 1997).

It was found that mph1 mutants are, if at all, only very slightly sensitive to X rays (Figure 4) and to UV light (Figure 5 and data not shown). They are also not sensitive to hydroxyurea and 6-azauracil (data not shown). They are, however, sensitive to MMS, EMS, 4-NQO, and camptothecin (Figures 2 and 3). This sensitivity is most likely not due to a defect in the G2/M DNA damage checkpoint (Paulovich et al. 1997), since the mph1 mutant did show G2 arrest (as judged microscopically from the accumulation of dumbbell cells) at least as efficiently as wild type after challenging cells with MMS (data not shown). This argues for an involvement of MPH1 in a pathway protecting the genome against lesions caused by these chemicals, either by preventing or repairing these lesions. It is particularly noteworthy that mph1-mutants are quite sensitive to 4-NQO but not to UV light.

REV3 dependence of mph1 mutator phenotype: The mutator phenotype of many DNA repair mutants results from channeling unprocessed or partially processed DNA lesions into the error-prone pathway of postreplicative repair (PRR), which results in the mutagenic bypass of these lesions by the Rev3/Rev7 DNA polymerase (Nelson et al. 1996b). Therefore, mph1 double mutants with rev3 were constructed and tested for spontaneous mutator phenotype in a canavanine forward mutation assay. The mutator phenotype of mph1 is abolished in mph1 rev3 double mutants (mutation rates: wild type, 1.5 × 10⁻⁷; rev3, 1.0 × 10⁻⁷; mph1, 1.5 × 10⁻⁶; mph1 rev3, 1.7 × 10⁻⁷). This result indicates that the mutator phenotype of mph1 mutants results from premutagenic lesions that are processed by error-prone PRR and that MPH1 is not required for enhancement of fidelity during replication.

Interaction with known repair pathways: The sensitivity of mph1 mutants to various DNA-damaging chemicals and the dependence of the spontaneous mutator phenotype on REV3 raises the question whether MPH1 is part of a known repair pathway. To investigate this, an epistasis analysis of mph1 mutants with mutants in base excision repair, nucleotide excision repair, postreplicative repair, and recombinogenic repair was carried out.

As representatives for base excision repair, MAG1, encoding a wide substrate specificity glycosylase removing alkylated adenines (Chen et al. 1990), and APN1, encoding the major AP-endonuclease in yeast (Popoff et al. 1990), were selected. Furthermore, interaction with MMS, which was identified by virtue of the MMS sensitivity of mutants (Xiao et al. 1998), but has no clearly assigned function yet, was tested. MMS-induced killing curves for the various single and double mutants were recorded (Figure 2).
The mph1 mutant is the least sensitive of all single mutants, followed by mms4, apn1, and mag1. All double mutants with mph1 were more sensitive than the respective single mutants, with mag1 mph1 being the most sensitive. The lack of epistasis in the double mutants argues for an involvement of MPH1 in a repair pathway different from the MAG1 APN1-dependent base excision repair pathway of alkylated adenes.

As representative for nucleotide excision repair RAD14, for recombinogenic repair RAD52, for both error-prone and error-free PRR RAD6 and for the error-free branch of PRR MMS2 and the error-prone branch REV3 was chosen. The rev3, mms2, rad6, rad14, and rad52 single mutants and the respective double mutants with mph1 were tested in a drop dilution plate assay for sensitivity against MMS, EMS, 4-NQO, and camptothecin. As can be seen in Figure 3, all mph1 double mutants are more sensitive than the respective single mutants, with the possible exception of the mph1 rad52 mutant on camptothecin. Since the sensitivities of the mph1 mutant are not epistatic to any of the repair mutants tested, it can be concluded that MPH1 is not involved in one of the known major repair pathways.

The above mutants were also tested for X-ray and UV light sensitivity. None of the double mutants with mph1 was more sensitive to X rays than the respective single mutants (Figure 4). UV light sensitivity was tested in a semiquantitative plate assay. As can be seen in Figure 5, double mutants of mph1 with mms2, rad6, and rad14 were slightly more sensitive to UV light than the respective single mutants.

To investigate whether an overlap exists for the premutagenic lesions leading to mutations in mph1 mutants and other repair mutants, an epistasis analysis for the spontaneous mutation rates to canavanine resistance was carried out with the mutants shown in Table 2. Mutation rates were determined by the method of the median (Lea and Coulson 1948). The data for rev3 confirms the data obtained before. The reduction of the mutation rate observed in rad6 mph1 compared to mph1 is expected, if the spontaneous mutations in mph1 mutants arise via the error-prone pathway of PRR, since rad6 is required for this pathway as well. The mutation rates for rad52 and mph1 are additive, indicating that the mutations arise from different kinds of premutagenic lesions. The mutation rates of mms2 and mph1 are lower than additive, but also not epistatic, whereas the mutation rates of rad14 and mph1 seem to be more than additive, but also not clearly synergistic. A simple interpretation of this behavior is difficult as long as neither the nature of the lesions leading to mutations in particular mutants nor the exact role of the genes is defined. But as for the other tests, no clear epistatic interaction of mph1 with any of the other mutants could be observed.

**Mph1 homologs:** A BLAST search comparing the deduced amino acid sequence of MPH1 with the databases (Altschul et al. 1997) gave the closest homologs putative proteins from the archaea Archaeoglobus fulgidus, Methanococcus januschii, Methanobacterium thermotrophicum, Pyrococcus abyssi, P. horikoshii, and Caenorhabditis elegans. Among the list of proteins with significant sequence homology were also matches to the transcription repair coupling factors of eubacteria and the proteins from the RecQ family, which include the yeast gene SGS1 (Watt et al. 1995) and the human disease genes BLM and WRN, which are required for genome stability (German 1993; Epstein et al. 1966).

A multiple sequence alignment of RecQ, Blm, Sgs1, and
DISCUSSION

In this study, we have characterized the new mutator gene MPH1 from S. cerevisiae. The deduced amino acid sequence of this gene reveals the key signature motifs characteristic for the DEAH family of RNA helicases. After determination of the structure of such a helicase from hepatitis C virus (Yao et al. 1997) the functions of many of the conserved motifs assigned previously by mutational analyses could also be explained on a structural basis (see also Introduction). Since we have demonstrated by introducing mutations into the conserved motifs that these motifs are necessary for the function of Mph1 in fidelity control and that this function does not reside in a different domain of the protein, the possibility of Mph1 being an RNA helicase has to be considered.

The effect of MPH1 is probably not exerted indirectly: The mutator phenotype of mph1 mutants can be elicited in two principal ways: either Mph1 partakes directly in a mechanism safeguarding genetic information.

Figure 3.—Sensitivity of various mutants to MMS, EMS, 4-NQO, and camptothecin. Isogenic derivatives of strain CEN.PK2-1C with the relevant genotypes, as indicated to the left of the figure, were spotted onto plates containing the chemicals, as indicated on top. The spots contained, from left to right, $10^5$, $10^4$, $10^3$, and $10^2$ cells, respectively. The YPD plate served as growth control. Each mutant and its respective mph1 double mutant were analyzed on the same plate. The concentration of the chemical in a particular plate is given in white letters between the respective pair.

Figure 4.—X-ray sensitivity of various mutant strains. Isogenic derivatives of strain CEN.PK2-1C with the relevant genotypes, as given in the figure, were irradiated with X rays at the doses indicated. The viable titer was determined and the percentage of survivors was calculated. The percentage of survivors was plotted on a logarithmic scale.
we found that four different RNAs tested were spliced to various chemicals for mutants from all repair pathways: (i) The mutation spectrum of mph1 is additive to that of msh6, a gene involved in DNA mismatch repair. Furthermore, no defect in the repair of heteroduplex DNA with defined mismatches was observed in a mph1 mutant. The dependence of the mutator phenotype of mph1 on REV3, which encodes a subunit of the polymerase \( \xi \) required for error-prone translesion synthesis (Nelson et al. 1996b), makes participation in any process that increases fidelity at the replication fork very unlikely, since most or all of the mutations in mph1 mutants seem to arise by mutagenic bypass of unrepaired lesions. But a participation in base excision, nucleotide excision, error-prone, and error-free postreplicative repair and recombinational repair can be largely excluded also, since representative mutants from all these pathways (maq1, apn1, rad14, rad6, rev3, mms2, and rad52) are less sensitive to chemical treatment than the respective double mutants with mph1.

**Possible functions of MPH1:** Thus, Mph1 is most likely not part of a hitherto known repair pathway. This raises the question of other possible functions. Three observations seem to be particularly valuable to arrive at a plausible speculation: (i) The mutation spectrum of mph1 is very similar to wild type. The only obvious difference is the underrepresentation of frameshift mutations. (ii) The lack of MPH1 increases the sensitivity to various chemicals for mutants from all repair pathways, i.e., base excision repair, nucleotides excision repair, error-free and error-prone postreplicative repair, and recombinational repair. (iii) mph1 mutants are not or only slightly sensitive to UV irradiation, but they are quite sensitive to the UV mimeticum 4-NQO.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Mutation rate (( \times 10^7 ))</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>mph1</td>
<td>14.8</td>
<td>12</td>
</tr>
<tr>
<td>mms2</td>
<td>16.1</td>
<td>13</td>
</tr>
<tr>
<td>mms2 mph1</td>
<td>22.6</td>
<td>18</td>
</tr>
<tr>
<td>rad6</td>
<td>3.2</td>
<td>2.7</td>
</tr>
<tr>
<td>rad6 mph1</td>
<td>6.1</td>
<td>5</td>
</tr>
<tr>
<td>rev3</td>
<td>0.57</td>
<td>0.5</td>
</tr>
<tr>
<td>rev3 mph1</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>rad14</td>
<td>5.4</td>
<td>4.5</td>
</tr>
<tr>
<td>rad14 mph1</td>
<td>36.3</td>
<td>30</td>
</tr>
<tr>
<td>rad52</td>
<td>19.4</td>
<td>16</td>
</tr>
<tr>
<td>rad52 mph1</td>
<td>38.1</td>
<td>32</td>
</tr>
</tbody>
</table>

Isogenic derivatives of strain CEN.PK2-1C with the relevant genotypes as given were grown in rich medium and mutation rates were determined by the method of the median (Lea and Coulson 1948) using nine parallel cultures for each strain.
Mutations in wild type arise by two major pathways: Errors during replication and mutagenic bypass of premutagenic lesions in the DNA. Typical errors originating during replication are frameshifts and base substitution mutations. Premutagenic lesions have—depending on their chemical structure—a very diverse, but often lesion-specific mutagenic potential. O6-methylguanine, for example, induces mainly G/C to A/T transitions (Eadie et al. 1984; Loechler et al. 1984) and the bypass of AP sites by Rev1 results in the insertion of a C opposite the AP site (Nelson et al. 1996a). The underrepresentation of frameshifts in the mph1 mutant is in accord with the notion derived from the REV3 dependence of the mutator phenotype that the error frequency during replication is not increased. Thus, the major source for the increased number of mutations in mph1 mutants should be an increased number of premutagenic lesions that are bypassed by the error-prone pathway. If, however, the mutation spectrum is not slanted toward a particular type of mutation as is found for other repair mutants (Kunz et al. 1998), the simplest explanation is not that

The distribution of the different types of premutagenic lesions is changed in mph1 mutants but rather the frequency of all of them. This assumption is also in accordance with the observation that the mutator phenotype is not epistatic with any of the mutants tested. The frequency of premutagenic lesions might be changed either by an altered rate of formation, an altered removal rate, or by changes in both of them.

It seems unlikely that MPH1 constitutes a new, independent repair pathway, which could correct all the various lesions. A more plausible explanation would be that MPH1 is acting either upstream of repair, decreasing the rate of lesion formation, or as an accessory factor for the known repair pathways, increasing the removal rate. Such an accessory function could be modulation or reestablishment of chromatin, which has been shown to affect repair efficiency (Smerdon and Conconi 1999). Furthermore, mutants in subunits of the chromatin assembly factor I required for reassembly of chromatin after replication are UV sensitive (Kaufman et al. 1997; Game and Kaufman 1999) and it has been shown...
that this factor also couples chromatin assembly to nucleotide excision repair in Xenopus (Gailhard et al. 1996). This would, however, not explain the apparent differences in processing of UV-induced damage vs. 4-NQO adducts, a phenotype also observed for Werner mutant cells (Fujitawa et al. 1977; Gbur et al. 1997). This could be explained if Mph1 affects the rate of lesion formation, e.g., by modulating the susceptibility of DNA to chemical attack. Mph1 may help to return DNA from a more vulnerable state, e.g., after replication or transcription, to a more protected state again. At the present state, any guesses must be speculative. It might be the compaction of chromatin, the amount of unwound DNA in a cell, or changes in the secondary structure of DNA. To reconcile the lack of UV and X-ray sensitivity in mph1 mutants with the increased sensitivity to MMS, EMS, and 4-NQO, the “vulnerable” state should affect the rate of lesion formation for the latter three treatments but to a lesser extent than that of the first two treatments. A variety of studies indicate that the rate of lesion formation is influenced differentially by chromatin structure depending on the DNA-damaging agents. Whereas, e.g., the distribution of UV-induced cyclobutane pyrimidine dimers is largely unaffected by chromatin (Williams and Friedberg 1979; Niggli and Cerutti 1982; Mitchell et al. 1990), most chemically induced DNA adducts show a pronounced preference for linker DNA (Ramanathan et al. 1976; Mertz et al. 1977; Kuo and Hu 1978; Jahn and Litman 1979; Suhaklar et al. 1979; Feldman et al. 1980; Kaneko and Cerutti 1980, 1982; Berko witz and Sil’k 1981). Thus, changes in chromatin structure in the mph1 mutant might be responsible for the different sensitivities.

At any rate, if mammals do possess Mph1 orthologous genes as strongly suggested by the available EST clones, defects in this gene in humans may greatly influence the susceptibility of affected individuals to genotoxic chemicals and thus increase cancer risk as a result of exposure to these chemicals.


Jahn, C. L., and G. W. Litman, 1979 Accessibility of deoxyribonu-


Walker, J. E., M. Saraste, M. J. Runswick and N. J. Gay, 1982 Distantly related sequences in the α- and β-subunits of ATP syn-
thetase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. 1: 945-951.


Communicating editor: L. S. Symington