Yeast *MPH1* Gene Functions in an Error-Free DNA Damage Bypass Pathway That Requires Genes From Homologous Recombination, but Not From Postreplicative Repair

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Manuscript received September 26, 2003
Accepted for publication December 31, 2003

ABSTRACT

The *MPH1* gene from *Saccharomyces cerevisiae*, encoding a member of the DEAH family of proteins, had been identified by virtue of the spontaneous mutator phenotype of respective deletion mutants. Genetic analysis suggested that *MPH1* functions in a previously uncharacterized DNA repair pathway that protects the cells from damage-induced mutations. We have now analyzed genetic interactions of *mph1* with a variety of mutants from different repair systems with respect to spontaneous mutation rates and sensitivities to different DNA-damaging agents. The dependence of the *mph1* mutator phenotype on *REV3* and *REV1* and the synergy with mutations in base and nucleotide excision repair suggest an involvement of *MPH1* in error-free bypass of lesions. However, although we observed an unexpected partial suppression of the *mph1* mutator phenotype by *rad5*, genetic interactions with other mutations in postreplicative repair imply that *MPH1* does not belong to this pathway. Instead, mutations from the homologous recombination pathway were found to be epistatic to *mph1* with respect to both spontaneous mutation rates and damage sensitivities. Determination of spontaneous mitotic recombination rates demonstrated that *mph1* mutants are not deficient in homologous recombination. On the contrary, in an *sgs1* background we found a pronounced hyperrecombination phenotype. Thus, we propose that *MPH1* is involved in a branch of homologous recombination that is specifically dedicated to error-free bypass.

ALL organisms studied in more detail so far possess a large arsenal of DNA repair systems to remove lesions that constantly arise from both endogenous and environmental sources. In most cases, a prerequisite for removal of a lesion without altering the informational content of the DNA is the availability of an undamaged copy, which is usually provided by the complementary strand. If, however, a hitherto unrepaired or new DNA damage appears in a replication fork, where both strands are separated, it cannot be repaired without taking additional measures. Such damage poses a severe threat to the survival of a cell, since many DNA lesions will arrest the replication machinery. Replicative DNA polymerases are very accurate enzymes with error rates usually in the range of 10^{-5} per replicated nucleotide (Schaaper 1993; Roberts and Kunkel 1999). The major discrimination factor between correct and incorrect base pairs seems to be the geometrical fit into the active site of the polymerase (Kunkel and Bebenek 2000; Kool 2002). From this it is conceivable that a modified template nucleotide can prevent further replication and hence cell division, which by definition is equivalent to cell death.

One cellular mechanism to cope with this problem is translesion synthesis (TLS) by specialized DNA polymerases (for review see, *e.g.*, Wang 2001; Friedberg *et al.* 2002; Goodman 2002). The ability of these polymerases to copy a damaged template is probably due to a relaxed binding site (Friedberg *et al.* 2001). On the other hand, these polymerases often have a strongly reduced fidelity in copying undamaged templates compared to replicative DNA polymerases (Kokoska *et al.* 2002). In yeast, Pol η (Rad30) and Rev1 have been identified as translesion polymerases, where Pol η is an enzyme that can catalyze error-free bypass of thymidine dimers (Johnson *et al.* 1999; Washington *et al.* 2000) and Rev1 has a dCMP transferase activity (Nelson *et al.* 1996a) that has been invoked in the bypass of apurinic/apyrimidinic sites (AP sites; Nelson *et al.* 2000), although this function is still debated (Haracska *et al.* 2001). Another enzyme involved in translesion synthesis is polymerase ζ, which consists of the two subunits Rev3 and Rev7. The catalytic subunit of Pol ζ is encoded by the *REV3* gene (Morrison *et al.* 1989). Pol ζ has an accuracy resembling that of other replicative polymerases (Johnson *et al.* 2000) and is thus, rather, a high-fidelity polymerase. Although Pol ζ is able to bypass lesions (Nelson *et al.* 1996b; Haracska *et al.* 2003), it

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does so with only low efficiency compared to human polymerase ι, for example (Johnson *et al.* 2000). However, Pol ζ quite efficiently elongates mismatched primers (Johnson *et al.* 2000; Haracska *et al.* 2001, 2003). Thus, the actual lesion bypass is apparently carried out by a specialized translesion or a replicative polymerase, whereas Pol ζ is employed for extension of mismatched termini (Prakash and Prakash 2002; Haracska *et al.* 2003).

Since many lesions are noninstructive, TLS will often result in mutations. Evidently, cells have also developed error-free mechanisms for the bypass of lesions during replication (for review of such mechanisms in *Escherichia coli*, see, *e.g.*, Cox 2001; Michel *et al.* 2001, McGlynn and Lloyd 2002a,b). The situation differs depending on whether the damage is on the template for the leading or the lagging strand. Lagging-strand synthesis can proceed with the synthesis of the next Okazaki fragment and the remaining gap (Svoboda and Vos 1995) may subsequently be filled by recombination. For the leading strand, however, primer synthesis is strictly regulated to once per cell cycle (Katayama 2001; Bell and Dutta 2002; Nasheuer *et al.* 2002). Therefore, resumption of replication after damage-induced polymerase arrest cannot be achieved by synthesis of a new primer downstream of the lesion, but replication can proceed only if the lesion is bypassed or removed. Apparently, arrest of the leading-strand polymerase does not cause an immediate fork arrest, but lagging-strand synthesis still continues (Svoboda and Vos 1995; Cordeiro-Stone *et al.* 1999; Pagés and Fuchs 2003). Hence, the terminated leading strand could be elongated with the lagging strand as template, which would allow error-free extension of the leading strand beyond the lesion. A possible intermediate in this process could be a D-loop resulting from invasion of the leading strand into the sister chromatid or a Holliday junction formed by fork regression (Fujiwara and Tatsumi 1976; Higgins *et al.* 1976). In *E. coli*, formation of such intermediates seems to require RecA (Robu *et al.* 2001; Lusetti and Cox 2002) and/or RecG (McGlynn and Lloyd 2000, 2002a). The decision to use either TLS or error-free bypass in yeast is at least in part governed by modification of PCNA by ubiquitin and SUMO (Hoege *et al.* 2002; Stelter and Ulrich 2003).

In this study we describe the analysis of genetic interactions of *mph1* mutants with a variety of mutants from different pathways of the cellular response to DNA damage. We have previously shown that *mph1* mutants have a *REV3*-dependent mutator phenotype and that *MPH1* is probably not a member of base excision or nucleotide excision repair (Scheller *et al.* 2000). The genetic interactions that we found in this study strongly suggest that Mph1 is involved in a pathway for error-free bypass and that this pathway requires components from the homologous recombination system, but is distinct from the Rad5-dependent error-free branch of the postreplicative repair pathway.

MATERIALS AND METHODS

Media:

YPD: 2% D-glucose (MERCK, Darmstadt, Germany, or Roth, Karlsruhe, Germany), 2% bacto peptone (Difco Becton Dickinson, Sparks, MD), and 1% yeast extract (Oxoid, Basingstoke, Great Britain) in water purified with a Milli-Q water purification system (Millipore, Bedford, MA), autoclaved for 20 min at 121°. For plates, 1.6% agar (agar bacteriological no. 1; Oxoid, Basingstoke, UK) was added before autoclaving.

Synthetic complete medium: 2% D-glucose (autoclaved separately or together with agar, if preparing plates), 0.17% Difco yeast nitrogen base without amino acids and without ammonium sulfate, 0.51% ammonium sulfate, and 680 mg/liter synthetic complete mixture. Synthetic complete mixture contained the following components weighed in as powder and added before autoclaving (final concentrations are indicated): adenine 40 mg/liter, L-arginine 30 mg/liter, L-histidine 20 mg/liter, L-isoleucine 20 mg/liter, L-leucine 30 mg/liter, L-lysine-HCl 30 mg/liter, L-methionine 20 mg/liter, L-phenylalanine 50 mg/liter, L-serine 100 mg/liter, L-threonine 150 mg/liter, L-tryptophane 30 mg/liter, L-tyrosine 30 mg/liter, uracil 20 mg/liter, L-valine 100 mg/liter.

Drop-out media: Synthetic complete medium lacking nutrilite supplements. For example, "uracil-less" medium is without uracil (SC −ura).

Canavanine medium: SC −arg containing 40 mg/liter canavanine, added as filter-sterilized 2% stock solution in water after autoclaving when medium was partially cooled.

5-Fluoroorotic acid plates: SC plates containing, in addition, 50 mg/liter uracil and 1 g/liter 5-fluoroorotic acid (5-FOA; added after autoclaving as powder when medium was partially cooled).

G418 plates: YPD plates containing G418 (200 μg/ml; Calbiochem, San Diego), added as powder after autoclaving when medium was partially cooled.

For sterile filtered media, all components were dissolved in water and filtered through Vacuflo PV 050/3 disposable sterile filter units (0.2-μm pore size; Schleicher & Schuell, Dassel, Germany).

Strains and plasmids: All mutant strains except those used for determination of mitotic recombination rates (see below) were constructed in a CEN.PK2-1C background (*ura 3-52 leu2-3,112 his3Δ1 trp1-289, MAL-2-8*^{c} *SUC2 MAT***a**, from Peter Kötter; Entian *et al.* 1999) by one-step gene disruption (Rothstein 1991). Transformations were carried out as described (Gietz *et al.* 1992). DNA fragments for deletion construction were obtained by either cleavage of plasmids listed in Table 1 or PCR (Wach *et al.* 1994, 1997). PCR primers contained 40 nucleotide tails from the 5′- or 3′-flanking region, respectively, of the gene to be deleted. Transformants were streaked for single cells on selective medium and single-cell colonies were tested by PCR for correct construction of the deletion. When the *hisG::URA3::hisG* cassette (Alani *et al.* 1987) was used, *ura3*$^-$ recombinants were selected by streaking for single cells on 5-FOA medium (Sikorski and Boeke 1991). Both 5′- and 3′-flanks were verified using primer pairs, where one primer was located in the selective marker and one primer in the flanking region of the deleted gene, but outside of the DNA fragment used for transformation. Primer sequences are available from the authors upon request. For storage, a freshly

TABLE 1

Isogenic derivatives of *S. cerevisiae* strain CEN.PK2-1c

Strain	Disruption	Disruption construct	Reference
apn1	*apn1::HIS3*	pSCP19A cut with *Eco*RI and *Bam*HI	Ramotar *et al.* (1991)
apn1 rev3	*rev3::hisG* in *apn::HIS3* background	pYPG101 cut with *Kpn*I	Morrison *et al.* (1989)
apn2	*apn2::hisG*	pBLUE-ETH1 cut with *Sac*II and *Xho*I	Bennett (1999)
lig4	*lig4::HIS3*	pGEMTScLig4 cut with *Pae*I and *Sal*I	Teo and Jackson (1997)
mag1	*mag1::hisG*		Scheller *et al.* (2000)
mgs1	*mgs1::HIS3MX6*	PCR based	Wach *et al.* (1997)
mms2	*mms2::kanMX4*		Scheller *et al.* (2000)
mph1	*mph1::hisG*		Scheller *et al.* (2000)
rad5	*rad5::kanMX4*	PCR based	Wach *et al.* (1994)
rad5 rad52	*rad52::URA3* in *rad5::kanMX4* background	pSM22 cut with *Bam*HI	Torres-Ramos *et al.* (1996)
rad6	*rad6::kanMX4*		Scheller *et al.* (2000)
rad18	*rad18::HIS3MX6*	PCR based	Wach *et al.* (1997)
rad26	*rad26::kanMX4*	PCR based	Wach *et al.* (1994)
rad28	*rad28::kanMX4*	PCR based	Wach *et al.* (1994)
rad51	*rad51::LEU2*	PCR using chromosomal DNA	Strain for template DNA obtained from Ian Hickson
rad52	*rad52::kanMX4*		Scheller *et al.* (2000)
rad55	*rad55::LEU2*	pSTL11 cut with *Hin*dIII	Lovett and Mortimer (1987)
rev1	*rev1::hisG-URA-hisG*	pSF3 cut with *Xho*I and *Sal*I	Larimer *et al.* (1989)
rev1 rad5	See single mutants	Mating and dissection	
rev3	*rev3::kanMX4*		Scheller *et al.* (2000)
ubc13	*ubc13::HIS3MX6*	PCR based	Wach *et al.* (1997)
yku70	*yku70::LEU2*	pGEM4Z-S-H-leu cut with *Sac*I and *Hin*dIII	Feldmann and Winnacker (1993)
yku80	*yku80::kanMX4*	pHDF2 kan cut with *Bam*HI and *Sal*I	Feldmann *et al.* (1996)

Double and triple mutants with *mph1* were constructed using CEN.PK2-1c *mph1* or the respective double mutant as transformation recipient instead of CEN.PK2-1c.

grown overnight culture in YPD was adjusted to 7% (v/v) DMSO and kept at −70°.

Mutation rates: Mutation rates were determined by the method of the median (Lea and Coulson 1948). Eleven 30-ml test tubes each containing 7 ml YPD were inoculated to a cell density of 20 cells/ml with an overnight culture of the respective strain. The tubes were incubated for 3 days (4 days for slow-growing mutant strains) at 30° with agitation. Aliquots from all 11 cultures were plated onto canavanine medium to determine the number of mutants in each culture. Viable titer was determined by plating appropriate dilutions of two randomly chosen cultures onto SC −arg plates. Cultures were stored at 4° and cell density of the median culture was determined in a hematocytometer. The mean of the viable titers was used in the calculation of the mutation rate. (Where indicated, hematocytometer counts were employed.) For all mutant strains, the mutation rate of the wild type and the *mph1* mutant was determined in parallel using the same batch of medium for growth. For calculating relative mutation rates, the mutation rate of the respective mutant was normalized to that of the wild type determined in parallel in that particular experiment.

Determination of induced mutation frequency: Cells were grown overnight in sterile filtered YPD. Cultures were diluted in fresh YPD to give a cell density of $\sim 1 \times 10^7$ cells/ml and incubated for 75 min at 30°. Cultures were arrested by adding α-mating factor to a final concentration of 4 μg/ml. After incubation at 30° for 90 min with agitation, the same amount of α-factor was added again. After an additional incubation for 60 min at 30°, cells where washed twice with water and resuspended in YPD to give a cell density of 5×10^6 cells/ml. Cultures were divided in 2-ml samples and incubated for 120 min with 4-nitroquinoline-1-oxide (4-NQO) added to the medium. Samples were washed with cold water, resuspended in cold YPD, and kept on ice to minimize cell growth. Aliquots were plated onto canavanine plates to determine the number of mutants and onto YPD plates to determine the viable titer.

Drop dilution assay to determine sensitivity to DNA-damaging agents: Methyl methanesulfonate (MMS) and 4-NQO were from Fluka Chemie GmbH (Buchs, Switzerland). For sensitivity tests, the respective strains were grown overnight in liquid YPD at 30°. In the morning, strains were diluted ~1:10 in fresh YPD and grown at 30° for another 4 hr. In the meantime, YPD plates containing the respective chemicals were prepared. Chemicals were not added before the medium had cooled to <60°. Cell density was determined with a hematocytometer and adjusted to 1×10^7 cells/ml. Three serial 1:10 dilutions were prepared (up to 1×10^4 cells/ml). A total of 10 μl of the adjusted cell suspension and of the serial dilutions (containing 10^5, 10^4, 10^3, and 10^2 cells, respectively) were spotted onto YPD plates without added chemicals as control and onto YPD plates containing MMS or 4-NQO. Plates were incubated for 2–3 days at 30°.

Mitotic recombination rates: Mitotic recombination rates were determined according to Dora *et al.* (1999). Strains used were NLBL1 [*MAT*α *ade5 met13-c* (temperature sensitive) *cyh2*^{R} *trp5 LEU1 CLY8 his7-1 tyr1-2 lys2-2 ade2-1 ura3-1 CAN1*] and NLBL3 (*MAT***a** *ADE5 met13-d CYH2 TRP5 leu1 ADE6 CLY8*

TABLE 2

Influence of defects in different repair pathways on the forward mutation rate of *mph1* mutants to canavanine resistance

Strain	Mutation rate × 10^7	Relative rate	Strain	Mutation rate × 10^7	Relative rate
Wild type	2.9 ± 0.42[1]	1	*mph1*	23.5 ± 2.7[1]	8.1
	1.6 ± 0.24[2]			12.4 ± 1.5[2]	7.8
	2.7 ± 0.40[3]			31.2 ± 3.6[3]	11.6
	3.1 ± 0.61[4]			46.2 ± 5.3[4]	14.9
	2.9 ± 0.43[5]			38.5 ± 4.2[5]	13.3
	2.9 ± 0.46[6]			32.2 ± 3.1[6]	11.1
mag1[a]	2.9 ± 0.42[1]	1.0	*yku70*	1.6 ± 0.25[3]	0.59
mag1 mph1[a]	41.1 ± 4.3[1]	14.2	*yku70 mph1*	29.4 ± 3.3[3]	10.9
rad26[a]	1.0 ± 0.25[1]	0.35	*yku80*	2.3 ± 0.38[4]	0.74
rad26 mph1[a]	27.3 ± 3.1[1]	9.4	*yku80 mph1*	35.0 ± 4.0[4]	11.3
rad28[a]	0.7 ± 0.11[2]	0.44	*lig4*	4.1 ± 0.59[5]	1.4
rad28 mph1[a]	9.3 ± 1.1[2]	5.8	*lig4 mph1*	36.1 ± 4.1[5]	12.4
mgs1	4.9 ± 0.77[6]	1.7			
mgs1 mph1	45.7 ± 5.5[6]	15.8			

Total cell numbers for calculation of mutation rates were determined from viable titer. Mutation rates for wild type and *mph1* were determined in each set of experiments. Relative rates were calculated by normalizing the mutant mutation rates to the wild-type rate determined in that particular experiment, which is indicated by the indices 1–6. The errors indicated are the quotients of the standard deviations of the number of mutations (Lea and Coulson 1948) and total cell numbers.

[a] For these strains, cell numbers were determined from hematocytometer counts.

his7-2 tyr1-1 lys2-1 ade2-1 ura3-1 can1^R). NLBL1 *mph1::hisG* was constructed according to Scheller *et al.* (2000) and NLBL3 *mph1::kanMX4* was constructed with a disruption cassette derived by PCR from pFA6a (Wach *et al.* 1994). NLBL1 *sgs1::kanMX4*, NLBL1 *sgs1::kanMX4 mph::hisG*, and NLBL3 *sgs1::kanMX4* were constructed by introducing a PCR-derived *sgs1::kanMX4* disruption cassette (Wach *et al.* 1994) into NLBL1, NLBL1 *mph1::hisG*, and NLBL3, respectively. NLBL3 *sgs1::kanMX4 mph1::hisG* was derived from NLBL3 *sgs1::kanMX4* according to Scheller *et al.* (2000). Diploids were selected on SC −leu −trp after mass mating of appropriate haploid strains on solid YPD and stored at −70° in 7% DMSO.

For determination of mitotic recombination, diploids from frozen stock cultures were streaked for single cells on SC −leu −trp plates and grown at 30°. A red colony from each diploid was picked, resuspended in sterile water, and cell density was determined in a hematocytometer. Eleven 30-ml test tubes, each containing 10 ml YPD$^+$ (YPD media supplemented with 75 μg/ml each of adenine, histidine, leucine, lysine, tryptophane, tyrosine, methionine, and uracil), were inoculated to a cell density of four cells/ml and incubated for 3 days at 30° with agitation. Appropriate dilutions were plated onto SC −lys, SC −his, and SC −met for heteroallelic recombinants and onto canavanine medium for single-site conversions. Viable titer was determined on SC plates. Colonies were counted after incubation for 4 days at 30° or 37° for SC −met plates. Mitotic recombination rates were determined by the method of the median (Lea and Coulson 1948).

RESULTS

In our previous analysis of the *MPH1* gene (Scheller *et al.* 2000) we could not detect epistatic relationships for any of the following repair pathways that were analyzed: base excision repair (BER), nucleotide excision repair (NER), postreplicative repair (PRR), or homologous recombination (HR), with the possible exception of *rad52* with respect to sensitivity to DNA-damaging agents. We suspected that the accessibility of DNA to genotoxic chemicals may be altered in *mph1* mutants. We therefore measured the amount of DNA damage by PCR in a polymerase blocking assay (Jennerwein and Eastman 1991; Jenkins *et al.* 2000), but we could not obtain any evidence for a significant increase of DNA damage in *mph1* mutants in comparision to wild type after treatment with 4-NQO, MMS, and UV (data not shown).

Genetic interactions with DNA repair pathways: In addition to the DNA repair mutants that already had been investigated in our previous study (Scheller *et al.* 2000), we extended this analysis to cover several repair pathways more thoroughly and to include some new repair pathways. Mutants studied here were from BER *mag1* and *apn1*, from transcription-coupled nucleotide excision repair (TC-NER) *rad26* and *rad28*, from the nonhomologous end-joining pathway (NHEJ) *yku70*, *yku80*, and *lig4*, and a mutant of the recently described *MGS1* gene that has been implicated in polymerase processivity (Hishida *et al.* 2001, 2002; Branzei *et al.* 2002). From postreplicative repair we analyzed *rad5*, *rad6*, *rad18*, *ubc13*, *mms2*, and *rev1* (Table 4) and from homologous recombination *rad51*, *rad52*, and *rad55* (Table 5). Spontaneous forward mutation rates to canavanine resistance of the other mutants are shown in Table 2. The rates for wild type and the relative increases for the *mph1* mutant vary between different experiments, as can also be seen in Tables 3–5. We ascribe this phenomenon in part to statistical fluctuation inherent in

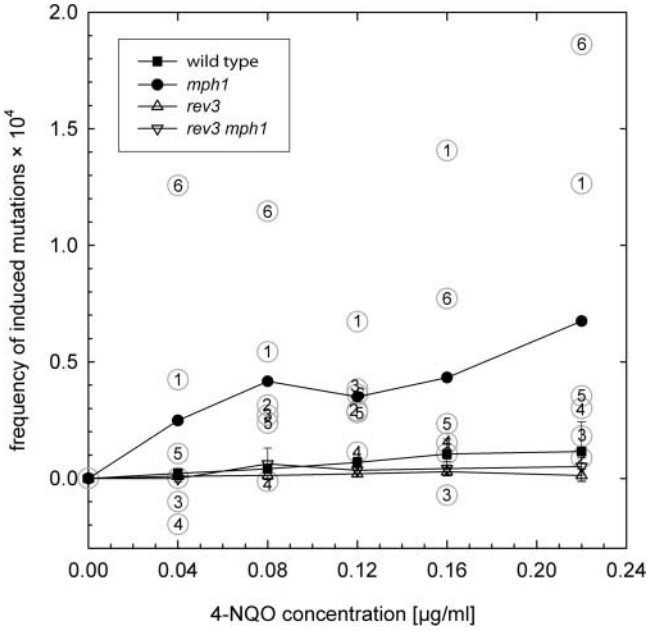

FIGURE 1.—The frequency of damage-induced mutations, determined as described in MATERIALS AND METHODS, is increased in *mph1* mutants. Values for wild type, *rev3*, and *rev3 mph1* are the means of three independent experiments each. Error bars are standard deviations. Values for *mph1* are the means of six independent experiments. The values for each experiment are indicated by the shaded outlines of circles, containing the number of the respective experiment.

the method of determining mutation rates, but also to differences in the exposure to environmental mutagens during growth. Since a large portion of spontaneous mutations, in particular in *mph1* mutants, arise by Rev3-mediated mutagenic bypass of DNA lesions (QUAH *et al.* 1980; SCHELLER *et al.* 2000), the number of mutations should correlate with the concentration of mutagenic agents in the growth medium (see Figure 1). Browning reactions like caramelization and the Maillard reaction (the complex reactions resulting from heating of mixtures of proteins and carbohydrates) are known to create mutagenic substances (POWRIE *et al.* 1986). Whereas *mph1* mutants grown in autoclaved medium showing considerable browning usually have an up to 12-fold increase in mutation rate, they displayed only an ~5-fold increase in mutation rate compared to wild type if grown in sterile filtered rich medium (mutation rates: wild type, 1.2×10^{-7}; *mph1*, 6.1×10^{-7}). Since these factors, as well as the exposure to other environmental mutagens such as oxygen, are difficult to control accurately, we always determined the mutation rate of the wild type and the *mph1* mutant in parallel using the same batch of medium and the same culturing conditions. Thus, although the absolute rates may vary from experiment to experiment, the internal relations between the mutation rates should remain preserved.

As can be seen in Table 2, no striking effect on the spontaneous mutation rates was observed for the *mph1*

TABLE 3

Synergy of *apn1* and *apn2* with *mph1* with respect to forward mutation rates to canavanine resistance

Strain	Mutation rate × 10^7	Relative rate
Wild type	6.3 ± 0.89^1	1
	3.2 ± 0.44^2	
mph1	39.9 ± 4.6^1	6.3
	19.4 ± 2.2^2	6.1
apn1	6.9 ± 0.99^1	1.1
apn1 mph1	117 ± 12.4^1	18.6
apn2	8.2 ± 1.1^1	1.3
apn2 mph1	32.4 ± 3.7^1	5.1
apn1 apn2	5.1 ± 0.91^1	0.81
apn1 apn2 mph1	212 ± 23.3^1	33.6
apn1 rev3	3.6 ± 0.53^2	1.1
apn1 rev3 mph1	3.4 ± 0.48^2	1.1

Total cell numbers for calculation of mutation rates were determined from viable titer except for the values with the index 2, where cell number was determined from hematocytometer counts. Relative rates were calculated by normalizing mutant mutation rates to the respective wild-type rate in that particular experiment, which is indicated by the indices 1 and 2. The errors indicated are the quotients of the standard deviations of the number of mutations (LEA and COULSON 1948) and total cell numbers.

double mutants with mutants from NHEJ (*yku70*, *yku80*, *lig4*) and from TC-NER (*rad26*, *rad28*), which seem to be approximately additive. The mutation rate was well below additivity only for the *rad28 mph1* double mutant. For all the single mutants mentioned above—with the exception of *lig4*—we found a reduced spontaneous mutation rate. This may indicate that *rad26* and *rad28* are also involved in translesion synthesis, but we have not yet followed this observation any further. The *mag1 mph1* and *mgs1 mph1* double mutants were slightly synergistic.

The number of mutations in *mph1* mutants is dependent on the amount of DNA damage: The probably synergistic mutator phenotype of the DNA repair mutants *mag1* and *rad14* (SCHELLER *et al.* 2000) with *mph1* could indicate that in *mph1* mutants the increased amount of DNA damage caused by the respective repair defect is preferentially channeled into translesion synthesis, whereas in *MPH1* cells it is processed in an error-free manner. Analysis of mutants in *APN1*, encoding the major AP endonuclease in yeast (POPOFF *et al.* 1990), supported this hypothesis. As can be seen in Table 3, the *apn1 mph1* double mutant displayed a considerable synergistic phenotype with respect to the spontaneous mutation rate. That the increase in spontaneous mutations in the double mutant is actually due to increased TLS is demonstrated by its dependence on *REV3* (Table 3). Furthermore, complementation of the phenotype by a plasmid-borne *MPH1* (data not shown) indicates that the effect is due to the deletion of the *MPH1* gene

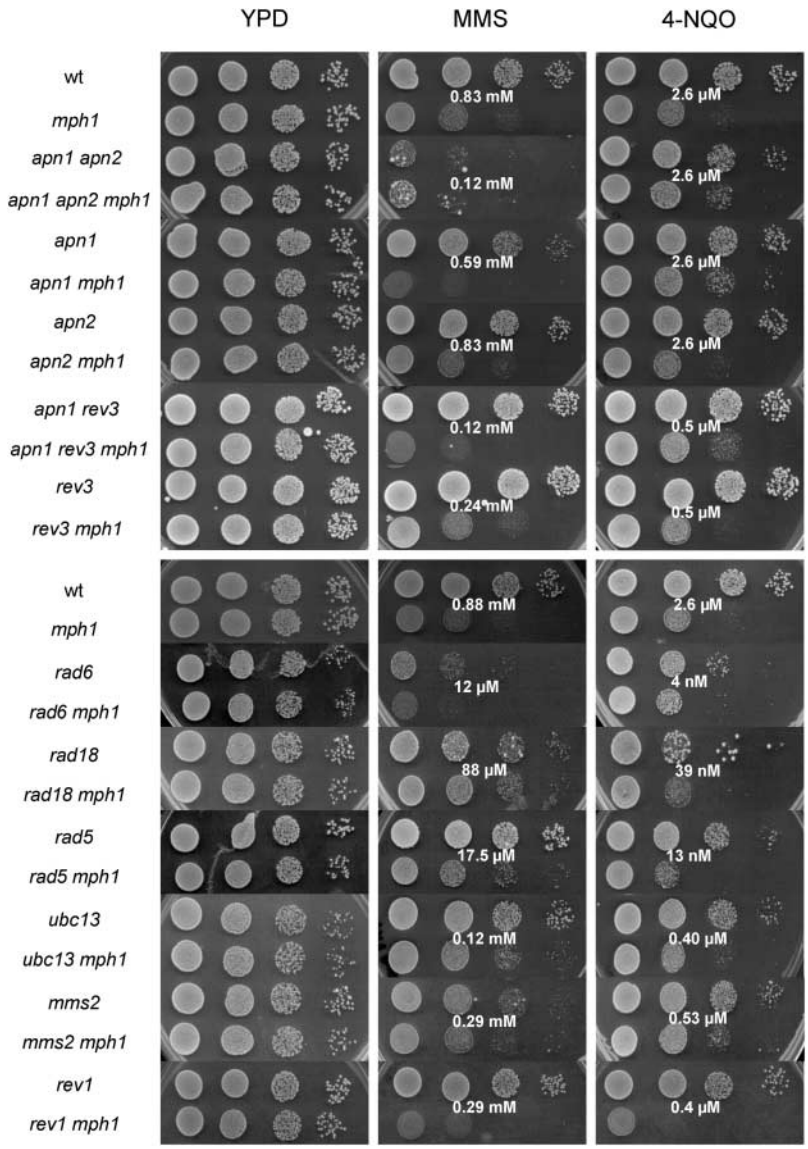

FIGURE 2.—Influence of an *mph1* mutation on the sensitivity of various *apn1*, *apn2*, and PRR mutants to MMS and 4-NQO. Drop dilution assays were carried out as described in MATERIALS AND METHODS. The concentration of the chemical in the plate for each pair of strains (without and with the *mph1* mutation) is given between the spots of the respective pair (1 mM MMS corresponds to 0.0085% and 1 μM 4-NQO to 0.19 μg/ml).

and not, *e.g.*, to some unwanted background mutation that might have occurred during strain construction.

A prediction from the hypothesis stated above would be that an additional deletion of *APN2*, also encoding an AP endonuclease, would further increase the synergistic effect, since even more unrepaired AP sites can be expected (JOHNSON *et al.* 1998). We therefore constructed several mutants with different combinations of *apn1*, *apn2*, and *mph1* and determined the spontaneous mutation rates to canavanine resistance as shown in Table 3. Whereas *apn2* alone does not exert any effect on the mutator phenotype of *mph1* mutants, the triple mutant *apn1 apn2 mph1* has by far the strongest mutator phenotype. Thus, apparently the increase of AP sites in the *apn1 apn2* double mutant results in a strong increase of mutagenic bypass in the absence of *MPH1*. One possible explanation for these effects would be that *MPH1* is an accessory factor to BER. This, however, is unlikely, since synergy was also observed with *rad14* defective in NER (SCHELLER *et al.* 2000) The conclusion is further supported by the analysis of the sensitivity to the DNA-damaging agents MMS and 4-NQO as shown in Figure 2. MMS is a methylating agent that produces primarily N7-methylguanine followed by N3-methyladenine (PEGG 1984), whereas 4-NQO predominantly forms aminoquinoline 1-oxide adducts with N2 and C8 of guanine and N6 of adenine (TURESKY 1994). First, the sensitivity of *apn1* and *mph1* to MMS is apparently synergistic. Second, whereas *apn1* and *apn2* are, as expected, not sensitive to 4-NQO, introduction of an additional *mph1* mutation confers a 4-NQO sensitivity that is comparable to that of the *mph1* single mutant.

From the lack of a spontaneous mutator phenotype of the various AP endonuclease mutants (Table 3) one may conclude that almost all spontaneously occurring AP sites can be processed by an *MPH1*-dependent error-free pathway. This seems also to be true for lesions that are subject to processing by Mag1 (Table 2), but to a

TABLE 4

Influence of mutations involved in PRR on the forward mutation rate of *mph1* mutants to canavanine resistance

Strain	Mutation rate × 10^7	Relative rate	Strain	Mutation rate × 10^7	Relative rate
Wild type	2.4 ± 0.39[1]	1	*rad5*	5.1 ± 0.89[1]	2.1
	3.8 ± 0.53[2]			9.8 ± 1.2[2]	2.6
	3.0 ± 0.41[3]			5.1 ± 0.77[3]	1.7
mph1	27.0 ± 3.3[1]	11.3	*rad5 mph1*	10.8 ± 1.6[1]	4.5
	35.1 ± 4.2[2]	9.2		17.6 ± 2.1[2]	4.6
	21.8 ± 2.4[3]	7.3		10.6 ± 1.3[3]	3.5
rad6	7.1 ± 1.1[1]	3.0	*ubc13*	14.8 ± 1.8[1]	6.2
rad6 mph1	2.6 ± 0.45[1]	1.1		15.9 ± 1.8[2]	4.2
rad18	11.8 ± 1.7[1]	4.9	*ubc13 mph1*	21.4 ± 2.6[1]	8.9
rad18 mph1	6.7 ± 0.95[1]	2.8		35.8 ± 3.9[2]	9.4
rev1	1.1 ± 0.27[3]	0.37	*mms2*	16.1 ± 2.2[1]	6.7
rev1 mph1	6.4 ± 0.81[3]	2.1	*mms2 mph1*	37.6 ± 4.4[1]	15.7

Relative rates were calculated by normalizing mutant mutation rates to the wild-type rate determined in that particular experiment, which is indicated by the indices 1–3. The errors indicated are the quotients of the standard deviations of the number of mutations (Lea and Coulson 1948) and total cell numbers.

lesser extent for lesions subject to NER, as indicated by the weak mutator phenotype of the *rad14* single mutant (Scheller *et al.* 2000).

The phenotypes of the mutants described above suggest a correlation between the number of DNA lesions and the number of mutations arising in *mph1* mutants. To test this more directly, we measured the dose response curves for induced mutations *vs.* concentration of 4-NQO. The cells were arrested in G_1 with α-factor and incubated after release of the arrest with different low concentrations of 4-NQO until the cells reached G_2. In this way we assured that the cells had completed one S-phase in the presence of the mutagen. The results are shown in Figure 1. Although the number of induced mutations varied considerably between single experiments, we generally found that the increase in the number of canavanine-resistant mutants with increasing mutagen concentrations was more pronounced in *mph1* cells than in wild type. The *REV3* dependence of induced mutations in both wild type and the *mph1* mutant demonstrates that the increased number of induced mutations in *mph1* mutants is due to translesion synthesis.

Interactions with mutants from postreplicative repair: PRR has long been discussed to be required for lesion bypass during replication. We therefore decided to analyze interactions with mutants from postreplicative repair in more detail. The results are shown in Table 4. *rad6* and *rad18* mutations virtually abolished the mutator phenotype of *mph1* mutants. Since both genes are required for *REV3*-dependent TLS (Xiao *et al.* 2000), a phenotype similar to a *rev3* deletion had to be expected. Surprisingly, however, a similar effect was observed for a *rad5* mutation. Although *RAD5* has alternatively been described as *REV2* (Lawrence and Christensen 1978), a gene involved in UV mutagenesis, later investigations placed it into the error-free pathway since no significant influence of a *rad5* mutation on most damage-induced mutations could be detected (Johnson *et al.* 1992). The Rad5 protein has been demonstrated to interact with the Mms2/Ubc13 dimer (Ulrich and Jentsch 2000; Ulrich 2003), which is a ubiquitin-conjugating enzyme assembling unusual K63-linked polyubiquitin chains (Hofmann and Pickart 1999) and is involved in error-free PRR (Broomfield *et al.* 1998; Hofmann and Pickart 1999). Whereas the effects of *mms2* and *mph1* are somewhat additive, as observed before (Scheller *et al.* 2000), *ubc13* was hypostatic to *mph1* with respect to mutator phenotype.

REV1 encodes a dCMP transferase, which has been implicated in the bypass of AP sites by both biochemical and genetic analysis (Nelson *et al.* 1996a, 2000). In another study, however, evidence was presented that Rev1 plays only a minor role in bypass of abasic sites (Haracska *et al.* 2001). Although the role of Rev1 for AP bypass is still debated, it seems clear that Rev1 has a more general function in the bypass of lesions (Bayntон *et al.* 1999; Nelson *et al.* 2000; Haracska *et al.* 2001). In our analysis, *rev1* had a phenotype similar to that of *rev3* (Scheller *et al.* 2000). The spontaneous mutation rate of a *rev1* mutant is lower than that of wild type, and an additional *rev1* mutation reduces the mutator phenotype of *mph1* to twice the wild-type level (Table 4). The strong synergism of sensitivity to DNA-damaging agents as shown in Figure 2 is similar to that observed for *rev3* (Figure 2; Scheller *et al.* 2000). From the synergistic interactions of the mutator phenotype of *mph1 apn1* and *mph1 apn1 apn2* mutants we conclude that lesions normally processed by an *MPH1*-dependent error-free pathway are channeled into translesion synthesis. The sensitivities of *rev1 mph1* and *rev3 mph1* double mutants indicate that additional blockage of TLS results in a significant increase of cell death in the presence of DNA damage, which is also supported by the

TABLE 5

Influence of mutants from homologous recombination on the *mph1* forward mutator phenotype to canavanine resistance

Strain	Mutation rate × 10^7	Relative rate	Strain	Mutation rate × 10^7	Relative rate
Wild type	3.0 ± 0.43[1]	1	*mph1*	25.5 ± 2.9[1]	8.5
	4.2 ± 0.56[2]			27.8 ± 3.0[2]	6.6
	4.0 ± 0.60[3]			21.2 ± 2.7[3]	5.3
	2.9 ± 0.44[4]			30.1 ± 3.6[4]	10.4
	3.6 ± 0.50[5]			24.4 ± 2.7[5]	6.7
	4.2 ± 0.57[6]			29.5 ± 3.3[6]	7.0
	1.0 ± 0.17[7]			4.6 ± 0.62[7]	4.6
rad51	28.3 ± 3.3[1]	9.4	*rad52*	30.8 ± 4.1[3]	7.7
	41.6 ± 4.5[2]	9.9		44.3 ± 5.5[4]	15.3
rad51 mph1	30.8 ± 3.6[1]	10.3		36.3 ± 4.2[5]	10.1
	37.4 ± 4.1[2]	8.9		37.6 ± 4.4[6]	8.9
rad55	30.5 ± 3.4[3]	7.6		5.5 ± 0.80[7]	5.5
rad55 mph1	30.9 ± 3.5[3]	7.7	*rad52 mph1*	18.4 ± 2.5[3]	4.6
				37.9 ± 4.7[4]	13.1
				34.2 ± 4.1[5]	9.5
				44.4 ± 5.2[6]	10.5
				7.2 ± 1.0[7]	7.2

Relative rates were calculated by normalizing mutant mutation rates to the wild-type rate determined in that particular experiment, which is indicated by the indices 1–7. The errors indicated are the quotients of the standard deviations of the number of mutations (Lea and Coulson 1948) and total cell numbers.

increase in sensitivity to MMS, but not to 4-NQO, that is conferred by an additional *apn1* mutation in an *mph1 rev3* background.

In summary, the analysis of the mutator phenotypes shows (sometimes incomplete) epistasis of *rad6*, *rad18*, *rev3*, *rev1*, and *rad5* to *mph1*. These epistatic relationships, however, do not pertain to the sensitivity to DNA-damaging agents. As shown in Figure 2, all the double mutants of *mph1* with mutants in PRR are considerably more sensitive to DNA damage than the respective single mutants. Therefore, this analysis shows that *MPH1* is not a member of the *RAD6* epistasis group with respect to DNA damage sensitivity.

Interaction with homologous recombination: We also analyzed the interaction of *mph1* with *rad51*, *rad52*, and *rad55* mutants affected in HR. The respective forward mutation rates to canavanine resistance are shown in Table 5. As can be seen, we found epistatic relationships for *rad51* and *rad55*. While we had reported an additive relationship for *rad52* in our previous analysis (Scheller *et al.* 2000), we now observed a mostly epistatic interaction of *rad52* to *mph1* in several repetitions of the experiment. Nevertheless, there was some variation in the relationship, with the mutation rate of the double mutant sometimes being slightly higher or lower than that of the *rad52* single mutant. At present, we cannot decide whether this is due to statistical fluctuations or to subtle experimental differences.

The epistasis of mutants from homologous recombination to *mph1* was also found for sensitivities to DNA-damaging agents as shown in Figure 3. The double mutants of *mph1* with *rad51*, *rad52*, and *rad55* display almost the same sensitivity as the respective *rad* single mutants.

Mitotic recombination rates: From these epistatic interactions one might suspect that Mph1 is an accessory factor to homologous recombination. We previously observed that spore survival coming from homozygous *mph1* diploids is not reduced (Scheller *et al.* 2000), arguing against an involvement in meiotic recombination. To test the effect of *mph1* on mitotic recombination, we determined spontaneous mitotic recombination rates for wild-type and homozygous *mph1* diploids. As shown in Table 6, the recombination rates for heteroallelic markers in *mph1* mutants were not reduced compared to wild type. The *mph1* mutant instead showed, if at all, a slightly increased recombination rate. In an *sgs1* background, however, *mph1* clearly conferred a hyperrecombination phenotype. *sgs1* mutants have been reported previously to exhibit a spontaneous hyperrecombination phenotype by themselves (Watt *et al.* 1996; Myung *et al.* 2001). The *sgs1 mph1* double mutant displayed a further strong increase in recombination rates. We therefore conclude that *mph1* mutants are not deficient in homologous recombination. (Circumstantial evidence for this is also provided by our nonquantitative observation that it is no more difficult to construct mutations by one-step gene disruption in an *mph1* background than in wild type.) On the contrary, in the system under investigation Mph1 instead may exert an antirecombinogenic effect, at least in an *sgs1* background.

Interaction among *mph1*, *rad5*, and *rad51*: The epi-

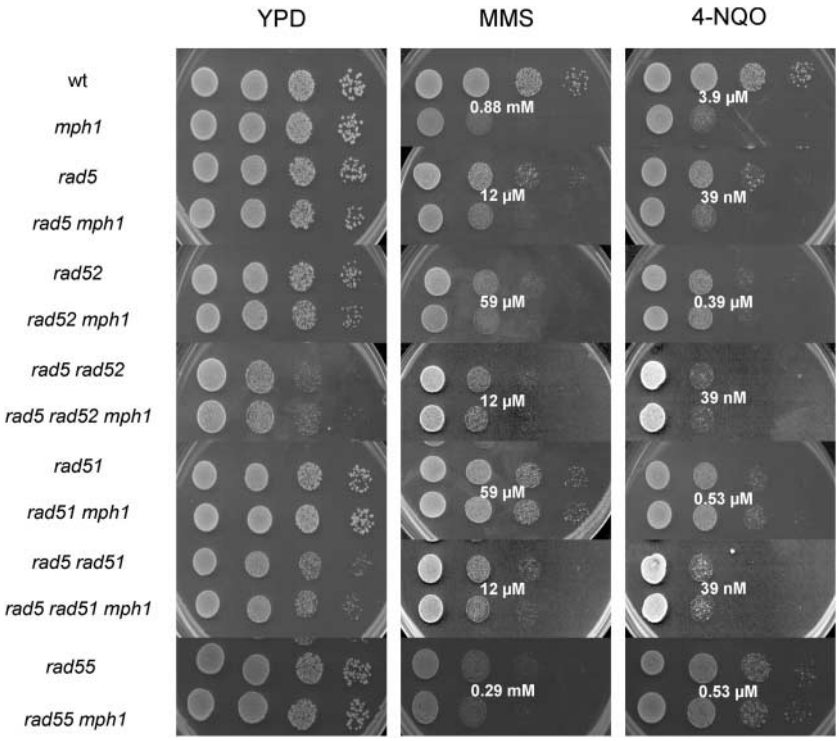

FIGURE 3.—Influence of an *mph1* mutation on the sensitivity of mutants from homologous recombination to MMS and 4-NQO. Drop dilution assays were carried out as described in MATERIALS AND METHODS. The concentration of the chemical in the plate for each pair of strains (without and with *mph1* mutation) is given between the spots of the respective pair (1 mM MMS corresponds to 0.0085% and 1 μM 4-NQO to 0.19 μg/ml).

static interactions observed with mutants from HR and *rad5* prompted us to analyze the genetic interactions between these genes. The forward mutation rates to canavanine resistance are shown in Table 7. *rad5* is epistatic not only to the mutator phenotype of *mph1* but also to that of *rad51*. The *rad5 rad51 mph1* triple mutant has a phenotype similar to that of the *rad5 rad51* double mutant. Therefore, the epistasis of *rad51* to *mph1* is also maintained in the absence of Rad5, which is also true for sensitivity to DNA-damaging agents, as shown in Figure 3. But again, as already observed for *mph1* (see Figure 2), the epistasis of *rad5* to *rad51* (and *rad52*) does not apply to DNA damage sensitivity, at least not to 4-NQO, which is in accord with the findings for UV sensitivity of *rad5 rad52* mutants (JOHNSON *et al.* 1992; ULRICH 2001). For sensitivity to MMS, *rad5* is not epistatic to *mph1* but possibly to *rad51* and *rad52*. This may mean that Mph1 acts after the enzymes of homologous recombination for MMS-induced lesions.

DISCUSSION

In the present study we have analyzed the genetic interactions of *mph1* mutants with mutants from BER and NER, from PRR, and from HR. The phenotypes analyzed were spontaneous mutation rates and sensitivity to MMS and 4-NQO.

The following genetic interactions seem particularly relevant to the better understanding of the cellular role of Mph1:

1. *mph1* was synergistic with respect to spontaneous mutator phenotype with the NER mutant *rad14* (SCHELLER *et al.* 2000) and the BER mutants *mag1* and *apn1*. The synergistic effect was most pronounced for *apn1 apn2* double mutants, where both known AP endonucleases of yeast are inactivated. For these mutants, the sensitivity to MMS (but not to 4-NQO) also was synergistic.
2. From PRR, *rad6*, *rad18*, *rad5*, *rev3*, and *rev1* were (at least partially) epistatic to *mph1* for the spontaneous mutator phenotype, but not for DNA damage sensitivity.
3. *rad51*, *rad52*, and *rad55* from HR were epistatic to *mph1* with respect to both spontaneous mutation rates and DNA damage sensitivity.
4. The DNA damage sensitivities of *rev3 mph1* and *rev1 mph1* double mutants were synergistic, as has been reported for *rad51 rev3* double mutants before (RATTRAY *et al.* 2002).
5. A *rad5* deletion, which partially suppressed the *mph1* mutator phenotype, had a very similiar effect on *rad51* mutants and the *rad5 rad51* double mutation was epistatic to *mph1*.
6. *mph1* mutants had slightly increased mitotic heteroallelic recombination rates, which were synergistic with *sgs1*.

Several conclusions can be drawn from these genetic interactions:

1. In the absence of Mph1, DNA lesions that are normally processed by Mph1 are channeled mainly into TLS. The spontaneous mutator phenotype of *mph1* mutants is due exclusively to TLS, since it is (almost)

TABLE 6

Mitotic recombination rates in wild-type and homozygous *mph1* and *sgs1* diploids

Marker	Diploid	Recombination rate × 10^7	Relative rate
his7	Wild type	1.9 ± 0.31	1
	mph1	2.2 ± 0.35	1.2
	sgs1	36.2 ± 4.6	19.1
	sgs1 mph1	67.0 ± 7.9	35.3
lys2	Wild type	9.7 ± 1.3	1
	mph1	17.6 ± 2.2	1.8
	sgs1	58.3 ± 6.1	6.0
	sgs1 mph1	133 ± 14	13.7
met13	Wild type	15.9 ± 1.9	1
	mph1	19.9 ± 2.4	1.3
	sgs1	108 ± 12	6.8
	sgs1 mph1	189 ± 20	11.9

Factors were calculated by normalizing mutant recombination rates to the wild-type rate. Haploid strains for construction of diploids were derivatives of NLBL1 and NLBL3. The errors indicated are the quotients of the standard deviations of the number of recombination events (equivalent to the number of mutations as described in Lea and Coulson 1948) and total cell numbers.

TABLE 7

Interaction of *rad5*, *rad51*, and *mph1* with respect to the forward mutator phenotype to canavanine resistance

Strain	Mutation rate × 10^7	Relative rate
Wild type	3.0 ± 0.43	1
mph1	25.5 ± 2.9	8.5
rad51	28.3 ± 3.3	9.4
rad51 mph1	30.8 ± 3.6	10.3
rad5	7.4 ± 0.99	2.5
rad5 mph1	11.5 ± 1.5	3.8
rad5 rad51	8.8 ± 1.3	2.9
rad5 rad51 mph1	6.1 ± 1.1	2.0

Relative rates were calculated by normalizing mutant mutation rates to the wild-type rate. The errors indicated are the quotients of the standard deviations of the number of mutations (Lea and Coulson 1948) and total cell numbers.

completely dependent on *REV3* and *REV1*. AP sites are processed predominantly by Mph1, since the synergism for both the spontaneous mutator phenotype and the sensitivity to MMS is very pronounced in *apn1* and *apn1 apn2* mutants and since these mutants do not exhibit a spontaneous mutator phenotype in the presence of *MPH1*. The synergistic MMS sensitivity also strongly suggests that Mph1 is not an accessory factor to BER. The most obvious conclusion from these observations is an involvement of Mph1 in error-free bypass of lesions.

2. *MPH1* does not belong to the PRR pathway, since all mutants tested (*rad6*, *rad18*, *rad5*, *mms2*, *ubc13*, *rev3*, and *rev1*) become more sensitive to DNA damage, if *MPH1* is deleted. The suppression of the (TLS-dependent) *mph1* mutator phenotype by *rad6*, *rad18*, *rev3*, and *rev1* can be ascribed to the TLS defect generated by these mutations. The partial suppression by *rad5*, however, is surprising, since *RAD5* belongs to the error-free branch of PRR.
3. The epistasis of mutations from HR to *mph1* shows that error-free bypass involving Mph1 requires HR functions. Also the similiarity in DNA damage sensitivity of *rad51 rev3* (Rattray *et al.* 2002) and *mph1 rev3* supports this conclusion.
4. Since *mph1* mutants are proficient in mitotic recombination, it can be concluded that Mph1 functions specifically in a branch of HR responsible for error-free bypass of DNA lesions, but not for general recombination. We suggest the term *MPH1*-HR for this pathway.

Relation of error-free PRR and *MPH1*-mediated error-free bypass: A central enzyme in error-free PRR is Rad5. *RAD5* belongs to the *RAD6* epistasis group and has been assigned to the error-free branch of PRR, since *rad5* mutants are not generally defective in UV-induced mutagenesis (Johnson *et al.* 1992). However, UV-induced reversion of several *ochre* alleles is markedly reduced in *rad5* mutants in an apparently allele-specific manner (Lawrence and Christensen 1978; Johnson *et al.* 1992). Rad5 possesses an ATPase activity that is stimulated by single-stranded DNA (Johnson *et al.* 1994) and a RING finger domain that is required for interaction with Ubc13 (Ulrich and Jentsch 2000; Ulrich 2003), which is a ubiquitin-conjugating enzyme that, in cooperation with Mms2, assembles unusual K63-linked polyubiquitin chains (Hofmann and Pickart 1999). Rad5 also interacts with Rad18, a protein with single-strand DNA-binding activity that recruits Rad6 to DNA (Bailly *et al.* 1994). These interactions allow a multimeric complex containing Rad6, Rad18, Rad5, Ubc13, and Mms2 to be formed (Ulrich and Jentsch 2000). Rad6, which is also a ubiquitin-conjugating enzyme (Jentsch *et al.* 1987), can monoubiquitinate PCNA at K164 (Hoege *et al.* 2002), which is a mandatory prerequisite for translesion synthesis to occur (Stelter and Ulrich 2003). The monoubiquitinated PCNA can be decorated by action of Rad5 and the Mms2/Ubc13 heterodimer with K63-linked polyubiquitin chains (Hoege *et al.* 2002), which, on the basis of the phenotypes of *mms2* and *ubc13* mutants, are thought to be required for error-free PRR. Both *MMS2* and *UBC13* have been genetically assigned to the error-free branch of PRR (Broomfield *et al.* 1998; Brusky *et al.* 2000). Interestingly, although *mms2* and *ubc13* were found to be epistatic to each other (Hofmann and Pickart 1999; Brusky *et al.* 2000), the phe-

notypes in combination with *rad6* were found to differ in one instance: Whereas *mms2* was hypostatic to *rad6* (Broomfield *et al.* 1998), *ubc13* was found to slightly suppress the UV and MMS sensitivity of *rad6* in one particular study (Brusky *et al.* 2000). We found that *mph1* and *mms2* were (sub)additive with respect to spontaneous mutation rates, whereas *ubc13* was hypostatic to *mph1*. Therefore, *ubc13* and *mms2* mutations may not be completely functionally equivalent, as one would have expected on the basis of the finding that the heterodimer is necessary for assembly of K63-linked polyubiquitin chains (Hofmann and Pickart 1999).

The additive UV sensitivity of *mms2* and *rad4* from NER (Broomfield *et al.* 1998) suggests that Mms2 is not involved in DNA repair but rather, like Mph1, in error-free bypass of lesions. The synergistic DNA damage sensitivity of both *mms2* and *ubc13* with *rev3* (Broomfield *et al.* 1998; Brusky *et al.* 2000; Xiao *et al.* 2000) also suggests that the major "rescue" pathway in case of failure is TLS, similar to the bypass involving Mph1 and homologous recombination. The *MMS2/UBC13* bypass seems to work independently from the *MPH1*-HR bypass, since the UV sensitivity of *mms2* and *ubc13* is approximately additive with that of *rad52* (Ulrich 2001). In cases where the *MMS2/UBC13* pathway is to be used, the *MPH1*-HR pathway might be suppressed by *SRS2*, which is very reasonable to assume in light of the ability of Srs2 to disrupt Rad51 filaments (Krejci *et al.* 2003; Veaute *et al.* 2003). This would explain the suppression of the spontaneous mutator phenotype of *mms2* and the MMS sensitivity of *mms2 rev3* by *srs2* (Broomfield and Xiao 2002) as well as the specificity of the suppression for the error-free branch of PRR (Ulrich 2001). This idea has been expressed previously in a less explicit manner to explain the dependence of the suppression of *rad6* sensitivity by *srs2* on homologous recombination (Schiestl *et al.* 1990). According to this line of reasoning, however, it follows from the mutator phenotypes of *mph1*, *rad51*, *rad52*, and *rad55* mutants that the *MPH1*-HR pathway is not always silenced by Srs2. At present it is unclear which conditions may lead to use of either one or the other pathway. Judging from the strong synergism of *mph1* with *apn1 apn2* in comparision with the other repair mutants (*mag1*, *rad14*), one might suspect that the nature of the lesion could play a role in this decision process.

The conclusion that can be drawn from these notions is that error-free PRR and *MPH1*-HR probably act in parallel, but not in intimately connected pathways. This leaves open the question of why a *rad5* mutation partially suppresses the mutator phenotype of *mph1* and *rad51*. Formally, two obvious possibilities can explain this phenomenon: Either an alternative error-free pathway is available in the absence of Rad5, whose operation would eliminate the necessity for TLS, or TLS is not fully active in the absence of Rad5. A possible candidate for an alternative, Rad5-independent error-free pathway is the damage-tolerant polymerase η, encoded by *RAD30*, which can bypass a number of lesions in a relatively accurate manner (Johnson *et al.* 1999; Haracska *et al.* 2000a,b; Washington *et al.* 2000; Minko *et al.* 2003). In fact, simultaneous deletion of *rad5* and *rad30* leads to a strong synergistic increase in the number of damage-induced mutations (McDonald *et al.* 1997), suggesting that in the absence of error-free PRR Rad30 counteracts the mutagenic effects of Rev3-dependent TLS. However, several observations also support a stimulatory contribution of Rad5 to TLS. On the basis of the *REV3*-dependent spontaneous mutator phenotype of *rad5* (Čejka *et al.* 2001) it is clear that Rad5 is not essential for TLS. This mutator phenotype, however, is considerably weaker than that of *mms2* and *ubc13*, which would be unexpected if the sole function of Rad5 was to act as a ubiquitin protein ligase for Mms2/Ubc13, but could be explained by a Ubc13- and Mms2-independent stimulatory effect of Rad5 on Rev3. In *mph1* or *rad51* mutants, this same effect would also be responsible for mutagenic repair of lesions normally processed in an error-free manner by the *MPH1*-HR pathway, thus explaining the partial suppression of the *mph1* and *rad51* mutator phenotypes by the *rad5* deletion. In addition, the proposed stimulation of TLS by Rad5 might also explain the reduction of UV-induced reversion rates for several *ochre* alleles in a *rad5* background (Lawrence and Christensen 1978; Johnson *et al.* 1992).

The stimulatory effect of Rad5 on TLS appears to contradict previous findings that the function of Rad5 in error-free PRR is to promote the multiubiquitination of PCNA (Hoege *et al.* 2002), while TLS requires PCNA monoubiquitination (Stelter and Ulrich 2003). However, our reasoning can perhaps be reconciled with this model on the basis of the fact that not only monoubiquitination, but also SUMO modification of PCNA stimulates Rev3-dependent spontaneous mutagenesis (Stelter and Ulrich 2003). Given that Rad5 directly interacts with the SUMO conjugating enzyme Ubc9 (Hoege *et al.* 2002), it is not unreasonable to expect Rad5 to exert a regulatory effect on the SUMO modification of PCNA, possibly by recruiting the modifying enzyme to a stalled replication fork. Assuming that direct interactions between Rad5 and the SUMO conjugation system indeed affect the activity of Rev3, inactivation of individual domains within the Rad5 protein would naturally have distinguishable consequences. Even the differential effects of Ubc13, which directly interacts with Rad5 by means of the Rad5 RING domain (Ulrich 2003), and of Mms2, whose contact to Rad5 is only indirectly mediated by means of Ubc13 (Ulrich and Jentsch 2000), would be expected, as Ubc13 could still associate with and affect Rad5 in the absence of Mms2, but not vice versa. Definitive conclusions about the influence of Rad5 on TLS, however, will have to await a molecular analysis of the protein and its various

interactions with other factors of the ubiquitin and SUMO conjugation system.

Possible function of Mph1 in *MPH1*-HR bypass: Speculations about the role of Mph1 in the *MPH1*-HR pathway have to take into account that *mph1* mutants probably display a slight hyperrecombination phenotype for mitotic heteroallelic recombination, which is clearly apparent in an *sgs1* background. This largely excludes the possibility that Mph1 is a general accessory factor for homologous recombination, since in this case a mitotic hyporecombination phenotype should be expected. It seems more likely that Mph1 functions specifically in a subbranch of HR that is dedicated to error-free bypass, which would most plausibly promote information transfer from a sister chromatid. Disruption of such a pathway may not affect interhomolog recombination in diploids or may actually increase it. The pronounced hyperrecombination phenotype of the *mph1* mutation in an *sgs1* background suggests that Sgs1 can prevent most of these events in *mph1* single mutants. We have observed that both the spontaneous deletion rate and the damage-induced deletion frequency of a direct duplication is reduced in a haploid *mph1* mutant (C. Rudolph, K. Schürer and W. Kramer, unpublished data), which is in line with the hypothesis of *MPH1* affecting recombination between sister chromatids. However, from the present data we are not able to clearly define a function for Mph1. It may also direct the processing of an intermediate created by proteins from homologous recombination into a productive form in terms of error-free bypass, whereas in its absence this intermediate may be processed to an unproductive form that may also lead—at least in the absence of Sgs1—to the formation of mitotic recombinants in diploids.

We thank Birgit Zeike for excellent technical assistance and R. A. Bennett, H. M. Feldmann, P. E. Gibbs, I. D. Hickson, S. P. Jackson, S. Lovett, K. Ohta, R. M. Ramirez, L. Symington, and W. Xiao for the generous gift of strains and plasmids. C.R. was supported by the Studienstiftung des Deutschen Volkes. This work was supported in part by the European Community's Human Potential Programme under contract HPRN-CT-2002-00240, Genome Stability and Checkpoint Control.

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Communicating editor: L. S. Symington