A Yeast Homologue of the Human Phosphotyrosyl Phosphatase Activator PTPA Is Implicated in Protection against Oxidative DNA Damage Induced by the Model Carcinogen 4-Nitroquinoline 1-Oxide*

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Dindial Ramotar‡, Edith Belanger, Isabelle Brodeur, Jean-Yves Masson, and Elliot A. Drobetsky

From the Hôpital Maisonneuve-Rosemont, Centre de Recherche, Université de Montréal, Montréal, Québec H3T 2M4, Canada

The model carcinogen 4-nitroquinoline 1-oxide (4-NQO) has historically been characterized as “UV-mimetic” with respect to its genotoxic properties. However, recent evidence indicates that 4-NQO, unlike 254-nm UV light, may exert significant cytotoxic and/or mutagenic potential via the generation of reactive oxygen species. To elucidate the response of eukaryotic cells to 4-NQO-induced oxidative stress, we isolated Saccharomyces cerevisiae mutants exhibiting hypersensitivity to the cytotoxic effects of this mutagen. One such mutant, EBY1, was cross-sensitive to the oxidative agents UVA and dianide while retaining parental sensitivities to 254-nm UV light, methyl methanesulfonate, and ionizing radiation. A complementing gene (designated yPTPA1), restoring full UVA and 4-NQO resistance to EBY1 and encoding a protein that shares 40% identity with the human phosphotyrosyl phosphatase activator hPTPA, has been isolated. Targeted deletion of yPTPA1 in wild type yeast engendered the identical pattern of mutagen hypersensitivity as that manifested by EBY1, in addition to a spontaneous mutator phenotype that was markedly enhanced upon exposure to either UVA or 4-NQO but not to 254-nm UV or methyl methanesulfonate. Moreover, the yptpA1 deletion mutant exhibited a marked deficiency in the recovery of high molecular weight DNA following 4-NQO exposure, revealing a defect at the level of DNA repair. These data (i) strongly support a role for active oxygen intermediates in determining the genotoxic outcome of 4-NQO exposure and (ii) suggest a novel mechanism in yeast involving yPtPA1p-mediated activation of a phosphatase that participates in the repair of oxidative DNA damage, implying that hPTPA may exert a similar function in humans.

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‡ To whom correspondence should be addressed: Hôpital Maisonneuve-Rosemont, Centre de Recherche, Université de Montréal, 5415 Boul. de l’Assomption, Montréal, PQ H1T 2M4, Canada. Tel.: 514-252-3400, Ext. 4884; Fax: 514-252-3430; E-mail: dramota@hmr.qc.ca.

1 The abbreviations used are: 4-NQO, 4-nitroquinoline 1-oxide; NER, nucleotide excision repair; AP, apurinic; MMS, methyl methanesulfonate; kb, kilobase(s).

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stable quinoline-purine monoadducts, i.e. at the exocyclic N-2 and N-6 positions of guanine and adenine, respectively (1, 2). In bacteria, yeast, and mammalian cells, these genotoxic “bulky” DNA lesions are processed largely by the nucleotide excision repair (NER) pathway in a manner analogous to classical dipyrimidine photoproducts (viz. cyclobutane pyrimidine dimers and (6–4) pyrimidine-pyrimidone photoproducts) generated by the model DNA-damaging agent 254-nm UV light (3, 4). As such, mutants that are deficient in NER are hypersensitive to the genotoxic effects of 254-nm UV light, as well as 4-NQO (3–6). The apparent strong similarity in modes of cellular processing for 254-nm UV light- and 4-NQO-induced DNA damage in diverse prokaryotic and eukaryotic systems has often resulted in categorization of the latter agent as “UV-mimetic” (7).

However, this designation may be inappropriate, because several recent investigations have clearly demonstrated that 4-NQO, unlike 254-nm UV light, can generate a substantial degree of intracellular oxidative stress. This may herald significant consequences for 4-NQO-exposed cells at the level of cell killing and mutagenesis. Indeed, it was shown that 4-NQO is a potent inducer of the Escherichia coli soxR/S regulon that responds to intracellular superoxide imbalances (8). It is believed that 4-NQO undergoes redox cycling to produce superoxide anion, which can be further converted into genotoxic reactive oxygen species (e.g. singlet oxygen and hydroxyl radicals) that engender modified bases and DNA strand breaks (8, 9). In fact, it has been shown in vitro that activated 4-hydroxyaminoquinoline 1-oxide can generate the highly mutagenic product 8-oxo-guanine, as well as strand breaks, in the presence of Cu(II) (9, 10). In E. coli, 8-oxoguanine is removed by the form-amidopyrimidine-DNA glycosylase (Fpg), which in turn generates apurinic (AP) sites as secondary lesions (11). AP sites are also highly mutagenic but can be efficiently removed from cellular DNA by AP endonucleases (12, 13). It is therefore not surprising that bacterial mutants lacking either Fpg or AP endonucleases are hypersensitive to the mutagenic effects of 4-NQO (but not of 254-nm UV light) (8, 14).

Based on the demonstrated ability of 4-NQO to induce oxidative stress that contributes to cell killing and mutagenesis in bacteria, we postulated that this agent may act similarly in eukaryotic cells. As such, using the model organism, Saccharomyces cerevisiae, the aim of the present investigation was to identify novel eukaryotic genes implicated in the cellular response to 4-NQO-induced oxidative DNA damage. Our strategy involved, sequentially (i) isolation of a large panel of yeast mutants exhibiting hypersensitivity to the cytotoxic effects of 4-NQO, (ii) categorical preclusion from further analysis of any mutants showing full cross-sensitivity to 254-nm UV light,
because these are likely to carry mutations in previously identified DNA repair genes, e.g., comprising the NER pathway known to participate in the processing of UV-type dipyrimidine photoproducts, as well as classical 4-NQO-induced bulky DNA adducts, and (iii) appropriate characterization of any remaining mutants expressing putative defects specifically in the processing of 4-NQO-induced oxidative DNA damage. In this manner, we identified a novel 4-NQO-sensitive yeast mutant that retains parental resistance to 254-nm UV light, while displaying certain hallmarks consistent with a deficiency in the repair of oxidative DNA lesions. A gene complementing this defective phenotype was isolated from a yeast genomic library, and was shown to correspond to a previously identified, but functionally uncharacterized, yeast gene with a predicted amino acid sequence that manifests significant identity with the human phosphotyrosyl phosphatase activator hPTPA. Our data strongly support a genotoxic role for reactive oxygen species in 4-NQO-exposed yeast, and provide novel in vivo evidence for the participation of cellular phosphatases in the repair of oxidative DNA damage in eukaryotic cells.

MATERIALS AND METHODS

Strains, Media, Genetic Analysis, and Transformation—The wild type S. cerevisiae strains used in this study were DBY747 (MATa, leu2-3, 112, his3-Δ1, trp1-289a, ura3-52, laboratory stock), DBY747-1 (isogenic to DBY747, except trp1::hisG), W303a (MATa, ade2-1, his3-11, leu2-3, 112, trp1-1, ura3-1, provided by Dr. M. Stark, University of Dundee, UK), FY68 (MATa, his3-Δ200, ura3-52, leu2Δ1, provided by Dr. Fred Winston, Harvard University), and MKp-o (MATa, can1-1000, ade2-1, lys2-1, ura3-52, leu2-3, 112, his3Δ200, trp1::hisG, provided by Dr. Bernard Kunz, Deakin University, Australia). The mutant strain EBY1 is isogenic to DBY747, except that it bears a mutated copy of the yPTPA1 gene (see below). EBY2, EBY3, and EBY4 were derived, respectively, from DBY747-1, MKp-o, and W303a by deleting a portion of the yPTPA1 coding region and replacing it with the selective marker LEU2 (see below). Likewise, CHY26 and CHY27 were derived, respectively, from DBY747-1 and EBY2 by deleting a portion of the coding region of the yPTPA2 gene and replacing it with the selective marker TRP1 (see below). The yeast strains DEY10-2C (isogenic to W303a, except phh21Δ::URA5), DEY142-4D (isogenic to W303a, except MATa, URA3, and Trp1::hisG), DEY224-3D (isogenic to W303a, except phh21Δ::HIS3, phen2::HIS3) were all generously provided by Dr. M. Stark (University of Dundee, UK). Yeast cells were grown in either complete yeast peptone dextrose (YPD) or minimal synthetic (SC) medium, to which nutritional supplements were added at 20 μg/mL (15). Standard genetic analysis and transformation were carried out as described previously (16, 17). The E. coli strain used for plasmid maintenance was HB101.

Isolation of Mutants Hypersensitive to 4-NQO—Exponentially growing wild type yeast (strain DBY747) were treated with 0.1% MMS for 1 h, and surviving colonies were streaked onto YPD agar plates containing 0.4 μg/mL of 4-NQO. This drug concentration permits 90–95% growth of wild type, and strains that did not sustain growth at this drug concentration were scored as mutants.

Construction of the yptpa1 and yptpa2 Deletion Mutants—A 1.5-kb Smal/HindIII yeast DNA fragment bearing the yptpa1 coding region and flanking 5′- and 3′-untranslated regions was derived from pDR1022 (see below) and subcloned into the pBlueScript vector KS/ to produce the plasmid pTV3. This plasmid was digested with SalI/SalI to release a 1.25-kb fragment from within the coding region of the yptpa1 gene. The released fragment was subcloned with the S. cerevisiae LEU2 selective marker, which was obtained as a 2.5-kb SalI/SalI fragment from the yeast plasmid YEp13 (16), to produce plasmid pTV4. A linear 3.2-kb fragment, yptpa1Δ::LEU2, was obtained from pTV4 by digestion with HindIII and ScaI and transformed directly into the wild type haploid strains DBY747, FY68, and MKp-o using the lithium acetate procedure (17). Leu" transformants bearing chromosomal deletion of the yptpa1 gene were verified by Southern blot analysis (16), using a 12P-labeled 1.0-kb fragment of the yptpa1 coding region as probe. To construct a yptpa2 deletion mutant, the entire yptpa2 gene was first isolated from strain DBY747 by polymerase chain reaction amplification using the upstream (5′-GAGGAACTGGTCGAAGATGAAATC-3′) and downstream (5′-GGAAAAGTACGTACCGGACG-3′) primers containing the restriction sites HindIII and SmaI (18). These primers amplified a 1.9-kb HindIII/SmaI DNA fragment, which was then subcloned into pBluescript digested with HindIII and Smal to produce pCH1. pCH1 was in turn digested with EcoRI, which removes 160 nucleotides from the N-terminal end of the yptpa2 coding region, and a 1.2-kb EcoRI fragment containing the Tryp1 selective marker was inserted at the EcoRI site of the plasmid pCH2. Digestion of pCH2 with HindIII and BamHI produced the yptpa2Δ::TRP1 3.0-kb DNA fragment, which was transformed into strains DBY747 and EBY2. yptpa2 deletion mutants were confirmed by Southern blot analysis.

Survival Curves—The sensitivities of the various yeast strains to 4-NQO, diamide, and methyl methanesulfonate (all purchased from Sigma Chemical Co., St. Louis, MO) were assessed using exponential phase cultures. Overnight cultures grown to saturation at 30 °C in YPD were diluted into fresh medium at an A600 of 0.2 (–2 × 107 cells/mL) and incubated at an A600 of 1.0. Aliquots were then treated with various concentrations of drugs at 30 °C with shaking (250 rpm) for 1 h. Relative survival was determined by immediately diluting the samples in sterile 20 mM potassium phosphate buffer (pH 7.0) and plating onto YPD agar. Colonies were counted after 3–4 days of growth at 30 °C. In the case of γ-irradiation treatment, exponential cells were irradiated in cold YPD medium using a Co60 source at a dose rate of 55.5 rad/min (experiments carried out in the laboratory of Dr. Michael Resnick, NIEHS, NIH).

For exposure to defined regions of the UV wavelength spectrum, mid-log phase yeast were irradiated in sterile distilled water using a cold NUV-UVB-W lamps emitting either UV-A (GE model F2578-BL), UVB (Spectroline model XX25B), or 254-nm UV light (GE model G2578). Cells were treated at relatively low density (105 cells/mL in 35-mm Petri dishes) and with constant shaking to avoid cellular shielding. The incident light was rigorously purified using 2-mm-thick glass filters (Schott, Mainz, Germany) to virtually eliminate contaminating wavelengths below 290 nm in the case of UVB (filter WQ 305) and below 320 nm for UVA (filter WG 345). The incident UBV (λ > 290 nm), UV (λ > 320 nm), and 254-nm UV light dose rates were measured using a Spectroline DRC 100X digital radiometer equipped with DIX 300, DIX 365, and DIX 254 sensors, respectively.

Gradient Plate Assay—This assay was performed as described previously (19). Briefly, cells were replicated as a thin line along the drug gradient, and after 2 days of growth at 30 °C the distance of growth of each strain was expressed as a percentage of the wild type. Growth along the gradient is considered to be 100%.

Mutation Rate Assay—Spontaneous mutation rates were determined using a fluctuation test as described previously (20). The measurement of the drug-induced mutation rate was done by directly adding the appropriate concentration of drug to the selective medium. For UVA treatment, a 24-well plate containing the culture was irradiated with UVA 2 days after the cells were exhausted of adenine, by which time they had grown from an initial inoculum of ~5,000 to a final density of ~1.0 × 107 cells.

Alkaline Sucrose Density Gradient—Chromosomal DNA from either untreated or 4-NQO-treated cells were isolated and analyzed as described previously (21).

Plasmids—The plasmids pEB1 and pEB2 were constructed by subcloning the 1.5-kb Smal/HindIII DNA fragment carrying the yptpa1 gene into the single copy vector YCplac33 and the 2μ multicopy vector YEplac195, respectively, cut with Smal and HindIII (22). Similarly, the plasmids pEB3 and pEB4 were constructed by subcloning the 1.9-kb HindIII/SmalI polymerase chain reaction fragment of the yptpa2 gene into YEplac33 and YEplac185, respectively, also cut with HindIII and Smal. The multicopy plasmids pPHH1 and pPHH2, respectively, bearing the entire PHH1 and PHH2 genes were provided by Dr. M. Stark.

Northern Blot—Total RNA was prepared by the rapid glass bead method (23). The nitrocellulose blot was probed with a random primed 32P-labeled 1.1-kb HindIII/SmalI fragment derived from the coding region of the yptpa1 gene.

Construction of yptpa1 Promoter Fusion to lacZ—The primers, 5′-CCTGTGGGCGAATTCATGCTC-3′ and 5′-ATCTCAGAATTCCAGAGAC-3′, containing the promoter region of the yptpa1 gene, were used to amplify a 632-bp fragment containing the promoter region of the yptpa1 gene from –615 to +17 basepairs. This PCR fragment was inserted into the multiple cloning site of the multi-copy vector pYES56R (provided by Dr. Fred Winston, Harvard Medical School, Boston, MA), such that the ATG start codon of the yptpa1 gene forms the initiation codon for the E. coli reporter lacZ gene encoding β-galactosidase.

DNA Sequence Analysis—To determine the location of the mutation in the yptpa1 gene derived from the mutant EBY1, the primers 5′-CCCTGGGCGAATTCATGCTC-3′ and 5′-TAAGCTGCTGATTGAT-3′...
CCACATTATA-3' were used to isolate a 1.5-kb DNA fragment of the \(yPTPA1\) gene, with the underlined EcoRI/BamHI sites, respectively, from strain EBY1 carrying the mutant allele. The fragment was subcloned into pBluescript, and only one strand of the mutant allele of the \(yPTPA1\) gene was entirely sequenced by the dideoxy chain termination method (24). All additional DNA fragments isolated by polymerase chain reaction were also sequenced by this method.

RESULTS

Characterization of Yeast Mutants Hypersensitive to 4-NQO—In an attempt to identify novel eukaryotic genes implicated in the repair of 4-NQO-induced oxidative DNA damage, a panel of 55 \(S.\ cerevisiae\) mutants exhibiting hypersensitivity to the cytotoxic effects of this agent was initially isolated. Fifty of the mutants among this collection were excluded from further analysis, because they displayed strong cross-sensitivity to 254-nm UV light, i.e. reflecting the UV-mimetic character of 4-NQO, therefore indicating the involvement in the observed 4-NQO hypersensitivity of previously identified genes comprising, e.g. the NER pathway (3–5). The remaining five 4-NQO-sensitive mutants all retained parental resistance to 254-nm UV light and MMS and were therefore deemed potentially deficient in the processing of oxidative DNA damage. One of these 4-NQO-sensitive, 254-nm UV light-resistant mutants, designated EBY1, was shown to be highly cross-sensitive to the oxidizing agents UVA (320–400 nm) and diamide (Fig. 1). Moreover, EBY1 displayed only marginal sensitivity to UVB (290–320 nm), which has a much smaller oxidative component relative to UVA (Fig. 1), as well as to hydrogen peroxide (data not shown). Finally, EBY1 also manifested parental resistance to ionizing radiation (Fig. 1) and bleomycin (data not shown), providing evidence that it is not defective in the Rad52 recombinational/double-strand break repair pathway (25).

The mutation conferring 4-NQO and UVA sensitivity to EBY1 was recessive, because a diploid strain constructed by crossing EBY1 (\(MATa\)) with a wild type strain FY86 (\(MATa\)) showed wild type resistance to 4-NQO (data not shown). Dissection of eight tetrads derived by sporulating the diploid EBY1 X FY86 produced 2:2 segregation of the 4-NQO sensitivity in the progeny, indicating that EBY1 carries a single mutation.

The EBY1-complementing Gene, Designated \(yPTPA1\), Is a Yeast Homologue of the Human Phosphatase Activator \(hPTPA\)—Because UVA, 4-NQO, and diamide all generate free radicals, we reasoned that the hypersensitivity of EBY1 to these agents may be because of a defect in a pathway that signals the repair of, or itself repairs, oxidatively damaged DNA.
The four highly conserved regions (I, II, III, and IV) are also present in yPtpa2p and in proteins predicted from related genes found in other species (see text).

Fig. 2. Comparison of the predicted amino acid sequence between S. cerevisiae yPTPA1p and human PTPA.

Because yPtpa2p shares significant identity with yPtpa1p, it is possible that the observed phenotype of strain EBY1 might also arise from a genetic defect in the yPTPA2 gene. To test this, we isolated the yPTPA1 and yPTPA2 genes from strain EBY1 using polymerase chain reaction and subjected both to DNA sequence analysis (18). No alteration was found in the nucleotide sequence of the yPTPA2 gene, as compared with DNA sequences in the S. cerevisiae data base. In contrast, a single base substitution was found in the yPTPA1 gene derived from strain EBY1. The mutation was located at codon 60, where TAC was mutated to the TAA stop codon. Thus, the phenotypes associated with EBY1 are because of a defective yPTPA1 gene and not its homologue yPTPA2.

yptpa1 Null Mutants Are Hypersensitive to UVA and 4-NQO—To directly confirm that the yPTPA1 gene is responsible for the observed phenotype in strain EBY1, we replaced a portion of the yPTPA1 coding region, with the selective marker LEU2 in the parental strain DBY747. The resulting mutant EBY2 (yptpa1Δ::LEU2) showed identical sensitivity to UVA and 4-NQO, as compared with EBY1 (Fig. 3, A and B). Deletion of the yPTPA1 gene in three additional parent strains FY68, MKp-o, and W303 also produced mutants with marked sensitivities to UVA and 4-NQO (data not shown), eliminating the possibility that the observed phenotype of strain EBY1 might also arise from a genetic defect in the yPTPA2 gene. To test this, we isolated the yPTPA1 and yPTPA2 genes from strain EBY1 using polymerase chain reaction and subjected both to DNA sequence analysis (18). No alteration was found in the nucleotide sequence of the yPTPA2 gene, as compared with DNA sequences in the S. cerevisiae data base. In contrast, a single base substitution was found in the yPTPA1 gene derived from strain EBY1. The mutation was located at codon 60, where TAC was mutated to the TAA stop codon. Thus, the phenotypes associated with EBY1 are because of a defective yPTPA1 gene and not its homologue yPTPA2.

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Although the above data confirm that \( \text{yPtpa1p} \) is essential for cellular resistance to UVA and 4-NQO, they do not completely exclude a role for \( \text{yPtpa2p} \). To directly test this, a mutant was constructed by deleting the \( \text{yPTPA2} \) gene from the parent strain DBY747 by replacing it with the auxothropic marker gene \( \text{TRP1} \). The resulting mutant CHY26 retained parental resistance to UVA, to 4-NQO, and to other DNA-damaging agents such as MMS (Fig. 3B; data shown only for 4-NQO). Furthermore, a \( \text{yptpa1 yptpa2} \) double mutant CHY27 was no more sensitive to 4-NQO than the single \( \text{yptpa1} \) mutant EBY2 (Fig. 3B). These data confirm that \( \text{yPTPA1} \) and not \( \text{yPTPA2} \) is required for UVA and 4-NQO tolerance. It should also be noted that 4-NQO resistance could not be restored to strain CHY27 by the plasmid pEB3, which bears a single copy of the \( \text{yPTPA2} \) gene (Fig. 3B). Interestingly, however, if the \( \text{yPTPA2} \) gene was inserted into the multicopy vector YCplac195, and the resulting plasmid pEB4 was introduced into strain CHY27, full parental 4-NQO resistance was restored (Fig. 3B). This latter observation may not be surprising in view of the high degree of homology between \( \text{yPtpa1p} \) and \( \text{yPtpa2p} \), but nonetheless confirms that the endogenous level or activity of \( \text{yPtpa2p} \) is insufficient to compensate for complete loss of \( \text{yPtpa1p} \).

\( \text{yPtpa1p Expression Is Not Inducible} \)—To determine whether

\( \text{yPtpa1p} \) is inducible by cellular stress, the putative \( \text{yPTPA1} \) promoter region (spanning positions –615 to +1) was fused to the reporter gene \( \text{lacZ} \), encoding \( \beta \)-galactosidase. Upon introduction of the fusion plasmid construct into either EBY2 or the wild type strain, each expressed the same basal level of \( \beta \)-galactosidase activity (120 units/mg protein). This activity could not be induced by exposure to either UVA or 4-NQO nor by stress conditions including heat and osmotic shock. It would therefore appear that the endogenous level of \( \text{yPtpa1p} \) is sufficient for its biological function.

The \( \text{yptpa1 Null Mutant Is Defective in the Recovery of High Molecular Weight DNA Following Treatment with 4-NQO} \)—Mutants deficient in any of the three major DNA repair pathways, i.e., the nucleotide excision, post-replication, and recombinational pathways, show hypermutability in response to a wide spectrum of DNA-damaging agents including 4-NQO. We therefore tested whether the hypersensitivity of EBY2 to 4-NQO might be attributable to a defect in DNA repair. The
parental and mutant strains were challenged, or not, with 0.5 μg/ml of 4-NQO for 1 h, followed by isolation of chromosomal DNA and quantitation of strand breaks by alkaline sucrose density gradient analysis (21). Undamaged DNA isolated from either the parent or the mutant were indistinguishable with respect to size sedimentation (Fig. 4A). In response to 4-NQO treatment, however, chromosomal DNA from both strains were fragmented and in each case appeared to sustain the same extent of initial damage (Fig. 4B). When the cells were washed free of 4-NQO and allowed to recover for 8 h in fresh growth medium, the parental DNA was completely restored to high molecular weight status, whereas only approximately 40% of the EBY2-derived DNA was fully restored to its native size (Fig. 4C). Higher doses of 4-NQO (1.0 μg/ml for 1 h) resulted in further accumulation of fragmented DNA only in the mutant, and at 2 μg/ml of 4-NQO for 1 h, no restoration of high molecular weight DNA was observed in the mutant, whereas 55% of the parental DNA had recovered (data not shown). These data are consistent with the notion that 4-NQO induces two types of DNA lesion, only one of which is repaired less efficiently by the yptpa1 mutant.

yptpa1 Null Mutants Exhibit a Hypermutable Phenotype—Because our data are consistent with the notion that YPTPA1 plays a role in the repair of 4-NQO-induced oxidative DNA lesions, this protein may be expected to act similarly for spontaneously occurring oxidative DNA lesions generated during aerobic growth. We therefore measured the reversion mutation rate at the ade-2 locus in a wild type strain MKP-o, and in the isogenic yptpa1 null mutant EBY3. The ade-2 allele bears an ochre stop codon that results in a truncated protein unable to support adenine biosynthesis, i.e. the strain depends on exogenous adenine for growth. Under normal growth conditions, EBY3 showed a 3-fold increased rate of reversion to Ade⁺, as compared with MKP-o (Table I). This suggests that YPTPA1 may participate in the repair of endogenously generated DNA lesions. The reversion rate to Ade⁺ was increased by as much as 9-fold when the mutant was irradiated with 150 kJ/m² of UVA light and up to 17-fold with 300 kJ/m², whereas this rate was increased only approximately 2-fold in wild type cells irradiated with 300 kJ/m² UVA. Exposure to sublethal doses of 4-NQO, i.e. 0.005 and 0.01 μg/ml, also dramatically increased the mutation rate in the yptpa1 mutant by 8- and 13-fold, respectively, as compared with an increase of only 2-fold in parental cells treated with 0.01 μg/ml of 4-NQO. There was no striking difference in the mutation rate between MKP-o and EBY3 upon exposure to MMS or 254-nm UV light, relative to untreated cells (Table I).

yptpa1 Null Mutant EBY3 showed a 3-fold increased rate of reversion to Ade⁺ compared with MKP-o (Table I). This suggests that YPTPA1 may participate in the repair of endogenously generated DNA lesion(s), which are consistent with the notion that 4-NQO induces two types of the parental DNA had recovered (data not shown). These data are consistent with the notion that 4-NQO induces two types of DNA lesion, only one of which is repaired less efficiently by the yptpa1 mutant. The results presented here provide novel evidence that the previously identified but functionally uncharacterized S. cerevisiae gene, herein designated YPTPA1, plays a significant role in cellular protection against the model DNA-damaging agent 4-NQO. Moreover, yptpa1 null mutants exhibit prominent phenotypic characteristics that are consistent with a defect in the repair of 4-NQO-induced oxidative DNA lesions rather than of classical 4-NQO-induced bulky adducts, including (i) wild type resistance to 254-nm UV light, which shares considerable genotoxic properties with 4-NQO but does not significantly alter the redox state of the cell with respect to cytotoxicity and mutagenesis (31); (ii) cross-sensitivity to the cytotoxic and mutagenic effects of UVA, an agent that acts virtually exclusively through the generation of reactive oxygen species (32, 33); (iii) only marginal sensitivity to killing by UVB, which has an oxidative component but nonetheless closely resembles 254-nm UV light in genotoxic properties (34, 35); (iv) a spontaneous mutator phenotype, which is characteristic of prokaryotic and eukaryotic mutants deficient in the cellular response to oxidative DNA damage (36); and (v) impaired recovery of high molecular weight DNA following 4-NQO exposure, strongly indicating a defect at the level of DNA repair. We note that the yptpa1 mutant is not sensitive to the powerful oxidant ionizing radiation, which acts principally through the formation of hydroxyl radicals, rather than via singlet oxygen that is implicated in the genotoxic effect of UVA (32, 33). The overall data strongly support the notion that 4-NQO-induced oxidative DNA damage, in addition to the well-characterized N-2 and N-6 adducts of guanine and adenine, respectively, can exert considerable cytotoxicity and premutagenic potential in eukaryotic cells.

The fact that yptpa1 mutants are cross-sensitive to the cytotoxic and mutagenic effects 4-NQO and UVA presumably reflects the production of a common oxidative DNA lesion(s). This lesion would not appear to be 8-oxoguanine or AP sites, because unlike the case for E. coli, yeast mutants lacking either the enzyme Ogg1p (corresponding to the bacterial Fpg protein) or the major AP endonuclease Apn1, are not sensitive to either UVA or 4-NQO. This difference between bacteria and yeast may be explained if the latter retains “backup” proteins to repair 4-NQO-induced oxidative DNA lesions. In support of this assumption, it has been shown that yeast expresses the protein Ogg2p, which displays similar activities to Ogg1, as well as another AP endonuclease, Pde1p (38, 39). In any case, the culpable premutagenic oxidative lesion(s) common to 4-NQO- and UVA-exposed cells and the yptpa1p-mediated pathway that alleviates its (their) genotoxic potential remain to be characterized.

**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MKP-o mutation rate/10⁶ cell/generation</th>
<th>EBY3 mutation rate/10⁶ cell/generation</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.4 ± 0.8</td>
<td>10.9 ± 1.6</td>
<td>3</td>
</tr>
<tr>
<td>UVA (150 kJ/m²)</td>
<td>4.8 ± 0.7</td>
<td>43.8 ± 3.1</td>
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<td>UVA (300 kJ/m²)</td>
<td>6.6 ± 0.9</td>
<td>110.8 ± 7.1</td>
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<td>4-NQO (0.005 μg/ml)</td>
<td>5.1 ± 1.1</td>
<td>39.8 ± 3.5</td>
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<td>4-NQO (0.01 μg/ml)</td>
<td>7.3 ± 1.7</td>
<td>95.2 ± 8.5</td>
<td>13</td>
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<tr>
<td>MMS (0.001%)</td>
<td>2.4 ± 0.5</td>
<td>8.4 ± 1.5</td>
<td>3</td>
</tr>
<tr>
<td>MMS (0.05%)</td>
<td>5.3 ± 0.9</td>
<td>13.4 ± 2.8</td>
<td>2</td>
</tr>
<tr>
<td>254-nm UV light (10 J/m²)</td>
<td>4.1 ± 0.7</td>
<td>7.9 ± 1.6</td>
<td>2</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results presented here provide novel evidence that the previously identified but functionally uncharacterized S. cerevisiae gene, herein designated YPTPA1, plays a significant role in cellular protection against the model DNA-damaging agent 4-NQO. Moreover, yptpa1 null mutants exhibit prominent phenotypic characteristics that are consistent with a defect in the repair of 4-NQO-induced oxidative DNA lesions rather than of classical 4-NQO-induced bulky adducts, including (i) wild type resistance to 254-nm UV light, which shares considerable genotoxic properties with 4-NQO but does not significantly alter the redox state of the cell with respect to cytotoxicity and mutagenesis (31); (ii) cross-sensitivity to the cytotoxic and mutagenic effects of UVA, an agent that acts virtually exclusively through the generation of reactive oxygen species (32, 33); (iii) only marginal sensitivity to killing by UVB, which has an oxidative component but nonetheless closely resembles 254-nm UV light in genotoxic properties (34, 35); (iv) a spontaneous mutator phenotype, which is characteristic of prokaryotic and eukaryotic mutants deficient in the cellular response to oxidative DNA damage (36); and (v) impaired recovery of high molecular weight DNA following 4-NQO exposure, strongly indicating a defect at the level of DNA repair. We note that the yptpa1 mutant is not sensitive to the powerful oxidant ionizing radiation, which acts principally through the formation of hydroxyl radicals, rather than via singlet oxygen that is implicated in the genotoxic effect of UVA (32, 33). The overall data strongly support the notion that 4-NQO-induced oxidative DNA damage, in addition to the well-characterized N-2 and N-6 adducts of guanine and adenine, respectively, can exert considerable cytotoxicity and premutagenic potential in eukaryotic cells.

The fact that yptpa1 mutants are cross-sensitive to the cytotoxic and mutagenic effects 4-NQO and UVA presumably reflects the production of a common oxidative DNA lesion(s). This lesion would not appear to be 8-oxoguanine or AP sites, because unlike the case for E. coli, yeast mutants lacking either the enzyme Ogg1p (corresponding to the bacterial Fpg protein) or the major AP endonuclease Apn1, are not sensitive to either UVA or 4-NQO. This difference between bacteria and yeast may be explained if the latter retains “backup” proteins to repair 4-NQO-induced oxidative DNA lesions. In support of this assumption, it has been shown that yeast expresses the protein Ogg2p, which displays similar activities to Ogg1, as well as another AP endonuclease, Pde1p (38, 39). In any case, the culpable premutagenic oxidative lesion(s) common to 4-NQO- and UVA-exposed cells and the yptpa1p-mediated pathway that alleviates its (their) genotoxic potential remain to be characterized.

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2 D. Ramotar, unpublished data.
We have noted, however, that yptpa1p manifests significant homology with human hPTPA, which was shown to stimulate the weak phosphotyrosine phosphatase activity of PP2A in an ATP- and Mg$^{2+}$-dependent manner (26, 27). However, the biological consequences of this phosphatase activation step in human cells remain unclear. Based on our present findings that reveal a novel role for yptpa1p in the repair of 4-NQO-induced oxidative DNA damage in yeast, we predict that hPTPA could perform a similar function in human cells. Indeed, considering the ubiquitous participation of reversible protein phosphorylation in diverse cellular processes (40, 41), including the overall response to genotoxic agents, e.g. cell cycle arrest (42) and apoptosis (43), it is reasonable to anticipate that protein phosphatases also play an essential role in DNA repair. Preliminary evidence for the involvement of cellular PP2A phosphatases in NER has been obtained in human cells (37). In this study, specific inhibitors of PP2A were shown to interfere with the NER activity of human HeLa cell extracts, an effect that could be fully reversed by the addition of purified PP2A protein (37). However, the exact component of the NER pathway that is dephosphorylated by PP2A to allow efficient repair in vitro is unknown, and certainly, any precise in vivo roles for specific protein phosphatases in the DNA repair process remain to be elucidated.

It will therefore be crucial to identify the cellular targets, presumably (but not necessarily) corresponding to a protein phosphatase(s), that interact with yptpa1p to protect against the genotoxic effects of 4-NQO/UVA exposure. In S. cerevisiae, there are two distinct families of serine/threonine protein phosphatases, PPP and PP2C (30). The PPP family consists of at least 12 members, Gkh7, Pph21, Pph22, Cna1, Cna2, Ppz1, Ppz2, Sa16, Pph3, Sit4, Ppg1, and Ppt1 (30), which are further subdivided on the basis of enzymatic properties into three classes, PP2A, PP2B, and PP1. The PP2A members, including Pph3, Pph21, Pph22, Sit4, and Ppg1, are trimeric holoenzymes consisting of a 35-kDa catalytic subunit, a 65-kDa constant regulatory subunit, and a variable regulatory subunit ranging from 54 to 74 kDa in size (30). The constant and variable regulatory subunits are important for controlling phosphatase activity, substrate specificity, and cellular localization.

In view of the aforementioned putative role of PP2A-type phosphatases in human NER and the fact that hPTPA is capable of activating these phosphatases in vitro (26–27), we hypothesized that certain PP2A-related enzymes in yeast might play a role in the cellular response to 4-NQO. However, it is unlikely that yptpa1p mediates its biological effect via Pph21 or Pph22, because mutants lacking these serine/threonine phosphatases did not manifest hypersensitivity to either UVA or 4-NQO. Furthermore, overproduction of these same phosphatases in the yptpa1 mutant did not confer increased resistance to UVA or 4-NQO. Full characterization of additional 4-NQO/UVA-sensitive mutants, coupled with direct investigation of proteins that interact with yptpa1p, may be expected to shed significant light on the precise protective pathway mediated by this protein in yeast.

We hypothesized that certain PP2A-related enzymes in yeast

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Yeast Tolerance to 4-NQO

A Yeast Homologue of the Human Phosphotyrosyl Phosphatase Activator PTPA Is Implicated in Protection against Oxidative DNA Damage Induced by the Model Carcinogen 4-Nitroquinoline 1-Oxide

Dindial Ramotar, Edith Belanger, Isabelle Brodeur, Jean-Yves Masson and Elliot A. Drobetsky

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