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Base excision of oxidative purine and pyrimidine DNA damage in *Saccharomyces cerevisiae* by a DNA glycosylase with sequence similarity to endonuclease III from *Escherichia coli*

(formamidopyrimidine/thymine glycol/*NTG1*/DNA damage inducibility)

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ABSTRACT **One gene locus on chromosome I in *Saccharomyces cerevisiae* encodes a protein (YAB5_YEAST; accession no. P31378) with local sequence similarity to the DNA repair glycosylase endonuclease III from *Escherichia coli*. We have analyzed the function of this gene, now assigned *NTG1* (*endonuclease three-like glycosylase 1*), by cloning, mutant analysis, and gene expression in *E. coli*. Targeted gene disruption of *NTG1* produces a mutant that is sensitive to H_2O_2 and menadione, indicating that *NTG1* is required for repair of oxidative DNA damage *in vivo*. Northern blot analysis and expression studies of a *NTG1–lacZ* gene fusion showed that *NTG1* is induced by cell exposure to different DNA damaging agents, particularly menadione, and hence belongs to the DNA damage-inducible regulon in *S. cerevisiae*. When expressed in *E. coli*, the *NTG1* gene product cleaves plasmid DNA damaged by osmium tetroxide, thus, indicating specificity for thymine glycols in DNA similarly as is the case for EndoIII. However, NTG1 also releases formamidopyrimidines from DNA with high efficiency and, hence, represents a glycosylase with a novel range of substrate recognition. Sequences similar to *NTG1* from other eukaryotes, including *Caenorhabditis elegans*, *Schizosaccharomyces pombe*, and mammals, have recently been entered in the GenBank suggesting the universal presence of *NTG1*-like genes in higher organisms. *S. cerevisiae* NTG1 does not have the [4Fe–4S] cluster DNA binding domain characteristic of the other members of this family.**

Common forms of DNA damage, for instance those induced spontaneously by reactive metabolites produced inside the cells, are handled by the base excision repair pathway (1, 2). Different types of oxidized and alkylated base residues are recognized by different DNA glycosylases that release the bases in a free form and leave abasic sites in the DNA. Abasic sites are subsequently removed by the combined action of an apuriniclapyrimidinic (AP)-endonuclease and a 5′-phosphodiesterase or, alternatively, by an AP-lyase and a 3′-phosphodiesterase. Base excision repair is completed by repair synthesis to fill the one nucleotide gap and ligation to restore the continuity of the DNA strand. Other protein factors are probably also involved in the base excision repair pathway *in vivo* (2).

The DNA glycosylases are responsible for damage recognition and as such are key enzymes in the repair process. These can be classified into a few different subtypes, mainly based on genetic and biochemical characterization of DNA glycosylases in bacteria (2). Two of these are primarily involved in the removal of oxidative DNA damage, i.e., endonuclease III (EndoIII) and the formamidopyrimidine/8-oxoguanine DNA glycosylase (Fpg), which in a broad sense are responsible for the removal of oxidized pyrimidine and purine damage, respectively (3–6). Both of these enzymes have intrinsic AP-lyase activity, whereas other enzymes involved in the removal of deaminated and alkylated base residues do not (2). Both EndoIII and Fpg have been extensively characterized from *Escherichia coli* and other bacteria; however, attempts to define the functional role of analogous enzymes in eukaryotes have been hampered by difficulties in cloning the corresponding genes. Enzyme activities similar to EndoIII and Fpg have been purified from yeast (7, 8) and mammalian cells (9, 10); however, limited amounts of the enzymes in such species have prevented purification to homogeneity and cloning by the approach of protein sequencing.

Since all DNA glycosylases cleave the N-glycosylic bond between the base and the deoxyribose moieties of the nucleotides, one might have expected to find a common sequence domain that would be responsible for the cleavage reaction. However, different DNA glycosylases are largely unrelated in terms of sequence with the exception of EndoIII and MutY, which are of common evolutionary origin (11). Nevertheless, local sequence alignments have identified a subgroup of DNA glycosylases, including EndoIII and MutY, that share a characteristic helix–hairpin–helix domain. This domain appears to be involved in determining the specificity of the enzymes for reacting with different types of DNA damage (2, 12). The MutY enzyme removes adenosines from A·8-oxoguanine (8-oxoG) mispairs to prevent mutations arising from the misincorporation of an adenosine opposite 8-oxoG in the template (13) whereas EndoIII releases modified or fragmented pyrimidine residues (4). Searches for this motif in the genome databases have shown that a similar domain also is present in other enzymes involved in DNA repair and recombination, and it has been suggested that such a motif may participate in DNA binding. In these searches, we have also identified an open reading frame from yeast that contains a sequence motif similar to *E. coli* EndoIII. In this communication, we have characterized the function of this gene in *Saccharomyces cerevisiae* and show that the gene indeed encodes a DNA glycosylase. The enzyme is involved in the repair of oxidative DNA damage; however, the specificity of this enzyme appears different from any other DNA glycosylase previously described.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* BH20 (*fpg*; ref. 14) and BW372 (*nth*::*kan*; ref. 15) were obtained from S. Boiteux and B.Weiss,

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Abbreviations: EndoIII, endonuclease III; AP, apuriniclapyrimidinic; 8-oxoG, 8-oxoguanine; Fpg, formamidopyrimidine/8-oxoguanine DNA glycosylase; MMS, methyl methanesulfonate; 4-NQO, 4-nitroquinoline-*N*-oxide; faPy, formamidopyrimidine residue.

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respectively. BK3001 (*nth*::*kan*) is a derivative of JM105 made by P1 transduction with the *nth*::*kan* marker of BW372.

Yeast Strains. The haploid *S. cerevisiae* strain FL200 (*MATα*, *ura3*) was obtained from Tordis B. Øyen, Biochemistry Department, Oslo University. The *NTG1* mutant was made by targeted gene disruption of *NTG1* in FL200. The *URA3* marker was inserted into the *Bgl*II restriction site of *NTG1* in pUC19. The insert was excised and partially degraded with BAL-31 to ensure homologous ends (16). Transformation of FL200 was done according to Hill *et al.* (17). The mutant genotype was checked by Southern blot analysis and PCR.

Plasmids and DNA Constructs. The *NTG1* gene coding region (1180 bp) was amplified by PCR from genomic yeast DNA using primers gcggatccCATGCAAAAGATCAGTAAATA (start codon underlined; linker sequence with lowercase type) and ccgaattcaagcttAGTCCTCTACTTTAACAG (complementary strand of stop codon underlined). The amplified fragment was cloned into the *Bam*HI and *Eco*RI restriction sites of pUC19 and *Bam*HI and *Hin*dIII of pQE31 (Qiagen). In pQE31 the coding region is expressed in-frame with a His_6 leader from a bacteriophage T5 promoter. The sequence of the insert was analyzed to eliminate any clones with mutations introduced by the PCR. The promoter region of *NTG1* (297 bp), including the start codon and 30 bp of downstream sequence, was amplified by PCR using primers tttggtaccTGATTGATAATAATGG and tttggatccATAGATGAGTATTTACTG (linker sequences in lowercase type). The amplified promoter fragment was cloned between the *Kpn*I and *Bam*HI restriction sites of the centromeric yeast/*E. coli* shuttle plasmid pHQ107 (provided by Louise Prakash, Texas University) in front of and in-frame with the *lacZ* gene of *E. coli.*

Northern Blot Analysis. Exponentially growing FL200 cells were exposed to methyl methanesulfonate (MMS, 2 mM), H_2O_2 (1.5 mM), 4-nitroquinoline-*N*-oxide (4-NQO; 5 mM) for 30 min at 37°C, pelleted, and lysed by vigorous vortex mixing with 5 g of RNase-free glass beads. The lysate was extracted twice with phenol/chloroform, 1:1(vol/vol)/10% SDS in extraction buffer (0.1 M Tris·HCl, pH 7.5/100 mM LiCl/10 mM dithiothreitol) at ice temperature. The RNA was precipitated with ethanol at −20°C overnight and resuspended in 50 μl of 0.02% solution of diethylpyrocarbonate in water with 34 units of rRNasin (Promega). Agarose gel electrophoresis was performed as described by Fourney *et al.* (18); the RNA was electroblotted essentially as described by Chomczynski (19) except that sarkosyl was omitted. Hybridization was according to Fourney *et al.* (18), except that the standard saline citrate (SSC) concentrations were doubled during washing, performed for three 5-min periods at room temperature followed by two 15-min periods at 50°C. The amounts of radioactivity on the filters were quantified by a Molecular Dynamics PhosphorImager model 445 SI.

Promoter Fusion Analysis. Exponentially growing FL200 cells harboring the *NTG1–lacZ* construct were exposed to MMS and menadione at the concentrations indicated for 2.5 h. Aliquots (1.5 ml) were pelleted and incubated in 250 μl of 1 M sorbitol/0.1% Triton/100 units of lyticase for 15 min at 30°C. The β-galactosidase reaction was carried out in Z buffer (0.75 ml; ref. 20) with chlorophenolred β-D-galactopyranoside (0.8 mg; Boehringer Mannheim) and the activity was measured by reading the absorbance at 574 nm. Incubation was for 60 min at 25°C and the reaction was stopped by adding 400 μl of 1 M Na_2CO_3. Values are corrected for variations in protein concentration in the extract and calculated relative to β-galactosidase activity in untreated control.

DNA Substrates. DNA containing ^{3}H-labeled formamidopyrimidine residues (faPy) was prepared as described (21), except that radiolabeled *N*-methyl-*N*-nitrosourea (740 GBq/mmol) was used instead of dimethyl sulfate. The specificity of the substrate was 4.7 kBq/mg of poly(dG-dC). DNA with thymine glycols was made by exposing 100 μg of pUC19 to 0.1% OsO_4 for 90 min at 70°C. Covalently closed circular DNA was purified by agarose gel electrophoresis followed by electroelution and dialysis. Duplex DNA substrate with 8-oxoG was prepared by ^{32}P end labeling of 14 pmol of the 15-mer oligonucleotide 5′-ATCACCGGC-[8-oxoG]-CCACA-3′ (provided by R. Fuchs, Centre National de la Recherche Scientifique, Strasbourg, France) followed by annealing to a 22-mer complementary oligonucleotide: 5′-CAGCTCGTGTGGCGCCGGTGAT-3′. The shorter 8-oxoG-containing strand was extended by synthesis with 1 unit of *Taq* polymerase. The double-stranded oligonucleotide was purified by 20% PAGE, electroelution, and dialysis. AP-DNA substrate was made in a similar fashion by annealing a 5′-^{32}P-labeled-uracil-containing oligonucleotide, 5′-GCTCATGCGCAGUCAGCCGTACTCG-3′, to a complementary 18-mer. The 18-mer was extended by primer extension and the double-stranded oligonucleotide was purified by gel electrophoresis, electroelution, and dialysis. To make the AP site, the double-stranded oligonucleotide was finally incubated with 1 unit of uracil DNA glycosylase for 1 h.

Enzymes. Purified *E. coli* proteins Fpg and EndoIII were provided by Serge Boiteux (Centre National de la Recherche Scientifique, Fontenay au Roses, France). Uracil DNA glycosylase was from Boehringer. Endonuclease IV (Nfo) from *E. coli* was purified using the His_6-tag Ni-affinity purification system (Qiagen). The *NTG1* gene product was expressed in different *E. coli* mutants transformed by the pQE-*NTG1* construct as indicated by adding 5 mM isopropyl β-D-thiogalactopyranoside and incubating for 2 h before the cells were lysed by the plasmolysis procedure (22). Protein concentration was determined by the method of Bradford (23) with bovine serum albumin as reference.

Enzyme Assays. All enzyme reactions were carried out in 70 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.5/1 mM dithiothreitol/1 mM EDTA/5% glycerol for 30 min at 37°C unless otherwise indicated. FaPy DNA glycosylase assays (50 μl) contained 7.1 μg of DNA of faPy substrate (2000 dpm). For HPLC, 10,000 dpm of faPy substrate was incubated with 120 ng of Fpg or 14 μg of cell extract from BH20 expressing NTG1 for 1 h at 37°C in 50 μl of reaction buffer without glycerol. Reverse-phase HPLC was performed on a RP-18 column (Brownlee Lab) using isocratic elution with 50 mM $NH_4H_2PO_4$, pH 4.5/5% methanol and a flow rate of 1.0 ml/min essentially according to Boiteux *et al.* (21). OsO_4 DNA nicking assay mixture (20 μl) contained 0.2 μg of DNA. The DNA was analyzed by agarose (0.8%) gel electrophoresis in ethidium bromide at 0.5 μg/ml at 75 V for 1.5 h. 8-oxoG assays (20 μl) contained 20 fmol of ^{32}P-labeled 8-oxoG-containing oligonucleotide. The cleavage products were analyzed on 20% denaturing PAGE. The gels were fixed for 1 h (20% ethanol/10% HAc) and dried. AP-DNA lyase assays contained 100 fmol of substrate and the DNA was analyzed on denaturing gels similar to those used for 8-oxoG-DNA.

Yeast Survival Experiments. Cells were grown in YEPG medium at 30°C for 7–8 h to $OD_{600} \approx 0.5$, washed three times in ice-cold water, and incubated on ice for 30 min prior to exposure. After treatment, cells were diluted in ice-cold water, plated on YEPG agar plates, and incubated at 30°C for 2–3 days. Treatment with different concentrations of H_2O_2 was done at 30°C for 30 min. UV-irradiation was from a 254-nm germicidal lamp at a fluency of 4 J per m^2 per s. γ-radiation was from a ^{137}Cs source (4.5 Gy/min). Cells were kept on ice during both UV- and γ-irradiation.

RESULTS

A Yeast Gene with Sequence Similarity to EndoIII from *E. coli*. The TBLAST program (24) was used to search the DNA sequence database for coding sequences matching a consensus of the helix–bend–helix motif as described (2). An open reading frame on chromosome I was identified that translated

into a protein (YAB5_YEAST; accession no. P31378) that contained a domain with a statistical significant match to the search motif. In particular, the sequence identified resembled that of endonuclease III (Nth) and to a smaller extent that of MutY from *E. coli* (Fig. 1). The similarity was confined to the predicted helix–hairpin–helix domain and could not be extended much beyond this region. In view of the sequence similarity and the functional characterization described below, we have reassigned this locus *NTG1*.

Mutant Analysis of *NTG1*. To investigate the *in vivo* function of the *NTG1* gene, a mutant strain was constructed by insertional inactivation of *NTG1* with *URA3*. The mutant was found to be sensitive to H_2O_2 and slightly sensitive to menadione but normally resistant to γ-irradiation, UV (Fig. 2), and MMS (data not shown). It thus appears that the *NTG1* function is involved in the repair of DNA damage induced by oxidative DNA damaging agents. Ionizing irradiation is also known to induce oxidative DNA base damage, but in this case survival most likely is limited by repair of other types of lesions (e.g., double-strand breaks) also known to be induced by the irradiation.

Induction of *NTG1* by DNA Damaging Agents. Several DNA repair genes in yeast are known to be DNA damage inducible. The inducibility of *NTG1* was investigated by Northern blot analysis as well as by gene promoter fusion to the bacterial *lacZ* reporter gene (Fig. 3). An approximate 2- to 3-fold increase in the amounts of transcripts were found following cell exposure to H_2O_2, MMS, and 4-NQO. For comparison, the same filter was also probed for transcripts of the DNA damage inducible *MAG* gene encoding 3-methyladenine DNA glycosylase (25). A 2- to 3-fold increase was also observed in the case of the *MAG* gene (data not shown). The level of the β-actin transcript, however, was unaffected by DNA damage exposure (Fig. 3*A*). In other experiments with promoter fusion of *NTG1* to the β-galactosidase reporter gene, a 9-fold increase of β-galactosidase activity was obtained by exposure to menadione and 2-fold increase was obtained by MMS exposure (Fig. 3*B*). The *NTG1* gene therefore seems to be part of the DNA damage inducible regulon of *S. cerevisiae* as has been shown previously for other DNA repair genes.

Incision of Osmium Tetroxide-Damaged DNA by NTG1. The *NTG1* gene was cloned into the pQE31 expression vector for analyzing any repair enzyme activity of the gene product. The pQE vectors express coding regions in-frame with a His_6 N-terminal leader that allows simple affinity purification on Ni columns. After such purification a major protein band of M_r 45,000 was observed upon SDS/PAGE (data not shown). However, no DNA glycosylase or endonuclease activity was found to be present in such preparations regardless of the substrate employed. We suspected that the purification as such might cause protein denaturation as is sometimes experienced with the Ni affinity purification system. Possible enzymatic activity was, therefore, tested in protein extracts after expression in *nth* mutant bacterial cells using as a substrate plasmid DNA treated with OsO_4. OsO_4 induces predominantly thymine glycols in the DNA; a good substrate for EndoIII from *E. coli* (16). Extracts from the *NTG1*-expressing cells caused cleavage of the OsO_4-treated DNA as was observed in controls with EndoIII from *E. coli* (Fig. 4). Extracts from cells containing only vector without the gene did not produce appreciable amounts of cleavage of the OsO_4-treated DNA, and nondamaged DNA was also resistant to enzymatic cleavage. These results indicate that *NTG1* expresses an endonuclease activity similar to EndoIII from *E. coli* with specificity for thymine glycols in DNA, presumably acting by the two-step mechanism of N-glycolytic base removal followed by AP-lyase strand cleavage at the AP site.

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NTG1_Yeast sTCkILqdQfssdVPaTinELlgLPGVGpKmAylLqkAwGKiegIcVD
NTH_Ecoli  KTCrILleQHnGeVPEdraaLeALPGVGRKTAnvvLntAfGw-ptIaVD
MUTY_Ecoli KaaqqvatlHgGkfPETfeEvaALPGVGRsTAgaiLslslGKhfpIldg
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FIG. 1. Local alignment of the putative helix–hairpin–helix motif of yeast NTG1 (YAB5_YEAST) and *E. coli* EndoIII and MutY.

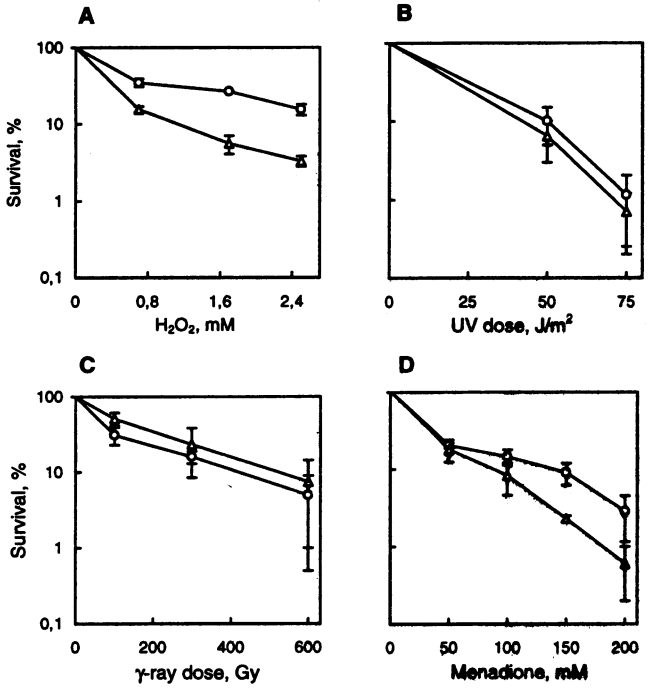

FIG. 2. Survival of the haploid yeast *ntg1*::*URA3* mutant exposed to H_2O_2 (*A*), UV (254 nm) (*B*), γ-radiation (*C*), and menadione (*D*). Each point represents the average of four measurements (two experiments) and error bars indicate the complete range of the data points. Symbols: ○, FL200 (wild type); and △, BKY301 (*ntg1*::*URA3*).

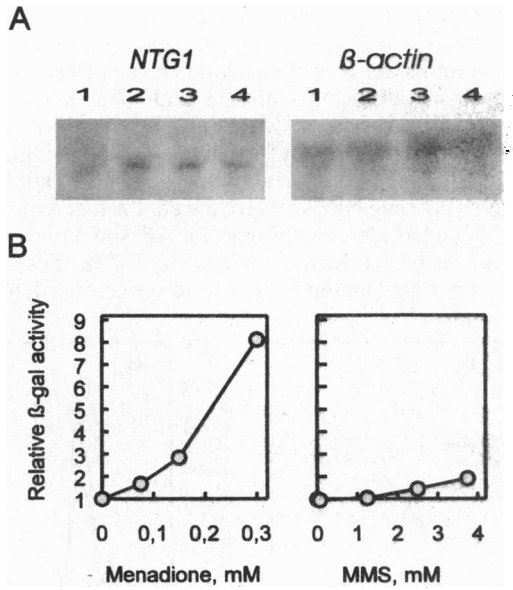

FIG. 3. Gene expression analysis of *NTG1* in *S. cerevisiae* exposed to DNA damaging agents. (*A*) Northern blot analysis. RNA blots from the haploid yeast strain FL200, isolated from undamaged control (lane 1) or from cells exposed to H_2O_2 (lane 2), MMS (lane 3), or 4-NQO (lane 4), were hybridized with the 1.2-kb *NTG1* probe. The filter was stripped and rehybridized with the 2.0-kb β-actin probe as control. (*B*) Promoter fusion analysis. The haploid yeast FL200 carrying the *NTG1–lacZ* fusion on a centromeric plasmid was exposed to increasing concentrations of menadione and MMS, incubated for 2.5 h, and assayed for β-galactosidase activity.

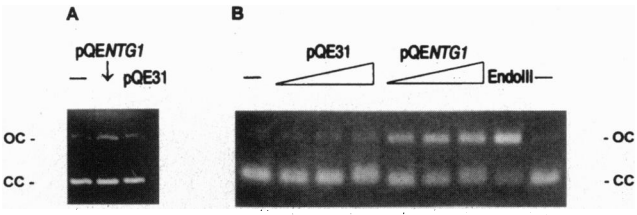

FIG. 4. Incision of OsO_4-DNA by NTG1. OsO_4-DNA and control DNA was incubated at 37°C for 30 min with different amounts of cell extracts from strain BK3001 (*nth*::*kan*) transformed by the expression vector pQE-*NTG1* and by pQE31 as control. (*A*) Nondamaged DNA. From the left, amounts used were no extract, 0.5 μg of pQE-NTG1 extract, and 0.5 μg of pQE31 extract, respectively. (*B*) OsO_4-DNA. From the left, amounts used were no extract; 0.1, 0.5, and 1.0 μg of extract of pQE31-transformed bacteria; 0.1, 0.5, and 1.0 μg of extract of pQE-*NTG1*-transformed cells; 25 ng of purified *E. coli* EndoIII; and no extract.

Excision of Formamidopyrimidines, but not 8-oxoG, by NTG1. In further characterization of the enzymatic function of NTG1, alkylated DNA and formamidopyrimidine-containing DNA were used in glycosylase assays with NTG1-expressing extracts. As expected, no release of methylated bases could be demonstrated (data not shown). However, quite unexpectedly, efficient release of formamidopyrimidines was observed (Fig. 5). In *E. coli*, such excision is not catalyzed by EndoIII; however, it is by the Fpg DNA glycosylase (14, 21). Formamidopyrimidine excision by NTG1 was compared with that obtained with purified Fpg, and the excision product appeared identical as analyzed by reverse-phase HPLC (Fig. 5*B*). In these experiments the NTG1 enzyme was expressed in *fpg* (*mutM*) mutant bacteria and controls with vector plasmid only were completely deficient in removal of formamidopyrimidines. These results confirm that the yeast NTG1 enzyme is a DNA glycosylase. Moreover, the substrate range specificity for this enzyme is clearly different from that of the homologous *nth* gene function in *E. coli*.

Since *E. coli* Fpg excises 8-oxoG as well as faPy, the expressed NTG1 was also tested for cleavage of oligonucleotides containing an 8-oxoG residue in a defined sequence position. In experiments with the end label in the strand containing the 8-oxoG, no strand cleavage activity could be demonstrated, irrespective of the type of base residing opposite 8-oxoG in the complementary strand (Fig. 6 and data not shown). All possible matches were tested. Further experiments with oligonucleotides containing an AP site indicated that NTG1 possesses AP-lyase activity (Fig. 7). In these experiments, only small amounts of extracts were used to avoid

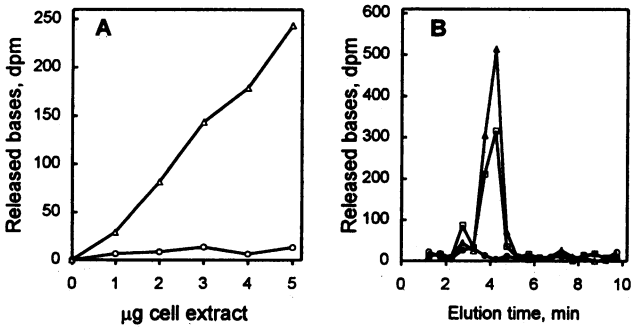

FIG. 5. FaPy DNA glycosylase activity of NTG1. (*A*) [^{3}H]faPy-poly(dG-dC) was incubated at 37°C for 30 min with various amounts of cell extracts of BH20 (*fpg*) carrying pQE31(○) or pQE-*NTG1*(△). After incubation, ethanol soluble radioactivity was determined. (*B*) Reverse-phase HPLC of the bases released. FaPy substrate was incubated at 37°C for 1 h with water (○), 14 μg of cell extract of BH20 carrying pQE-*NTG1* (△), or 0.12 μg of purified Fpg (□).

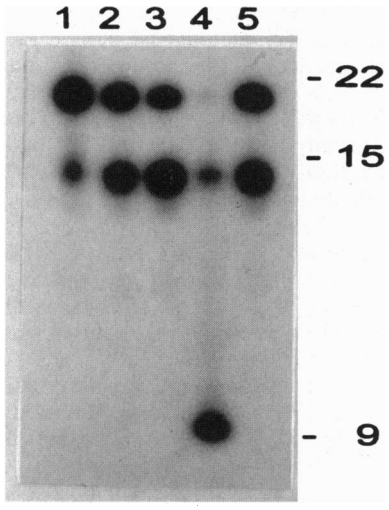

FIG. 6. Denaturing PAGE of 8-oxoG containing oligonucleotide incubated with yeast NTG1. The 8-oxoG 15-mer oligonucleotide strand was labeled with ^{32}P at the 5′ end and annealed to a complementary 22-mer with cytidine opposite 8-oxoG. A full 22-mer duplex was made by primer extension with the *Taq* polymerase. The extension was not fully effective as indicated by the bands at the position of the 15-mer. Prior to analysis the oligonucleotide was incubated at 37°C for 30 min as follows: no extract (lane 1), 1.3 μg of extract from BH20 (*fpg*) carrying pQE31 (lane 2) or pQE-*NTG1* (lane 3), 40 ng of purified *E. coli* Fpg (lane 4), and 25 ng of purified EndoIII (lane 5).

interference from background AP-endonuclease or AP-lyase activities present in the *E. coli* extract. Under these conditions, only extracts from cells transformed by the NTG1 expression vector produced significant cleavage of the AP-DNA. The cleaved DNA migrated to the same position as that produced by EndoIII and not further as is the case for the fragment created by δ-elimination product of purified Fpg or 5′ hydrolysis by purified Endonuclease IV (Nfo) from *E. coli* (data not shown). It thus appears that yeast NTG1 resembles the *E. coli* Fpg enzyme only in its affinity for removal of formamidopyrimidines and, however, differs from Fpg in lacking the activity for removal of 8-oxoG.

DISCUSSION

Much progress has been made in recent years in the characterization of the gene functions involved in the nucleotide

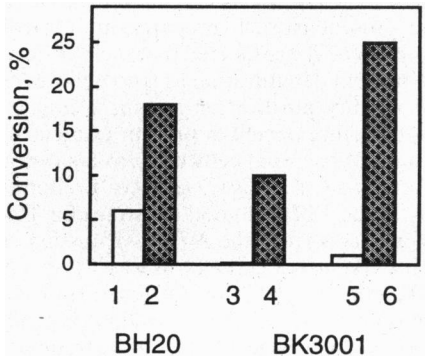

FIG. 7. Incision of AP-DNA by yeast NTG1. A 25-mer duplex DNA with a single AP site in the 5′-end-labeled strand was incubated at 37°C for 30 min with extracts from *E. coli* mutants carrying pQE (open bars) or pQE-*NTG1* (solid bars). The DNA was analyzed on denaturing 20% PAGE and the fraction of cleaved DNA versus noncleaved DNA was quantified by PhosphorImager scanning. Mutant extracts used were BH20 (*fpg*) at 25 ng of protein (bars 1 and 2) and BK3001 (*nth*::*kan*) at 20 ng (bars 3 and 4) or 50 ng of protein (bars 5 and 6).

excision repair pathway in eukaryotic cells. Less effort has been made toward the cloning and characterization of the genes involved in base excision repair. In spite of that, these functions are likely to be very important if not essential for protecting against endogenous DNA damage. We have characterized a yeast homologue of EndoIII in bacteria and show that this enzyme is a DNA glycosylase acting at thymine glycol and faPys in DNA. The yeast NTG1 thus appears to partially combine the functional properties of the oxidative DNA damage repair glycosylases EndoIII and Fpg in *E. coli*. However, no activity is observed against 8-oxoG by NTG1, implying that NTG1 does not fully cover the functional role of both of these enzymes. Studies of the *ntg1*::*URA3* mutant show that NTG1 is of importance for survival of yeast toward oxidative DNA damaging agents such as H_2O_2 and menadione. To our knowledge, this appears to be the first experimental evidence for base excision being important for the protection against the cytotoxic effects of such compounds *in vivo*. Neither the *nth* nor the *fpg* mutant of *E. coli* show reduced survival after exposure to oxidative DNA damaging agents. However, this has been ascribed to the presence of alternative repair pathways in *E. coli*. For instance, a second minor DNA glyocosylase activity similar to EndoIII has been detected in this organism (26). Such activity may be responsible for the low background of OsO_4 DNA nicking observed in our extract studies (Fig. 4*B*, lanes 2–4). Further characterization of the *in vivo* role of *NTG1* should involve the construction of double mutant strains also carrying defects in *RAD* genes of the epistasis groups 3 (nucleotide excision repair) and 6 (postreplication repair) to evaluate the relative significance of different repair pathways in the recovery from oxidative DNA damage.

DNA glycosylases for removal of thymine glycols, redoxyendonuclease (7), and faPy DNA glycosylase (8) have previously been purified from *S. cerevisiae*. Mostly based on the presumed analogy with the *E. coli* system, these have been considered to be separate enzymes. However, their chromatographic behavior is similar as are their molecular masses (40–45 kDa) as determined by gel filtration. The molecular mass of NTG1 is 45 kDa and, therefore, clearly within the range of the other enzyme activities. In view of the enzymatic properties of NTG1, it seems possible that all three enzymes could be identical. Further biochemical characterization of NTG1 will help to elucidate this question. It was originally suggested that the yeast faPy DNA glycosylase described also could remove 8-oxoG. However, this activity was quite low and it was unclear whether this activity was due to a contaminating enzyme in the purified faPy enzyme preparation (8).

Transcription of *NTG1* (termed *FUN33*) has been demonstrated by Barton *et al.* (27) who made a survey of all the genes present in this region on chromosome I in *S. cerevisiae*. The transcript of *NTG1* was estimated to about 1.3 kb and the level of the transcript was found to be fairly low. They also found that viability was not affected by deleting this region from the genome, proving that *NTG1* is a nonessential gene. Our *nth1* mutant grows normally and does not show any obvious phenotype apart from the sensitivity to oxidative DNA damaging agents. We also find that *NTG1* is expressed at a low level that, however, is increased by exposure to DNA damaging agents. The inducibility appears particularly pronounced after menadione exposure, which could imply that there is some specificity for the DNA damage inducibility of *NTG1*.

The presence of an EndoIII-like function in yeast raises the question as to whether similar functions also exist in other eukaryotic cells. Searches made with *NTG1* toward the complete genome database now reveal that several entries have been made in the last few months of expressed sequence tags and genomic DNA translating into protein sequences clearly homologous to NTG1 (Fig. 8). A multiple alignment of such sequences, also including those from several bacterial species, shows that the prokaryotic and the eukaryotic sequences

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YEAST    MQKISKYSSMAILRKRPLVKTETGPESELLPEKRTKIKQEEVVPQPVDIDWVKSLPNKQY

HAEIN                                                    MNKTKRIEILTRLR--
ECOLI                                                    MNKAKRLEILTRLR--
BACSU                                                    MLNLKQIEFCLDKIG--
YEAST    FEWIVVRNGNVPNRWATPLDPSILVTPASTKVPYKFQETYARMRVLRSKILAPVDIIGGSS
SCHPO                                MSKDYGTPPENWREVYDEICKMKAKVVAPVDVQG--
CAEEL                                                    MRKDMIAPVDTMG--

HAEIN    --------EQNPHPTTELQYNSPFELLIAVILSAQATDKGVNKATEKLFPVANT------
ECOLI    --------ENNPHPTTELNFSSPFELLIAVLLSAQATDVSVNKATAKLYPVANT------
BACSU    --------DMFPHAECELVHSNPFELVVAVALSAQCTDALVNRVTKTLFQKYKR------
YEAST    IPVTVASKCGISKE-QISPRDYRLQVLLGVMLSSQTKDEVTAMAMLNIMRYCIDELHSEE
SCHPO    --------CHTLGE-RNDPKKFRFQTLVALMLSSQTKDIVLGPTMRNLKEKLAGGLC---
CAEEL    --------CHKLADPLAAPPVHRFQVLVALMLSSQTRDEVNAAAMKRLKDHGLS------

HAEIN    ---PQAILDLGLDGLKSYIKTIGLFNSKAENIIKTCRDLIEKHNGEVPENREALEALAGV
ECOLI    ---PAAMLELGVEGVKTYIKTIGLYNSKAENIIKTCRILLEQHNGEVPEDRAALEALPGV
BACSU    ---PEDYLAVPLEELQQDIKSIGLYRNKAKNIQKLSKMIIEDYGGEVPRDRDELVKLPGV
YEAST    GMTLEAVLQINETKLDELIHSVGFHTRKAKYILSTCKILQDQFSSDVPATINELLGLPGV
SCHPO    ---LEDIQNIDEVSLNKLIEKVGFHNRKTIYLKQMARILSEKFQGDIPDTVEDLMTLPGV
CAEEL    ---IGKILEFKVPDLETILCPVGFYKRKAVYLQKTAKILKDDFSGDIPDSLDGLCALPGV

HAEIN    GRKTANVVLNTAFG-HPTIAVDTHIFRVCNRTNFAAGKD---VVKVEEKLLKVVPNEFKV
ECOLI    GRKTANVVLNTAFG-WPTIAVDTHIFRVCNRTQFAPGKN---VEQVEEKLLKVVPAEFKV
BACSU    GRKTANVVVSVAFG-VPAIAVDTHVERVSKRLGICRWKDS--VLEVEKTLMRKVPKEDWS
YEAST    GPKMAYLTLQKAWGKIEGICVDVHVDRLTKLWKWVDAQKCKTPDQTRTQLQNWLPKGLWT
SCHPO    GPKMGYLCMSIAWNKTVGIGVDVHVHRICNLLHWCNT---KTEEQTRAALQSWLPKELWF
CAEEL    GPKMANLVMQIAWGECVGIAVDTHVHRISNRLGWIKT---STPEKTQKALEILLPKSEWQ
RAT      .....................HRIANRLKWTKKMT-KSPEETRRNLEXWLPRVLWS
HUMAN    ..............................................ALEEWLPRELWH

HAEIN    DVHHWLILHGRYTCIARKPRCGSCII--EDLCEYKEKVEF
ECOLI    DCHHWLILHGRYTCIARKPRCGSCII--EDLCEYKEKVDI
BACSU    VTHHRLIFFGRYHCKAQSPRCAECPL--LSLCREGQKRDKKGLVKR
YEAST    EINGLLVGFGQIITKSRNLGDMLQFLP-PDDPRSSLDWDLQSQLYKEIQQNIMSYPKWVK
SCHPO    ELNHTLVGFGQTICLPRGRRCDMCTLSSKGLCPSAFKEKSGITITKRKVKTIKRVKKRPA
CAEEL    PINHLLVGFGQMQCQPVRPKCGTCLC--RFTCPSSTAKNVKSETEETSTSIEVKQEVEDE
RAT      EINGLLVGFGQXICLPVHPRCQACLX--KALCPAAQGL
HUMAN    EINGLLVGFGQQTCLPVHPRCHACLN--QALCPAAQGL

YEAST    YLEGKRELNVEAEINVKHEEKTVEETMVKLENDISVKVED
SCHPO    SESPPLSPLSLPTDDLYYQSIEDKSLIKLEDLDPVDSISHMNEPLKKEPAADIDVDQKPP
CAEEL    FEDEKPAKKIKKTRKTRTKIEVKTESET

SCHPO    VAFHSTTKETRSLRRSKRVAKKSSQYFSQQSLQDIEDLV
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FIG. 8. Multiple alignment of the *S. cerevisiae* NTG1/*E. coli* EndoIII gene family in pro- and eukaryotic cells. The alignment was made by the CLUSTAL W program of the GCG (University of Wisconsin) sequence analysis program. The sequences are grouped as pro- and eukaryotes and identical residues conserved in all members of each group are presented in boldface type. GenBank accession numbers are as follows: *Haemophilus influenzae*, L46319; *E. coli*, J02857; *Bacillis subtilis*, U11289; *S. cerevisiae* (yeast), L05146; *S. pombe*, Z67961; *Caenorhabditis elegans* Z50874; rat H33255; human cells, F04657.

assemble into two different groups. Each group have a characteristic sequence signature, although there are also many conserved residues common to gene members from all species. A characteristic feature of EndoIII revealed from the structural analysis is the presence of the [4Fe–4S] cluster motif, which is proposed to be important for DNA binding (28). This domain appears to be conserved in all member of the EndoIII family except for *S. cerevisiae* NTG1. However, the yeast enzyme has a long unique positively charged N-terminal end that could be an alternative motif for DNA binding. Toward the C-terminal end, where other gene family members have the [4Fe–4S] cluster, the yeast NTG1 has an acidic domain that perhaps could be involved in protein–protein interactions. It has recently been shown that removal of thymine glycols is coupled to transcription and that this depends on *RAD2* but not *RAD1* (29). Studies of a double mutant carrying *ntg1* and *rad2* or *rad1* might indicate whether NTG1 would be responsible for transcription coupled removal of thymine glycols in yeast and elucidate the question of whether this mechanism involves the base excision repair pathway.

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