Published online January 2, 2004

Nucleic Acids Research, 2004, Vol. 32, No. 1 ***115–126***
DOI: 10.1093/nar/gkh151

The major role of human AP-endonuclease homolog Apn2 in repair of abasic sites in *Schizosaccharomyces pombe*

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Received September 23, 2003; Revised and Accepted October 31, 2003

ABSTRACT

The abasic (AP) sites, the major mutagenic and cytotoxic genomic lesions, induced directly by oxidative stress and indirectly after excision of damaged bases by DNA glycosylases, are repaired by AP-endonucleases (APEs). Among two APEs in *Saccharomyces cerevisiae*, Apn1 provides the major APE activity, and Apn2, the ortholog of the mammalian APE, provides back-up activity. We have cloned *apn1* and *apn2* genes of *Schizosaccharomyces pombe*, and have shown that inactivation of Apn2 and not Apn1 sensitizes this fission yeast to alkylation and oxidative damage-inducing agents, which is further enhanced by Apn1 inactivation. We also show that Uve1, present in *S.pombe* but not in *S.cerevisiae*, provides the back-up APE activity together with Apn1. We confirmed the presence of APE activity in recombinant Apn2 and in crude cell extracts. Thus *S.pombe* is distinct from *S.cerevisiae*, and is similar to mammalian cells in having Apn2 as the major APE.

INTRODUCTION

Mitochondria continuously generate reactive oxygen species (ROS) as respiration by-products. ROS are genotoxic and induce a multitude of DNA damage, including oxidized bases, abasic (AP) sites due to direct glycosylic bond cleavage in DNA, and strand breaks caused by fragmentation and oxidation of deoxyribose in the DNA backbone with generation of 3′-phosphoglycolaldehyde and 3′-phosphoglycolate termini (1). AP sites are also generated as repair intermediates after excision of alkylated and oxidatively damaged bases by DNA glycosylases and are produced due to spontaneous depurination (2,3). Oxidized base-specific DNA glycosylases possess intrinsic AP-lyase activity, cleaving the DNA backbone at AP sites and producing 3′-phospho-α,β unsaturated aldehyde or 3′-phosphates. AP sites, arguably the most abundant endogenous lesions in DNA, have been shown to be cytotoxic and mutagenic (3–5). Repair of AP sites and single-strand breaks with 3′-blocked termini occurs primarily via the DNA base excision repair (BER) pathway, which is essential for mammalian survival. Inactivation of some BER proteins including the major AP-endonuclease (APE), DNA polymerase β (Polβ) or XRCC1 protein in the mouse causes embryonic lethality (6). Repair of AP sites is initiated with an APE which cleaves the DNA strand 5′ to the AP site and generates 3′-OH and 5′-phosphodeoxyribose termini. The resulting 5′-phosphodeoxyribose group is removed by the lyase activity of Polβ to generate a 1 nt gap. Subsequently, Polβ fills in the gap, and DNA ligase seals the single-strand nick to complete repair [reviewed in Mitra *et al*. (7)]. APEs also have intrinsic 3′-phosphodiesterase activity responsible for removing the 3′-blocking groups generated at strand breaks caused by either ROS directly or due to AP lyase activity of some DNA glycosylases (8). Two major classes of APEs are present in organisms ranging from the bacteria to mammals whose prototypes are Xth (exonuclease III), representing ~90% of the total AP repair activity, and Nfo (endonuclease IV) in *Escherichia coli*. In *Saccharomyces cerevisiae*, Apn1, the first APE to be cloned, belongs to the *E.coli* Nfo family (9), while Apn2 shares homology with *E.coli* Xth and human APE1 and APE2 (10,11). Both Apn2 and Apn1 possess APE and DNA 3′-phosphodiesterase activities (12,13). However, the *apn1* but not the *apn2* mutant is sensitive to the methylating genotoxicant methyl methanesulfonate (MMS), while the *apn1*Δ *apn2*Δ double mutant is much more sensitive to MMS than the *apn1*Δ mutant (10,11,14). These results confirm the earlier conclusion that Apn1 is the major APE in *S.cerevisiae*, and that Apn2 has a back-up role in repair of AP sites in the absence of Apn1.

Fission and budding yeasts diverged early in evolution and often have distinct functional differences. Phylogenetically, the fission yeast *Schizaosaccharomyces pombe* is generally believed to be closer to mammalian cells than *S.cerevisiae*, particularly in functions of many regulatory pathways (15,16). However, the BER pathway has not been studied in detail. Although the candidate genes for Apn1 and Apn2 were identified in the *S.pombe* genome, and the Apn1 gene was cloned (17), the APE activity in the recombinant protein was not shown, presumably because of the presence of an intron in the genomic clone. Although Uve1, a UV photoproduct-specific endonuclease identified in *S.pombe*, but not *S.cerevisiae*, was shown to possess APE activity (18), attempts to demonstrate APE activity in cell-free extracts of *S.pombe*

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Table 1. *Schizosaccharomyces pombe* strains used in this study

Strain	Genotype	Source
PO4	*leu1-32 h⁻*	TCP1 Invitrogen
PO10	*his4-239 ura4-D18 h⁻*	M. Sipiczki
PO20	*lys4-95 ura4-D18 h⁺*	M. Sipiczki
PO29	*arg3-D 4ura4-D18 leu1-32 ade7-152 h⁻*	V. Bashkirov
PO24	*apn2::ura4⁺lys4-95 leu1-32 h⁺*	This study
PO61	*apn1::ura4⁺his4-239 h⁻*	This study
PO60	*apn1::ura4⁺apn2::ura4⁺leu1-32 his4-239 h⁺*	This study
PO89	*uve1::ura4⁺ ura4-D18 ade6-210 leu1-32 h⁻*	P. Doetsch
PO74	*apn1::ura4⁺apn2::ura4⁺uve1::ura4⁺ his4-239 h⁺*	This study
PO90	*apn2::ura⁺4 uve1::ura4⁺h⁺*	This study

were unsuccessful (17). Although Apn2 was identified in the *S.pombe* genome database (10,11), its repair activity was not shown. Based on these observations, it was suggested that *S.pombe* does not have active APE (17). We show in this report that *S.pombe* does possess active Apn1 and Apn2 in addition to Uve1. Furthermore, we show a distinct difference in APE activity of the fission and budding types in that, in contrast to the situation in *S.cerevisiae*, Apn2 is the major APE in *S.pombe*. Moreover, the UV photoproduct-specific Uve1, present in *S.pombe* but not in *S.cerevisiae*, provides back-up activity along with Apn1 in repair of AP sites.

MATERIALS AND METHODS

Strains and media

The genotypes of strains used in this study are listed in Table 1. Standard genetic techniques were used for construction and growth of strains (19). *Schizosaccharomyces pombe* were routinely grown on yeast extract medium (YE) or EMM minimal medium containing appropriate supplements (19).

Deletion of *apn1* and *apn2* genes

A microhomology-mediated PCR targeting method was used (20), following PCR-mediated generation of the entire *ura4⁺* gene cassette flanked by 80 bp segments from the 5′ and 3′ regions of the gene to be disrupted. Oligonucleotides used to generate disruption cassettes for *apn1⁺* are: 'DEL1', TAC TTG ATG AGT CAA GAA GAA GAA ATT AAA GAA AGT TCT CCT GAA AAG GAC CTT AAA CAA GAG GAT TCTGAG GAA AAG CTT AGC TAC AAA TCC CAC TGG C; and 'DEL2', AAC AGC GTT GCT CAT GGG AAA TGG TAA AAT GAT AAA ATT ATA AGT AAT GTA TCG TTA TAT GAT TAA AAA TTC CGT ATT CCA GTG AAG CTT GTG ATA TTG ACG AAA; and for *apn2⁺* are 'DELAPN2F', TTT AGA AAG AAT CAT TAG ATT GTT TAC AGT TGT AAC TCA ATA TTA GTC AAT CTA GAA TTT TTT TGA AAA TCA ACA TTC AAA AGC TTA GCT ACA AAT CCC ACT GGC; and 'DELAPN2B', GTC CGG TGT GCA GAA GAA AAG TAC ATC CAA ACA AAG TTA TAT GTT TGG AAA TGA TGC TCG GAT CTC AAA AAA AGA AAT CTA AGC TTG TGA TAT TGA CGA AAC TTT. Following transformation of haploid strains *his4 ura4-D18 h⁻* (PO10) and *lys4 ura4-D18h⁺* (PO20), *ura⁺* progeny colonies were screened for the correct integration pattern by diagnostic PCR using primer pairs spanning the presumptive recombination sites, and used for further genetic crossing. The genotype of resulting progeny was verified by PCR analysis.

MMS and phleomycin D1 treatment

Cells were grown overnight in YE or EMM medium for survival studies after treatment with MMS and phleomycin D1 (Zeocin; Invitrogen, Carlsbad, CA). The cells were washed with distilled water and resuspended (2×10^7/ml) in phosphate-buffered saline (PBS) before addition of the appropriate amount of MMS. Aliquots were removed at the indicated times and, after inactivating MMS with 10% Na-thiosulfate, the suspensions were serially diluted and plated on solid growth media. In separate studies, the yeast cells were treated with various concentrations of phleomycin D1 at 30°C for 1 h with shaking. Cell suspensions were then diluted 10-fold with cold water, and appropriate dilutions were plated on YEA for viability determination. In all cases, colonies were counted after 4–6 days of growth at 30°C. The data shown here are representative of three independent experiments.

Assay of MMS-induced *can*^{r} mutagenesis

Cells grown overnight in YE medium were washed with PBS, treated with MMS for 30 min and then appropriate dilutions were spread on YEA plates for measuring viability, and also on EMM plates containing glutamate as the sole nitrogen source, and 75 μg/ml canavanine for *can*^{r} colonies which were counted after 8 days of incubation at 30°C (21).

Growth inhibition assay

The agar diffusion assay for examining complementation (22) was used with the following modification. Transformed cells were grown overnight in EMM medium lacking leucine and containing thiamine, and 5 μl (10^6 cells) aliquots were streaked in parallel lines on a thiamine-containing EMM plate (square-form Petri dish). Then 10% MMS (v/v)-soaked filter paper strips were placed orthogonally on the streaks. After incubation for 3 days at 30°C, the growth inhibition zone was measured. The data represent results of an average of three or more experiments.

Plasmids

The wild-type *apn1* cDNA was generated by PCR amplification of an *S.pombe* cDNA library (Machmaker Library,

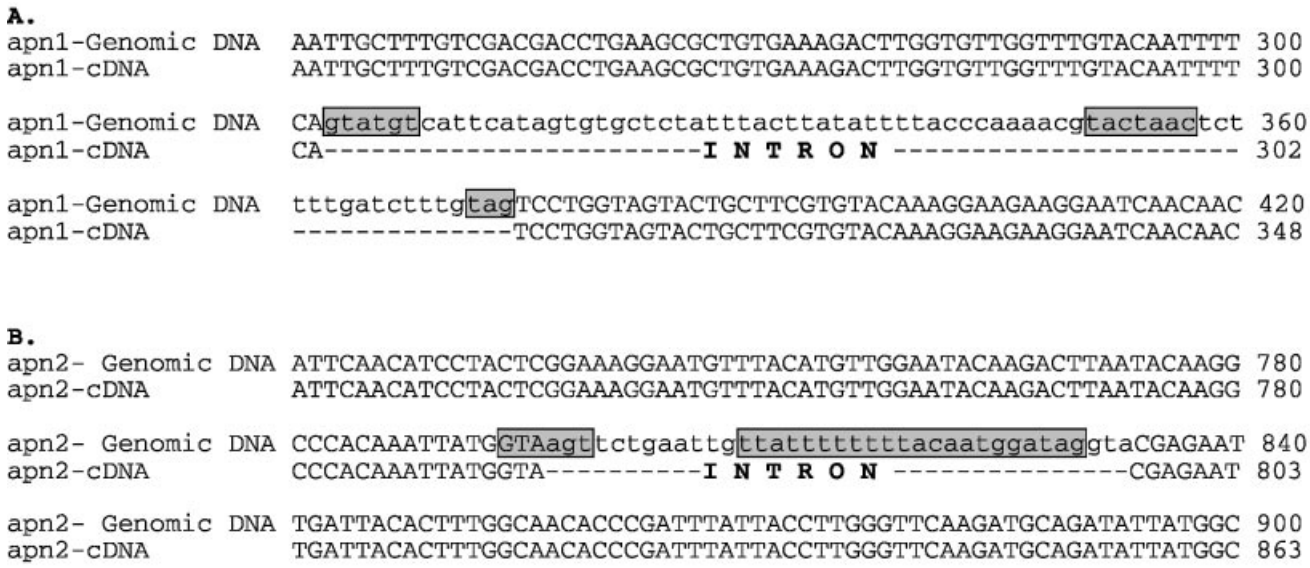


Figure 1. Intron-containing region of the *apn1*$^+$ (**A**) and *apn2*$^+$ (**B**) genes. The intron sequences are shown in lower case. The consensus sequences for the 5′ splice, branch and 3′ splice sites are boxed [(17) and http://www.sanger.ac.uk/Projects/S_pombe].

Clontech, Palo Alto, CA). *apn2* cDNA was cloned by RT–PCR amplification using total RNA isolated (23) from wild-type *S.pombe* (Perkin Elmer GENE Amp RNA PCR Kit). The amplified insert was cloned into pNMT1-TOPO vector in-frame with a His_6 tag (Invitrogen, Carlsbad, CA). The recombinant plasmids were checked by PCR with primer pairs flanking the intron region, in order to distinguish cDNA clones from those resulting from contaminating genomic DNA or non-spliced mRNA. After confirming the cDNA sequence, the expression plasmids pNBR117 for Apn1 and pNBR110 for Apn2 were generated. Two point mutations in the putative proliferating cell nuclear antigen (PCNA)-binding motif (F402A and F403A), and three point mutations in the putative TOP3-binding domain (P456A, L457A and C458A) were generated in the Apn2 cDNA in pNBR110 by PCR using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The following oligonucleotide primers were used. For PCNA: F402A, F403A (PCN-F-5′ TCG AAG CTT CTT TCA *GCC GCC* GCG AAG CAA AAG GAA 3′, PCN-B-5′ TTC CTT TTG CTT CGC GGC GGC TGA AAG AAG CTT 3′) and for TOP3: P456A, L457A, C458A (TOP-F-5′ AGT GGA CGA GCT CCT *GCC GCC GCC* GAG GGT CAC AAG GAA 3′, TOP-B-5′ TTC CTT GTG ACC CTC GGC GGC GGC AGG AGC TCG TTC ACT 3′).

The authenticity of the mutant plasmids, named pNBR114 (*apn2A*^{402}*A*^{403}) and pNBR115 (*apn2A*^{456}*A*^{457}*A*^{458}), was verified by sequencing.

To over-express GST–Apn2 fusion protein, we subcloned the Apn2 cDNA from pNBR110 into pESP1 vector (Stratagene, La Jolla, CA) using PCR amplification, and generated plasmid pEBR116.

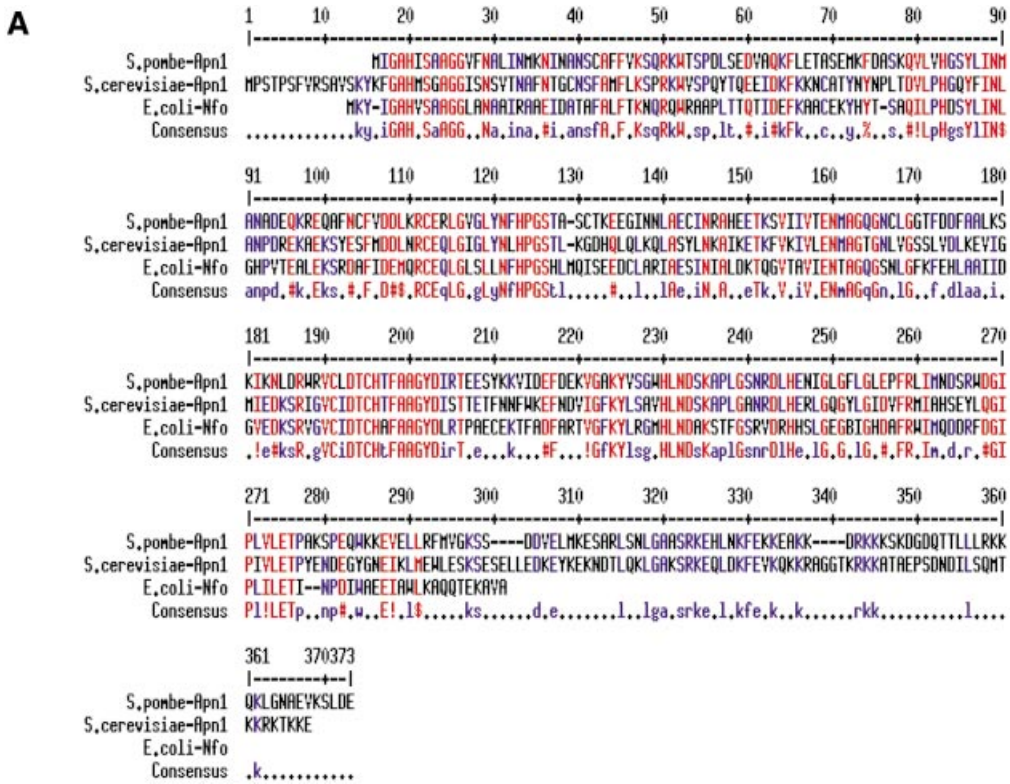
The human APE1 and APE2 cDNAs were gifts of Drs S. Seki and K. Akiyama, Okayama University, Japan. The APE1 cDNA was subcloned into pREP3XHIS vector (23) using BamHI and SalI restriction sites, to produce pRBR113. The cDNA of APE2 was subcloned into pNMT1-TOPO vector using PCR amplification to generate pNBR103.

Expression and purification of *S.pombe* Apn2

The GST–Apn2 fusion protein was over-expressed from the plasmid pEBR116 in the *S.pombe apn1*Δ*apn2*Δ mutant (PO60). Cells were grown to stationary phase in EMM medium containing 5 μM thiamine and lacking leucine. The culture was washed three times with water and diluted in fresh EMM medium to an OD_{600} of 0.05. After additional incubation for 16 h at 30°C with shaking (180 r.p.m.), the cells were collected by centrifugation, and resuspended in cell breakage buffer containing 0.3 M sorbitol, 0.1 M NaCl, 5 mM $MgCl_2$, 10 mM Tris–HCl pH 7.4, with protease inhibitor mixture (Mini-Complete, Roche, Indianapolis, IN), and disrupted in a French press. After removing cell debris by centrifugation (20 000 *g*, 30 min) and filtration, the extract was loaded on a gluthatione–Sepharose 4B column [Amersham Pharmacia (12)] which was then washed extensively with cell breakage buffer supplemented with 1 M NaCl. The GST–Apn2 protein was finally eluted from the column with the buffer containing 20 mM gluthatione. The peak fractions containing GST–Apn2 were pooled, and concentrated in a Centricon 30 concentrator.

Assay of AP-endonuclease

A 75mer synthetic nucleotide, 5′-AGC TAC CAT GCC TGC CTC AAG AGT TCG TAA XAT GCC TAC ACT GGA GTA CCG GAG CAT CGT CGT GAC TGG GAA AAC-3′, containing a single tetrahydrofuran (THF), an AP site analog, at position 31 (indicated by X), was purchased from IDT (12). The complementary 75mer contained C opposite THF. The THF-containing oligo was ^{32}P labeled at the 5′ end, using T4 polynucleotide kinase and [γ-^{32}P]ATP, and then annealed to the complementary oligo before using in an APE assay. In Figure 7, a 43mer THF-containing oligo was used as before



(24). The assay mixture (10 µl) contained 20 mM Tris–HCl pH 7.4, 2 mM $MgCl_2$, 0.5 mM EDTA, 30 mM KCl, 0.1 mg/ml bovine serum albumin (BSA) and 1 mM dithiotreithol (DTT). ^{32}P-labeled duplex oligo (50 fmol) was incubated with 300 ng of GST–Apn2, at 30°C for various times. After stopping the reaction by addition of formamide/EDTA gel loading buffer, the cleaved oligo was separated by electrophoresis in a denaturing polyacrylamide gel (12%) containing 7 M urea, and the radioactivity was quantitied by PhosphorImager analysis using the ImageQuant Software (Molecular Dynamics, Inc., Sunnyvale, CA).

RESULTS

Existence of an intron in the *apn1* gene of *S.pombe*, and cloning of Apn1 cDNA

Ramotar *et al.* (17) predicted the presence of a 72 bp intron sequence in the *apn1* gene of *S.pombe*, flanked by 5′- and 3′-splice sites, which is in-frame with the open reading frame (ORF) of the putative coding sequence. To confirm the coding sequence, we cloned Apn1 cDNA from a cDNA library as described in Materials and Methods. The cDNA contained an ORF with 1029 bp, and lacked the intron (Fig. 1). Apn1, with predicted M_r of 40 kDa, shares 46% identity with *S.cerevisiae* Apn1 and 43% with *E.coli* Nfo (Fig. 2A) (25).

The X-ray crystallographic structure of *E.coli* Nfo showed it to be a single domain protein with eight parallel β-strands surrounded by eight peripheral α-helices. Its active site contains a trinuclear Zn center (26). These residues and the flanking sequences are highly conserved in *S.pombe* Apn1 and other members of the Nfo family.

Cloning of *S.pombe* Apn2 cDNA

The *apn2/xth*-like sequence (SPBC3D6.10) was identified in the *S.pombe* genome database by homology search using human APE1 (10,11,27). Using PCR, we isolated cDNA clones containing the predicted ORF from the Machmaker cDNA library. As in the case of Apn1, we identified a putative intron sequence, based on the genomic sequence, by using primer pairs flanking the intron region. Surprisingly, all (>40) clones were found to contain the intron sequence, which could have been derived from either genomic DNA contamination or unspliced transcripts. It is clear that the intron-containing cDNA does not encode active APE because of the presence of an in-frame stop codon in the intron. We then succeeded in cloning mature cDNA, by using RT–PCR of total RNA, although nearly half of the clones still contained the intron sequence. The Apn2 cDNA contains 1572 bp, corresponding to a polypeptide with 523 amino acid residues and predicted M_r of 58 kDa (Fig. 1).

Schizosaccharomyces pombe Apn2 is a member of the Xth family

Schizosaccharomyces pombe Apn2 shares several conserved and essential residues with *E.coli* Xth, human APE1 and *S.cerevisiae* Apn2 (Fig. 2B.) (10,11). The hAPE1 polypeptide with 318 amino acid residues contains two distinct domains, of which the N-terminal domain is dispensable for the endonuclease activity, but is required for its regulatory function (24).

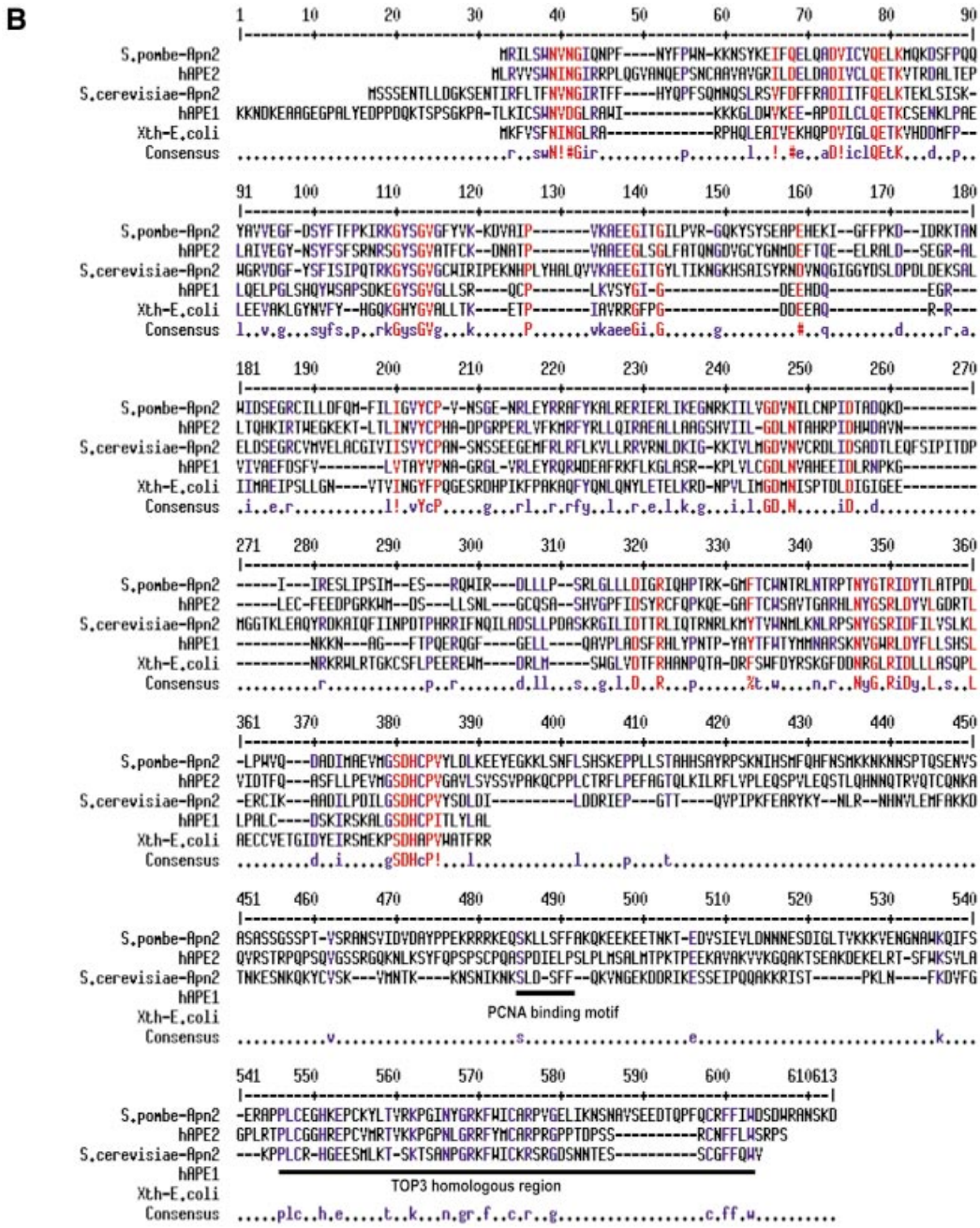


Figure 2. (**A**) Apn1 sequence alignment of *S.pombe* and *S.cerevisiae* Apn1with Nfo of *E.coli*. (**B**) Apn2 sequence alignment of *S.pombe* and *S.cerevisiae*, human APE2, human APE1 and *E.coli* Xth proteins. Identical and highly conserved residues are indicated in red. Spaces indicate gaps introduced to optimize alignment by the MultiAlin program (23): http://prodes.toulouse.inra.fr/multalin/multalin.html. GenBank accession numbers AY483157 for *apn1*$^{+}$ and AY483158 for *apn2*$^{+}$.

This region is absent in yeast Apn2 (Fig. 2B). The C-terminal sequence of APE1, essential for the endonuclease activity, is homologous to Apn2 (24,28).

The C-terminal regions of yeast Apn2/hAPE2-type proteins contain a PCNA-binding motif (29) and a sequence homologous to one present in the C-terminal region of the DNA topoisomerase III (TOP3) family (29,30). The C-terminal region of *S.cerevisiae* Apn2 is not required for its APE activity *in vitro*, but is required for *in vivo* repair of AP sites (12). Unk *et al*. subsequently showed physical and functional interaction of Apn2 with PCNA, which stimulates the 3′-phophodiesterase activities of Apn2 *in vitro* (31).

Apn1 and Apn2 are not essential proteins

The *apn1* and *apn2* mutants of *S.pombe* showed no obvious phenotype with regard to growth rate or viability, which indicates that both *apn1* and *apn2* are dispensable under normal conditions, as was also observed for *S.cerevisiae* (11).

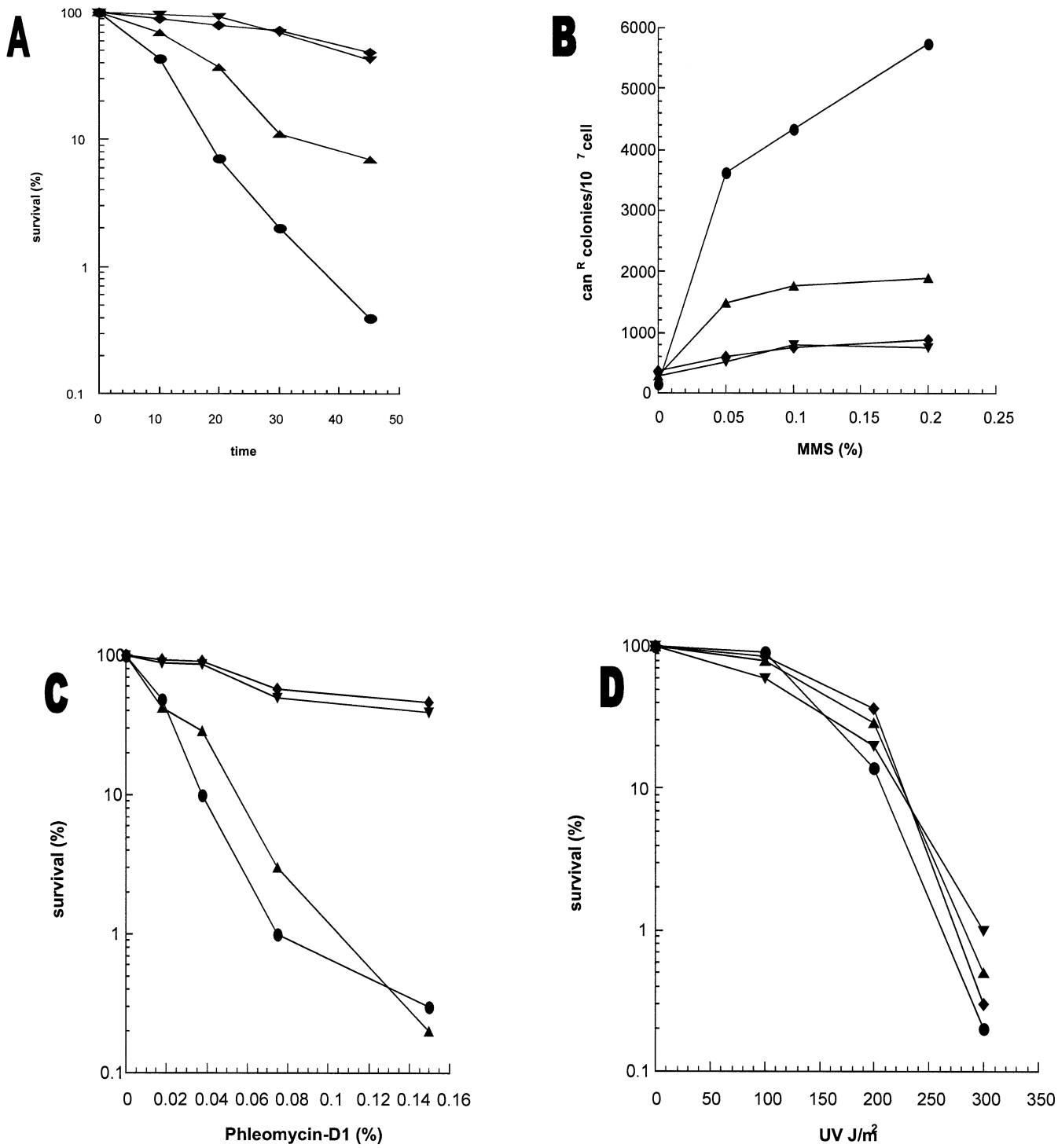


Figure 3. (**A**) MMS sensitivity of *apn1*Δ and *apn2*Δ strains. Cells grown overnight in YEL medium were treated with 0.2% MMS for various times. (**B**) MMS-induced mutagenesis at the *can1* gene of *S.pombe*. The details are described in Materials and Methods. (**C**) Phleomycin D1 toxicity. Cells grown overnight in YEL medium were treated with phleomycin D1 for 1 h before spreading on YEA plates. (**D**) UV sensitivity of *S.pombe apn1*Δ and *apn2*Δ mutant strains. Sensitivity of the strains to UV was measured after exposure to various UV doses. PO29, wild type (diamonds); PO61, *apn1*Δ (inverted triangles); PO24, *apn2*Δ (upright triangles); PO60, *apn1*Δ*apn2*Δ (circles).

MMS sensitivity of *apn* mutants

APEs play a central role in repair of methylated bases, e.g. 3-methyladenine and 7-methylguanine which are induced by MMS (32). Excision of the alkyl base adducts with mammalian *N*-methylpurine-DNA glycosylase, MPG (32), and *mag1* in *S.pombe* (33), should generate AP sites whose repair requires APE. Thus MMS sensitivity of APE mutants is

a measure of the *in vivo* repair activity of APEs. Figure 3A shows that the *apn2*Δ mutant was moderately more sensitive to MMS than the wild type, while *apn1*Δ mutation alone had no effect on MMS sensitivity. However, the *apn1*Δ*apn2*Δ double mutant showed significantly higher sensitivity to MMS than the *apn2*Δ mutant.

These results strongly suggest that Apn2 and not Apn1 plays a rate-limiting role in protecting cells from alkylation damage. However, in the absence of Apn2, Apn1 provides protection and has a minor role in repair of AP sites. Thus there is an unexpected role reversal of Apn1 and Apn2 in *S.pombe* relative to *S.cerevisiae* in which Apn2 is completely dispensable in alkylation damage repair in the presence of Apn1 (11).

MMS-induced mutagenesis in the *apn1*Δ *apn2*Δ mutant

Because non-instructional AP sites are highly mutagenic, a deficiency in APE leads to enhanced mutation frequency after treatment with genotoxic agents such as MMS (34). We determined the frequency of MMS-induced forward mutation to canavanin resistance (*can*^{r}*1*) in *apn* mutants. Figure 3B shows that the *apn1*Δ mutant and wild-type strain had a similar mutagenic response, while significant enhancement in frequency of *can*^{r}*1* mutation was observed in the *apn2*Δ mutant; the frequency was even higher in the *apn1*Δ*apn2*Δ double mutant. These mutagenesis data, which mirror the survival data, clearly show that the APE activity of Apn2, and to a lesser extent Apn1, is needed to repair AP sites and prevent mutations.

Sensitivity of the *apn2*Δ mutant to phleomycin D1

We assessed the relative 3′-phosphodiesterase activity of Apn1 and Apn2 *in vivo* by examining the sensitivity of various *S.pombe* mutants to phleomycin D1, a glycopeptide antibiotic structurally similar to bleomycin. These antibiotics react with deoxyribose to cleave phosphodiester bonds, and generate 3′-phosphoglycolate, and oxidized AP sites (1). Figure 3C shows that mutation in *apn1* had no effect on phleomycin sensitivity, while the *apn2*Δ mutant or the *apn1*Δ*apn2*Δ double mutant was ~3-fold more sensitive to this drug than the wild-type strain. In contrast, *apn2*Δ mutation did not sensitize *S.cerevisiae* at all to the antibiotic (10). These results strongly suggest that Apn2 is the major contributor of not only APE but also DNA 3′-phosphodiesterase activity in *S.pombe*.

Inactivation of an alternative excision repair pathway enhances sensitivity of the *apn2*Δ mutant to alkylation damage

Uve1, expressed in *S.pombe* but absent in *S.cerevisiae*, was shown to repair UV photoproducts on the basis of its ability to phenotypically complement UV sensitivity of the *E.coli uvrA recA phr* mutant (35,36). In addition to its function as a mismatch-, and UV photoproduct-specific endonuclease (18,21,37), Uve1 possesses APE activity (18). We tested the contribution of Uve1 in repair of AP sites by measuring MMS and UV sensitivity of *apn* and *uve1* mutants. The observed lack of UV sensitivity in *apn1*Δ and *apn2*Δ mutants was expected because UV damage repair does not involve BER in which Apns play a key role (Fig. 3D). The *uve1*Δ mutant was shown to be moderately sensitive to UV, which is consistent with its role in UV damage repair (38). On the other hand,

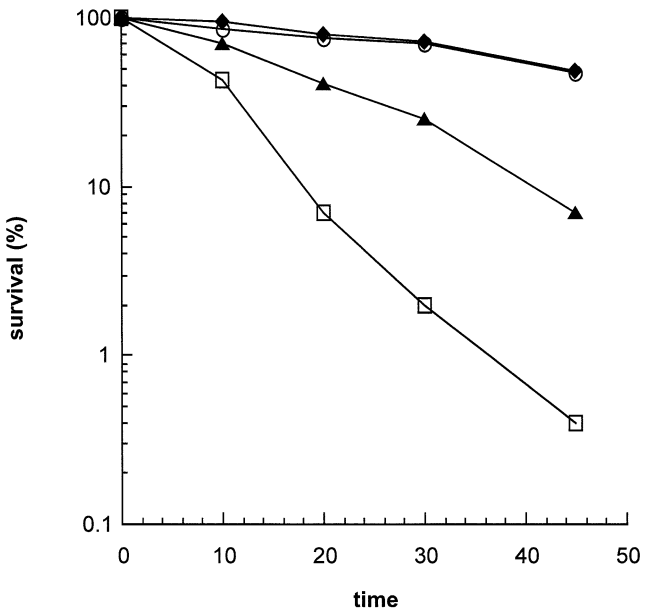


Figure 4. Effect of *uve1*Δ mutation on MMS sensitivity of the *apn2*Δ mutant. Other details are as in Figure 3. PO29, wild type (diamonds); PO24, *apn2*Δ (triangles); PO89, *uve1*Δ (open circles); PO90, *apn2*Δ*uve1*Δ (open squares).

mutation in *uve1* did not sensitize *S.pombe* cells to MMS (Fig. 4D). However, this mutation acted like *apn1* mutation in enhancing MMS sensitivity of the *apn2*Δ mutant. This indicates that both Uve1 and Apn1 provide back-up functions in repairing AP sites in the genome.

Phenotypic complementation of the *apn1*Δ*apn2*Δ mutant with *S.pombe* Apn2 and human APE1

We tested phenotypic complementation of the *apn1*Δ*apn2*Δ mutant with *S.pombe* Apn2 or human APE1, using their expression plasmids in pNMT1 and pESP1 vectors with the thiamine-repressible *nmt1* promoter, as described in Materials and Methods. Leaky expression of these proteins was observed in the presence of thiamine (39). In any case, as shown in Figure 5A, both Apn2 and human APE1 restored MMS resistance of the *apn1*Δ*apn2*Δ mutant to the wild-type level. In contrast, human APE2 or *S.pombe* Apn1 could not complement *apn1 apn2* deficiency (Fig. 5A and B). We conclude from these results that *S.pombe* Apn2 and human APE1 have a similar range of activity in repairing AP sites, and hence human APE1 could fully complement Apn2 deficiency.

PCNA-binding and TOP3-homologous region in Apn2 are dispensable for *in vivo* repair of alkylation damage

The C-terminal region in *S.cerevisiae* Apn2 contains a PCNA-binding motif which was shown to be required for its binding to PCNA. Such binding activates the 3′-phosphodiesterase activity of Apn2 (31). Because *S.pombe* and *S.cerevisiae* Apn2 have extensive sequence homology, including the PCNA-binding and TOP3-like motifs, we tested the role of these motifs in MMS resistance of *S.pombe*. We generated alanine substitution mutants at positions 402 and 403 in the

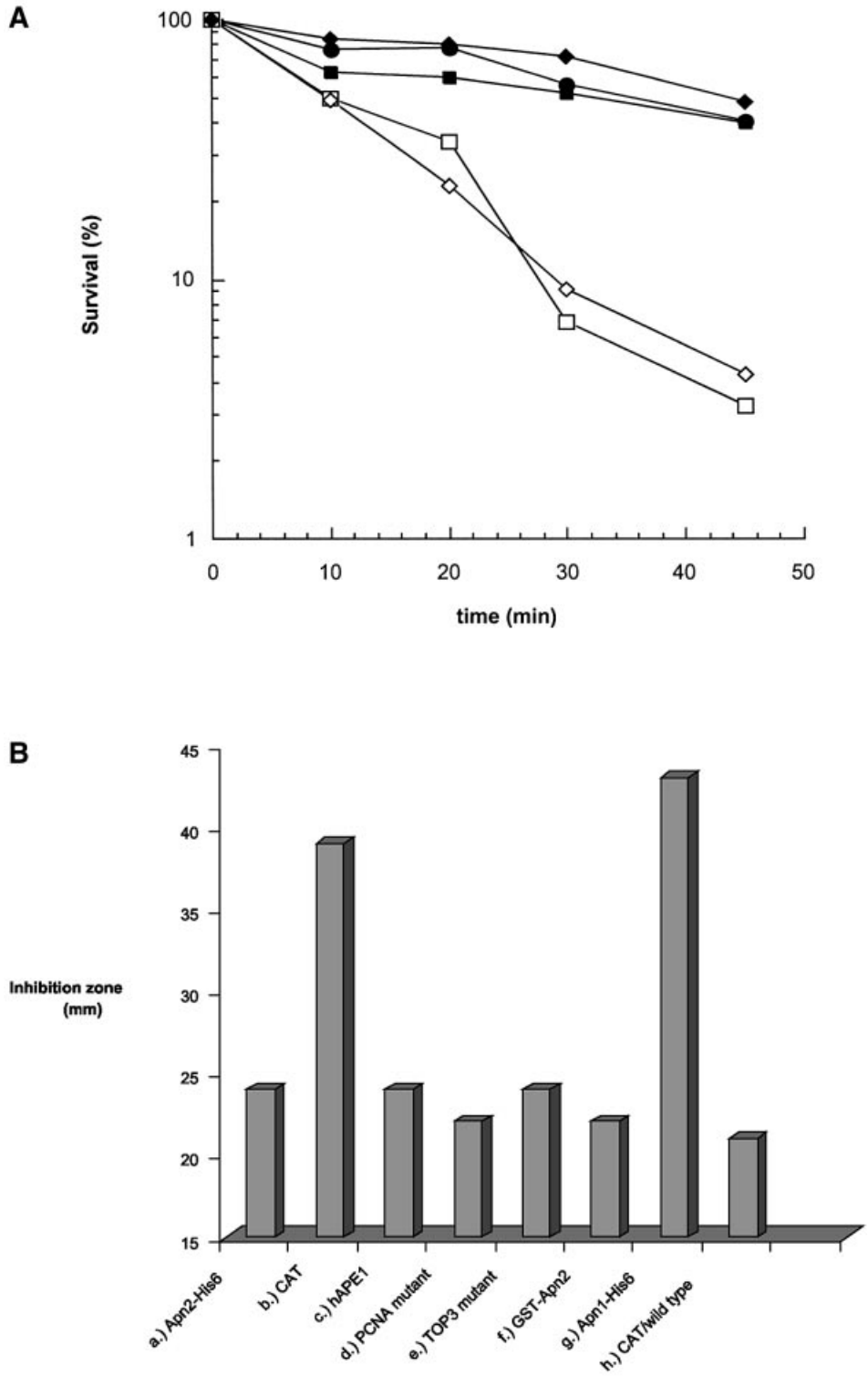


Figure 5. (**A**) Complementation of MMS sensitivity of the *apn1Δapn2Δ* (PO60) strain with wild-type *S.pombe* Apn2-His_6 (pNBR110) (filled squares); human APE1 (pRBR113) (filled circles); and human APE2 (pNBR103) (open squares); wild-type control, *leu1-32* (PO4), with control CAT expression vector (pNMT1-CAT; Invitrogen) (filled diamonds); sensitivity of *apn1Δapn2Δ*: (PO60) transformed with pNMT1-CAT (open diamonds). Other details are described in Materials and Methods. (**B**) Agar diffusion assay of complementation of *apn1Δapn2Δ* (PO60) and wild-type (PO4) strains as described in Materials and Methods. Data represent results from an average of three or more experiments. (a) Wild-type Apn2-His_6 on plasmid pNBR110 in PO60; (b) control pNMT1-CAT plasmid in PO60; (c) human APE1 on plasmid pRBR113 in PO60; (d) Apn2-$A^{402}A^{403}$ mutation (PCNA) on plasmid pNBR114 in PO60; (e) Apn2-$A^{456}A^{457}A^{458}$ mutation on plasmid pNBR115 in PO60; (f) wild-type GST–Apn2 on plasmid pEBR116 in PO60; (g) wild-type Apn1-His_6 on plasmid pNBR117 in PO60; (h) pNMT1-CAT plasmid in PO4

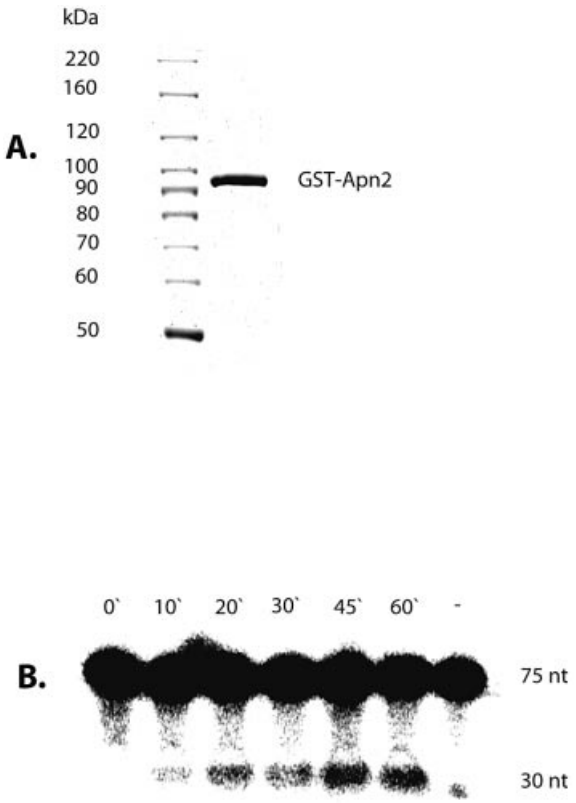


Figure 6. (**A**) Purification of GST–Apn2. Eluted GST–Apn2 fusion protein was analyzed on a 7.5% denaturing polyacrylamide gel and stained with Coomassie blue. Lane 1, molecular weight standards; lane 2, 3 μg of eluted GST–Apn2 protein. (**B**) Kinetics of APE activity of GST–Apn2; lane (–) is the no-protein control. The details are described in Materials and Methods.

PCNA-binding motif, and at positions 456–458 included in the TOP3-like motif in the Apn2 expression plasmid. These mutants were as effective in phenotypic complementation of the *apn1*Δ*apn2*Δ mutant as wild-type Apn2 (Fig. 5B). Thus, even if the binding of PCNA to Apn2 occurs *in vivo*, this interaction, unlike in the case of *S.cerevisiae*, does not modulate Apn2 activity in *S.pombe*.

APE activity of recombinant Apn2

The *in vivo* studies described so far strongly suggest that Apn2 is active *in vivo* as an APE and a 3′-phosphodiesterase. We then directly tested recombinant Apn2 for APE activity. We attempted to over-express GST–Apn2 and His_6-Apn2 fusion polypeptides in the *S.pombe apn1*Δ *apn2*Δ mutant. The recombinant Apn2-His_6 was found to be mostly insoluble (data not shown). Furthermore, overexpression of the GST–Apn2 fusion protein appeared to be toxic because the transformed *S.pombe* stopped growing and became elongated (data not shown). Nevertheless, we were able to purify the GST–Apn2 fusion polypeptide in soluble form by affinity chromatography from extracts of these cells early after transformation. GST–Apn2 showed significant APE activity with THF-containing DNA oligo as described in Materials and Methods. The formation of the predicted 30 nt product indicated that Apn2 catalyzed hydrolysis of DNA 5′ to the abasic site linearly for up to 1 h (Fig. 6B). There are several possible reasons for very low specific activity of the Apn2 fusion protein. One obvious possibility is that even though present in a soluble form, a large fraction of the protein is not correctly folded, and the folding problem was exacerbated by the presence of the GST.

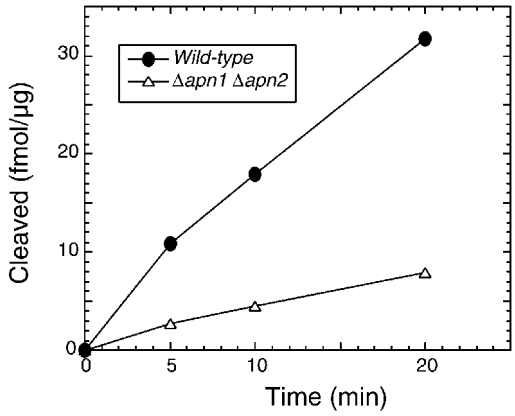


Figure 7. Analysis of APE activity in *S.pombe* extract. Wild-type and mutant cells were broken by French press and centrifuged. Crude cell extract (1 μg) was mixed with 50 fmol of the oligonucleotide containing the AP site in reaction mixture (20 μl) containing 20 mM Tris pH 8.0, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT and 100 μg/ml BSA. After incubation at 37°C, the 5′-labeled 30mer product was identified with PhosphorImager (Molecular Dynamics) after electrophoretic separation on 20% polyacrylamide gels containing 7 M urea (26,45).

AP-endonuclease activity in *S.pombe* extracts

An earlier report raised the unlikely possibility that APE is absent in *S.pombe* in spite of the presence of *apn* genes (17). We examined cell-free extracts of wild-type and mutant *S.pombe* for APE activity. The APE activity was easily detectable in the wild-type cell extract which was stable at 37°C, and maintained linearity up to 20 min to generate the expected cleavage product (Fig. 7). As expected, the total APE activity was significantly decreased in the *apn1*Δ*apn2*Δ mutant, and the residual activity could be due to Uve1 or other unidentified APEs.

DISCUSSION

Although both *S.cerevisiae* and *S.pombe* have been extensively used as model eukaryotes for elucidating various cellular processes, *S.pombe*, in spite of its closer similarity to human cells, has not been extensively investigated for DNA repair processes. Among various excision repair pathways, BER is involved in repair of endogenous oxidation and alkylation damage in which APE plays a central role. While two APEs in *S.cerevisiae*, Apn1 and Apn2, have been extensively characterized, much less is known about the Apns in *S.pombe*. One major problem in examining *S.pombe apn* genes is the presence of small introns and low efficiency of the cells to splice out these introns, especially in the case of *apn2*.

Ramotar *et al*. (17) predicted, but did not show experimentally, that the *S.pombe apn1* gene contains a 72 bp intron. We confirmed the absence of this intron in cloned Apn1 cDNA. The Apn1 of *S.pombe* shares 46% identical residues with

S.cerevisiae Apn1 which accounts for >90% of the APE/3′-diesterase activity in this yeast (11). This is consistent with the observed increase of spontaneous mutations as well as MMS sensitivity when the *APN1* gene was inactivated in the budding yeast. In contrast, deletion of the *apn1* gene in *S.pombe* did not affect its MMS sensitivity. Thus it appears that Apn1 is not the major contributor of APE activity. In our attempts to clone the *S.pombe* Apn2 cDNA, we discovered that due to inefficient splicing, the cDNA library contained mostly copies of Apn2 primary transcripts. This suggests that splicing plays a regulatory role in maintaining the Apn2 protein level. Similar regulation at the splicing level was observed for the *mes1* gene in *S.pombe* whose product is developmentally regulated (40).

Deletion of *apn1* and *apn2* genes in *S.pombe* did not affect its growth and viability. Deletion of the *apn2* gene in *S.pombe* caused MMS sensitivity, in contrast to a lack of phenotype of *APN2* mutant in *S.cerevisiae*. Although *apn1*Δ mutation alone did not confer sensitivity to MMS in *S.pombe*, it enhanced MMS sensitivity of the *apn2*Δ mutant. These results strongly argue for the primary role of Apn2 in the removal of AP sites, and indicate that Apn1 provides the back-up activity in the absence of Apn2 in the fission yeast. An analogous situation was observed in *E.coli* in which *nfo* mutants do not show increased sensitivity to MMS, unlike the *xth* mutant. Xth is the major APE in *E.coli* and Nfo appears to provide the back-up activity. Accordingly, the *nfo/xth* double mutant is more sensitive to alkylating and oxidizing agents than the *xth* mutant (41). As expected, inactivation of *apn1* and *apn2* did not sensitize the cells to UV (254 nm, 300 J/m^2) because the BER pathway is not involved in repair of UV-induced pyrimidine photoproducts.

Structure modeling shows that Uve1 and *E.coli* Nfo have similar secondary structure (42). Even though *in vitro* studies have indicated that Uve1 generates DNA strand breaks at AP sites (18,43), its function in BER was unknown. The present studies provide the first evidence for the ability of Uve1 to repair AP sites *in vivo*. As in the case Apn1, inactivation of Uve1 alone did not confer sensitivity to MMS. However, deletion of the *uve1* gene in the *apn2*Δ mutant significantly increased MMS sensitivity, suggesting that Uve1 functions independently of Apn2 in removal of AP sites to enhance cell survival. A low level of APE activity was detected in the extract of a *apn1*Δ*apn2*Δ*uve1*Δ triple mutant, suggesting the presence of other unidentified APEs in *S.pombe* (data not shown). Interestingly, the *apn1*Δ*apn2*Δ*uve1*Δ triple mutant was no more sensitive to MMS than the *apn2*Δ*uve1*Δ double mutant (unpublished observation). This suggests a common downstream pathway in repair of AP sites initiated by Uve1 and Apn1, which is distinct from the Apn2-initiated process. In any case, Apn2 appears to be the predominant APE in *S.pombe* while both Uve1 and Apn1 provide the back-up functions in BER. However, it should be noted that it was recently shown that the *apn1*Δ*apn2*Δ double mutant of *S.cerevisiae* is viable but not in the absence of Rad1/Rad10. This suggests alternative repair of AP sites involving this nucleotide excision repair-specific endonuclease (44). An analogous situation may be present in *S.pombe*.

An earlier attempt to demonstrate APE activity in *S.pombe* extracts was unsuccessful (17). Because the present studies predict the presence of APE activity in Apn polypeptides, we have shown directly that the recombinant Apn2 is an authentic APE. We were also able to measure APE activity in *S.pombe* extracts, in contrast to an earlier report (17). Although the reason for the discrepancy between the previous study and our experiments is not clear, it could be due to a difference in extract preparation. While we detected the APE activity in extract produced in a French press, the extract prepared with beating beads was inactive (data not shown). It is likely that Apns are easily inactivated during preparation. More importantly, a significant amount of APE activity in the *apn1*Δ *apn2*Δ double mutant could be due to Uve1, and other unknown APEs. Thus our results provide the first evidence for APE-dependent BER in the fission yeast, in line with other organisms.

The *E.coli* Xth family of APEs, including yeast Apn2 and human APE1, share critical active site motifs. A newly discovered human APE, hAPE2, shares significant sequence homology with yeast Apn2 (30). Although the recombinant hAPE2 was reported to have very weak APE activity (30), we were unable to detect such activity in the recombinant protein purified from insect cells (unpublished experiment). We examined the *in vivo* activity of hAPE1 and hAPE2 in a phenotypic complementation assay using the *S.pombe* *apn1*Δ*apn2*Δ mutant. Expression of hAPE1 in the mutant was indicated by enhanced APE activity in cell-free extract (data not shown), and increased MMS resistance of the mutant. On the other hand, no complementation was observed when hAPE2 was expressed in this mutant strain (Fig. 5A). Thus, while hAPE2 may have some unknown enzymatic activity, it does not appear to function as an APE *in vivo*. The reason for the lack of complementation of the *apn1*Δ *apn2*Δ mutant with *S.pombe* Apn1 is not obvious. However, it is possible that Apn1 does not possess the same level of distinct activities of Apn2 or hAPE1 needed for repair of MMS-induced damage. Furthermore, the low sensitivity of the growth inhibition assay may mask the small extent of complementation with Apn1.

The C-terminal regions in proteins of the APE2 family contain a consensus sequence for a PCNA-binding motif that was shown to be functional in human APE2 (29). Unk *et al.* (12) reported that the *C*-terminal region of Apn2 of *S.cerevisiae* is dispensable for its APE activity *in vitro*, but not for *in vivo* suppression of MMS sensitivity of the *apn1*Δ*apn2*Δ mutant. In contrast, we found in *S.pombe* that mutation in the putative PCNA-binding and TOP3-type motifs of Apn2 did not affect MMS resistance. These results suggest that the PCNA-binding and TOP3-type motifs of Apn2 in *S.pombe* are not directly involved in the repair of abasic sites *in vivo*.

Phleomycin D1 produces oxidized AP sites and strand breaks with 3′-phosphoglycolate termini which prevent DNA replication or repair by DNA polymerases (1). In *S.cerevisiae*, the *apn1*Δ*apn2*Δ double mutant, but not the single mutants, displays enhanced sensitivity to phleomycin D1, indicating that these enzymes functionally overlap in repair of oxidized AP sites and in the repair of strand breaks with 3′-terminal lesions (10). We have shown here that Apn2 contributes significantly to repair of phleomycin D1-induced DNA damage in *S.pombe*, based on enhanced sensitivity of the *apn2* mutant to the drug (Fig. 4). The sensitivity did not increase further when cells were also deficient in *apn1*. These

results and identification of 3′-phospodiesterase and 3′–5′ exonuclease activities in *S.cerevisiae* Apn2 strongly suggest that *S.pombe* Apn2 possesses both of these activities.

In summary, the present study provides the first evidence for the presence of active APEs in *S.pombe*, and for distinct functions of these APEs in *S.pombe* and *S.cerevisiae*. In the fission yeast, as in mammals but unlike in *S.cerevisiae*, Apn2, an APE1 ortholog, is the major APE/3′-phosphodiesterase for repairing DNA damage caused by oxidation and alkylation. Furthermore, at least three APE genes, *apn1*, *apn2* and *uve1*, are functional *in vivo*. Whether expression of these genes is regulated by ROS, and how the proteins interact with other BER components needs to be addressed in order to elucidate the BER process in the fission yeast.

ACKNOWLEDGEMENTS

We thank Dr D. Konkel for careful editing of the manuscript, Drs V. Bashkirov, P. Doetsch, W. Heyer and M. Sipiczki for *S.pombe* strains, and Drs. S. Seki and K. Akiyama for the human APE1 and APE2 cDNA plasmids. This work was supported by USPHS grants R01 CA53791, ES08457 and P50 ES06676. B.R. is grateful to the Soros Foundation for a Fellowship award and to the Hungarian Science and Technology Foundation, for a travel grant.

REFERENCES

1. Breen,A.P. and Murphy,J.A. (1995) Reactions of oxyl radicals with DNA. *Free Radical Biol. Med.*, **18**, 1033–1077.
2. Mitra,S., Hazra,T.K., Roy,R., Ikeda,S., Biswas,T., Lock,J., Boldogh,I. and Izumi,T. (1997) Complexities of DNA base excision repair in mammalian cells. *Mol. Cell*, **7**, 305–312.
3. Haracska,L., Unk,I., Johnson,R.E., Johansson,E., Burgers,P.M.J., Prakash,S. and Prakash,L. (2001) Roles of yeast DNA polymerases δ and ζ and of Rev1 in the bypass of abasic sites. *Genes Dev.*, **15**, 945–954.
4. Lindahl,T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.
5. Nakamura,J. and Swenberg,J.A. (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res.*, **59**, 2522–2536.
6. Lindahl,T., Barnes,D.E., Klungland,A., Mackenney,V.J. and Schar,P. (1997) Repair and processing events at DNA ends. *Ciba Found. Symp.* **211**, 198–205.
7. Mitra,S., Izumi,T., Boldogh,I., Bhakat,K.K., Hill,J.W. and Hazra,T.K. (2002) Choreography of oxidative damage repair in mammalian genomes. *Free Radical Biol. Med.*, **33**, 15–28.
8. Demple,B. and Harrison,L. (1994) Repair of oxidative damage to DNA: enzymology and biology. *Annu. Rev. Biochem.*, **63**, 915–948.
9. Popoff,S.C., Spira,A.I., Johnson,A.W. and Demple,B. (1990) Yeast structural genes (APN1) for the major apurinic endonuclease: homology to *Escherichia coli* endonuclease IV. *Proc. Natl Acad. Sci. USA*, **87**, 4193–4197.
10. Bennett,R.A. (1999) The *Saccharomyces cerevisiae* ETH1 gene, an inducible homolog of exonuclease III that provides resistance to DNA-damaging agents and limits spontaneous mutagenesis. *Mol. Cell. Biol.*, **19**, 1800–1809.
11. Johnson,R.E., Torres-Ramos,C.A., Izumi,T., Mitra,S., Prakash,S. and Prakash,L. (1998) Identification of APN2, the *Saccharomyces cerevisiae* homolog of the major human AP endonuclease HAP1 and its role in the repair of abasic sites. *Genes Dev.*, **12**, 3137–3143.
12. Unk,I., Haracska,L., Johnson,R.E., Prakash,S. and Prakash,L. (2000) Apurinic endonuclease activity of yeast Apn2 protein. *J. Biol. Chem.*, **275**, 22427–22434.
13. Unk,I., Haracska,L., Prakash,S. and Prakash,L. (2001) 3′-Phosphodiesterase and 3′→5′ exonulcease activities of yeast Apn2 protein and requirement of these activities for repair of oxidative DNA damage. *Mol. Cell. Biol.*, **21**, 1656–1661.
14. Ramotar,D., Popoff,S.C., Gralla,E.B. and Demple,B. (1991) Cellular role of yeast Apn1 apurinic endonuclease/3′-diesterase: repair of oxidative and alkylation DNA damage and control of spontaneous mutation. *Mol. Cell. Biol.*, **11**, 4537–4544.
15. Forsburg,S.L. (1999) The best yeast? *Trends Genet.*, **15**, 340–344.
16. Sipiczki,M. (2000) Where does fission yeast sit on the tree of life? *Genome Biol.*, **1**, 1011.
17. Ramotar,D., Vadnais,J., Masson,J.Y. and Tremblay,S. (1998) *Schizosaccharomyces pombe* apn1 encodes a homologue of the *Escherichia coli* endonuclease IV family of DNA repair proteins. *Biochim. Biophys. Acta*, **1396**, 15–20.
18. Kanno,S., Iwai,S., Takao,M. and Yasui,A. (1999) Repair of apurinic/apyrimidinic sites by UV damage endonuclease; a repair protein for UV and oxidative damage. *Nucleic Acids Res.*, **27**, 3096–3103.
19. Alfa,C., Fantes,P., Hyams,J., Mcleod,M. and Warbrick,E. (1993) *Experiments with Fission Yeast. A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
20. Manivasakam,P., Weber,S.C., McElver,J. and Schiestl,R.H. (1995) Micro-homology mediated PCR targeting in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **23**, 2799–2800.
21. Kaur,B., Fraser,J.L., Freyer,G.A., Davey,S. and Doetsch,P.W. (1999) A Uve1p-mediated mismatch repair pathway in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.*, **19**, 4703–4710.
22. Maris,A.F., Kern,A.L., Picada,J.N., Boccardi,F., Brendel,M. and Henriques,J.A. (2000) Glutathione, but not transcription factor Yap1, is required for carbon source-dependent resistance to oxidative stress in *Saccharomyces cerevisiae*. *Curr. Genet.*, **37**, 175–182.
23. Ribar,B., Grallert,A., Olah,E. and Szallasi,Z. (1999) Deletion of the *sep1*$^{(+)}$ forkhead transcription factor homologue is not lethal but causes hyphal growth in *Schizosaccharomyces pombe*. *Biochem. Biophys. Res. Commun.*, **263**, 465–474.
24. Izumi,T. and Mitra,S. (1998) Deletion analysis of human AP-endonuclease: minimum sequence required for the endonuclease activity. *Carcinogenesis*, **19**, 525–527.
25. Corpet,F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res.*, **16**, 10881–10881.
26. Hosfield,D.J., Guan,Y., Haas,B.J., Cunningham,R.P. and Tainer,J.A. (1999) Structure of the DNA repair enzyme endonuclease IV and its DNA complex: double-nucleotide flipping at abasic sites and three-metal-ion catalysis. *Cell*, **98**, 397–408.
27. Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.
28. Xanthoudakis,S., Smeyne,R.J., Wallace,.D. and Curran,T. (1996) The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice. *Proc. Natl Acad. Sci. USA*, **93**, 8919–8923.
29. Tsuchimoto,D., Sakai,Y., Sakumi,K., Nishioka,K., Sasaki,M., Fujiwara,T. and Nakabeppu,Y. (2001) Human APE2 protein is mostly localized in the nuclei and to some extent in the mitochondria, while nuclear APE2 is partly associated with proliferating cell nuclear antigen. *Nucleic Acids Res.*, **29**, 2349–2360.
30. Hadi,M.Z. and Wilson,D.,III (2000) Second human protein with homology to the *Escherichia coli* abasic endonuclease exonuclease III. *Environ. Mol. Mutagen.*, **36**, 312–324.
31. Unk,I., Haracska,L., Gomes,X., Burgers,P.M.J., Prakash,S. and Prakash,L. (2002). Stimulation of 3′–5′ exonuclease and 3′-phosphodiesterase activities of yeast Apn2 by proliferating nuclear antigen. *Mol. Cell. Biol.*, **22**, 6480–6486.
32. Roy,R., Brooks,C. and Mitra,S. (1994) Purification and biochemical characterization of recombinant N-methylpurine-DNA glycosylase of the mouse. *Biochemistry*, **33**, 15131–15140.
33. Memisoglu,A. and Samson,L. (1996) Cloning and characterization of a cDNA encoding a 3-methyladenine DNA glycosylase from the fission yeast *Schizosaccharomyces pombe*. *Gene*, **177**, 229–235.
34. Loeb,L.A. and Preston,B.D. (1986) Mutagenesis by apurinic/apyrimidinic sites. *Annu. Rev. Genet.*, **20**, 201–230.
35. McCready,S.J., Osman,F. and Yasui,A. (2000) Repair of UV damage in the fission yeast *Schizosaccharomyces pombe*. *Mutat. Res.*, **451**, 197–210.
36. Takao,M., Yonemasu,R., Yamamoto,K. and Yasui,A. (1996) Characterization of a UV endonuclease gene from the fission yeast *Schizosaccharomyces pombe* and its bacterial homolog. *Nucleic Acids Res.*, **24**, 1267–1271.
37. Bowman,K.K., Sidik,K., Smith,C.A., Taylor,J.S., Doetsch,P.W. and Freyer,G.A. (1994) A new ATP-independent DNA endonuclease from

Schizosaccharomyces pombe that recognizes cyclobutane pyrimidine dimers and 6–4 photoproducts. *Nucleic Acids Res.*, **22**, 3026–3032.
38. Yonemasu,R., McCready,S.J., Murray,J.M., Osman,F., Takao,M., Yamamoto,K., Lehmann,A.R. and Yasui,A. (1997) Characterization of the alternative excision repair pathway of UV-damaged DNA in *Schizosaccharomyces pombe*. *Nucleic Acids Res.*, **25**, 1553–1558.
39. Forsburg,S.L. (1993) Comparison of *Schizosaccharomyces pombe* expression systems *Nucleic Acids Res.*, **21**, 2955–2956.
40. Kishida,M., Nagai,T., Nakaseko,Y. and Shimoda,C. (1994) Meiosis-dependent mRNA splicing of the fission yeast *Schizosaccharomyces pombe mes1*$^{+}$ gene. *Curr. Genet.*, **25**, 497–503.
41. Cunningham,R.P., Saporito,S.M., Spitzer,S.G. and Weiss,B. (1986) Endonuclease IV (nfo) mutant of *Escherichia coli*. *J. Bacteriol.*, **168**, 1120–1127.
42. Aravind,L., Walker,D.R. and Koonin,E.V. (1999) Conserved domains in DNA repair proteins and evolution of repair systems. *Nucleic Acids Res.*, **27**, 1223–1242.
43. Avery,A.M., Kaur,B., Taylor,J.S., Mello,J.A., Essigmann,J.M. and Doetsch,P.W. (1999) Substrate specificity of ultraviolet DNA endonuclease (UVDE/Uve1p) from *Schizosaccharomyces pombe*. *Nucleic Acids Res.*, **27**, 2256–2264.
44. Guillet,M. and Boiteux,S. (2002) Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2 and Rad1/Rad10 in *Saccharomyces cerevisiae*. *EMBO J.*, **21**, 2833–2841.
45. Izumi,T., Malecki,J., Chaudhry,M.A., Weinfeld,M., Hill,J.H., Lee,J.C. and Mitra,S. (1999) Intragenic suppression of an active site mutation in the human apurinic/apyrimidinic endonuclease. *J. Mol. Biol.*, **287**, 47–57.