Trans-complementation by human apurinic endonuclease (Ape) of hypersensitivity to DNA damage and spontaneous mutator phenotype in *apn1*[−] yeast

David M. Wilson III, Richard A. O. Bennett, John C. Marquis, Parswa Ansari and Bruce Demple*

Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA 02115, USA

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ABSTRACT

Abasic (AP) sites in DNA are potentially lethal and mutagenic. 'Class II' AP endonucleases initiate the repair of these and other DNA lesions. In yeast, the predominant enzyme of this type is Apn1, and its elimination sensitizes the cells to killing by simple alkylating agents or oxidants, and raises the rate of spontaneous mutation. We investigated the ability of the major human class II AP endonuclease, Ape, which is structurally unrelated to Apn1, to replace the yeast enzyme *in vivo*. Confocal immunomicroscopy studies indicate that ~25% of the Ape expressed in yeast is present in the nucleus. High-level Ape expression corresponding to ~7000 molecules per nucleus, equal to the normal Apn1 copy number, restored resistance to methyl methanesulfonate to near wild-type levels in Apn1-deficient (*apn1*[−]) yeast. Ape expression in *apn1*[−] yeast provided little protection against H₂O₂ challenges, consistent with the weak 3'-repair diesterase activity of the human enzyme. Ape expression at ~2000 molecules per nucleus reduced the spontaneous mutation rate of *apn1*[−] yeast to that seen for wild-type cells. Because Ape has a powerful AP endonuclease but weak 3'-diesterase activity, these findings indicate that endogenously generated AP sites can drive spontaneous mutagenesis.

INTRODUCTION

Endogenous reactive chemicals are generated by normal cellular processes and from exposure to environmental mutagens (1–3). Reactive oxygen species such as the hydroxyl radical have been implicated in several human diseases, carcinogenesis, and ageing, potentially through the modification of genetic material (4,5). Apurinic/aprimidinic (AP) sites and single-strand breaks with 3'-deoxyribose fragments are two common forms of oxidative DNA damage (6). AP sites are also generated by spontaneous base loss or through the removal of modified bases by specific

DNA repair glycosylases (6,7). Both abasic sites and 3'-damages can be lethal if left unrepaired, and AP sites have mutagenic potential (8). To counteract the harmful effects of these DNA lesions, cells have developed mechanisms that prevent AP site formation or repair abasic sites and 3'-blocking fragments (4,6).

The major cellular enzymes responsible for initiating the repair of AP sites are class II AP endonucleases (6,9). These enzymes cleave immediately 5' to an abasic site to produce a normal 3'-OH nucleotide and a 5'-deoxyribose-5-phosphate (abasic) moiety. In addition, class II AP endonucleases possess repair diesterase activity for several 3'-damages in DNA, including 3'-phosphoglycolate esters, 3'-deoxyribose-5-phosphate esters, and 3'-phosphates (6). The use of repair-deficient strains has demonstrated the importance of these activities in the maintenance of genetic stability and protection against a variety of DNA-damaging agents in both prokaryotes (10) and eukaryotes (11).

In *Escherichia coli*, the major proteins involved in the correction of AP sites and 3'-DNA fragments are exonuclease III and endonuclease IV (12,13). Exonuclease III comprises ~90% of the total for both repair activities, and endonuclease IV accounts for much of the rest (12,14,15). These bacterial proteins also represent the two known families of class II AP endonucleases (6). The major (and perhaps sole) class II AP endonuclease/3'-diesterase of *Saccharomyces cerevisiae* is Apn1, an endonuclease IV homolog (11,16). Exonuclease III, endonuclease IV and Apn1 display their endonuclease and diesterase activities at roughly equal levels (12,13,17). The major AP endonuclease of mammalian cells, Ape [an exonuclease III homolog; (18,19)], possesses only weak 3'-diesterase activity (~1/200th of its AP endonuclease activity; 20,21), but is a powerful hydrolytic AP endonuclease, displaying this activity at a ~10-fold higher level than the bacterial enzymes or Apn1 (6).

Yeast strains lacking Apn1 (*apn1*[−]) exhibit an elevated spontaneous mutation rate (11) that includes a ~60-fold increase in the rate of AT to CG transversions (22). Genetic studies suggest that this mutator effect is driven by AP sites generated endogenously (22,23). However, the possibility remains that oxidative strand breaks that require processing by a 3'-repair

* To whom correspondence should be addressed

diesterase contribute to the *apn1*⁻ mutator phenotype. It seemed that the enzymatic properties of the human AP endonuclease might provide the means to assess the relative contribution of AP sites and oxidative strand breaks to the *apn1*⁻ mutator effect. We therefore developed specific yeast expression constructs for Ape and determined the impact of the human protein on resistance to DNA-damaging agents and the spontaneous mutation rate in *apn1*⁻ strains.

MATERIALS AND METHODS

Yeast strains

The yeast strains used were the wild-type MKP-o (*MATα can1-100 ade2-1 lys2-1 ura3-52 leu2-3,112 his3-Δ200 trp1-Δ901*) and FY86 (*MATα ura3-52 leu2Δ1 his3-Δ200 GAL2+*). The respective isogenic *apn1*⁻ derivatives (*apn1-Δ1::HIS3*) DRY373 and DRY377 have been described (11,24).

APE expression plasmids

A *HindIII*–*BamHI* DNA fragment containing the *APE* cDNA was isolated from pCW26 (18). This fragment was purified from an agarose gel and subcloned into the yeast galactose-inducible expression vector, pYES2 (Invitrogen), which had been cleaved with the same enzymes (25). The resulting recombinant plasmid was termed pYAPE (Fig. 1), with *APE* expression expected to be under *GAL* control.

For construction of pDEXAPE (Fig. 1), a *BamHI*–*KpnI* DNA fragment containing the Ape coding sequence was isolated from pDW26.3; this plasmid is identical to pCW26 (18) but with the *APE* cDNA in the opposite orientation within the *EcoRI* site. The purified *APE* fragment was subcloned into *BamHI*–*KpnI* cleaved p2UG (26; kindly provided by Dr K. R. Yamamoto, U.C. San Francisco) to generate pDEXAPE. Plasmid pGN795, which expresses the rat glucocorticoid receptor protein (26), was also a generous gift of Dr K. R. Yamamoto.

Plasmids were transformed into yeast cells using the lithium acetate procedure described previously (27).

Induction of APE expression

FY86 and DRY377 yeast cells were used for experiments involving pYES2 and pYAPE. For galactose induction of cells bearing pYAPE or pYES2, yeast were inoculated into YPD medium (28) and grown overnight at 30°C. Cells were harvested by centrifugation at 1000 g for 15 min and washed three times with distilled water and once with complete minimal (CM) medium lacking uracil (Ura⁻) and containing 2% glycerol instead of glucose. The cells were then resuspended in glycerol-containing CM Ura⁻ medium, and the cultures grown overnight at 30°C. After washing as described above with water and CM Ura⁻ medium containing 2% galactose, the cells were resuspended in galactose-containing CM Ura⁻ medium, grown overnight at 30°C, and processed the following day (see below).

Yeast strains MKP-o (phenotypically *Gal*⁻; unpublished data) and DRY373 were used for the induction of Ape protein from the glucocorticoid-inducible vector, pDEXAPE. Yeast cultures were grown at 30°C for 48 h with shaking (300 r.p.m.) in CM Ura⁻

medium lacking tryptophan and containing 2% glucose. To induce Ape expression, saturated cultures were diluted 10-fold in the same medium, and dexamethasone [Sigma; 20 mM stocks were prepared in 80% ethanol (v/v) and stored at -20°C] was added to the indicated final concentration. Cultures were grown under inducing conditions for the indicated times at 30°C before processing (see below).

Protein extracts and repair enzyme assays

After treatment with the appropriate inducing agent, yeast were harvested by centrifugation at 1000 g for 15 min. The pellet was washed once with 50 mM HEPES–KOH, pH 7.5, 150 mM KCl, 10% glycerol, 1 mM DTT. The cells were resuspended in the same buffer containing protease inhibitors (1 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 μg/ml leupeptin, 100 μg/ml PMSF) and mixed with 0.1 vol of acid-washed glass beads (Sigma, 0.5 mm diameter). The cells were lysed by vortexing eight times at maximum setting, each time for 20 s, alternating with chilling on ice. Debris and intact cells were removed by centrifugation at 14 000 g for 5 min at 4°C, and the supernatants were assayed for AP endonuclease activity as previously described (20,29). Protein concentrations of the crude extracts were determined by the BioRad Protein Dye system.

Indirect immunofluorescence and confocal microscopy

Purified recombinant Ape fusion protein (GST-Ape; 30) was bound to an Aminolink column according to the specifications of the manufacturer (Pierce), and the resulting Ape-linked column was used to prepare affinity purified antiserum (31). Following the appropriate induction scheme, yeast cells were prepared for immunofluorescence and probed with a 1:50 dilution of this primary antibody solution for 2 h at room temperature (32). Goat anti-rabbit IgG secondary antibody conjugated to FITC (Sigma) was used for visualization of anti-Ape cross-reacting material (32). Immunostained yeast were mounted in medium containing 10 mg/ml *p*-phenylenediamine (32) and analyzed by confocal microscopy with the assistance of Dr Bruce Ekstein.

Gradient plate analysis and survival curve experiments

Strain sensitivities to MMS (Sigma) and H₂O₂ (Sigma) were determined by gradient plate analysis (10,24) or survival curves (11,24).

Determination of spontaneous mutation rates

Spontaneous mutation rates were determined by the fluctuation test (33). Overnight cultures of MKP-o or DRY373 containing the p2UG vector or pDEXAPE were grown at 30°C in CM Ura⁻, lacking tryptophan and containing 2% glucose. For Ape induction, dexamethasone was added to a final concentration of 25 μM, and the cultures were incubated for 8 h at 30°C. Reversion of ochre mutations was measured for the *ade2-1* and *lys2-1* alleles of MKP-o and DRY373 using at least 120 1-ml cultures that were grown in CM Trp⁻ Ura⁻ medium (28) supplemented with 2.5 μM dexamethasone and limiting for adenine (0.75 μg/ml) or lysine (1.0 μg/ml), respectively (11). The initial cell titer was ~2500 cells/ml, and the final cell density in the unverted wells was ~2.0 × 10⁶ cells/ml.

Figure 1. Ape expression constructs. A *HindIII*–*BamHI* DNA fragment containing the Ape cDNA was subcloned into the pYES2 vector, downstream of the *GAL1* promoter (*GAL1p*), to generate pYAPE (left). The Ape coding region was subcloned into the *BamHI* and *KpnI* restriction sites of p2UG to generate pDEXAPE (right). Ape expression from this construct is under the transcriptional control of the *CYC1* promoter and is inducible through the activity of three glucocorticoid-response elements (GREs). Both expression plasmids are multicopy (2-micron-based) and contain a selectable marker *URA3*⁺.

RESULTS

Ape expression in yeast

To analyze the biological activity of the human Ape AP endonuclease in a eukaryotic cell, yeast expression constructs containing the *APE* cDNA were generated (Fig. 1; see Materials and Methods). Expression of Ape from these recombinant plasmids was expected to be inducible by treatment with either galactose (pYAPE, Fig. 1) or dexamethasone (pDEXAPE, Fig. 1). The constructs were transformed into wild-type and *Apn1*-deficient (*apn1-ΔI*) strains, and the AP endonuclease activity of the cell-free protein extracts was measured to determine the amount of functional Ape protein present.

Galactose treatment of FY86 (wild-type) and DRY377 (*apn1-ΔI*) containing pYAPE increased the level of AP endonuclease activity of these cells ~40-fold over the levels measured for FY86 cells harboring the pYES2 vector (Table 1). This finding shows that Ape protein was expressed in an active form in yeast. Some leaky, non-induced expression was observed in extracts from DRY377 containing pYAPE, while DRY377 containing pYES2 possessed ≤0.5% of the AP endonuclease activity of wild-type cells (Table 1). The high level of AP endonuclease activity for the former extract is consistent with the higher turnover number of Ape relative to *Apn1* (17,20). Based on this comparison, and the estimate that wild-type yeast contain ~7000 molecules of *Apn1* per cell (34), we calculate that pYAPE directs the synthesis of 20 000–30 000 Ape molecules per cell.

Ape expression from pYAPE in MKP-o or DRY373 yeast was unresponsive to galactose (Table 1). Therefore, analysis of Ape function in these cells required the construction of pDEXAPE (Fig. 1B). The pDEXAPE plasmid was co-transformed with pGN795, which encodes the rat glucocorticoid receptor (26), into MKP-o (wild-type) and DRY373 (*apn1-ΔI*). Time course and dose–response experiments were performed to determine the optimal conditions for induction by dexamethasone. For significant Ape induction (>10-fold over wild-type), dexamethasone was required at a final concentration of ~25 μM (Fig. 2A). Higher concentrations of dexamethasone gave no significant increase (*apn1*[–] strain) or even a modest decrease (<2-fold; wild-type

yeast) in the level of AP endonuclease activity (Fig. 2A). For unknown reasons, Ape was expressed from pDEXAPE at a consistently higher level in *apn1*[–] than in wild-type cells (Table 2; Fig. 2A). For induction by 25 μM dexamethasone, the optimal time was found to be ~8 h (Fig. 2B). The AP endonuclease activity of MKP-o or DRY373 containing p2UG was unchanged by different concentrations of dexamethasone or induction times (data not shown). For all subsequent experiments, the treatment used for Ape induction in MKP-o and DRY373 gave AP endonuclease activities 8–12-fold higher than wild-type yeast bearing the plasmid vector p2UG (Table 2).

Table 1. AP endonuclease activity of yeast expressing Ape from pYAPE

Strain	AP endonuclease activity (U/mg)	
	Non-induced	Induced
FY86 + pYES2	6 ± 3	5 ± 1
FY86 + pYAPE	7 ± 4	250 ± 35
DRY377 + pYES2	< 0.03	< 0.03
DRY377 + pYAPE	3 ± 2	248 ± 43
MKP-o + pYES2	10 (1 expt)	12 ± 6
MKP-o + pYAPE	nd	nd
DRY373 + pYES2	<0.04	<0.03
DRY373 + pYAPE	7 ± 3	4 ± 1

AP endonuclease activity was measured for protein extracts of cultures grown in galactose (induced) or dextrose (non-induced) as described in Materials and Methods. The results displayed are averages of at least two experiments. nd, not determined.

Table 2. AP endonuclease activity of yeast expressing Ape from pDEXAPE

Strain	Plasmid	AP endonuclease activity (U/mg)	
		Non-induced	Induced
MKP-o	p2UG	6 ± 2	8 (1 expt)
MKP-o	pDEXAPE	4 ± 0.5	46 ± 17
DRY373	p2UG	nd	<0.06 (1 expt)
DRY373	pDEXAPE	5 ± 0.5	72 ± 19

AP endonuclease activity was determined for protein extracts that were prepared from MKP-o or DRY373 cells containing p2UG or pDEXAPE. Specific activity was measured for cultures grown for 12 h in the presence (induced) or absence (non-induced) of 25 μM dexamethasone (see Materials and Methods). Results represent the mean of at least two experiments. nd, not determined.

Intracellular localization of Ape

Immunofluorescence microscopy with affinity-purified antibodies to Ape revealed that the human protein was not localized to any specific subcellular compartment in galactose-treated FY86/pYAPE and DRY377/pYAPE cells (Fig. 3). A similar, but less intense, staining pattern was detected with dexamethasone-induced MKP-o/pDEXAPE and DRY373/pDEXAPE cells, while only background staining was visible with the vector controls (data not shown). From these images (Fig. 3), we estimate that the nucleus composes ~25% of the total cell volume, so that ~25% of the Ape produced in these cells resides in the nucleus (based on the roughly uniform distribution of cross-reacting material). This estimate is consistent with the results of

Figure 2. Glucocorticoid-dependent expression of Ape in *S. cerevisiae* strains MKP-o (*APN1*⁺) and DRY373 (*apn1*-Δ1). Overnight cultures were diluted 10-fold in fresh medium prior to the induction. (A) Determination of optimal dexamethasone concentration. The cultures were grown 12 h in the indicated concentration of dexamethasone. For inductions (see Materials and Methods), dexamethasone was added to the final concentration indicated. The cells were incubated for 12 h before preparation of the protein extracts and activity analysis. The values shown are the means of at least two independent determinations (except for the untreated cells and the *APN1*⁺ strain treated with 5 μM dexamethasone), with the standard errors indicated. (B) Determination of optimal induction time. The cultures were grown for the indicated times in the presence of 25 μM dexamethasone. Protein extracts were then prepared and assayed for AP endonuclease activity. AP endonuclease activity was not determined for MKP-o/pDEXAPE at 16 h or DRY373/pDEXAPE at 4 h.

cell fractionation experiments (data not shown). Thus, FY86 cells expressing induced levels of Ape have ~7000 molecules of the human protein in the nucleus, and dexamethasone-induced MKP-o/pDEXAPE yeast contain ~2000 nuclear Ape molecules.

Protection against MMS and H₂O₂ toxicity by Ape

Prior experiments demonstrated cross-complementation of the MMS hyper-sensitivity of AP endonuclease-deficient bacteria by Ape (18,19). In those experiments, however, neither nuclear transport nor recognition of damage in eukaryotic chromatin was required. To address whether Ape is capable of functioning *in vivo* in a defined eukaryotic system, *APN1*⁺ and *apn1*⁻ yeast strains were transformed with pYAPE or pDEXAPE.

Following treatment with the appropriate inducing agent, the sensitivity of these cells to MMS was determined by gradient plate analysis. Induced expression of Ape by galactose in

DRY377 restored nearly wild-type MMS resistance, while similar treatment of DRY377 bearing only the vector (pYES2) failed to restore resistance (Fig. 4A). Similarly, dexamethasone-induced expression of Ape in DRY373 containing pDEXAPE restored near wild-type MMS resistance (Fig. 4B). Non-induced Ape expression did result in a minor (<10%) increase in MMS-resistance for DRY377 cells containing pYAPE (data not shown). Survival curve experiments confirmed that Ape can confer resistance to MMS in *Apn1*-deficient yeast (Fig. 5A).

Ape expression from pYAPE did not significantly increase the resistance of *apn1*⁻ cells to H₂O₂ (Fig. 5B), which induces the formation of 3'-blocking fragments in DNA (12). This finding is consistent with *in vitro* data showing that Ape possesses a relatively poor 3'-repair diesterase activity (20,21). However, the slightly higher survival of Ape-expressing cells seen in Figure 5B was observed consistently.

Reduced spontaneous mutation frequency associated with Ape expression

Apn1-deficient strains exhibit a high spontaneous mutation rate, detectable in DRY373 as increased reversion of the *ade2-1* (~6-fold over *APN1*⁺) and *lys2-1* (~3-fold over *APN1*⁺) ochre alleles (Table 3). The fraction of pseudorevertants (due to suppressor mutations) differs for the two alleles (11). DRY373 and MKP-o were co-transformed with pGN795 and either pDEXAPE or p2UG, and the role of Ape in preventing spontaneous mutations in these cells was investigated. Induced expression of Ape in DRY373 (*apn1*-Δ1) returned the rate of both *ade2-1* and *lys2-1* reversion to wild-type levels (Table 3). The increased levels of AP endonuclease activity in *APN1*⁺ cells (MKP-o) expressing Ape protein did not significantly alter the spontaneous mutation rates for the *ade2-1* and *lys2-1* alleles (Table 3). Therefore, the amount of *Apn1* protein in wild-type yeast seems sufficient to handle AP sites (or other lesions) that are generated spontaneously.

Table 3. Reversal of *apn1*-Δ1 mutator by expression of Ape

		Reversion rates (per 10 ⁸ cells per generation)	
		Lys ⁺	Ade ⁺
MKP-o	p2UG	3.9 ± 1.1	1.3 ± 0.8
MKP-o	pDEXAPE	5.9 ± 1.9	0.8 ± 0.5
DRY373	p2UG	11.9 ± 2.2	7.7 ± 1.2
DRY373	pDEXAPE	3.5 ± 1.1	1.7 ± 0.8

Spontaneous reversion rates for *lys2-1* and *ade2-1* were measured as described in Materials and Methods. Each value shown was determined from at least 120 1-ml cultures as described by von Borstel (33).

DISCUSSION

The *in vitro* activities of Ape are reflected in the pattern of complementation by the human protein expressed in *apn1*-Δ1 yeast. As seen for AP endonuclease-deficient *E. coli* (18,19), Ape complements the hypersensitivity of *apn1*⁻ yeast to MMS relatively well, but contributes little to H₂O₂ resistance, consistent with the weak 3'-repair diesterase activity of Ape (20). In contrast, expression in *apn1*⁻ yeast of *E. coli* endonuclease IV, an enzyme with robust 3'-repair diesterase activity, provides

Figure 3. Intracellular localization of Ape. Yeast bearing pYAPE were induced with galactose, prepared for immunofluorescence, and probed with affinity-purified antibodies to Ape (see Materials and Methods). After incubation with fluoroisothiocyanate-conjugated secondary antibody, the cells were analyzed by confocal fluorescence microscopy. (A–C), FY86/pYAPE cells; (D–F), DRY377 (*apn1*[−])/pYAPE cells. A and D, fluorescence superimposed over cell images; B and E, transmitted light images of cells; C and F, fluorescence only. The bar indicates 5 μ m.

resistance to the cytotoxicity of both MMS and H₂O₂ (D. Ramotar and B. Demple, submitted).

Most notably, expression of Ape abrogated the mutator phenotype of *Apn1*-deficient yeast (11,22). Endonuclease IV equipped with a nuclear targeting signal can also suppress the *apn1*- Δ I mutator effect (D. Ramotar and B. Demple, submitted). These findings indicate that mutagenic lesions arising *in vivo* are repaired in common by Ape, *Apn1* and endonuclease IV, and the relative specificity of Ape for AP sites (20,21) provides a molecular basis for proposing endogenous mutagenic lesions. The results presented here strongly implicate AP sites as the *in vivo* DNA lesions responsible for much of the increased spontaneous mutation in *apn1*- Δ I cells. The above conclusion is consistent with the observation that the Mag1 DNA glycosylase generates mutagenic products (presumable AP sites) that are eliminated by *Apn1* (22,23). However, a portion of the extra mutagenesis in *apn1*- Δ I cells could be potentiated by other types of abasic sites, such as oxidized forms of deoxyribose that arise from free-radical attack (1). Note in this context that the absolute mutation rate for *apn1*[−] cells decreased ~3-fold upon transfer to anaerobic

growth, although there was still a mutator effect relative to wild-type yeast cultured anaerobically (11).

Previous experiments showed that suppression of the MMS hypersensitivity of *apn1*- Δ I cells requires localization of the complementing repair protein to the nucleus (22). Ape is strongly localized to the nucleus of human and mouse cells (18,31), but the present results reveal that localization of the human protein to the yeast nucleus is only partial, at least at the highest levels of expression. These observations prompt a consideration of the amount of AP endonuclease activity needed to maintain a normal mutation rate in yeast. By our estimates, ~2000 molecules of Ape in the nucleus are sufficient to repair the mutagenic DNA damages that arise from endogenous sources in *apn1*[−] yeast (Table 3). It is unclear how this copy number for Ape should be compared with the situation in wild-type yeast. Ape has a turnover number as an AP endonuclease ~10-fold higher than that of *Apn1* (17,20), such that 1500 Ape molecules in the nucleus would provide an overall repair activity at least equal to that of wild-type yeast. Alternatively, the number of repair protein molecules per nucleus could

Figure 4. Gradient plate analysis of MMS resistance in yeast expressing Ape. (A) Followed by induction with galactose, yeast cells harboring pYES2 or pYAPE were plated on MMS gradient plates and the growth determined (see Materials and Methods). (B) Gradient plate analysis was performed as above with yeast containing p2UG or pDEXAPE, except that induction was carried out with 25 μ M dexamethasone for 8 h. The results displayed are representative experiments that were performed with 0.025% MMS in the bottom layer of the gradient plate (see Materials and Methods).

be the most important feature, in which case wild-type yeast would harbor a number of Apn1 molecules ≤ 5 -fold greater than necessary to limit spontaneous mutagenesis. It does appear that the amount of Apn1 in wild-type cells is not limiting for genetic stability, because the additional expression of Ape in wild-type yeast (Table 3) or the 20-fold overexpression of Apn1 did not diminish the spontaneous mutation rate detectably (D. Ramotar and B. Demple, submitted). A corollary of this conclusion is that spontaneous mutation in wild-type yeast is driven by lesions other than conventional AP sites in duplex DNA. This does not rule out the possible involvement of AP sites that are refractory to repair, as in single-stranded DNA (6).

It is noteworthy that Ape was unable to restore wild-type MMS resistance to Apn1-deficient yeast. This finding could be interpreted in several ways. The incomplete targeting of Ape to the yeast nucleus might render AP endonuclease activity limiting for repair. Alternatively, Ape may not interact well with damages in yeast chromatin. There may be an AP site derivative or MMS-induced lesion that is an effective substrate for Apn1, but which is poorly repaired by Ape. Indeed, Apn1 and Ape, which represent different AP endonuclease families, possess distinct

Figure 5. Effect of Ape expression on the resistance of Apn1-deficient yeast to DNA-damaging agents. (A) Trans-complementation for resistance to MMS. Galactose-induced cultures of FY86/pYES2, DRY373/pYES2 and DRY373/pYAPE were challenged with the indicated concentrations of MMS as described in Materials and Methods. After the challenge, the cells were diluted, plated on YPD agar, and colonies were counted after 2–3 days of growth at 30°C. (B) Lack of trans-complementation for resistance to H₂O₂. Cell growth, challenge and plating was as described for (A), except that H₂O₂ was used at the indicated concentrations instead of MMS (see Materials and Methods). The values indicated represent the means and standard errors of at least two independent experiments.

preferences for some substrates *in vitro* (6,32; D.M.W., E. Kim and B.D., unpublished data). Addressing this last possibility will require new methods, currently under development, for quantitating specific abasic lesions in genomic DNA.

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