Saccharomyces cerevisiae Apn1 and Escherichia coli endonuclease IV are homologous enzymes that initiate the repair of abasic (AP) sites or oxidative DNA strand breaks. Yeast lacking Apn1 (apn1') are hypersensitive to simple alkylating agents (which produce many AP sites) and to oxidants and display an elevated spontaneous mutation rate due to endogenous damages. We explored whether the prokaryotic repair enzyme could substitute for its yeast counterpart. Plasmid constructs were generated that expressed endonuclease IV at 1/20 to 10-fold the AP endonuclease activity of wild-type yeast; some of these plasmids expressed hybrid forms of endonuclease IV equipped with the C-terminal nuclear localization signal of Apn1. Although hybrid endonuclease IV-Apn1 (but not native endonuclease IV) was selectively localized to the yeast nucleus, expression of this chimeric protein at 25% of the normal Apn1 level did not restore alklation or oxidative resistance to apn1' yeast, but it did partially counteract the mutator phenotype of apn1' yeast. Expression of either the hybrid protein or native endonuclease IV at ~10 times wild-type Apn1 levels restored wild-type resistance to methyl methanesulfonate and near-wild-type H₂O₂ resistance. High level expression of native endonuclease IV also restored the normal spontaneous mutation rate to apn1' yeast. These data place limits on the amounts of AP endonuclease activity necessary for repair of DNA damages caused by both endogenous and environmental agents and point to a direct role of spontaneous AP sites as potentially mutagenic lesions.

Ionizing radiation, chemical oxidants, and aerobic metabolism produce toxic oxygen derivatives such as superoxide, hydrogen peroxide, and hydroxyl radical (von Sonntag, 1987; Im- lislism produce toxic oxygen derivatives such as superoxide, hy- droxyl radical (von Sonntag, 1987; Im-

MMS, methylmethanesulfonate; PBS-BSA, phosphate-buffered saline-

pombe et al., 1991), plants (Babiychuk et al., 1991; Seki et al., 1994), and in the fission yeast Schizosaccharomyces pombe,² but their biological roles remain undetermined. No homolog of exonuclease III has yet been reported for S. cerevisiae, but exonuclease III-related enzymes have been found in mammalian cells (Demple et al., 1991; Robson and Hickson, 1991; Robson et al., 1991; Seki et al., 1991), Dro sophila (Sander et al., 1991), plants (Babiychuk et al., 1994), and Gram-positive bacteria (Puyet et al., 1989).

The alkylation and oxidation resistance conferred in yeast by Apn1 is due, respectively, to efficient repair of alkylation-induced AP sites and blocked oxidative 3'-termini in DNA (Ramotar et al., 1991b). The mutator phenotype of Apn1-deficient (apn1-11) strains seems largely due to the generation of AP sites by endog-

⁵ The abbreviations used are: AP, apurinic/apyrimidinic; SD, minimal synthetic medium; PCR, polymerase chain reaction; bp, base pair(s); MMS, methyl methanesulfonate; PBS-BSA, phosphate-buffered saline-

bovine serum albumin solution.

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‡ D. Ramotar, J. Vadnais, J.-Y. Masson, and S. Tremblay, manuscript in preparation.
In an earlier report, we demonstrated that Apn1 expressed in E. coli can substitute specifically for endonuclease IV in the repair of damaged DNA in vivo (Ramotar et al., 1991a). It thus appears that Apn1 and endonuclease IV share in vivo substrate specificity in DNA repair consistent with their homology and similar in vitro properties (J ohnson and D emple, 1988b; L evin et al., 1988). We show here that native E. coli endonuclease IV can functionally substitute for Apn1 in yeast when expressed at high but not low levels and is not localized to any specific organelle. A derivative of endonuclease IV equipped with the distal two basic clusters of Apn1 accumulated in the yeast nucleus and lowered the apn1-1 mutation rate even when expressed at a lower level.

**MATERIALS AND METHODS**

Strains and Media—The APN1 + strains, and their derivatives, were constructed by PCR (Ramotar et al., 1991b) and were provided by S. Cunningham and D. Demple (1989). The E. coli strain strain AB1157 (str - raf - 1) was kindly provided by D. D emple, 1986; strain BW256 (lactose-producing, nuc - 1) was kindly provided by D. B eiss (U niversity of M ichigan, A nn A rbor). E. coli strain H8101 (Sambrook et al., 1989) was used for plasmid maintenance.

Construction of plac-EndoV and plac-EndoV'—The plasmid pRPC124 (generously provided by D. R. Cunningham, A lbaney, N Y) contains the entire coding region of the E. coli nfo gene and the flanking 5' - and 3'-untranslated DNA sequences (Cunningham et al., 1986). This plasmid was used as the template to amplify by polymerase chain reaction (PCR) (Sambrook et al., 1989) the sequence from -21 to +977 (numbering with respect to the first A of the nfo start codon) using the primers DR1 (5' - GGGT TAA CAGGG GTATCC GATGAA TAC-3') and DR2 (5' - TTTT GC TCG GTG GATG TTC TAA -3') to amplify the 3' untranslated region of the nfo gene.

Attachment of Apn1 C-terminal Basic Clusters to Endonuclease IV—The 3' end of the APN1 gene (base pairs +940 to +1435) was amplified by PCR using the primers DR4 (5' - ATG AA TGGT AATG ATC TTAA -3') and DR5 (5' - TAC GTG GTG TTG GATG AAC GGA AATC -3') bearing restriction sites for BamHI and SalI, respectively. The PCR-amplified 500-bp fragment encodes Apn1 basic clusters 2 and 1, which together span 50 amino acid residues (317-GAK-RRKTKKEON ENKLKRK -326) and Apn1 reference sequences (Fig. 1). The amplified fragments were ligated with BamHI, blunt-ended using S1 nuclease, and digested with EcoRV to remove cluster 2 sequences precisely; the resulting linear blunt-ended fragment was circularized to generate plasmid plac-EndoV297, which bears only cluster 1, fused in-frame with endonuclease IV.

**Construction of Plasmids for Endonuclease IV Expression in Yeast—** Plasmids plac-EndoV, plac-EndoV', plac-EndoV335, and plac-EndoV297 were digested with KpnI and XhoI (sites located in the multiple cloning site of pKEn2) to release the DNA fragment encoding endonuclease IV and its derivatives containing Apn1 sequences. These fragments were subcloned directly next to the GAL1 promoter in the yeast expression vector pYES2.0 (Ramotar et al., 1993) to produce pGAL-Endo IV335, pGAL-Endo IV297, pGAL-Endo IV100, pGAL-Endo IV27, and pGAL-Endo IV. The expression vectors pGAL-Endo IV335 and pGAL-Endo IV297 also lacks the normal stop codon such that translation is expected to terminate at positions 5'335 and 5'297, respectively. This construct was predicted to encode endonuclease IV with an additional 55 amino acid residues at the C terminus.

**Enzyme Assays and Activity Gels—** Enzyme assays were generally performed as described by Bernelot-Moens and D emple (1989) as modified by Ramotar et al. (1991a). Cellular Sensitivity Measurements—The sensitivity of yeast strains to hydrogen peroxide or to methyl methanesulfonate (MMS) was measured by standard survival curves using exponential phase cultures (Ramotar et al., 1991b) or by gradient plate assays (Cunningham et al., 1986; Ramotar et al., 1993). In the plates, the bottom layer (30 ml) contained 0.4 mmol (for E. coli) or 0.13 mmol (for yeast) of MMS. For tert-butylhydroperoxide, 3.9 mmol was used in the bottom layer (30 ml) for gradient plates with E. coli. Mutation Assay—Spontaneous mutation rates in yeast were measured using the fluctuation test described by V on Borstel (1978). For each test, 24 independent 1-ml cultures were grown in 24-well plates (Costar) in SD medium (Sherman et al., 1983) with limiting concentrations of adenine (0.75 μg/ml) for ade2-1 or lysine (1.0 μg/ml) for lys2-1. The initial cell density was typically ~4000 cells/ml and reached a density of ~1.0 × 10^8 cells/ml when the medium in the wells was exhausted. For induction, 1 ml of the SD medium contained 2% galactose instead of glucose. Calculation of the spontaneous mutation rates and experimental errors was also according to the formulae given by V on Borstel (1978).

**Immunological Methods—** Rabbit polyclonal antibodies specific for endonuclease IV were used at a dilution of 1:1000 in 1 ml of Tris-HCl buffer (pH 7.5), 150 mM NaCl, and 4% powdered milk (Gershoni and Palade, 1983). Ten ml of this solution was used to probe each nitrocellulose filter (8 × 10 cm) blotted from SD-polycrylamide gels. Goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio/Can Scientific, Inc., Ontario, Canada) was used as the secondary antibody at a dilution of 1:5000. Immunoreactive polypeptides were detected using 4-chloro-1-naphthol (Bio-Rad).
containing 2% galactose, and grown overnight at 30 °C to permit induction of endonuclease IV. The cells were then fixed in 4.4% formaldehyde for 90 min at room temperature. After fixation, the cells were permeabilized by a 20-min incubation with zymolyase, washed twice with 1 M sorbitol, 10 mM Tris-HCl, pH 7.5, and resuspended in 50 μl of the same buffer. Ten μl of the resulting spheroplasts was placed on a polylysine-coated glass microscope slide and incubated overnight at room temperature with 20 μl of anti-endonuclease IV antisera at a dilution of 1:1000 in 137 mM NaCl, 2.6 mM KCl, 7.9 mM Na2HPO4, 1.4 mM KH2PO4, pH 7.3, 1 mg/ml bovine serum albumin (PBS-BSA; Pringle et al. 1991). The odsIs were then washed for 30 min with 10 drops of PBS-BSA, and the secondary antibody (fluorescein-conjugated “Affini-pure” donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories)) was added to the cells as 20 μl of a 1:50 dilution, and the incubation continued for 2 h at room temperature. The cells were then washed for 1 h with 20 drops of PBS-BSA, and 20 μl of 200 μg/ml propidium iodide was added to each well for 10 min. Cells were then washed only twice, each time with 2 drops of PBS-BSA (excessive washing removed the propidium iodide and weakened the DNA staining). The results were documented using a Zeiss fluorescence microscope equipped with an Axioptot camera.

RESULTS

A plasmid was constructed to express E. coli endonuclease IV in yeast by placing the nfo gene behind the yeast galactose-inducible GAL1 promoter in the multicopy vector pYES2.0 (Ramotar et al., 1993). The resulting construct (pGAL-EndoV) was transformed into the yeast strain FY66 and its apn-1 derivative DRY377, and the transformants were examined for expression of AP endonuclease activity. AP endonuclease activity was not detectable in cell-free extracts of the apn-1 mutant harboring the vector pYES2.0, while the expected activity (~60 units/mg) was seen in the APN1+ strain for cells grown in either glucose or galactose (Table I). The plasmid pGAL-EndoV directed the synthesis of AP endonuclease activity in the apn-1 strain at ~25% of the level for glucose-grown, wild-type yeast, with an induction of ~50-fold by galactose (Table I). Under the same conditions, plasmid pDRE containing the APN1+ gene under pGAL1 control (Ramotar et al., 1993) gave both basal and induced AP endonuclease levels in DRY377 at ~6-fold higher than observed for pGAL-EndoV (Table I). We do not know whether this difference reflects differences in the synthesis or the stability of endonuclease IV and Apn1 or a failure of the bacterial enzyme to be generated in the fully active form in yeast. A more remote possibility is that endonuclease IV is somehow poorly extracted from yeast cells under our conditions. The lower induced activity of endonuclease IV in yeast extracts was not due to the presence of an inhibitor, because the addition of purified endonuclease IV to apn-1 extracts did not alter the activity of the bacterial enzyme (data not shown).

The expression in yeast of active, full-length endonuclease IV was confirmed by activity gel analysis. In this approach, enzymatic cleavage of a synthetic 3’-[32P]phosphoglycoaldehyde substrate releases the label, which then diffuses out of the gel to leave a clear band upon autoradiography (Bernelot-Moens and Demple, 1989; Ramotar et al., 1993a). Extracts prepared from the apn-1 strain failed to form a detectable band, while the presence of plasmid pGAL-EndoV gave rise to a clear activity band (Fig. 2, lane 3) at the position expected for endonuclease IV (lane 7). Growth of this strain in galactose allowed the endonuclease IV activity band to be easily detected with 20-fold less protein (lane 3 versus 4). Samples of wild-type yeast (lane 1) or strains bearing plasmid pDRE (lanes 5 and 6) produced bands at the position expected for Apn1 (lane 8), in proportion to the amount of AP endonuclease activity detected in the quantitative enzymatic assay (Table I).

Functional Complementation of Yeast apn1-1 Mutants by E. coli Endonuclease IV—We tested whether endonuclease IV, which harbors both AP endonuclease and 3’-repair activities similar to Apn1, could functionally substitute for the yeast enzyme in vivo to provide cellular resistance to oxidative or alkylation damage. Under noninducing conditions (growth in glucose), the apn1-1 strain DRY377 harboring pGAL-EndoV remained hypersensitive to both MMS (Fig. 3A) and H2O2 (Fig. 3C). Upon growth in galactose, however, wild-type resistance to MMS (Fig. 3B) and near-wild-type resistance to H2O2 was provided by pGAL-EndoV. The APN1+ plasmid pDRE gave wild-type MMS and H2O2 resistance to the apn1-1 strain even under noninducing conditions (Fig. 3, A and C), and this resistance was not further enhanced by the strong overproduction of Apn1 resulting from growth in galactose (Fig. 3, B and D). Thus, the normal, wild-type level of Apn1 is not limiting for the repair of either alkylation-induced AP sites or oxidative 3’-damage.

Yeast cells that lack Apn1 accumulate mutations at a high rate, as measured using two different ochre alleles, ade2-1 and lys2-1 (Ramotar et al., 1991b). Basal expression of endonuclease IV from pGAL-EndoV in glucose failed to suppress this mutator phenotype (Table II). However, pGAL-EndoV expressed in galactose-containing medium eliminated the excess spontaneous mutagenesis for both the ade2-1 and the lys2-1 alleles in strain DRY373 (Table II). It is noteworthy that the
substantial overproduction of either endonuclease IV (Table I) or of Apn1 from pDR6 (data not shown) did not significantly lower the spontaneous mutation rate below that of wild-type levels. However, the results clearly indicate that endonuclease IV can functionally replace Apn1 in repairing both the endogenously generated DNA lesions that potentiate spontaneous mutagenesis (Kunz et al., 1994), as well as lethal lesions produced by treatment with exogenous DNA damaging agents.

Activity of Endonuclease IV-Apn1 Hybrid Proteins—Although pGAL-EndoIV expressed active endonuclease IV in yeast (Table I), this level of activity was insufficient at the basal level to substitute for Apn1 in providing resistance to the DNA-damaging agents MMS and H$_2$O$_2$ in yeast (Fig. 3, A and C). Since endonuclease IV bears no obvious nuclear localization signal, it seemed likely that basal expression of endonuclease IV did not yield sufficient enzyme in the yeast nucleus to effect DNA repair; this insufficiency is evidently overcome by the overproduction of endonuclease IV. Thus, if endonuclease IV were modified to achieve targeting to the yeast nucleus, basal expression of the bacterial enzyme might provide significant capacity for DNA repair.

Previously, we reported that basic cluster 1 of the Apn1 C terminus (Fig. 1) is essential for efficient targeting of the protein to the yeast nucleus (Ramotar et al., 1993). We tested whether basic cluster 1, or a combination of clusters 1 and 2, could act as nuclear transport signals for endonuclease IV. A small segment was not expected to alter the molecular size of a single immunoreactive polypeptide of the same size as purified endonuclease IV (Fig. 3; compare lanes 5 and 6), while pGAL-EndoIV$_{297}$ gave rise to two polypeptides of ~38 and ~35 kDa in size (Fig. 5, lane 4); the slower migrating polypeptide of ~38 kDa was of the size predicted for EndoIV$_{333}$ and accounted for most of the cross-reacting material. The cross-reacting polypeptide of ~35 kDa was likely a proteolytic fragment of EndoIV$_{333}$ that arose during extractions rather than an endogenous product (see below).

Intracellular Localization of Endonuclease IV and Derivatives—The intracellular localization of endonuclease IV and its derivatives was determined using monoclonal antibodies. Endonuclease IV encoded by plasmid pGAL-EndoIV was not detectable in yeast grown in glucose medium (data not shown). A series of plasmids was constructed (see "Materials and Methods") to express various endonuclease IV-Apn1 hybrid proteins (Fig. 1). The plasmid series plac-EndoV, plac-EndoV$_{297}$, and plac-EndoV$_{297}$ were designed for expression of these hybrid proteins in E. coli, under control of the lac promoter. When expressed even under noninducing conditions in the AP endonuclease-deficient, lac$^+$ strain BW528, all three plasmids directed the synthesis of substantial AP endonuclease activity (Table III). All three plasmids also conferred resistance in strain BW528 to both the alkylating agent MMS and the oxidant t-butyl hydroperoxide (Fig. 4). A slightly truncated (by two residues) endonuclease IV derivative, EndoIV*, produced during the construction of the chimeric proteins (see "Materials and Methods"), also retained functional activity in E. coli (Table III, Fig. 4).

For expression in yeast, EndoV$_{333}$ and EndoV$_{297}$ were positioned next to the GAL1 promoter to produce, respectively, pGAL-EndoV$_{333}$ and pGAL-EndoV$_{297}$ (see "Materials and Methods"). These plasmids were transformed into the yeast apn1-11 mutant DRY377, and the expression of endonuclease IV derivatives was assayed by assaying AP endonuclease activity (Table I). Both pGAL-EndoV$_{297}$ and pGAL-EndoV$_{333}$ directed basal AP endonuclease expression at ~15–20% the level detected for wild-type yeast and galactose-induced levels ~10-fold higher than wild-type (Table I).

The expression in yeast of endonuclease IV and the endonuclease IV-Apn1 hybrid proteins was confirmed by immunoblot analysis using anti-endonuclease IV monoclonal antibodies. Extracts of strain DRY377/pGAL-EndoIV expressed a single immunoreactive polypeptide of the same size as purified endonuclease IV (Fig. 5; compare lanes 2 and 6), while pGAL-EndoIV$_{333}$ gave rise to two polypeptides of ~38 and ~35 kDa in size (Fig. 5, lane 4); the slower migrating polypeptide of ~38 kDa was of the size predicted for EndoIV$_{333}$ and accounted for most of the cross-reacting material. The cross-reacting polypeptide of ~35 kDa was likely a proteolytic fragment of EndoIV$_{333}$ that arose during extractions rather than an endogenous product (see below).

Crude extracts of strain DRY377/pGAL-EndoV$_{297}$ expressed a single polypeptide of ~31 kDa in nearly the same amount as native endonuclease IV (Fig. 5, compare lanes 5 and 2). Since cluster 1 contains only 12 residues, this small segment was not expected to alter the molecular size of EndoV$_{297}$ significantly relative to native endonuclease IV. By the same token, immunoblotting could not establish that cluster 1 was retained in EndoV$_{297}$.

Intracellular Localization of Endonuclease IV and Derivatives—The intracellular localization of endonuclease IV and its derivatives was determined using endonuclease IV-specific monoclonal antibodies. Endonuclease IV encoded by plasmid pGAL-EndoIV was not detectable under our conditions by immunofluorescence in cells grown in glucose medium (data not shown).
TABLE III
Activity of endonuclease IV and derivatives expressed in E. coli

| Strain             | Plasmid                | AP endonuclease | MMS resistance |
|--------------------|------------------------|-----------------|----------------|
| AB1157 (xth^−, nfo^−) | pKEN2                 | 3.6             | ++++           |
| BW528 (xth^−, nfo^−)  | pKEN2                 | 0.02            | +             |
| BW528               | pGAL-EndoIV^333       | 76              | ++++           |
| BW528               | pGAL-EndoIV^297       | 41              | ++++           |
| BW528               | pGAL-EndoIV^333       | 37              | ++++           |
| BW528               | pGAL-EndoIV^297       | 75              | ++++           |

Fig. 4. Resistance conferred by pGAL-EndoIV and its derivatives in AP endonuclease-deficient E. coli. Gradient challenge plates were prepared and scored as indicated under “Materials and Methods.” Bars 1–6 in each panel represent the following strains: 1, AB1157/pKEN2; 2, BW528/pKEN2; 3, BW528/pGAL-EndoIV; 4, BW528/pGAL-EndoIV^297; 5, BW528/pGAL-EndoIV^333; and 6, BW528/pYES2.0.

Fig. 5. Immunoblot analysis of extracts of yeast expressing endonuclease IV and its derivatives. Lanes 1–5 each panel represent the following strains: A, FY86 (APN1^+); B, FY86 (apn1^-); C, FY86 (apn1^-); D, FY86 (apn1^-); E, FY86 (apn1^-); F, FY86 (apn1^-).

Fig. 6. Intracellular localization of endonuclease IV and its derivatives in yeast. All the strains were grown in galactose-containing SD medium. A, and B, strain DRY377/pGAL-EndoIV; C, and D, strain DRY377/pGAL-EndoIV^333; E, and F, strain DRY377/pGAL-EndoIV^297. A, C, and E show staining for DNA with propidium iodide. B, D, and F show staining with endonuclease IV-specific polyclonal antiserum.

A. MMS
B. t-BH

Fig. 7. Resistance to MMS conferred by pGAL-EndoIV and its derivatives in yeast apn1^-1 cells. Gradient plates were prepared and analyzed as described under “Materials and Methods”; “100% growth” corresponds to the full length of the tested gradient. Bars 1–6 in each panel represent the following strains: 1, FY86 (APN1^+); 2, DRY377 (apn1^-); 3, DRY377 (apn1^-); 4, DRY377 (apn1^-); 5, DRY377 (apn1^-); 6, DRY377 (apn1^-).

A. Glucose
B. Galactose

Functional Expression of Endonuclease IV In Yeast

Table III shows the activity of endonuclease IV and its derivatives expressed in E. coli. The table lists the strains used, the plasmids they carry, and the activity of the endonuclease, measured in units/mg of protein. The MMS resistance of the strains is also indicated. The results indicate that the plasmids containing endonuclease IV derivatives provided detectable MMS resistance to the yeast strains.

Figures 4 and 5 depict the resistance conferred by pGAL-EndoIV and its derivatives in AP endonuclease-deficient E. coli. The resistance is measured using gradient challenge plates. Figure 4 shows the growth of different strains on these plates, while Figure 5 provides an immunoblot analysis of extracts of yeast expressing endonuclease IV and its derivatives.

Figure 6 illustrates the intracellular localization of endonuclease IV and its derivatives in yeast. The localization is observed using fluorescence staining for DNA and endonuclease IV-specific polyclonal antiserum.

Figure 7 presents the resistance to MMS conferred by pGAL-EndoIV and its derivatives in yeast apn1^-1 cells. The resistance is measured using gradient challenge plates, and the results are shown for different strains.

The text discusses the functional expression of endonuclease IV in yeast, including the intracellular localization of the enzyme and its derivatives. It also mentions the resistance to MMS conferred by the expression of endonuclease IV and its derivatives in yeast.

The basal expression of pGAL-EndoIV^333 did not affect cell survival in the face of high-level damage by MMS, an effect on the apn1^-1 mutator phenotype was observed. The uninduced level of the EndoIV^333 hybrid protein diminished the spontaneous mutator rate in Apn1-deficient cells >50%, while induced expression of this protein provided a wild-type mutation rate (Table IV). The pronounced antimutator effect of EndoIV^333 confirms that the hybrid protein is efficiently transported to the yeast nucleus, although some proportion of EndoIV^333 could remain in the cytoplasm.

Although the basal expression of pGAL-EndoIV^333 did not affect cell survival in the face of high-level damage by MMS, an effect on the apn1^-1 mutator phenotype was observed. The uninduced level of the EndoIV^333 hybrid protein diminished the spontaneous mutator rate in Apn1-deficient cells >50%, while induced expression of this protein provided a wild-type mutation rate (Table IV). The pronounced antimutator effect of EndoIV^333 confirms that the hybrid protein is efficiently transported to the yeast nucleus, although some proportion of EndoIV^333 could remain in the cytoplasm.
The results presented here demonstrate that E. coli endonuclease IV is actively expressed in yeast and can functionally substitute for its S. cerevisiae homolog Apn1. Both endonuclease IV and Apn1 were shown previously to repair damaged DNA containing AP sites and blocked 3'-termini (Levin et al., 1988; Johnson et al., 1988b; Ramotar et al., 1991b). Thus, the observed substitution of yeast Apn1 by E. coli endonuclease IV is likely a direct effect of endonuclease IV acting in vivo to repair damaged chromosomal DNA in yeast cells. The cross-species complementation by both endonuclease IV and Apn1 (Ramotar et al., 1991a) suggests that their dual enzymatic activities, AP endonuclease and 3'-diesterase, were selected and conserved during evolution. Moreover, the need for these enzymes to repair spontaneous DNA lesions strengthens the likelihood that similar functions will be present in more complex eukaryotic cells.

The ability of EndoV_333 to diminish the spontaneous mutation rate in Apn1-deficient cells, even when expressed at only ∼1000 molecules per cell (Table I), indicates that this effect occurs via repair of major substrate(s) of the enzyme. Since the high-level expression of either native endonuclease IV or EndoV_333 restored normal spontaneous mutation rates to apn1-Δ1 strains, a mutagenic DNA damage that is a substrate for both the bacterial and the yeast enzyme is formed spontaneously in vivo. A major contributor is evidently the endogenous production of AP sites, since about half of the observed apn1-Δ1 mutator effect depends on a DNA glycosylase encoded by the yeast MAG1 gene (Xiao and Samson, 1993; Kunz et al., 1994). Oxidative DNA strand breaks could still constitute potentially mutagenic damages in vivo (Ramotar et al., 1991b), but such a hypothesis is undermined by the recently observed ability of the human APE AP endonuclease to restore normal mutation rates in Apn1-deficient yeast (Wilson et al., 1995). APE protein exhibits powerful AP endonuclease activity, but only weak 3'-repair diesterase (Chen et al., 1991).

Despite its active 3'-repair diesterase, endonuclease IV did not completely replace the function of Apn1 in providing resistance to the oxidant hydrogen peroxide, which produces a variety of DNA lesions including modified bases, oxidized abasic sites, and single strand breaks with blocked 3'-termini (Sonntag, 1987). One explanation for the only partial restoration of H_2O_2 resistance in yeast apn1-Δ mutants expressing endonuclease IV is that this agent may produce lesions in vivo that are not efficiently repaired by endonuclease IV, but no such damage has yet been identified (Johnson and Demple, 1988b; Levin et al., 1988; Demple and Harrison, 1994). Recently, it has been shown that endonuclease IV cleaves the oxidative DNA lesion α-deoxyadenosine (Ide et al., 1994), which arises by abstraction at C1' of deoxyribose and subsequent hydrogen donation by compounds such as glutathione (Sonntag, 1987). A number of other DNA repair enzymes, including E. coli exonuclease III, endonuclease III, endonuclease VIII, Fpg glycosylase, and T4 endonuclease V do not cleave at α-deoxyadenosine, but Apn1 was not tested (Ibe et al., 1994). Other candidate oxidative lesions include deoxyribonolactone (1'-oxidized) and deoxyxynos-4-ulose (4'-oxidized) residues, the latter actually cleaved preferentially by endonuclease IV (Häring et al., 1994). The activity of Apn1 against these oxidative abasic lesions is unknown. It is also possible that partial complementation of H_2O_2 resistance in apn1-Δ1 yeast is due to an inability of the bacterial protein to gain access to all of the oxidative damages present in yeast chromatin or by a failure of endonuclease IV to mediate productive interactions with (unknown) accessory repair proteins in yeast.

E. coli endonuclease IV lacks a eukaryotic nuclear localization signal and hence was not concentrated in the yeast nucleus. In this study, we achieved nuclear targeting of endonuclease IV in yeast by attaching both basic clusters 2 and 1 of Apn1 to the bacterial protein; cluster 1 by itself was insufficient to mediate such localization. Together with the delocalizing effect of deleting only cluster 1 from Apn1 (Ramotar et al., 1993), this result indicates that nuclear transport of Apn1 is achieved by a bipartite nuclear localization signal, analogous to the native signals reported for a variety of nuclear proteins (Robbins et al., 1991).

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REFERENCES

Babychuk, E., Kushnir, S., van Montagu, M., and Iuze, D. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3299–3303.

Bernier-Moens, C., and Demple, B. (1989) Nucleic Acids Res. 17, 587–600.

Chen, D., Herman, T., and Chen, D. (1991) Nucleic Acids Res. 19, 5907–5914.

Cunningham, R. P., Saporito, S. M., Spitzer, S. G., and Weiss, B. (1986) J. Bacteriol. 168, 1120–1127.

Demple, B., and Harrison, L. (1994) Annu. Rev. Biochem. 63, 915–948.

Demple, B. J., Ohson, A. W., and Fung, D. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 7731–7735.

Demple, B., Herman, T., and Chen, D. S. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 11450–11454.

Fraser, C. M., Gocayne, J. D., White, O., Adams, M. D., Clayton, R. A., Fleischmann, R. D., Bult, C. J., Kerlavage, A. R., Sutton, G., Kelley, J. M., Fritschman, J. L., Weidman, J. F., Small, K. V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T. R., Saudek, D. M., Phillips, C. A., Merrick, J. M., Tomb, J. F., Dougherty, B. A., Boyt, K. F., Hu, C.-L., Ludwig, T. S., Peterson, S. N., Smith, H. O., Hutchinson, C. A., and Venter, J. C. (1995) Science 270, 397–403.

Grossman, J. J., and Palade, G. E. (1983) Anal. Biochem. 131, 1–15.

Härting, M., Rüdiger, H., Demple, B., Boletzus, S., and Epe, B. (1994) Nucleic Acids Res. 22, 2010–2015.

Henner, W. D., Grunberg, S. M., and Haseltine, W. A. (1983) J. Biol. Chem. 258, 15198–15208.

Honore, N., Bergh, S., Chanteau, S., Doucet-Populaire, F., Elgmeier, K. G., Garnier, T., Georges, C., Launois, P., Limpaiboon, T., Newton, S., Niang, K., Del Portillo, P., Ramesh, G. R., Reddi, P., Ridel, P. R., Sittsonbut N., Wu-Hunter, S., and Cole, S. T. (1993) J. Med. Microbiol. 7, 207–214.

Hutchinson, F. (1985) Proc. Natl. Acad. Sci. U.S.A. 32, 115–154.

Ibe, H., Tadzuka, K., Shimagu, H., Shimura, C., Tsurumi, A. A., Wallace, S. S., and Kow, Y. W. (1994) Biochemistry 33, 7842–7847.
Functional Expression of Endonuclease IV In Yeast

Imlay, J. A., and Linn, S. (1988) Science 240, 1302–1309
Johnson, A. W., and Demple, B. (1988a) J. Biol. Chem. 263, 18009–18016
Johnson, A. W., and Demple, B. (1988b) J. Biol. Chem. 263, 18017–18022
Kunz, B. A., Henson, E. S., Roche, H., Ramotar, D., Nunoshiba, T., and Demple, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8165–8169
Levin, J. D., and Demple, B. (1990) Nucleic Acids Res. 18, 5069–5075
Levin, J. D., Johnson, A. W., and Demple, B. (1988) J. Biol. Chem. 263, 8066–8071
Lindahl, T. (1993) Nature 362, 709–715
Loeb, L. A., and Preston, B. D. (1986) Annu. Rev. Genet. 20, 201–230
Popoff, S. C., Spra, A. S., Johnson, A. W., and Demple, B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4193–4197
Pringle, J. R., Adams, A. E. M., Drubin, D. G., and Haarer, B. K. (1991) Methods Enzymol. 194, 565–602
Puyet, A., Greenberg, B., and Lacks, S. (1989) J. Bacteriol. 171, 2278–2286
Ramotar, D., Popoff, S. C., and Demple, B. (1991a) Mol. Microbiol. 5, 149–155
Ramotar, D., Popoff, S. C., Gralla, E. B., and Demple, B. (1991b) Mol. Cell. Biol. 11, 4537–4544
Ramotar, D., Kim, C., Lillis, R., and Demple, B. (1993) J. Biol. Chem. 268, 20533–20539
Robbins, J., Dilworth, S. M., Laskey, R. A., and Dingwall, C. (1991) Cell 64, 615–623
Robson, C. N., and Hickson, I. D. (1991) Nucleic Acids Res. 19, 5519–5523
Robson, C. N., Milne, A. M., Pappin, D. J. C., and Hickson, I. D. (1991) Nucleic Acids Res. 19, 1087–1092
Sambrook, J., Maniatis, T., and Fritsch, E. (1989) Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY
Sander, M., Lowenhaupt, K., Lane, W. S., and Rich, A. (1991) Nucleic Acids Res. 19, 4523–4529
Seki, S., Akiyama, K., Watanabe, S., Hatsushika, M., Ikeda, S., and Tsutsui, K. (1991) J. Biol. Chem. 266, 20797–20802
Sherman, F., Fink, G., and Hicks, J. (1983) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
von Borstel, R. C. (1978) Methods Cell Biol. 20, 1–24
von Sonntag, C. (1987) The Chemical Basis of Radiation Biology, Taylor and Francis, London
Warner, H. R., Demple, B., Deutsch, W. A., Kane, C. M., and Linn, S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 4602–4606
Wilson, D. M., III, Bennett, R. A. O., Marquis, J. C., Ansari, P., and Demple, B. (1995) Nucleic Acids Res. 23, 5027–5033
Xiao, W., and Samson, L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2117–2121
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