The EMBO Journal vol.15 no.9 pp.2306–2312, 1996

A *Drosophila* ribosomal protein contains 8-oxoguanine and abasic site DNA repair activities

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Ionizing radiation and normal cellular respiration form reactive oxygen species that damage DNA and contribute to a variety of human disorders including tumor promotion and carcinogenesis. A major product of free radical DNA damage is the formation of 8-oxoguanine, which is a highly mutagenic base modification produced by oxidative stress. Here, *Drosophila* ribosomal protein S3 is shown to cleave DNA containing 8-oxoguanine residues efficiently. The ribosomal protein also contains an associated apurinic/apyrimidinic (AP) lyase activity, cleaving phosphodiester bonds via a β,δ elimination reaction. The significance of this DNA repair activity acting on 8-oxoguanine is shown by the ability of S3 to rescue the H_2O_2 sensitivity of an *Escherichia coli mut*M strain (defective for the repair of 8-oxoguanine) and to abolish completely the mutator phenotype of *mut*M caused by 8-oxoguanine-mediated G→T transversions. The ribosomal protein is also able to rescue the alkylation sensitivity of an *E.coli* mutant deficient for the AP endonuclease activities associated with exonuclease III (*xth*) and endonuclease IV (*nfo*), indicating for the first time that an AP lyase can represent a significant source of DNA repair activity for the repair of AP sites. These results raise the possibility that DNA repair may be associated with protein translation.

Keywords: AP lyase/DNA repair/mutagenesis/oxidative stress/8-oxoguanine

Introduction

The formation of reactive free radicals is a direct consequence of normal oxygen metabolism. Such radicals are also formed by ionizing radiation, and are capable of interacting with a large array of biomolecules, including DNA. An abundant form of DNA damage produced by reactive oxygen species is 7,8-dihydro-8-oxoguanine (8-oxoguanine). The persistence of this lesion in DNA during DNA replication can result in the misincorporation of adenine opposite to 8-oxoguanine (Wood *et al.*, 1990; Shibutani *et al.*, 1991; Moriya *et al.*, 1991), leading to G→T transversions (Cabrera *et al.*, 1988; Wood *et al.*, 1990; Moriya *et al.*, 1991; Cheng *et al.*, 1992) which could be a significant source of mutations for tumor promotion and carcinogenesis (Floyd, 1990). In order to prevent the mutagenic effects of 8-oxoguanine, recent studies in *Escherichia coli* have shown that the removal of this lesion from DNA is mediated primarily through the action of formamidopyrimidine-DNA glycosylase (FPG; Tchou *et al.*, 1991), which is encoded by the *E.coli* mutator locus *mut*M (Michaels *et al.*, 1991) and possesses both DNA *N*-glycosylase and apurinic/apyrimidinic (AP) lyase activities (Bailly *et al.*, 1989). In eukaryotic organisms, we reported recently that glutathione *S*-transferase fusion constructs of *Drosophila* ribosomal protein S3 (GST–S3) also contained AP lyase activity (Wilson *et al.*, 1994). Since all known AP lyases identified in prokaryotes and eukaryotes also contain associated DNA glycosylase activity (Boiteux *et al.*, 1990; Doetsch and Cunningham, 1990), we further tested the ability of GST–S3 to act on a heavily UV-irradiated DNA substrate. It was found that the GST–S3 protein cleaved the irradiated DNA at a guanine photoproduct which was later determined to be 2,6 diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) (our unpublished results and Doetsch *et al.*, 1995).

In order to eliminate the possibility of trace contamination of the overexpressed GST–S3 fusion protein with FPG, GST–S3 was generated in an *E.coli mut*M strain (*fpg*$^-$) defective for the repair of 8-oxoguanine and formamidopyrimidines such as FapyGua (Michaels *et al.*, 1991). Experiments utilizing DNA substrates containing either an 8-oxoguanine or AP site show that the purified fusion protein is highly active on these lesions. The observed GST–S3 activity is comparable with that detected for other prokaryotic or eukaryotic repair enzymes that act on these sites of DNA damage. These *in vitro* results are supported by the ability of S3 to suppress *in vivo* the deleterious effects of two different mutagens in exposed *E.coli* strains deficient for the repair of either 8-oxoguanine or AP sites.

Results

Characterization of GST–S3 on a DNA substrate containing 8-oxoguanine

We utilized a 5′ end-labeled DNA duplex oligonucleotide that contained a single 8-oxoguanine residue (8-oxoG-37mer). The purified GST–S3 fusion construct was incubated with 8-oxoG-37mer, and the products of the reaction subsequently analyzed on a 20% polyacrylamide DNA sequencing gel. The purified GST–S3 protein was found specifically to introduce DNA scissions at the site of the 8-oxoguanine residue in reactions where product formation was dependent on both the time of incubation with GST–S3 (Figure 1, lanes 2–4) and on the amount of GST–S3 (Figure 2, lanes 1–4). No activity was detected on undamaged DNA (not shown). In addition, parallel

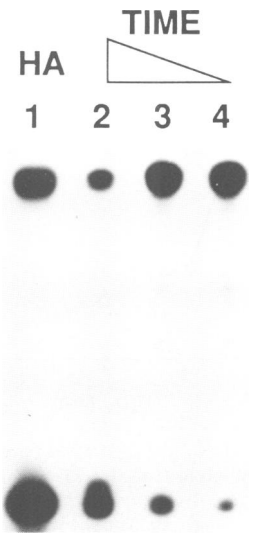

Fig. 1. Time dependence of GST–S3-mediated cleavage at sites of 8-oxoguanine. Incubations contained 1 pmol of 8-oxoG-37mer and 100 pg of GST–S3. The DNA reaction products were separated on a urea-containing 20% polyacrylamide gel and analyzed by autoradiography. Lane 1, hot piperidine (HA) to generate a β,δ elimination product. Incubations with GST–S3 were for 10 min (lane 4), 20 min (lane 3) and 30 min (lane 2).

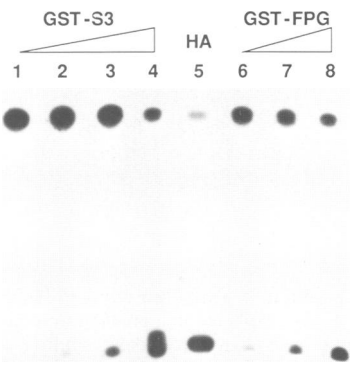

Fig. 2. Activity on 8-oxoguanine-containing DNA of different amounts of GST–S3 and GST–FPG. Reactions were for 30 min at 37°C and contained 1 pmol of 8-oxoG-37mer. The results presented are a combination of two DNA sequencing gels, where the final analysis of the 8-oxoG-37mer reaction products was by autoradiography. Lanes 1–4, incubations with GST–S3 with protein amounts of 10, 25, 50 and 100 pg, respectively; lane 5, hot piperidine; lanes 6–8, incubations with GST–FPG at protein concentrations of 25, 50 and 100 pg, respectively.

purifications of GST protein alone lacked activity on the 8-oxoG-37mer (not shown).

GST–S3 preparations used immediately following purification were calculated to have a turnover number (k_{cat}) of 6/min. This k_{cat} value compares with that obtained for GST–FPG (Figure 2, lanes 6–8), which is ~10/min under similar reaction conditions. The activity of GST–S3 is lost rapidly upon storage, showing a 15-fold decrease within 72 h after purification (not shown).

Characterization and mechanism of action of GST–S3 on a DNA substrate containing an AP site

We next compared the AP lyase activity of GST–S3 with other known AP lyases or AP endonucleases from both

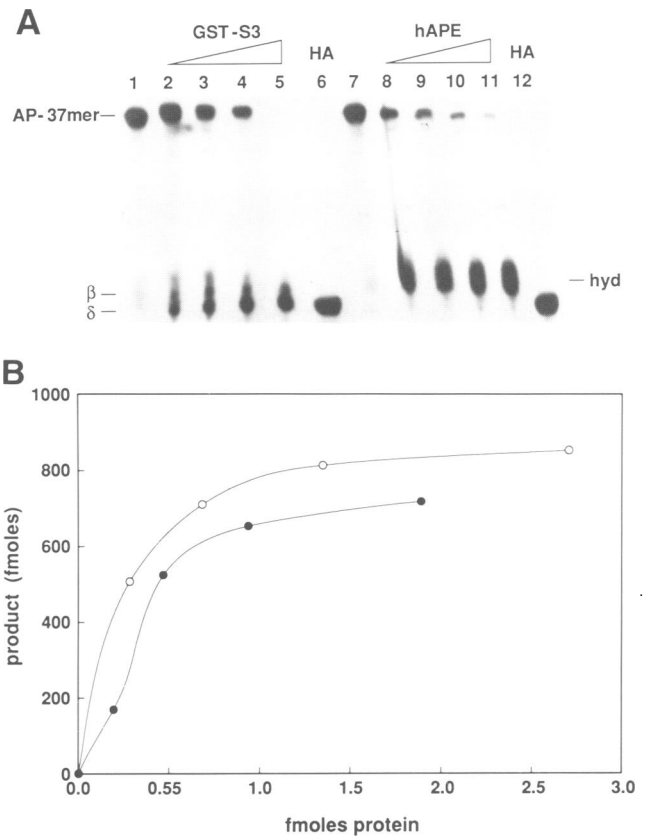

Fig. 3. Activities of GST–S3 and hAPE on abasic site-containing DNA. (**A**) The DNA cleavage products generated from the AP-37mer were separated on a 16% polyacrylamide DNA sequencing gel and analyzed by autoradiography. Incubations with GST–S3 (lanes 2–5) and hAPE (lanes 8–11) contained total protein amounts of 10 pg (lanes 2 and 8), 25 pg (lanes 3 and 9), 50 pg (lanes 4 and 10) and 100 pg (lanes 5 and 11); lanes 1 and 7, AP-37mer alone; lanes 6 and 12, hot piperidine (HA) treatment of AP-37mer. The electrophoretic mobilities of the uncleaved AP-37mer, and DNA cleavage products corresponding to a hydrolytic (hyd), β and δ elimination reactions are indicated. (**B**) Activity of GST–S3 and hAPE on AP-37mer. The data in (A) were quantified (Materials and methods) and plotted as the amount of AP-37mer cleavage product (fmol) produced following incubations with increasing amounts (fmol) of either GST–S3 (●) or hAPE (○).

eukaryotes and prokaryotes. These experiments utilized a 5′ end-labeled DNA fragment containing a single abasic site (AP-37mer), in which comparisons were originally made between GST–S3 and the major AP endonuclease in humans (hAPE; Demple *et al.*, 1991). The human AP endonuclease is known to cleave DNA at abasic sites via a hydrolytic mechanism, producing strand scissions 5′ to an AP site (Kane and Linn, 1981). As can be seen in Figure 3A, purified hAPE generated cleavage products with an electrophoretic mobility consistent with it acting 5′ to an AP site (Figure 3A, lanes 8–11). GST–S3 was found to be efficient in processing the AP-37mer (Figure 3A, lanes 2–5) with a k_{cat} value estimated to be 37/min (Figure 3B). GST–S3 was somewhat less efficient than hAPE, where the human enzyme was estimated to have a k_{cat} of 62/min (Figure 3B) under our standard reaction conditions. The DNA scission products produced by GST–S3 are different from hAPE, but similar to those generated by hot alkali treatment (Figure 3A, lanes 6 and 12), which is known to produce a β,δ elimination product (Doetsch and Cunningham, 1990).

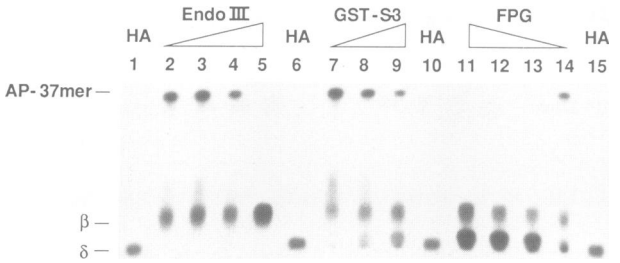

Fig. 4. Mechanism of action on abasic site-containing DNA. Reactions contained 1 pmol of AP-37mer, and were for 30 min at 37°C with *E.coli* endonuclease III (lanes 2–5) at protein amounts of 100, 150, 200 and 400 pg, respectively, or GST–S3 (lanes 7–9) at 20, 40 and 80 pg, respectively, or *E.coli* FPG (lanes 11–14) at 160, 120, 80 and 40 pg, respectively; lanes 1, 6, 10 and 15, hot piperidine (HA) treatment of the AP-37mer. The reaction products were separated on a 16% polyacrylamide DNA sequencing gel. The electrophoretic mobilities of the uncleaved AP-37mer and DNA cleavage products corresponding to β and δ elimination reactions are indicated.

We have concluded previously that GST–S3 cleaved DNA at AP sites via a β-elimination reaction (Wilson *et al.*, 1994). However, the results presented in Figures 2 and 3 suggest that, under certain conditions, δ elimination may also occur. In order to understand more fully the mechanism by which GST–S3 cleaves an AP site in DNA, GST–S3 was compared with homogeneous preparations of *E.coli* endonuclease III, a known β elimination catalyst (Bailly and Verly, 1987), and *E.coli* FPG, which carries out a concerted β,δ elimination reaction (Bailly *et al.*, 1989; O'Connor and Laval, 1989). The results obtained are in agreement with previous findings (Bailly and Verly, 1987; Bailly *et al.*, 1989), in which increasing the amounts of the FPG protein (Figure 4, lanes 11–14) resulted in equal quantities of the faster migrating δ elimination product and the β elimination product equivalent to that generated by *E.coli* endonuclease III (lanes 2–5). The GST–S3 fusion protein also produced a β,δ elimination product, but it is only observed at relatively high protein concentrations (lanes 7–9), suggesting that GST–S3 undergoes an additional encounter following β elimination at the AP site to generate the δ elimination product. Thus, the AP lyase activity of S3 may be mechanistically different from the AP lyase activities of *E.coli* endonuclease III and FPG.

S3 complementation of an E.coli mutM strain

The above experiments indicate that DNA repair activities are associated with the S3 ribosomal protein *in vitro*, however they do not address the question of whether these activities represent a significant source of DNA repair *in vivo*. We therefore attempted to exploit the sensitivity of a *mut*M strain to H_2O_2 by substituting the defective gene encoding FPG with S3 and determining H_2O_2 resistance. A plasmid expressing S3 was able to increase significantly the survival of *mut*M exposed to H_2O_2 (Figure 5). In addition, as revealed by H_2O_2 gradient plates (Figure 6), GST–S3 (lane 3) was comparable with *E.coli* FPG (GST–FPG, lane 2) for rescuing a *mut*M strain (lane 5) from H_2O_2 sensitivity. Comparisons were also made with eukaryotic methylpurine-DNA glycosylase (GST–MPG), which has been shown previously to possess an activity against 8-oxoguanine residues in DNA (Bessho *et al.*, 1993). GST–S3 was comparable with GST–MPG (lane 4) for

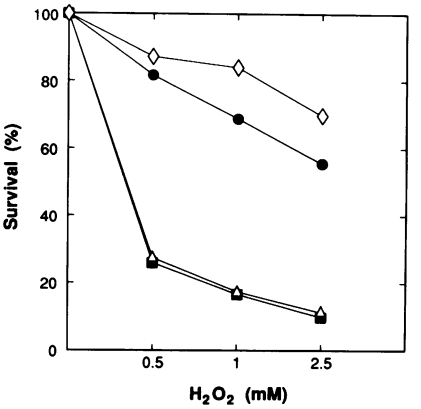

Fig. 5. Survival of *mut*M exposed to H_2O_2 in culture. Exponentially growing cells were exposed to H_2O_2 for 15 min, diluted, and plated on LB agar. The symbols are: ◇—◇, wild-type parental strain CC104; ●—●, *mut*M/pSE-S3; △—△, *mut*M/pSE; ■—■, *mut*M.

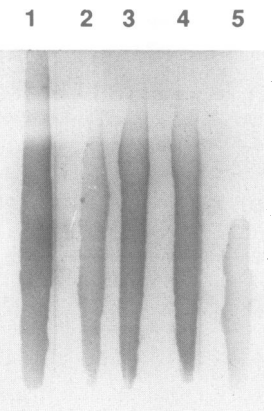

Fig. 6. Gradient plate test for *mut*M sensitivity to H_2O_2. The agar contained 1 mM H_2O_2 distributed in a gradient from bottom to top. The length of cell growth along the gradient is a measure of the strain's resistance to H_2O_2. Lane 1, CC104; lane 2, *mut*M/GST–FPG; lane 3, *mut*M/GST–S3; lane 4, *mut*M/GST–MPG; lane 5, *mut*M.

reversing the sensitivity of *mut*M to H_2O_2. Survival of each of the strains containing recombinant plasmids (lanes 2–4) was ~80% of the wild-type *mut*M parental strain CC104 (lane 1), whereas a 45% survival was observed for *mut*M after exposure to 1 mM H_2O_2 distributed in a gradient.

Additionally, *mut*M strains have a high rate of formation of lac^+ revertants when tested against a *lac*Z allele that can only revert to lac^+ by a specific 8-oxoguanine-mediated G·C to T·A transversion event (Cabrera *et al.*, 1988). Table I shows that the expression of ribosomal protein S3 reduced the number of *lac*$^+$ revertants in *mut*M to the level observed for the wild-type parental strain CC104.

S3 complementation of an E.coli strain lacking the major 5′ AP endonucleases

Some doubt exists as to whether AP lyases such as those associated with *E.coli* endonuclease III and FPG represent significant DNA repair activities *in vivo* that are distinct from their *N*-glycosylase activities. Therefore, it was of interest to determine if the AP lyase activity associated

Table I. Complementation of a *mut*M strain

Strain	Plasmid	Lac$^+$ revertants
CC104 (wild-type)	–	2 ± 1
*mut*M	–	45 ± 8
*mut*M	pSE420	42 ± 8
*mut*M	pSE420-S3	2 ± 1

Log phase cultures of *mut*M, *mut*M containing the control plasmid pSE420 (Invitrogen), or pSE420-S3, and wild-type CC104 (Michaels *et al.*, 1991) were plated on minimal lactose media. Revertants to Lac$^+$ were counted from 15 independent cultures. The average reversion frequencies and standard deviation are expressed per 10^8 cells.

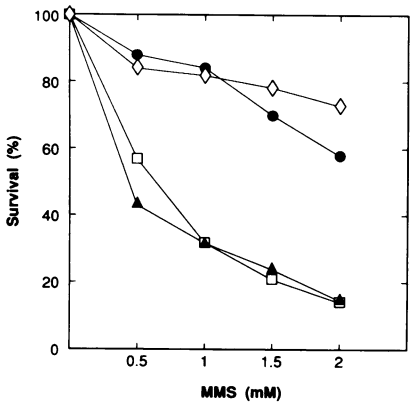

Fig. 7. Survival of RPC501 (*xth, nfo*) exposed to MMS in culture. See Figure 5 for the conditions used. The symbols are: ◇—◇, wild-type parental strain AB1157; ●—●, RPC501/GST–S3; ▲—▲, RPC501/GST; □—□, RPC501.

with S3 could protect *E.coli* mutants, deficient for the major hydrolytic AP endonuclease activities, from methyl methane sulfonate (MMS), an alkylating agent which produces AP sites in DNA (Cunningham *et al.*, 1986). The *E.coli* mutant strain chosen for transformation with either GST–S3 or GST was RPC501 (Cunningham *et al.*, 1986), deficient for the hydrolytic AP endonuclease activities associated with exonuclease III (*xth*), and endonuclease IV (*nfo*). GST–S3, but not GST, was able to reverse the sensitivity of RPC501 to MMS at low concentrations (Figure 7).

Discussion

Ribosomal protein S3 as a DNA repair protein

Original tests to determine whether *Drosophila* S3 acts on DNA led to the surprising discovery that the ribosomal protein contained AP lyase activity (Wilson *et al.*, 1994). Because of the ability of certain peptides and polyamines to carry out an apparently non-specific β elimination reaction on AP DNA (Doetsch and Cunningham, 1990), it was important to determine whether or not the S3 AP lyase activity was associated with an activity (presumably *N*-glycosylase) towards some modified DNA base. We subsequently observed that S3 was acting at FapyGua. An intermediate in the formation of FapyGua is 8-oxoguanine (Steenken, 1989; Doetsch *et al.*, 1995), which, unlike FapyGua, represents an abundant form of DNA damage caused by oxidative stress (Shigenaga *et al.*, 1989; Gajewski *et al.*, 1990). We therefore focused our attention on this form of DNA damage. Our results demonstrate that ribosomal protein S3 is highly active on this mutagenic DNA lesion, presumably due to an *N*-glycosylase activity associated with S3 that is liberating 8-oxoguanine to form an AP site. If this notion is correct, then S3 would fall into the category of AP lyases that participate in base excision repair by a combined *N*-glycosylase/AP lyase mechanism. Such an activity would prepare the DNA for several subsequent processing steps, including the removal of the AP site. With regard to the manner in which S3 cuts the phosphodiester backbone adjacent to an AP site, it appears to represent a mechanism distinct from *E.coli* AP lyases thus far characterized that catalyze either a β or a concerted β,δ elimination reaction. The AP lyase activity of S3 does resemble that recently reported for T4 endonuclease V, which acts as an *N*-glycosylase/AP lyase on pyrimidine dimers formed by UV light (Latham and Lloyd, 1995). For instance, both S3 and T4 endonuclease V promote a δ elimination reaction under circumstances where the appearance of this product is dependent on both the time of incubation and the protein concentration. These and other observations led Latham and Lloyd (1995) to propose that once T4 endonuclease V catalyzes a β elimination reaction, it dissociates from the incised DNA, and on a second encounter then cleaves the $C_{(5)}$-O-P bond 5′ to the abasic site by δ elimination. The β,δ elimination reaction mediated by S3 and T4 endonuclease V would therefore leave a one nucleoside gap bordered by a 3′ and a 5′ phosphoryl group. This mechanism may explain the surprising results with regard to S3 protecting RPC501 from cell killing by MMS. In such a situation, cells may be more able to cope with a 3′ phosphoryl group rather than a 3′-modified deoxyribose produced by β elimination catalysts such as *E.coli* endonucleases III.

The complementation data presented here firmly establish that the DNA repair activities mediated by S3 are able to act *in vivo* to protect cells from H_2O_2 and MMS toxicity. Although it could be argued that the overexpression of a ribosomal protein might somehow provide a selective advantage to cells exposed to various mutagens, this possibility was ruled out by the ability of S3 to reduce to wild-type levels the 8-oxoguanine-mediated G·C to A·T transversion mutation frequency in *E.coli mut*M and therefore strongly supports the notion that S3 is participating in the repair of DNA containing 8-oxoguanine.

Multifunctional proteins with roles in DNA repair

The results presented here demonstrate that ribosomal protein S3 has DNA repair activities directed towards the mutagenic lesions 8-oxoguanine and abasic sites in DNA. The presence of a nuclear localization signal and its tight association with the nuclear matrix and chromation (Wilson *et al.*, 1994) suggest that S3 may mediate functions in addition to its established ribosomal function in protein translation (Westermann *et al.*, 1979; Tolan *et al.*, 1983; Bommer *et al.*, 1991). Other multifunctional proteins have now been recognized as DNA repair proteins, thus providing a precedent for the possible involvement of S3 in DNA repair. For example, the major human AP endonuclease (hAPE) is also a transcription factor (Ref-1) which regulates Fos and Jun binding to their target DNA sequences (Xanthoudakis *et al.*, 1992). The coupling of

transcription to DNA repair provides yet another example of where a helicase encoded by *ERCC-2* (xeroderma pigmentosum complementation group D or XP-D) has been shown in yeast to be essential for both transcription by RNA polymerase II (Guzder *et al.*, 1994) and also for nucleotide excision repair (Wang *et al.*, 1994). Perhaps similar to the multifunctional role we propose here for S3 is the observation that the human excision repair gene *ERCC-3* that is associated with the hereditary DNA repair diseases XP and Cockayne's syndrome (Friedberg *et al.*, 1995) has as its yeast homolog *SSL2*, which is also involved in ribosomal binding and scanning of mRNA (Gulyas and Donahue, 1992).

Concluding remarks

Our results suggest the possibility that S3 may participate in both base excision repair and protein translation. The same might apply to ribosomal protein PO which possesses a 5′-acting AP endonuclease activity (Kelley *et al.*, 1989; Grabowski *et al.*, 1991). Curiously, elevated levels of mRNA corresponding to S3 and PO ribosome-associated proteins have been detected in human transformed cells from adenomatous polyps and colorectal carcinomas (Pogue-Geile *et al.*, 1991). In view of our results, it may be possible, therefore, for neoplastic cells to not only bear the characteristics of increased ribosomal protein production and activity, but may also provide these cells with a selective advantage if the same proteins contribute to an elevated DNA repair capacity.

Human S3 has also been implicated in the etiology of both XP and Fanconi's anemia (FA; Kim *et al.*, 1995). FA is a clinically diverse inherited disease with a high predisposition towards leukemia and solid tumors (Friedberg *et al.*, 1995). Interestingly, we find decreased levels of nuclear S3 in FA (Kelley, Deutsch and Clapp, our unpublished observations) which may explain the increased formation of 8-oxoguanine found in these patients (Takeuchi and Morimoto, 1993). The identification and characterization of multifunctional proteins with roles in DNA repair may begin to bring a better understanding of the wide array of clinical symptoms of human diseases with compromised systems of DNA repair.

Materials and methods

Cloning and expression of the E.coli formamidopyrimidine-DNA glycosylase (FPG), rat methylpurine-DNA glycosylase (MPG) and Drosophila ribosomal S3 genes

The FPG gene was isolated by PCR cloning using DNA isolated from HB101 *E.coli* cells. Oligonucleotides used in the PCR (start and stop codons are underlined) corresponded to the 5′ end (CUA_4 ATG CCT GAA TTA CCC GAA GTT G) and 3′ end (CAU_4 TTA CTT CTG GCA CTG CCG AC) of the previously sequenced gene (Boiteux *et al.*, 1987), with either CUA or CAU added to the oligonucleotides for cloning into pAMP (BRL; Wilson *et al.*, 1992; Carney *et al.*, 1995). Positive colonies were assessed for inserts and sequenced in their entirety to confirm the correct FPG gene sequence. One of these clones was then used as a template for PCR cloning into pGEX4T-1 (Pharmacia) at the *Eco*RI and *Sal*I sites using the above oligonucleotides, but replacing the CUA or CAU with *Eco*RI and *Sal*I respectively. This clone was also sequenced in its entirety to confirm both the correct sequence of the FPG gene and that the correct reading frame was used. This construct was then used for expression of the FPG protein as a GST fusion construct (GST–FPG). The cDNA for rat MPG was cloned in a similar manner to the above procedure for the FPG gene. RNA from rat Nb2 cells (immature T cell line) was reversed transcribed, and this cDNA was used in a PCR with oligonucleotides from the 5′ end (GAA GGT CGT GGG ATCC CCC ATG CCA GCG CCG CTT CAC GAA) and 3′ end (CAG TCA CGA TGA ATT CCC CTA TCT ACC ACA CTG ACC CAT) of the MPG cDNA (O'Connor and Laval, 1990) which included a *Bam*HI or *Eco*RI cloning site for insertion into the pGEX3X cloning vector at these sites for in-frame fusion protein expression (restriction sites and start and stop codons are underlined in the above oligonucleotide sequences). Clones were analyzed and one positive clone was sequenced in its entirety to verify the correct MPG coding sequence. This construct was used as a GST–MPG- expressing clone for the experiments described in this manuscript. The cloning and construction of *Drosophila* GST–S3 fusion constructs have been described previously (Wilson *et al.*, 1993, 1994).

Bacterial strains for overexpression or complementation

The *E.coli* strains used in this study were: CC104 [*ara*,Δ(gpt-lac)5, *rpsL* (F′*lacI378*, *lacZ461*, *proA*$^+$*B*$^+$)] and its derivative TT101 that has *mut*M:: mini-*tet* (Michaels *et al.*, 1991); AB1157 and its derivative *nfo*-1:: *kan* Δ(*xth-pncA*) 90 (RPC501; Cunningham *et al.*, 1986).

Overexpression of GST fusion constructs and purification

Overnight bacterial cultures were diluted in pre-warmed LB media to an OD_{595} = 0.1. When cultures reached an OD_{595} = 0.5, isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.1 mM, and growth at 27°C continued for 3 h. Bacteria were pelleted by centrifugation and resuspended in phosphate-buffered saline (PBS) containing 10 μg/ml aprotinin, 10 μg/ml leupeptin and 10 μg/ml antipain (Boehringer Mannheim). Bacteria were sonicated for three 20 s pulses and cellular debris removed by centrifugation. The soluble supernatant was then applied to a glutathione–agarose (Sigma) affinity column, washed with 10 column volumes of PBS, and the fusion construct eluted (1 ml fractions) with 10 mM reduced glutathione in 50 mM Tris–HCl, pH 7.5 and 1 mM dithiothreitol (DTT). This resulted in a homogeneous preparation of fusion construct as judged by SDS–PAGE and Coomassie staining. Individual fractions containing the fusion construct were combined and then dialyzed for 3 h against 30 mM HEPES, pH 7.5 and 35 mM NaCl. This procedure resulted in the expression and purification of only soluble fusion constructs, as opposed to previous methods that relied on the resolubilization of GST–S3 insoluble proteins (Wilson *et al.*, 1994).

Activity on 8-oxoguanine or abasic site-containing DNA

A 37 bp 5′ ^{32}P end-labeled duplex DNA fragment [5′-CTTGGAACTGG-ATGTCGGCACXAGCGGATACAGGAGCA-3′ where X = 8-oxoguanine (8-oxoG-37mer) or uracil at nucleotide position 21 (labeled strand)] was used as a substrate either directly (8-oxoG-37mer), or treated (20 pmol) with *E.coli* uracil-DNA glycosylase (Epicentre, 2 units) to form an apyrimidinic (AP) site in place of the uracil (Lindahl, 1980). Following phenol/chloroform extraction, the AP site-containing oligonucleotide (AP-37mer) was precipitated with cold ethanol.

Reaction mixtures (10 μl) contained ~1 pmol of 5′ end-labeled 37mer; in addition, reactions for GST–S3 contained 30 mM HEPES, pH 7.4, 50 mM KCl, 1 μg/ml bovine serum albumin (BSA), 0.05% Triton X-100, 1 mM DTT and 0.5 mM EDTA. For the human AP endonuclease, reactions contained 50 mM HEPES, pH 7.5, 50 mM KCl, 1 μg/ml BSA, 10 mM $MgCl_2$ and 0.05% Triton X-100. Reactions for *E.coli* endonuclease III contained 15 mM KH_2PO_4, pH 6.8, 10 mM EDTA, 10 mM β-mercaptoethanol and 40 mM KCl. The *E.coli* GST–FPG reactions contained 15 mM HEPES, pH 7.5, 50 mM KCl, 10 mM β-mercaptoethanol and 0.5 mM EDTA. The DNA reaction products were then separated on a polyacrylamide gel containing 7 M urea. Dried gels were subjected to autoradiography for visualization and video densitometric analysis (Lynx Densitometer, Applied Imaging Corp., Santa Clara, CA).

Complementation of mutM and RPC501

Overnight cultures were diluted to an OD_{595} = 0.1, and grown to an OD_{595} = 0.5, at which time IPTG (0.1 mM) was added, and growth allowed to continue for an additional 60 min at 27°C. Cell densities were then normalized to an OD_{595} = 0.5. For the survival of cells in growth media, either H_2O_2 or MMS was added to the culture media for 15 min at 27°C (without shaking). Cultures were then diluted to 10^{-6} in minimal M9 medium, and 50 μl of each culture plated on LB agar and incubated for 18–24 h at 37°C. For analysis of cell survival on gradient plates, the procedure of Cunningham *et al.* (1986) was followed. Briefly, after diluting induced bacterial cultures to an OD_{595} = 0.5 (see above),

50 μl of bacterial culture was diluted further in 2 ml of molten soft agar a 42°C and spread in a thin layer on a microscope side. Gradient plates were inoculated with the bacterial suspension by dipping the edge of a warm microscope slide in the suspension and then touching the surface of the gradient plate. The gradient of H_2O_2 was established by adding 25 ml of molten LB agar containing H_2O_2 (1 mM) to a 9×9 cm square Petri dish elevated at one edge. After the agar hardened, the plate was placed on a flat surface and overlaid with molten LB agar (25 ml). To visualize bacterial growth, plates were stained with acridine and photographed under UV light.

Acknowledgements

The authors wish to thank Dr J.Miller for the *mut*M strain and Dr R.Cunningham for the RPC501 strain and purified *E.coli* endonuclease III. Purified human AP endonuclease (hAPE) was a generous gift from the laboratory of Dr B.Demple. Purified FPG was kindly provided by Drs R.Rieger and A.Grollman, who also supplied an 8-oxoguaine-containing oligonucleotide used in our early studies. For the oligonucleotides used in the present study, we wish to acknowledge the Wells Center Oligonucleotide Synthesis Facility supported by the Riley Memorial Association. This work was supported by grants from the National Institutes of Health to M.R.K. and W.A.D. (RRO9884) and P.W.D. (CA55896), the American Cancer Society (CN126 to P.W.D.), and the Council for Tobacco Research (W.A.D. and M.R.K.).

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Received on October 26, 1995; revised on January 5, 1996