Conversion of the Bifunctional 8-Oxoguanine/β-δ
Apurinic/Apyrimidinic DNA Repair Activities of Drosophila
Ribosomal Protein S3 into the Human S3 Monofunctional
β-Elimination Catalyst through a Single Amino Acid Change*

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The Drosophila S3 ribosomal protein has important roles in both protein translation and DNA repair. In regards to the latter activity, it has been shown that S3 contains vigorous N-glycosylase activity for the removal of 8-oxoguanine residues in DNA that leaves baseless sites in their places. Drosophila S3 also possesses an apurinic/apyrimidinic (AP) lyase activity in which the enzyme catalyzes a β-elimination reaction that cleaves phosphodiester bonds 3’ adjacent to an AP lesion in DNA. In certain situations, this is followed by a δ-elimination reaction that ultimately leads to the formation of a single nucleotide gap in DNA bordered by 5’ and 3’-phosphate groups. The human S3 protein, although 80% identical to its Drosophila homolog and shorter by only two amino acids, has only marginal N-glycosylase activity. Its lyase activity only cleaves AP DNA by a β-elimination reaction, thus further distinguishing itself from the Drosophila S3 protein in lacking a δ-elimination activity. Using a hidden Markov model analysis based on the crystal structures of several DNA repair proteins, the enzymatic differences between Drosophila and human S3 were suggested by the absence of a conserved glutamine residue in human S3 that usually resides at the cleft of the deduced active site pocket of DNA glycosylases. Here we show that the replacement of the cleft of the deduced active site pocket of DNA glycosylase that cleaves the glycosylic bond between the sugar and damaged base, resulting in an apurinic/apyrimidinic (AP) site. For most prokaryotic and eukaryotic enzymes that act on 8-oxoG, the glycosylase step is followed by a β-elimination lyase reaction that results in a 3’-phosphodiester cleavage adjacent to the AP site. Mechanistically, the coupling of these two activities has been thought to be mediated through an ε-amine nucleophile of lysine that attacks the C-1’ of the damaged nucleotide sugar (7–10). This results in the loss of the base and the formation of a covalent attachment of the enzyme to the 2’-deoxyribose sugar moiety of the abasic site, forming an imine (Schiff) base intermediate that can conveniently be trapped in vitro by sodium borohydride (11). The formation of a Schiff base is followed by abstraction of the 2’-H and cleavage of the 3’-C=O bond through an elimination reaction (12–14). In yeast and humans, the ε-amine group of lysine occupies a place in a helix-hairpin-helix-GPD domain considered critical for non-sequence-specific binding to DNA (see Fig. 1). Also within

Aerobic respiration produces electrophilic oxidants known as reactive oxygen species, which are potentially deleterious to the stability and integrity of DNA. Beyond the internal cellular production of reactive oxygen species, the burden of free radical attack on cellular DNA is increased through exposure to ionizing radiation (1), cigarette tar and smoke (2), and the recently discovered presence of stable free radicals associated with particulate matter generated by combustion (3).

The impact that free radicals have on DNA is varied and profound. For example, single- and double-strand breaks, along with the production of baseless sites in DNA, are a common result of free radical damage to DNA (4). Oxidatively damaged DNA bases also result from the presence of free radicals; the most notable of such bases is 7,8-dihydro-8-oxoguanine (8-oxoG). This lesion frequently mispairs with adenine, resulting in G:C → T:A transversion mutations (5) that are exceedingly common in somatic mutations found in human cancers and, importantly, are abundant in the tumor suppressor gene p53 (6).

The formation of oxidative damage to DNA is subject to repair by the base excision repair pathway, which exists as a ubiquitous collection of enzymes that involves the removal of altered bases, followed by several steps to return the damaged DNA to its original state. The liberation of oxidatively damaged DNA bases is the initial step in this process, which is catalyzed by an N-glycosylase that cleaves the glycosylic bond between the sugar and damaged base, resulting in an apurinic/apyrimidinic (AP) site. For most prokaryotic and eukaryotic enzymes that act on 8-oxoG, the glycosylase step is followed by a β-elimination lyase reaction that results in a 3’-phosphodiester cleavage adjacent to the AP site. Mechanistically, the coupling of these two activities has been thought to be mediated through an ε-amine nucleophile of lysine that attacks the C-1’ of the damaged nucleotide sugar (7–10). This results in the loss of the base and the formation of a covalent attachment of the enzyme to the 2’-deoxyribose sugar moiety of the abasic site, forming an imine (Schiff) base intermediate that can conveniently be trapped in vitro by sodium borohydride (11). The formation of a Schiff base is followed by abstraction of the 2’-H and cleavage of the 3’-C=O bond through an elimination reaction (12–14). In yeast and humans, the ε-amine group of lysine occupies a place in a helix-hairpin-helix-GPD domain considered critical for non-sequence-specific binding to DNA (see Fig. 1). Also within
this active site pocket domain are an aspartic acid residue that is also essential for catalysis and a glutamine residue that resides at the mouth of the active site pocket and is believed to be involved in nucleotide flipping.

For Drosophila S3 (15), Escherichia coli formamidopyrimidine DNA glycosylase (mutM) (16), and T4 UV endonuclease (11, 17), the $\beta$-elimination reaction is followed by a $\delta$-elimination reaction that results in a one-nucleotide gap bordered by a 3'-P, 5'-P terminus. The formamidopyrimidine protein is an example of an enzyme that efficiently removes 8-oxoG through a concerted N-glycosylase/$\beta$-activity. Conversely, the Drosophila S3 and T4 UV endonuclease V activities appear to carry out a $\delta$-elimination reaction through a second encounter with an abasic DNA substrate.

In Drosophila, two genes have been identified that are involved in the removal of 8-oxoG (15, 18), one being the aforementioned S3 that is involved in both protein translation and the removal of 8-oxoG from DNA. Studies on the human S3 show that, even though it is 80% identical to the Drosophila protein and only two amino acids shorter, it catalyzes an extremely inefficient in removing 8-oxoG from oxidatively damaged DNA.

Based on previous studies, S3 appeared to be an orphan among other eukaryotic 8-oxoG DNA glycosylases. However, hidden Markov model-based analysis suggested structural similarity between the S3 sequence family and, among others, the E. coli repair proteins endonuclease III (7, 20), MutY (21), and AlkA 3-methyladenine DNA glycosylase (22, 23). Both Drosophila and human S3 possess conserved amino acids noted above and known to be important for substrate binding and/or catalysis (Fig. 1). One obvious difference between the human and Drosophila S3 protein revealed by this analysis, however, was a glutamine residue (Fig. 2) that rests at the cleft of the deduced active site pocket (Fig. 1) and is considered important for the “flicking out” of the modified base (24). We reasoned that the absence in human S3 of this glutamine residue and its marginal N-glycosylase activity for the removal of 8-oxoG might be linked to one another and that they therefore offered an excellent model for testing the importance of this amino acid in performing the N-glycosylase step in base excision repair. We therefore changed, by site-directed mutagenesis, the glutamine residue for Drosophila S3. As predicted from our hidden Markov analysis, a change in glutamine results in the loss of N-glycosylase activity.

EXPERIMENTAL PROCEDURES

Materials—Oligonucleotides containing 8-oxoguanine and uracil were purchased from Operon Technologies and Midland Certified Reagent Co., respectively. The QuickChange site-directed mutagenesis kit was from Stratagene (La Jolla, CA), and the pGEXXX vector was from Amersham Pharmacia Biotech. Glutathione-agarose and sodium borohydride were purchased from Sigma; aprotinin and leupeptin were from Roche Molecular Biochemicals; and isopropyl-1-thio-β-D-galactopyranoside was from Life Technologies, Inc. DNA-damaging agents MMS and hydrogen peroxide were from Fluka and Mallinkrodt, respectively. E. coli uracil DNA glycosylase was purchased from Epicentre (Madison, WI).

Site-directed Mutagenesis—The Drosophila ribosomal protein S3 gene was mutated at Gln-59 to Ala and Gln-59 to Asn using a QuickChange site-directed mutagenesis kit following the protocol provided by the manufacturer. The mutagenic primer sets used for Q59A and Q59N were 5'-GGCCAAAGACCCAGGAGGTGCTGGGCGAGA-AGG-3' and 5'-CCCTTCGCCCCGACACCCTGGTCTTGTGGCC-C-3' and 5'-GGCCAAAGACCCAGGAGGTGCTGGGCGAGA-AGG-3' and 5'-CCCTTCGCCCCGACACCCTGGTCTTGTGGCC-C-3', respectively. The S3 gene in pGEX3 was used as the template. The constructs plasmids with desired mutations were picked, and the mutations were confirmed by DNA sequencing.

Bacterial Strains for Overexpression or Complementation—AB1157 and its derivative RPC501 nfo-1::kanΔ(xth-pncA) 90 (25) were used in the protocols described here and elsewhere (15) for the characterization of S3.

Overexpression of GST Fusion Constructs and Purification—Overnight bacterial cultures of RPC501 transformed with Drosophila GST-S3, human GST-S3, and Drosophila mutants GST-S3-Q59A and GST-S3-Q59N were induced in liquid cultures with isopropyl-1-thio-β-D-galactopyranoside at a final concentration of 0.1 and grown at 37 °C until cultures reached an A$_{600}$ of 0.5; then isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.1 mM, and growth continued for 3 h at 27 °C. Bacteria were pelleted by centrifugation and resuspended in phosphate-buffered saline containing 10 μg/ml aprotinin and 10 μg/ml leupeptin. Bacteria were sonicated three times at 15-s intervals with constant pulse, and the sonicate was removed by centrifugation. The soluble supernatant was then applied to a glutathione-agarose affinity column and washed with 10 column volumes of phosphate-buffered saline, and the fusion construct was eluted (1-mL fractions) with 10 mM reduced glutathione in 8-oxoG or AP 37-mer, 30 mM HEPES, pH 7.4, 50 mM KCl, and Triton X-100. Reaction mixtures were incubated at 37 °C for 90 min. Incubation of 0.1 mM, and growth continued for 3 h at 7 °C. Bacteria were diluted in phosphate-buffered saline containing 10 μg/ml aprotinin and 10 μg/ml leupeptin. Bacteria were sonicated three times at 15-s intervals with constant pulse, and the sonicate was removed by centrifugation. The soluble supernatant was then applied to a glutathione-agarose affinity column and washed with 10 column volumes of phosphate-buffered saline, and the fusion construct was eluted (1-mL fractions) with 10 mM reduced glutathione in 50 mM Tris-HCl, pH 7.5. This resulted in a homogeneous preparation of fusion construct, as judged by SDS-polyacrylamide gel electrophoresis and Coomassie staining.

Activity on 8-Oxoguanine or Abasic Site-containing DNA—A 37-base pair 5'-P end-labeled duplex DNA fragment (5'-CTT GGA CGT GAT GTC GGC ACC AGC GGA TAC AGG AGC A-3', where X = 8-oxoguanine (8-oxoG 37-mer) or uracil at nucleotide position 21 (labeled 8-oxoG 37-mer) or uracil at nucleotide position 21 (labeled 8-oxoG 37-mer) or uracil at nucleotide position 21 (labeled 37-mer)) was used as a substrate either directly or treated (20 pmol) with E. coli uracil DNA glycosylase (2 units) to form an AP site in place of the uracil (26). Following phenol/chloroform extraction, the AP site-containing oligonucleotide (AP 37-mer) was precipitated with cold ethanol.

Reaction mixtures (10 μl) contained ~1 pmol of 5'-end labeled 37-mer; in addition, reactions for GST-S3 contained 30 mM HEPES, pH 7.4, 50 mM KCl, 1 μg/ml bovine serum albumin, 0.05% Triton X-100, 1 mM diethylthioptitol, and 0.5 mM EDTA. The DNA reaction products were separated on a 16% polyacrylamide gel containing 7 M urea. Dried gels were subjected to autoradiography for visualization and densitometric analysis (ChemilmagerTM 4000, Alpha Innotech Corporation).

Complementation of RPC501—Overnight cultures of AB1157, RPC501, and RPC501 transformed with Drosophila GST-S3, human GST-S3, and Drosophila mutants GST-S3-Q59A and GST-S3-Q59N were diluted to an A$_{600}$ of 0.1 and grown at 37 °C to an A$_{600}$ of 0.5, at which time isopropyl-1-thio-β-D-galactopyranoside (0.1 mM) was added, and growth was allowed to continue for an additional 3 h at 27 °C. The cultures were normalized to an A$_{600}$ of 1, and serial dilutions were applied as spots (20 μl) to untreated and MMS- and H$_2$O$_2$-treated LB agar plates.

Formation of Coadjutant Complexes in the Presence of NaBH$_4$—The fusion proteins (10 pmol) were combined with NaBH$_4$ (30 mM) and immediately transferred to reaction mixtures containing 20 pmol of 8-oxoG or AP 37-mer, 30 mM HEPES, pH 7.4, 50 mM KCl, and Triton X-100. Reaction mixtures were incubated at 37 °C for 90 min. Incubations were terminated with the addition of DNA loading dye, and samples were loaded on a 10% non-denaturing gel. After electrophoresis, gels were dried under vacuum and exposed to autoradiography.

Hidden Markov Modeling—An iterative procedure for hidden Markov model-based analysis of protein sequence families was em-
To examine the possible role of Gln in the removal of 8-oxoG, we mutated the residue to Ala or Asn. The site-directed Drosophila mutants were subsequently placed in pGEX3X for overexpression and purification in a bacterial strain defective for AP endonuclease activity (RPC501) (25). The final purification product resulted in apparently homogenous GST fusion construct protein preparations (as judged by Coomassie staining) that had molecular weights identical to that for the wild-type Drosophila GST-S3 protein (data not shown). Tests for 8-oxoG activity were then performed on a 5′-end-labeled oligonucleotide containing a single 8-oxoG residue at position 21 (15). Results show that a change from a Gln residue to an Ala amino acid completely abolished N-glycosylase activity (Fig. 3A, lanes 5–7 as compared with lanes 9–11, respectively). A more conservative substitution with an Asn residue (lanes 1–3) also failed to show activity except at higher protein concentrations. Notably, these site-directed changes resemble the activity possessed by wild-type human S3 (Fig. 3B, lanes 5–7), in which little if any activity is observed on 8-oxoG residues.

Gln-59 → Ala Abolishes Drosophila S3 8-OxoG DNA Glycosylase Activity—In Drosophila, the structural equivalent of E. coli endonuclease III Gln-41 is Gln-59. To examine the possible role of Gln-59 in the removal of 8-oxoG, we mutated the residue to Ala or Asn. The site-directed Drosophila mutants were subsequently placed in pGEX3X for overexpression and purification in a bacterial strain defective for AP endonuclease activity (RPC501) (25). The final purification product resulted in apparently homogenous GST fusion construct protein preparations (as judged by Coomassie staining) that had molecular weights identical to that for the wild-type Drosophila GST-S3 protein (data not shown). Tests for 8-oxoG activity were then performed on a 5′-end-labeled oligonucleotide containing a single 8-oxoG residue at position 21 (15). Results show that a change from a Gln residue to an Ala amino acid completely abolished N-glycosylase activity (Fig. 3A, lanes 5–7 as compared with lanes 9–11, respectively). A more conservative substitution with an Asn residue (lanes 1–3) also failed to show activity except at higher protein concentrations. Notably, these site-directed changes resemble the activity possessed by wild-type human S3 (Fig. 3B, lanes 5–7), in which little if any activity is observed on 8-oxoG residues.

Gln-59 → Ala Alters the Drosophila β-δ-Elimination Reaction at Abasic Sites to a Human S3-like β-Elimination—We next tested whether the change at Gln-59 had an effect on the activity of Drosophila S3 to act on an abasic site existing in a 5′-end-labeled oligonucleotide. Surprisingly, the Gln to Ala change resulted in the Drosophila protein catalyzing a single β-elimination reaction (Fig. 4A, lanes 9–11). This is in contrast to the wild-type Drosophila S3 activity that carries out a β-δ-elimination (Fig. 4A, lanes 5–7) but is similar to that observed for the human S3 (Fig. 4B, lanes 5 and 6), which, like the Drosophila Gln to Ala change, catalyzes a single β-elimination reaction. When the Drosophila Gln residue was changed to an Asn residue (Fig. 4A, lanes 1–3), activity appeared on the abasic substrate that was greater than that observed for the change to Ala but nevertheless appeared to lack δ-elimination activity under the enzymatic conditions employed. Multiple bands were, however, observed using the Gln to Asn change. When reaction conditions were chosen in which there was an excess amount of protein (2 pmol), these multiple products appeared to be entirely driven into a δ-elimination product (Fig. 5, A and B, lane 4). Excess amounts of ds3Q59A, on the other hand, failed to act on 8-oxoG (Fig. 5A, lane 3) and generated a single δ-elimination product when acting on the abasic-containing DNA substrate (Fig. 5B, lane 3). It should be noted that the δ-elimination product generated by ds3Q59A was susceptible to δ-cleavage by wild-type Drosophila GST-S3 (data not shown), suggesting that absence of this activity in the mutant was due to it somehow masking the site from cleavage.

Sodium Borohydride-mediated Trapping of Human S3 and Drosophila S3 and Mutants on 8-OxoG and Abasic DNA Substrates—A characteristic of virtually all N-glycosylase/AP lyases is that they form an imine intermediate that can be trapped by sodium borohydride (29). Using this approach, we tested whether the lack of N-glycosylase activity exhibited by the Gln to Ala change could be traced to an inability of the mutant to bind to pre-existing 8-oxoG or AP sites in synthetic oligonucleotides. As expected, an electromobility shift assay

![Multiple sequence alignment of Drosophila S3 (Swiss-Prot code RS3 DROME), human S3 (RS3 HUMAN), and E. coli endonuclease III (END3 ECOLI). Residues conserved in all three structures are in yellow. The secondary structure elements for END 3ECOLI are based on the crystal structure (2ABK) and are color-coded using the scheme in Fig. 1.](image)

![Activity on 8-oxoguanine-containing DNA for different amounts of GST-dS3, GST-dS3Q59A, GST-dS3Q59N, and GST-hS3. Reactions contained 1 pmol of 8-oxoG 37-mer incubated with enzyme for 30 min at 37 °C. A, lanes 1–3, incubations with 0.1, 0.2, and 0.4 pmol of GST-dS3, respectively; lanes 5–7, incubations with 0.1, 0.2, and 0.4 pmol of GST-dS3Q59A, respectively; lanes 9–11, incubations with 0.1, 0.2, and 0.4 pmol of GST-dS3Q59A, respectively; B, lanes 2–4, incubations with 0.1, 0.2, and 0.4 pmol of purified GST-dS3, respectively; lanes 5–7, incubations with 0.1, 0.2, and 0.4 pmol of GST-hS3, respectively. The results presented are representative of 4 independent trials.](image)
showed that the Drosophila S3 protein containing a Gln to Ala change failed to bind the 8-oxoG DNA substrate (Fig. 6A, lane 4). It also lacked DNA binding activity for an oligonucleotide containing an abasic site (Fig. 6B, lane 3). Surprisingly, the more conservative change to an Asn residue resulted in the retention of significant DNA binding activity to both DNA substrates (Fig. 6, A, lane 5 and B, lane 4), even though its catalytic activity toward these DNA substrates is severely compromised (Figs. 3 and 4). Notably, human S3 was found to efficiently bind a substrate containing 8-oxoG (Fig. 6, lane 3), although it lacks the catalytic activity to liberate this base from DNA.

**Do Drosophila S3, Its Site-directed Mutants, and Human S3 Protect Cells from DNA-damaging Agents?**—An important test for substantiating our in vitro observations was whether they could be reproduced in an in vivo setting where the individual mutants were challenged to rescue bacterial cells from known DNA-damaging agents. We utilized a bacterial strain, RPC501, which predominantly produce abasic and oxidatively damaged sites in DNA, respectively. Wild-type AB1157 cells and RPC501 containing the genes shown in Fig. 7 were grown to mid-log phase, at which time isopropyl-1-thio-β-d-galactopyranoside was introduced. After 3 h, the cells were normalized and then serially diluted onto plates containing MMS (2 mM) or hydrogen peroxide (0.25 mM). Exposure to MMS showed that RPC501 harboring human S3 was extremely sensitive to the effects of this DNA-damaging agent. Both the Gln to Ala and Gln to Asn mutants of Drosophila S3 also showed sensitivity to MMS when compared with RPC501 containing the wild-type S3 gene. Perhaps most revealing were the effects that hydrogen peroxide had on RPC501 harboring the genes depicted in Fig. 7, in which the Gln to Ala change was slightly less protective than the Gln to Asn mutant, which supports our in vitro observations that the asparagine change is not as deleterious as a change to alanine. The human S3 appears to be the least capable of protecting cells from the DNA-damaging effects of both MMS and hydrogen peroxide.

**DISCUSSION**

The Drosophila S3 gene and its encoded protein were first characterized by our group because of its highly unusual behavior in human cells. For example, in examining the presence of AP endonucleases in human cells, Kane and Linn (30) found two species that could be resolved by separation on phosphocellulose chromatography, one of which was retained and subsequently determined to be not only the major AP endonuclease in human cells but also a protein later found for controlling the redox status of a number of important transcription factors such as Fos and Jun (31). The other AP endonuclease characterized by Linn and co-workers (32) flowed through phosphocellulose and was subsequently found to be lacking in cell lines of xeroderma pigmentosum group-D fibroblasts. This protein was later purified to homogeneity and found to be ribosomal protein S3 (19).

Although the human S3 gene is not defective in xeroderma pigmentosum-D, its expression nevertheless seems to have a curious relationship with other human diseases. For example, it is overexpressed in human colorectal cancers (33). In addition, in attempts to clone genes defective for Fanconi’s anemia and its sensitivity to MMC, the S3 gene was commonly found to complement the MMC sensitivity. The biochemical characterization of human S3 showed that it acted upon AP sites and on DNA that was heavily exposed to UV irradiation, by creating a phosphodiester break between adjacent thymine residues in a pyrimidine dimer configuration (19).

**Drosophila S3** has been found to be a much more versatile enzyme than its human counterpart. For example, it has vigorous N-glycosylase activity for the removal of 8-oxoG residues in DNA that human S3 lacks (15, 34). It also has a novel deoxyriboendonuclease activity (35) that can remove 5′-AP sites, similar to that found for E. coli formamidopyrimidine (36) and human β-DNA polymerase (37). Notably, Drosophila S3 has also been shown to hydrolytically catalyze the removal of a 3′-AP site (35), suggesting for the first time that an AP lyase can be an efficient catalyst for the priming of a single nucleotide insertion into a site generated by a DNA repair protein.

Although both human and Drosophila S3 exhibit DNA repair activities, neither have any obvious sequence similarity to eukaryotic and bacterial genes known to be involved in base excision repair. However, hidden Markov model-based analysis indicated similarity between the S3 family and other proteins possessing an 8-oxoG DNA glycosylase domain, most notably *E. coli* endonuclease III, MutY, and 3-methyladenine DNA glycosylase.

Especially relevant to our studies was the information available through the generation of crystal structures of the aforementioned *E. coli* DNA repair proteins that aided in identifying those amino acids suspected to be involved in the N-glycosylase step. Recall that human S3 lacks the ability to remove 8-oxoG, whereas Drosophila S3 possesses the exceptional ability to remove this lesion and at catalytic rates comparable with other
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examines the activity of saturating amounts of GST-dS3, GST-dS3Q59A, GST-dS3Q59N, and GST-hS3 on 8-oxoG-containing DNA and AP site-containing DNA. A, 1 pmol of 8-oxoG 37-mer was incubated with 2 pmol of GST-dS3 (lane 1), GST-hS3 (lane 2), GST-dS3Q59A (lane 3), and GST-dS3Q59N (lane 4). B, 1 pmol of abasic site-containing DNA was incubated with 2 pmol of GST-dS3 (lane 1), GST-hS3 (lane 2), GST-dS3Q59A (lane 3), and GST-dS3Q59N (lane 4). The results presented are representative of four independent trials.

Fig. 6. NaBH₄-mediated trapping of GST-dS3, GST-hS3, GST-dS3Q59A, GST-dS3Q59N, and 8-oxoG 37-mer (A) and AP 37-mer (B), respectively. Individual reaction mixtures (40 μl) contained 8-oxoG (20 pmol) or AP 37-mer (20 pmol) and purified fusion proteins GST-dS3, GST-hS3, GST-dS3Q59A, and GST-dS3Q59N (10 pmol). Reaction samples were applied to a 10% non-denaturing gel, electrophoresed, vacuum-dried, and subjected to autoradiography. The control reaction (A, lane 1) was incubated in the absence of NaBH₄. The results presented are representative of three independent trials.

Fig. 7. Survival of wild-type AB1157 and RPC501 exposed to MMS and H₂O₂. The plates contained 0 and 2 mM MMS and 0 and 0.25 mM H₂O₂. Serial dilutions of AB1157 and RPC501 transformed with GST-dS3, GST-hS3, GST-dS3Q59A, and GST-dS3Q59N were plated as spots (20 μl), and the plates were incubated at 37 °C for 2–3 days. The results presented are representative of three independent trials.

eukaryotic and prokaryotic 8-oxoG DNA glycosylases (15). Examination of an S3/8-oxoG DNA glycosylase multiple sequence alignment in the context of known 8-oxoG DNA glycosylase domain structures pinpointed one possible reason for this difference, which was a glutamine residue proposed from other studies to be important for the flipping out of a modified or nonconventional base in DNA (24), which was preserved in Drosophila S3 but modified to asparagine in human S3. This difference therefore gave us a unique opportunity to test the importance of a single amino acid for the glycolytic removal of a modified base, using as a comparison an enzyme that is highly homologous but nevertheless incapable of efficiently carrying out this step in base excision repair.

What can clearly be suggested from our studies is that a glutamine residue in Drosophila S3, which presumably rests at...
the mouth of the active site pocket, as predicted from the structure of other DNA repair proteins, is involved in the removal of 8-oxoG. Although others have identified a lysine residue as participating in this important function (8, 9), those studies lacked the ability to compare highly homologous proteins in different organisms in which one lacked the N-glycosylase step. Indeed, drawing conclusions from a single amino acid change can lead to erroneous interpretations without a model system such as the one we have been able to exploit.

The helix-hairpin-helix-GPD motif has been concluded to be a trademark of a “superfamily” of DNA glycosylases (8). Crystallographic studies suggest that the interaction of the helix-hairpin-helix-GPD domain with DNA is mediated by amino acids located in the strongly conserved loop (LPGV) and at the N-terminal end of the second helix. Although the sequence of the equivalent *Drosophila* loop differs quite considerably (IMES), the protein is clearly able to remove 8-oxoG residues in DNA, as well as to liberate 5'- and 3'-modified termini at abasic sites in DNA (35).

Unexpectedly, when the N-glycosylase activity of *Drosophila* S3 was abrogated with a Gln to Ala change, a corresponding loss in the δ-elimination reaction was observed, thus creating an enzyme that behaves identical to that seen for the human S3 protein. This implies that the same amino acid may be involved both in the liberation of a nonconventional base in DNA and in the δ-cleavage of the phosphodiester bond 5′ and adjacent to an abasic site.

Our *in vitro* results were supported by experiments executed *in vivo* that showed that a change in Gln converted the *Drosophila* S3 protein product into one that poorly protected RPC501 from the toxic effects of MMS and H₂O₂. The amino acid changes to Ala and Asn more closely resembled the protective ability conveyed by human S3, which at the very least was no better than RPC501 on its own. Experiments comparing *Drosophila* S3 and human S3 in rescuing the MMC sensitivity of Fanconi’s anemia offer another example in which the expression of *Drosophila* S3 far exceeds that of human S3 (38). The power of S3 to efficiently protect cells from a variety of different DNA lesions *in vivo* may lie in its ability to hydrolytically liberate a 3′-abasic site and thereby result in a one-nucleotide gap in DNA that would be susceptible to filling by β-DNA polymerase.

We and others have mechanistically concluded that the δ-elimination reaction may involve a second encounter with the remaining abasic site after a concerted N-glycosylase/AP lyase steps of base excision repair and, upon completion of those studies, to determine whether the second encounter hypothesis is indeed valid.

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