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Conversion of the Bifunctional 8-oxoguanine/β-δ AP 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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Summary

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 a baseless site in its place. *Drosophila* S3 also possesses an apurinic/apyrimidinic (AP) lyase activity in which the enzyme catalyzes a β-elimination reaction that cleaves phosphodiester bonds 3' and 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 2 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 *Drosophila* glutamine by an alanine residue leads to the complete loss of glycosylase activity. Unexpectedly, the δ-elimination reaction at AP sites was also abrogated by a change in the *Drosophila* glutamine residue. Thus, a single amino-acid change converted the *Drosophila* activity into one that is similar to that possessed by the human S3 protein. In support of this were experiments executed *in vivo* which showed that human S3 and the *Drosophila* site-directed glutamine changes performed poorly when compared to *Drosophila* wild-type S3 and its ability to protect a bacterial mutant from the harmful effects of DNA damaging agents.

Introduction

Aerobic respiration produces electrophilic oxidants, known as reactive oxygen species (ROS),which are potentially deleterious to the stability and integrity of DNA. Beyond the internal cellular production of ROS, the burden of free radical attack on cellular DNA is increased through the 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 notably being the formation of 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 (BER), 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 identity of the ε-amine group of

lysine occupies a place in a helix-hairpin-helix-GPD (HhH) domain considered critical for non-sequence specific binding to DNA (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 (Fpg; *mut*M) (16), and T4 UV endonuclease (11,17) the β-elimination reaction is followed by a δ-elimination reaction that results in a one nucleotide gap bordered by a 3'-P, 5'P terminus. The Fpg protein is an example of an enzyme that efficiently removes 8-oxoG through a concerted N-glycosylase/β-δ activity. Conversely, the *Drosophila* S3 and T4 UV endonuclease V activities appear to carry out a δ-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 2 amino acids shorter, it catalyzes an entirely different enzymatic reaction. For example, human S3 acts primarily on heavily UV-irradiated DNA, and it catalyzes only a single β-elimination reaction at abasic sites residing in DNA (19). The human S3 protein, unlike *Drosophila* S3, is 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 (OGGs). However, hidden Markov model-based analysis suggested structural similarity between the S3 sequence family and, amongst others, the *E*. *coli* repair proteins endonulease 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 "flipping 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 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 acitivity.

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 pGEX3X vector from Pharmacia. Glutathione-agarose and Sodium Borohydride were purchased from Sigma, aprotinin and leupeptin from Boehringer Mannheim and IPTG from Gibco BRL. DNA damaging agents MMS and Hydrogen peroxide were from Fluka and Mallinckrodt respectively. *E. coli* Uracil DNA glycosylase was purchased from Epicentre (Madison, WI).

Site-directed mutagenesis. The *Drosophila* ribosomal protein S3 gene was mutated at Q59 to alanine (A), and Q59 to asparagine (N) using QuikChange Site-Directed Mutagenesis Kit following the protocol provided by the manufacturer. The mutagenic primer sets used for Q59A and Q59N were 5'-GGCCACCAAGACCCAGGAGGTGCTGGGCGAGAA GG -3' and 5'-CCTTCTCGCCCAGCACCGCCTGGGTCTTGGTGGCC -3'; 5'-GGCCACCAAGACCCAGANCGTGCTGGGCGAGAAGG -3' and 5'-CCTTCTCGCCCAGCACGTTCTGGGTCTTGGTGGCC -3' respectively. The S3 gene in pGEX3 was used as the template. The colonies containing 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 studies 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-S3Q59A and GST-S3Q59N were diluted in pre-warmed LB+ampicilin media to an OD_{595}=0.1 and grown at 37^{o}C until cultures reached an OD_{595}=0.5, then isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.1 mM, and growth continued for 3 hours at 27^{o}C. Bacteria were pelleted by centrifugation and resuspended in phosphate-buffered saline (PBS) containing 10 μg/ml aprotinin and 10 μg/ml leupeptin. Bacteria were sonicated three times at 15 sec intervals with constant pulse and cellular debris removed by centrifugation. The soluble supernatant was then applied to a glutathione-agarose 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. This resulted in a homogeneous preparation of fusion construct as judged by SDS-PAGE and Coomassie staining.

Activity on 8-oxoguanine or abasic site containing DNA. A 37 bp 5' ^{32}P end-labeled duplex DNA fragment [5'-CTT GGA CTG GAT GTC GGC ACX AGC GGA TAC AGG AGC A-3' where X=8-oxoguanine (8-oxoG-37mer) or uracil at nucleotide position 21 (labeled strand) was used as a substrate either directly, or treated (20 pmol) with *E.coli* uracil DNA glycosylase (2 units) to form an apyrimidinic (AP) site in place of the uracil (26). 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 37 mer; 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% TritonX-100, 1 mM DTT and 0.5 mM EDTA. The DNA reaction products were separated on a 16% polyacrylamide gel containing 7M urea. Dried gels were subjected to autoradiography for visualization and densitometric analysis (ChemiImager^{TM} 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-S3Q59A and GST-S3Q59N were diluted to an OD_{595}=0.1, and grown at 37^{o}C to an OD_{595}=0.5, at which time IPTG (0.1 mM) was added, and growth allowed to continue for an additional 3 hours at 27^{o}C. The cultures were normalized to an OD_{595}=1 and serial dilutions were applied as spots (20 μl) to untreated, MMS and H_2O_2 treated LB agar plates.

Formation of Covalent 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^{o}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 (HMM) based analysis of protein sequence families was employed (27). The training set for the domain OGG HMM consisted of bacterial, archaeal, organellar, and eukaryotic S3's together with bacterial endonuclease III, mutY and 3-methyladenine DNA glycosylase. Model training and analysis was performed using SAM suite of programs (see http://www.cse.ucsc.edu/research/compbio/sam.html and references therein). Figures for the HMM- generated alignment and ribbon diagram were generated using ALSCRIPT (28).

Results

Gln59 →Ala abolishes *Drosophila* S3 8-oxoG DNA glycosylase activity

In *Drosophila*, the structural equivalent of *E. coli* endonuclease III Q41 is gln Q59. To examine the possible role of gln59 (Q) in the removal of 8-oxoG, we mutated the residue to ala (A) or asn (N). The site-directed *Drosophila* mutants were subsequently placed in pGEX3X for over-expression 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 (not shown). Tests for 8-oxoG activity were then performed on a 5'end-labled 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 to 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 that possessed by wild-type human S3 (Fig. 3B, lanes 5-7), in which little if any activity is observed on 8-oxoG residues.

Gln59→Ala converts the *Drosophila* β-δ elimination reaction at abasic sites to a human S3-like β-elimination

We next tested what effect the change at gln59 had on the ability of *Drosophila* S3 to act on an abasic site existing in a 5'end-labled 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 reaction (lanes 5-7), but similar to that observed for the human S3 (Fig. 4B, lanes 5-6) which, like the *Drosophila* gln→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. 5A & B, lane 4). Excess amounts of dS3Q59A, on the other hand, still failed to act on 8-oxoG (Fig. 5A, lane 3), and generated but a single β-elimination product when acting on the abasic-containing DNA substrate (Fig 5B, lane 3). It should be noted that the β-elimination product generated by dS3Q59A was susceptible to δ-cleavage by wild-type *Drosophila* GST-S3 (not shown), suggesting that absence of this activity in the mutant was not 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 (EMSA) 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 6A, lane 5 and Fig. 6B, lane 4), even though its catalytic activity towards 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.

Does *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, that is severely compromised in its ability to repair DNA damage produced by exposure to MMS and hydrogen peroxide (15), which predominantly produce abasic and oxidatively-damaged sites in DNA, respectively. Wild-type AB1157cells, and RPC501 cells alone, or RPC501 containing genes shown in Figure 7, were grown to mid-log phase, at which time IPTG was introduced. After 3 hrs, 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→ala and gln→asn mutants of *Drosophila* S3 also showed sensitivity to MMS when compared to RPC501 containing the wild-type S3 gene. Perhaps most revealing were the effects that hydrogen peroxide had on RPC501 harboring the genes depicted in Figure 7, in which the gln→ala change was slightly less protective than the gln→ 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 was 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, Stuart Linn and his laboratory found two species (30) 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 as well 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 the Linn group flowed through phosphocellulose, and was subsequently found to be lacking in cell lines of xeroderma pigmentosum (XP) group-D fibroblasts (32). This protein was later purified to homogeneity, and found to be ribosomal protein S3 (19).

While the human S3 gene is not defective in XP-D, its expression nevertheless seems to have a curious relationship with other human diseases. For example, it is overexpressed in human colorectal cancers (33). And 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 on AP sites, and on DNA that was heavily exposed to UV-irradiation, by it 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 dRPase activity (35) that can remove 5'-AP sites similar to that found for *E. coli* Fpg (36) and human β-DNA polymerase (37). Notably, *Drosophila* S3 has been shown to also 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 had 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 OGG 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 to other eukaryotic and prokaryotic OGGs (15). Examination of an S3/OGG multiple sequence alignment in the context of known OGG 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), that was preserved in *Drosophila* S3, but modified to asparigine 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. While 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 HhH-GPD domain with DNA is mediated by amino acids located in the

strongly conserved loop (L-P-G-V) and at the N-terminal end of the second helix. Although the sequence of the equivalent *Drosophila* loop differs quite considerably (I-M-E-S), the protein is clearly able to remove 8-oxoG residues in DNA, as well as 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→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 would imply that the same amino acid could be involved in both 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 H_2O_2. 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 offers another example in which the expression of *Drosophila* S3 far exceeds that provided by 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 concluded that mechanistically the δ-elimination reaction may involve a second encounter with the remaining abasic site after a concerted N-glycosylase/AP lyase activity generates an AP site with a phosphodiester break 3' to the abasics site (11,15). Our studies tend to point to the involvement of a gln residue for both the N-glycosylase and δ-elimination activities possessed by *Drosophila* S3. However, our results do not rule out the participation of other active-site amino acids previously shown to be critical for the base excision repair of 8-oxoG. Clearly, additional studies are warranted to determine the identity of those amino acids within the active site pocket that might also be involved in the N-glycosylase/AP lyase steps of

base excision repair, and whether the second encounter hypothesis is indeed valid upon completion of those studies.

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Figure Legends

Figure 1. Ribbon diagram of *E. coli* endonuclease III (RCSB code 2ABK). The helix-hairpin-helix domain is shown in red. The side chains of residues important for catalysis (D138 and K120) and the glutamine examined in this work (Q41) are depicted.

Figure 2. 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 cyan. The secondary structure elements for END_3ECOLI) are based on the crystal structure (2ABK) are colored-coded usinf the scheme in Figure 1.

Figure 3. Activity on 8oxoguanine containing DNA for different amounts of GST-dS3, GST-dS3Q59A, GST-dS3Q59N and GST-hS3. Reactions contained 1 pmol of 8oxoG-37mer incubated with enzyme for 30 min at 37^{o}C. **(A)** Lanes 1-3, incubations with 0.1, 0.2, and 0.4 pmol of GST-dS3Q59N respectively; lanes 5-7, incubations with 0.1, 0.2, and 0.4 pmol of GST-dS3, respectively and 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; and 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.

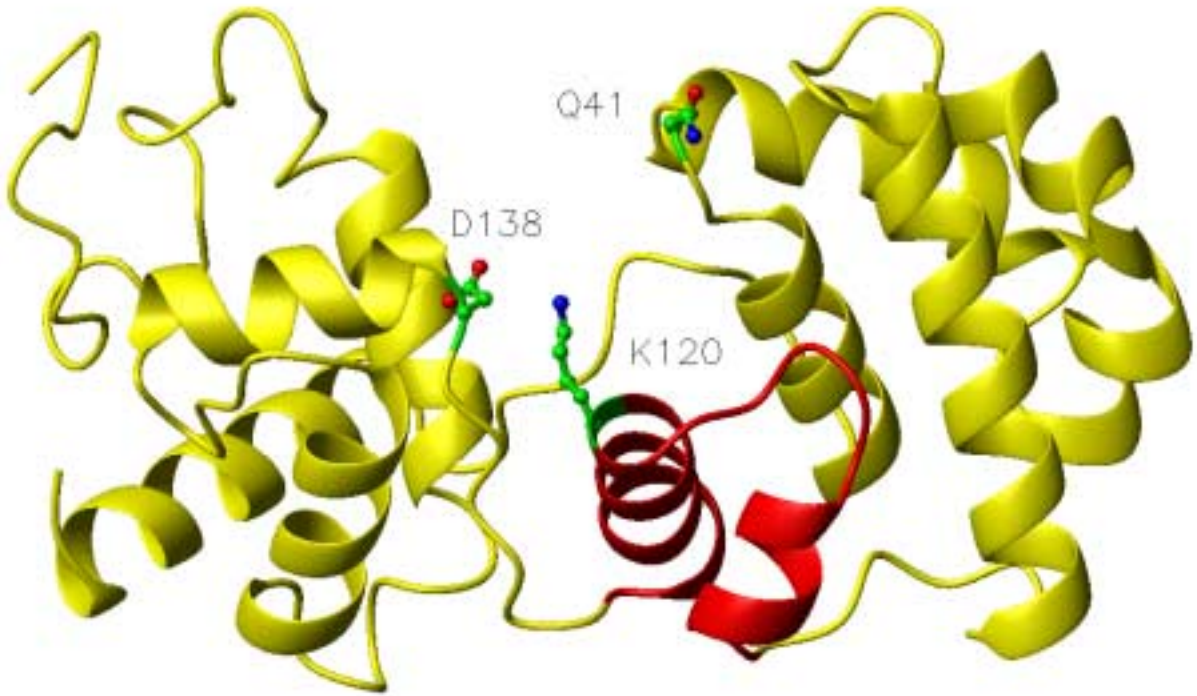
Figure 4. Activities of GST-dS3, GST-dS3Q59A, GST-dS3Q59N and GST-hS3 on abasic site containing DNA. The DNA cleavage products generated from the AP-37mer were separated on a 16% polyacrylamide DNA sequencing gel and analyzed by autoradiography. **(A)** Incubations with GST-dS3Q59N (lanes 1-3); with GST-dS3 (lanes 5-7); and with GST-dS3Q59A (lanes 9-11) contained total protein amounts of 0.1 pmol (lanes 1,5, and 9), 0.2 pmol (lanes 2, 6, and 10) and 0.4 pmol (lanes 3,7, and 11); lane 4, AP-37mer alone and lane 8, hot piperdine (HA) treatment of AP-37mer to generate a

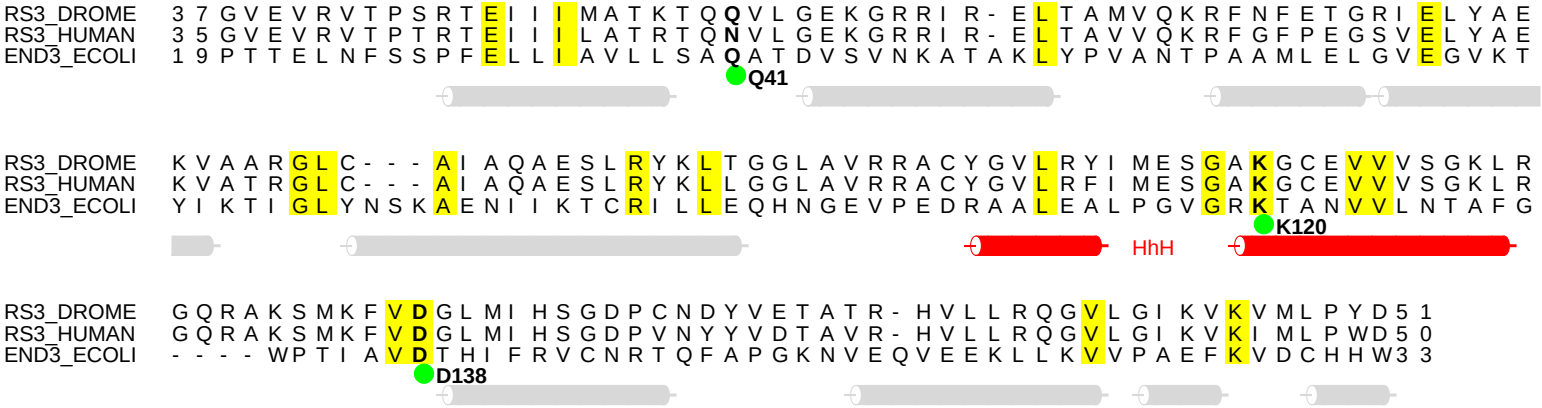
β, δ elimination product. **(B)** Incubations with 0.1, 0.2, and 0.4 pmol of GST-dS3 (lanes 2-4) and incubations with 0.1 and 0.2 pmol of GST-hS3 (lanes 5-6). The electrophoretic mobilities of the DNA cleavage products corresponding to β and δ elimination reactions are indicated. The results presented are representative of 4 independent trials.

Figure 5. 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 8oxoG-37mer was incubated with 100 ng of GST-dS3 (lane1), GST-hS3 (lane2), GST-dS3Q59A (lane3), and GST-dS3Q59N (lane 4). **(B)** 1 pmol abasic site containing DNA was incubated with 100 ng of GST-dS3 (lane 1), GST-hS3 (lane 2), GST-dS3Q59A (lane 3), and GST-dS3Q59N (lane 4). The results presented are representative of 4 independent trials.

Figure 6. $NaBH_4$-mediated trapping of GST-dS3, GST-hS3, GST-dS3Q59A, GST-dS3Q59N and 8oxoG-37mer **(A)** and AP-37mer **(B)**, respectively. Individual reaction mixtures (40 μl) contained 8-oxoG (20 pmol) or AP-37mer (20 pmol) and purified fusion proteins GST-dS3, GST-hS3, GST-dS3Q59A, GST-dS3Q59N (10 pmol). Reaction samples were applied to a 10% non-denaturing gel, electrophoresed, vacuum dried and subjected to autoradiography. Control reaction (panel **(A)** lane 1) was incubated in the absence of $NaBH_4$. The results presented are representative of 3 independent trials.

Figure 7. Survival of wild-type AB1157 and RPC501 exposed to MMS and H_2O_2. The plates contained 0, and 2 mM MMS; and 0, and 0.25 mM H_2O_2. 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 incubated at 37^{o}C for 2-3 days. The results presented are representative of 3 independent trials.


Q41
D138
K120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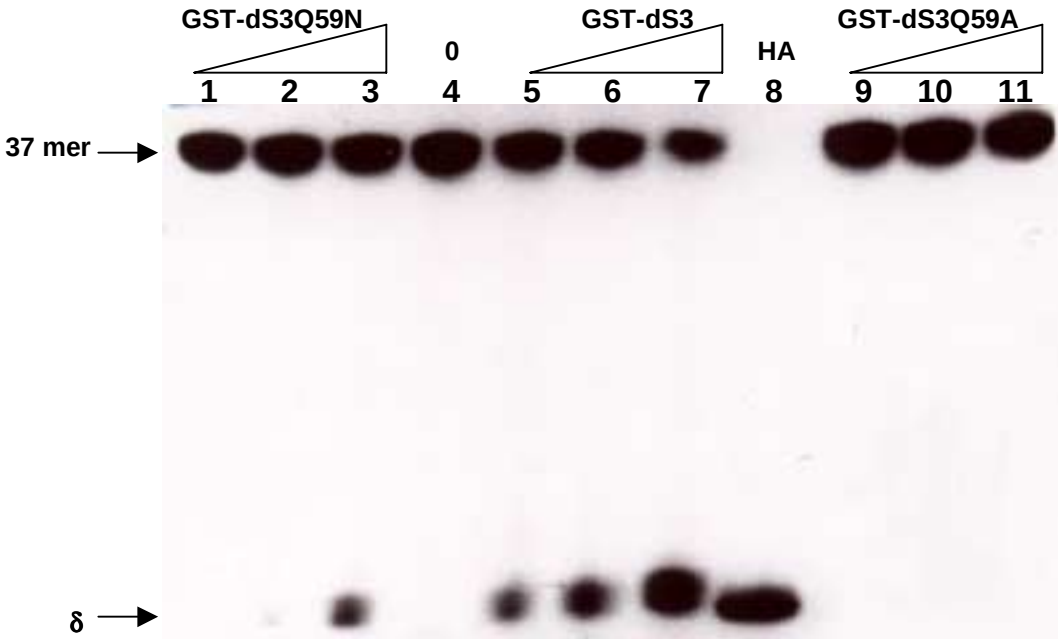
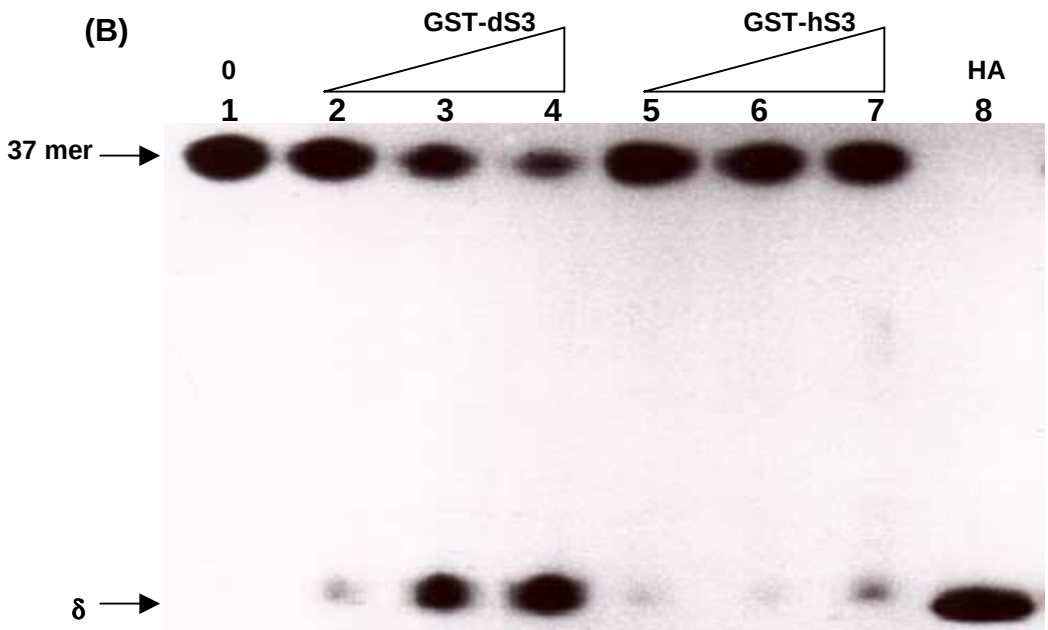
RS3_HUMAN 3 5 G V E V R V T P T R T E I I I L A T R T Q N V L G E K G R R I R - E L T A V V Q K R F G F P E G S V E L Y A E
END3_ECOLI 1 9 P T T E L N F S S P F E L L I A V L L S A Q A T D V S V N K A T A K L Y P V A N T P A A M L E L G V E G V K T
Q41
RS3_DROME K V A A R G L C - - - A I A Q A E S L R Y K L T G G L A V R R A C Y G V L R Y I M E S G A K G C E V V V S G K L R
RS3_HUMAN K V A T R G L C - - - A I A Q A E S L R Y K L L G G L A V R R A C Y G V L R F I M E S G A K G C E V V V S G K L R
END3_ECOLI Y I K T I G L Y N S K A E N I I K T C R I L L E Q H N G E V P E D R A A L E A L P G V G R K T A N V V L N T A F G
K120
HhH
RS3_DROME G Q R A K S M K F V D G L M I H S G D P C N D Y V E T A T R - H V L L R Q G V L G I K V K V M L P Y D 5 1
RS3_HUMAN G Q R A K S M K F V D G L M I H S G D P V N Y Y V D T A V R - H V L L R Q G V L G I K V K I M L P W D 5 0
END3_ECOLI - - - - W P T I A V D T H I F R V C N R T Q F A P G K N V E Q V E E K L L K V V P A E F K V D C H H W 3 3
D138

(A)


GST-dS3Q59N
0
GST-dS3
HA
GST-dS3Q59A
1 2 3 4 5 6 7 8 9 10 11
37 mer
δ

(B)


0
GST-dS3
GST-hS3
HA
1 2 3 4 5 6 7 8
37 mer
δ

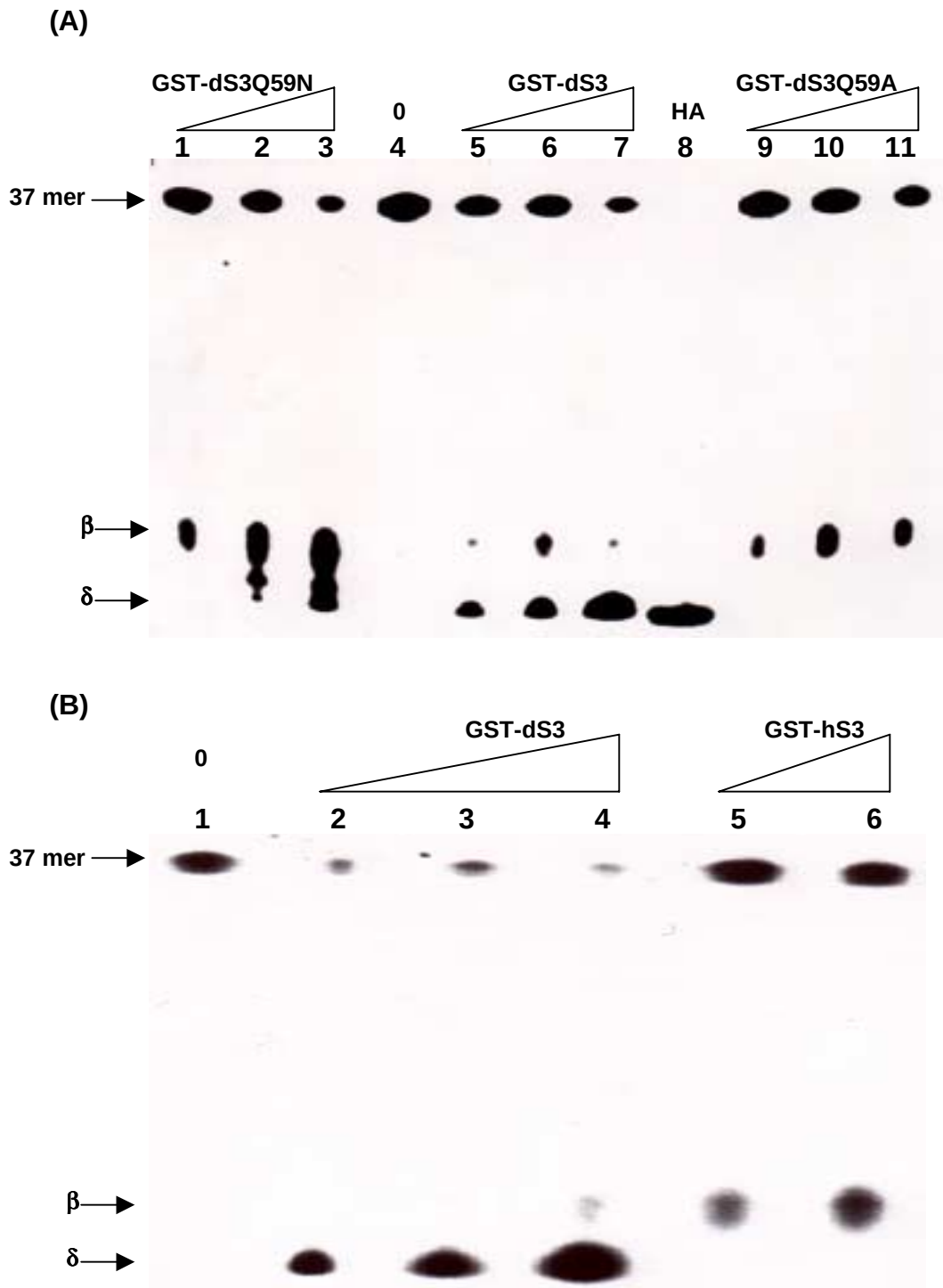

(A)
GST-dS3Q59N
0
GST-dS3
HA
GST-dS3Q59A
1
2
3
4
5
6
7
8
9
10
11
37 mer
β
δ
(B)
0
GST-dS3
GST-hS3
1
2
3
4
5
6
37 mer
β
δ

Figure5

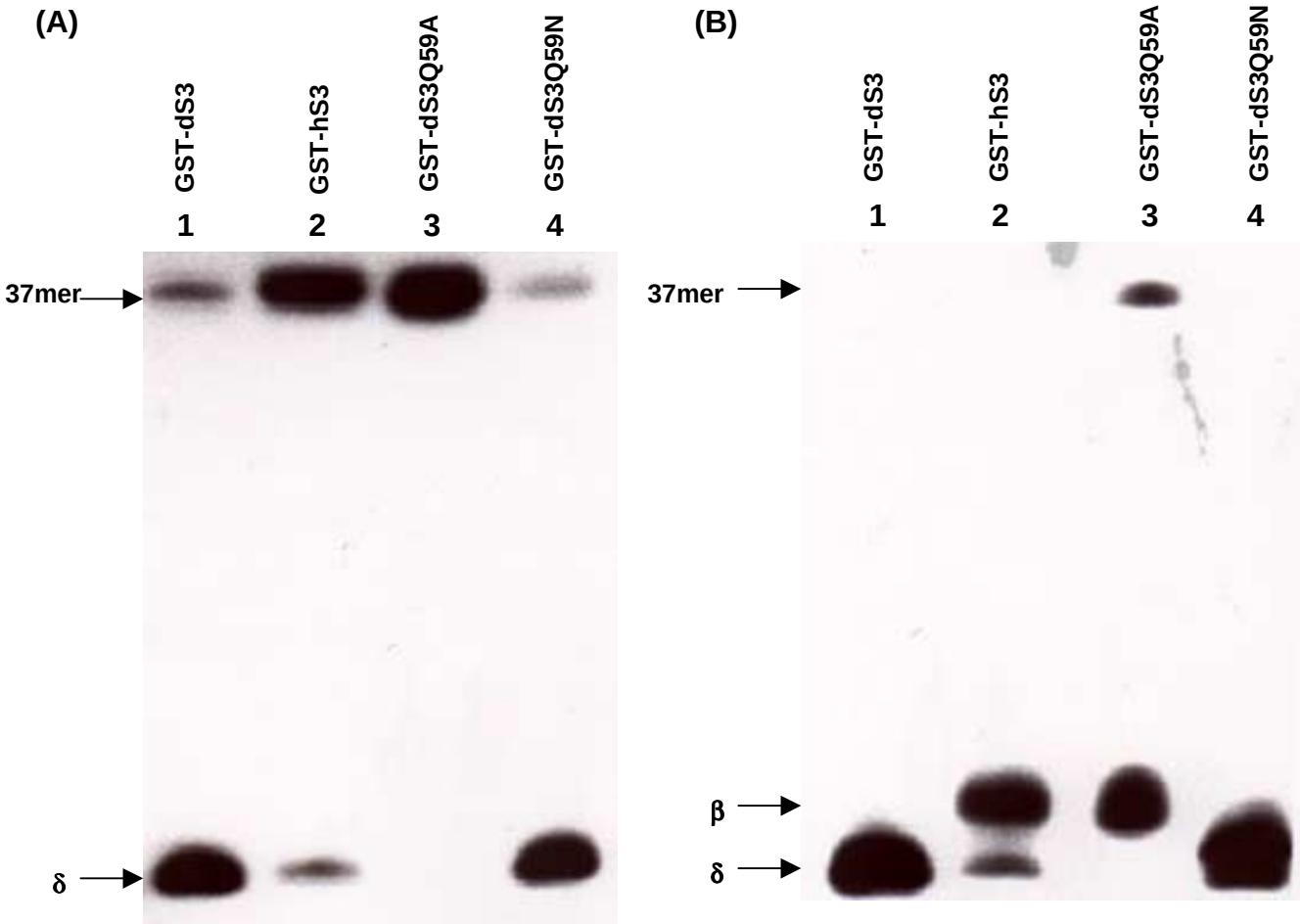
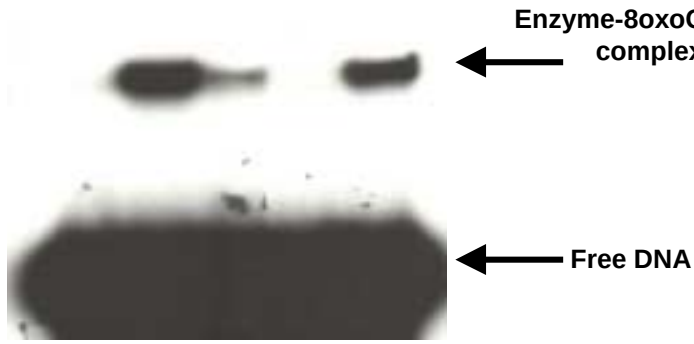

(A)
GST-dS3
GST-hS3
GST-dS3Q59A
GST-dS3Q59N
1
2
3
4
37mer
δ
(B)
GST-dS3
GST-hS3
GST-dS3Q59A
GST-dS3Q59N
1
2
3
4
37mer
β
δ

Figure 6

(A)

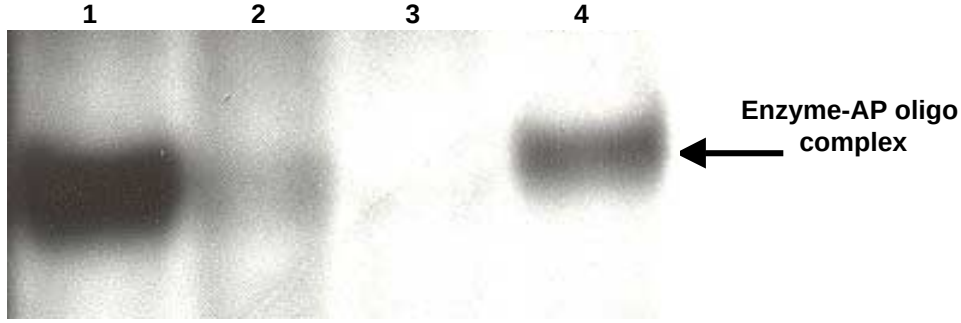
Enzyme	+	+	+	+	+
Oligo	+	+	+	+	+
$NaBH_4$	-	+	+	+	+
	1	2	3	4	5



Control | GST-dS3 | GST-hS3 | GST-dS3Q59A | GST-dS3Q59N

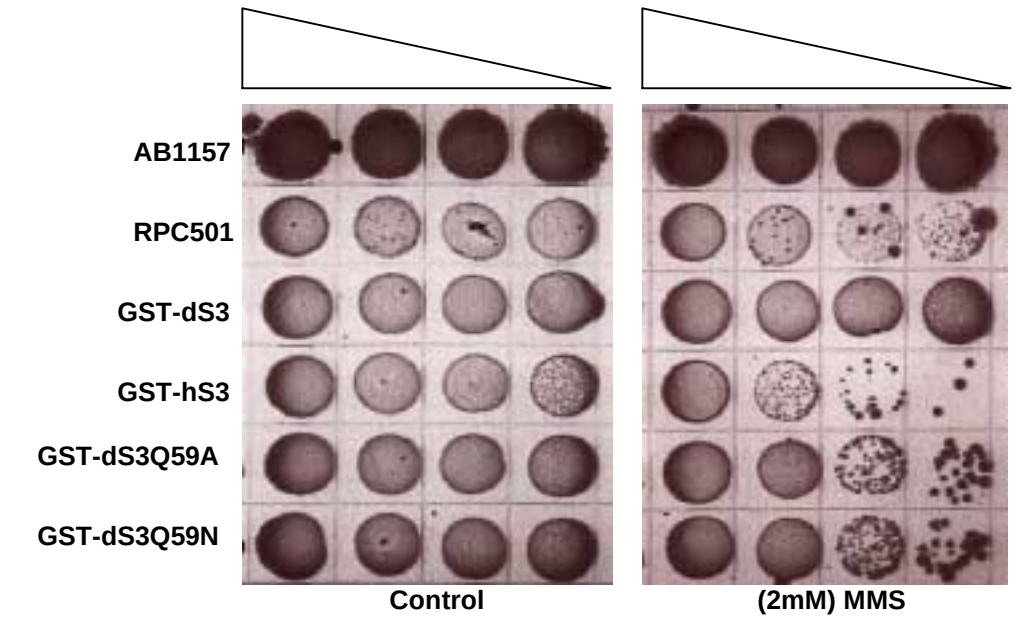
(B)

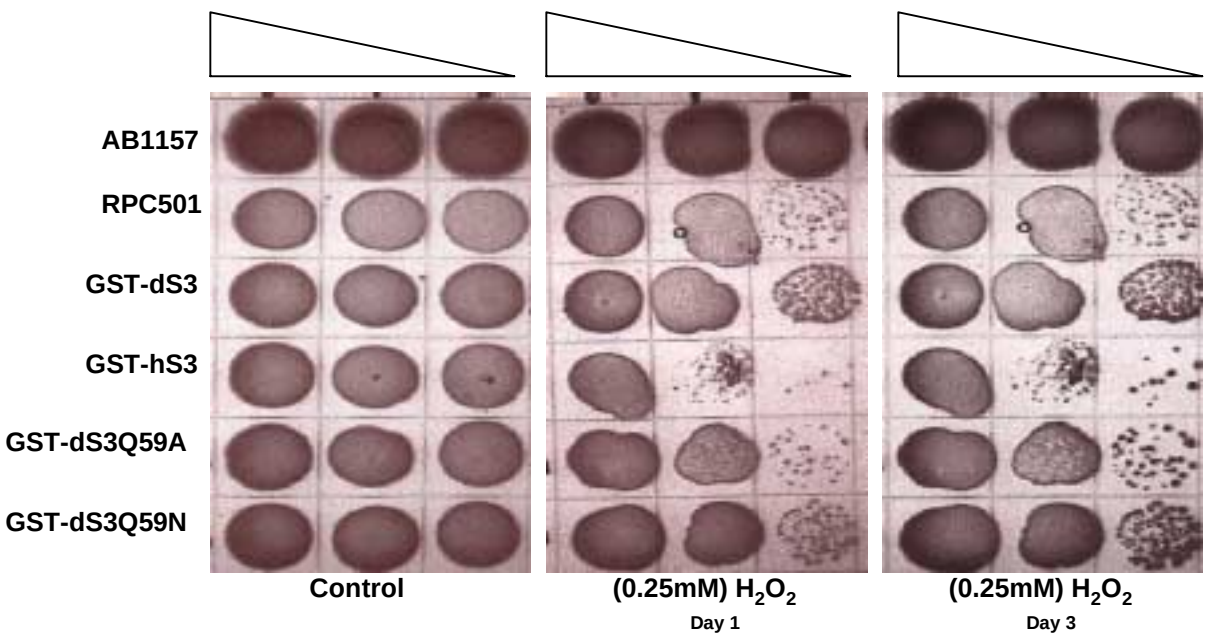
Enzyme	+	+	+	+
Oligo	+	+	+	+
$NaBH_4$	+	+	+	+
	1	2	3	4



GST-dS3 | GST-hS3 | GST-dS3Q59A | GST-dS3Q59N

Figure 7


AB1157
RPC501
GST-dS3
GST-hS3
GST-dS3Q59A
GST-dS3Q59N
Control
(2mM) MMS


AB1157
RPC501
GST-dS3
GST-hS3
GST-dS3Q59A
GST-dS3Q59N
Control
(0.25mM) H2O2
Day 1
(0.25mM) H2O2
Day 3