Defective Human MutY Phosphorylation Exists in Colorectal Cancer Cell Lines with Wild-type MutY Alleles*

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Oxidative DNA damage can generate a variety of cytotoxic DNA lesions such as 8-oxoguanine (8-oxoG), which is one of the most mutagenic base lesions formed from oxidation of genomic DNA because 8-oxoG can readily mispair with either cytosine or adenine. If un repaired, further replication of A8-oxoG mispairs results in C to A T transversions, a form of genomic instability. We reported previously that repair of A-8-oxoG mispairs was defective and that 8-oxoG levels were elevated in several microsatellite stable human colorectal cancer cell lines lacking MutY mutations (human MutY homolog gene, hmyh, MYH MutY homolog protein). In this report, we provide biochemical evidence that the defective repair of A-8-oxoG may be due, at least in part, to defective phosphorylation of the MutY protein in these cell lines. In MutY-defective cell extracts, but not with functional MutY, A-8-oxoG repair was increased by incubation with protein kinases A and C (PKA and PKC) and caesin kinase II. Treatment of these defective cells, but not cells with functional MutY, with phorbol-12-myristate-13-acetate also increased the cellular A-8-oxoG repair activity and decreased the elevated 8-oxoG levels. We show that MutY is serine-phosphorylated in vitro by the action of PKC and in the MutY-defective cells by phorbol-12-myristate-13-acetate but that MutY is already phosphorylated at baseline in proficient cell lines. Finally, using antibody-isolated MutY protein, we show that MutY can be directly phosphorylated by PKC that directly increases the level of MutY catalyzed A-8-oxoG repair.

Repair of DNA damage is an essential process required for maintaining genomic stability and cellular function. One form of DNA damage, oxidation, can be caused by exposure to reactive oxygen species, routinely generated as byproducts of the respiratory chain, during inflammation, by the exposure to ionizing radiation, or other oxidative stress conditions. Oxidation of DNA causes a wide variety of damage including strand breaks, abasic (Apurinic/Apyrimidinic) sites and oxidized bases, which are important for amino acid substitutions, among others (1, 2). One the most abundant and mutagenic bases formed from oxidation of genomic DNA is 8-oxoguanine (8-oxoG), one of the most mutagenic bases formed from oxidation of genomic DNA because 8-oxoG can readily mispair with either cytosine or adenine. If un-repaired, further replication of A-8-oxoG mispairs results in C to A T transversions, a form of genomic instability. We reported previously that repair of A-8-oxoG mispairs was defective and that 8-oxoG levels were elevated in several microsatellite stable human colorectal cancer cell lines lacking MutY mutations (human MutY homolog gene, hmyh, MYH MutY homolog protein). In this report, we provide biochemical evidence that the defective repair of A-8-oxoG may be due, at least in part, to defective phosphorylation of the MutY protein in these cell lines. In MutY-defective cell extracts, but not with functional MutY, A-8-oxoG repair was increased by incubation with protein kinases A and C (PKA and PKC) and caesin kinase II. Treatment of these defective cells, but not cells with functional MutY, with phorbol-12-myristate-13-acetate also increased the cellular A-8-oxoG repair activity and decreased the elevated 8-oxoG levels. We show that MutY is serine-phosphorylated in vitro by the action of PKC and in the MutY-defective cells by phorbol-12-myristate-13-acetate but that MutY is already phosphorylated at baseline in proficient cell lines. Finally, using antibody-isolated MutY protein, we show that MutY can be directly phosphorylated by PKC that directly increases the level of MutY catalyzed A-8-oxoG repair.

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• The abbreviations used are: 8-oxoG, 8-hydroxyguanosine; CRC, colorectal cancer; PKC, protein kinase C; PKA, protein kinase A; CKII, caesin kinase II; MSS, microsatellite stable; PMA, phorbol-12-myristate-13-acetate; RPA, replication protein A; LC/MS/MS, liquid chromatography and tandem mass spectroscopy.
after treatment with shrimp alkaline phosphatase. These data suggest that MutY may be phosphorylated, at least under certain conditions. In another line of investigation, a number of studies have reported decreased protein kinase C (PKC) activity levels in human and rodent colonic tumors (14). We therefore hypothesized that defective phosphorylation might, at least in part, be responsible for the defective MutY activity in our MSS CRC cell lines. We show here that repair of an A8-oxoG mispaired oligonucleotide can be increased in these defective cell lines by the action of protein kinases on cell extracts and by PMA treatment, known to induce PKC activity, of intact cells. There were no significant increases in A8-oxoG repair in MutY-proficient cell lines. In addition, the PMA-induced increase in activity was inhibited by preincubation of the cells with a PKC inhibitor. We show that PMA-induced increase in repair activity correlates with decreased levels of elevated genomic 8-oxoG in the MutY-defective cell lines and not MutY-proficient cell lines that contain background 8-oxoG levels. We show that MutY, in the MutY-defective cell lines, is phosphorylated in vitro by the action of PKC and in the cell by PMA, but that it is already phosphorylated in MutY-proficient cell lines. Finally, using antibody-isolated MutY protein, we show that MutY can be directly phosphorylated by PKC that directly increases the level of MutY catalyzed A8-oxoG repair.

MATERIALS AND METHODS

Cell Lines and Preparation of Whole Cell Extracts—The five human MSS CRC cell lines, VACO411, VACO425, VACO429, VACO489, and VACO9M, used in this study were isolated and generously provided by Dr. James Willson (Ireland Cancer Center and Case Western Reserve University), and SW837 and SW480 were purchased from the American Tissue Culture Center (ATCC). All cell lines were grown as described (15). Construction of SW480/vector and SW480/AS-mutY (SW480 transfected with vector alone or antisense mutY cDNA) will be described elsewhere. Both transfected cell lines were maintained as described above in regular growth medium containing 600 μg/ml G418 sulfate (Invitrogen). Cells were harvested as reported previously (12).

A8-oxoG Glycosylase Assay—A8-oxoG glycosylase activity was measured as described previously (12), except that 50 μg of cell extracts were used. Equal lane loading was confirmed with SDS-PAGE and

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\( A\) and \( B\) are graphs showing the percentage increase in adenine excision for different cell lines and treatments.\( A\) shows the effect of PKC, PKA, and CKII treatments on MutY-deficient and MutY-proficient cell lines. \( B\) shows the effect of PKC and CKII treatments on two specific cell lines, VACO411 and VACO425.

Fig. 1. In vitro repair of A8-oxoG mispair is increased by phosphorylation in MutY-defective cells extracts. In A, cell extracts of the MutY-defective cell lines VACO411, VACO425, VACO429, and VACO489 and the MutY-proficient cell lines VACO9M and SW837 were incubated alone or with PKC, PKA, or CKII. Some PKC-treated extracts were also subjected to MutY immunodepletion. Aliquots were then taken and assayed for changes in glycosylase activity. Asterisks represent statistically significant values of \( p < 0.05 \) (Student’s \( t \) test) when compared with the control without kinase added, or for MutY immunodepleted PKC-treated extracts, when compared with non-immunodepleted PKC-treated extracts. \( B\) shows the example of equal loading in lanes for VACO411 and VACO425. Approximately 40 μg of the same samples used in \( A\) were resolved in a separate SDS-PAGE gel and stained to monitor equal loading of the lanes. \( U\) represents untreated, \( C\) is treated with PKC, \( A\) is treated with PKA, and \( KII\) is treated with CKII.
Coomassie Blue staining. The amount of cleavage was similar to that reported previously (12).

In Vitro Phosphorylation and A/H18528 8-oxoG Repair—To examine the potential effects of in vitro phosphorylation on A/H18528 8-oxoG repair, 50 μg of whole cell extracts were incubated with protein kinase A (PKA) (New England Biolabs, Beverly, MA), PKC (Promega, Madison, WI), or caesin kinase II (CKII) (New England Biolabs) in TGED buffer (40 mM Tris-HCl pH 7.4, 10% glycerol, 1 mM EDTA, 0.5 mM dithiothreitol) with 10 mM MgCl₂, 0.3 mM CaCl₂, 500 μM ATP, and 2 units of the appropriate kinase. The reactions were incubated for 90 min at 30 °C and stored on ice. Aliquots were then taken and assayed for changes in glycosylase activity.

Induction of Cellular PKC with PMA and Inhibition with Bisindolylmaleimide IX—The cell lines were seeded in 150-cm² media flasks at ~50% confluency (~1 × 10⁶ cells) in MEM2 media (16) and incubated at 37 °C for 1–2 days. Cells were incubated with 1.0 μM PMA (Sigma) in serum-free MEM2 media for 6 h and harvested by centrifugation. In some experiments, cells were incubated for 1 h with 5 μM bisindolylmaleimide IX (RO-31–8220, Sigma) before and during PMA treatment.

PKC Activity—The PKC activity in cell extracts (20 μg) was tested using the PepTag® PKC assay kit (Promega) following the manufacturer’s instructions. The PepTag® C1 fluorescent peptide (RLSRTLVAK, 500 ng) was phosphorylated by the cell line extracts (treated with or without PMA) or purified PKC in PKC reaction buffer (20 mM HEPES pH 7.4, 1.3 mM CaCl₂, 1 mM dithiothreitol, 10 mM MgCl₂, and 1 mM ATP) with 250 μM phosphatidyl serine. Phosphorylation changes the net charge of the peptide substrate from +1 to −1. The phosphorylated and unphosphorylated versions of the substrate were then separated on a 1% agarose gel. The negatively charged bands from the cell lines were excised from the gels and heated to 95 °C. For controls, regions of the gels were excised that contained no peptides. The mixture was diluted with gel solubilization solution (Promega) and glacial acetic acid, and the absorbance was recorded at 570 nm.

Liquid Chromatography and Tandem Mass Spectroscopy (LC/MS/MS) Measurement of 8-Hydroxyguanosine—LC/MS/MS was performed as described (12) with the following exceptions: the mobile phase consisted of 1% acetic acid as eluent A and an Atlantis C18 column (150 mm × 2.1 mm, 3 μM, Waters Corp., Milford, MA) was used. The flow rate with this solvent and column was increased to 400 μl/min with a desolvation temperature of 550 °C. In addition, 5-N15–8-oxoG from N15 algal genomic DNA (Spectra Stable Isotopes, Columbia, MD) was used as an internal standard. This was accomplished by using the standard hydrolysis procedure on the DNA, isolating 5-N15–2dG and reacting it with methylene blue to create 5-N15–8-oxoG. The N15–2dG and N15–8-oxoG were then separated on a Luna C18 column (4.6 × 150 mm) (Phenomenex, Torrance, CA). Fractions were collected and pooled, and then lyophilized and brought to an appropriate volume such that 10 μl added to a sample vial contained ~50 fmol of N15–8-oxoG. With these improvements, the detection limit for 8-oxoG was increased to 5 fmol on the column with less standard deviation due to co-elution with the

![Figure 2](image-url)
Phosphorylation Up-regulates 8-oxoG Repair

RESULTS

A8-oxoG Repair Is Up-regulated in vitro Phosphorylation of Cell Extracts—Because of the possibility that MutY might be regulated by phosphorylation, we investigated the potential involvement of hypo-phosphorylation in defective A8-oxoG repair. We treated cell extracts from four defective MSS CRC cell lines, VACO411, VACO425, VACO429, and VACO489, and two MutY-proficient cell lines, VACO9 M and SW837 (12), with three protein kinases and monitored the effect they had on the cleavage of a 20-bp A8-oxoG oligonucleotide duplex. All three kinases, PKA, PKC, and CKII, are serine/threonine kinases and have been known to regulate the activities of several DNA repair proteins such as apurinic/apyrimidinic endonuclease 1 (APE1), coxcheyne syndrome B protein, mutS homolog 2 (MSH2), and replication protein A (RPA) by phosphorylation (17–20). In vitro phosphorylation of the extracts from all four defective cell lines with these kinases increased the cleavage of the A8-oxoG oligonucleotide considerably, with the most significant increase observed with PKC treatment (Fig. 1A). The repair activity was not significantly increased in the MutY-proficient cell lines after phosphorylation. MutY immunodepletion of the PKC-treated cell extracts using an anti-MutY antibody showed marked decreases in cleavage activity relative to the PKC-treated extracts, suggesting that the increase in cleavage activity stimulated by PKC was mainly attributable to the specific increase in the activity of defective human MutY. Equal loading of each lane was monitored by resolving the same samples in separate SDS-PAGE gels and detecting with Coomassie Blue staining (an example is shown in Fig. 1B).

Human MutY Serines Are Phosphorylated in vitro—Since MutY is the major protein involved in repair of A8-oxoG mismatches, we asked whether phosphorylation of MutY correlated with the increase in A8-oxoG repair observed after treatment of the MutY-defective cell extracts with protein kinases. Evaluation of a MutY antibody, using the MutY-proficient cell line SW480/vector, shows that immunoprecipitation with the human MutY antibody and Western blot analysis produced only one protein band of ~59 kDa (Fig. 2A, lane 1). In the absence of anti-MutY antibody, essentially no MutY protein was detected in the precipitates (lane 2). Furthermore, when the immunoprecipitation was carried out using a MutY-defective cell line, stably transfected with mutY antisense cDNA, the 59-kDa protein band was also not detected, regardless of whether the MutY antibody was used or not (lanes 3 and 4). These data lead us to conclude that the MutY antibody specifically reacts with and directly immunoprecipitates MutY protein and therefore is suitable for immunoprecipitation studies.

Since PKC treatment of the MutY-defective cell extracts provided the largest increase in cleavage activity and VACO411 possessed the largest increase in cleavage activity in response to PKC treatment (Fig. 1A), we used this cell line and the MutY-proficient cell line SW837 to investigate whether human MutY is phosphorylated, as suggested previously (13). As expected, there was no apparent difference in the levels of MutY immunoprecipitated in the extracts treated with and without PKC (Fig. 2B, compare lanes 1 with lanes 2). Using these immunoprecipitates, we tested whether human MutY was phosphorylated in vitro, by Western blotting using monoclonal antibodies specific for phosphorylated serine, tyrosine,
or threonine residues. After treatment with PKC, phosphoserine protein bands the same size as human MutY were detected in both cell lines, but although a phosphoserine band was observed in SW837 in the absence of PKC treatment, no band was observed in VACO411 without PKC treatment. This suggested that serine residues were not phosphorylated in the MutY-defective cell line VACO411 but could be phosphorylated in vitro with PKC (lanes 3 and 4). Phosphothreonine protein bands were detected with and without PKC treatment in both cell lines (lanes 7 and 8), and no bands were evident either before or after treatment when probed with the anti-phosphotyrosine antibody (lanes 5 and 6).

The use of cell extracts does not definitely prove that MutY is phosphorylated. For a more direct analysis of MutY phosphorylation, we used the antibody-purified MutY from untreated cell lines to test whether it could be directly phosphorylated.
in vitro and could therefore be directly responsible for the increase in 8-oxoG repair. Immunoprecipitation of MutY was performed from the MutY-defective cell line VACO411 and treated with or without PKC (Fig. 3A). Treatment of antibody-purified MutY with PKC increased the levels of 8-oxoG repair ~3-fold. Using the MutY-defective SW480/AS-mutY cell line and the MutY-proficient SW480/vector cell line as controls, we also show that no increases in 8-oxoG repair were evident in either cell line after incubation with PKC, suggesting that the increased 8-oxoG activity levels in VACO411 after PKC treatment were relatively specific for MutY phosphorylation.

Since the results from Fig. 2B suggest that MutY is hypophosphorylated at serine residues in MutY-defective cell lines and that treatment of antibody-isolated MutY with PKC increases 8-oxoG repair (Fig. 3A), we tested whether PKC treatment would directly phosphorylate MutY serine residues. Fig. 3B shows that PKC treatment of MutY from VACO411 increases the phosphorylation of serine residues (compare lanes 1 with lanes 2) but does not alter the phosphorylation status of tyrosine or threonine residues. As expected, there were no bands detected in the MutY-defective antisense cell line (compare lanes 3 and 4 with lanes 5 and 6), and in the MutY-proficient cell line SW480/vector, there is no change in the phosphorylation status of serines, tyrosines, or threonines as observed in cell-free extracts. When one considers the increase in functional activity after phosphorylation by PKC (Fig. 1) and the demonstration of MutY phosphorylation in cell extracts (Fig. 2B) and when antibody-purified (Fig. 3B), we conclude that the MutY is most likely directly phosphorylated at one or more serine residues and that this process may be defective in the VACO411 CRC cell line.

A-8-oxoG Repair Is Up-regulated by PMA Treatment of MutY-defective Cells—The in vitro data suggested that MutY activity could be regulated by its phosphorylation state, so we hypothesized that MutY may be regulated by phosphorylation in the cell. Since PKC produced the largest increase in activity in vitro (Fig. 1), phosphorylated MutY in cell extracts (Fig. 2), and antibody-purified MutY (Fig. 3B) in vitro and since some colon cancers are known to have a decrease in PKC, we tested whether treatment of the MutY-defective cells with the tumor-promoting agent phorbol-12-myristate-13-acetate (PMA) would increase 8-oxoG repair since it is known to induce PKC activity (21). The four MSS CRC cell lines and two MutY-proficient cell lines were exposed to PMA for 6 h, and the extracts were examined for changes in PKC activity. As shown in Fig. 4A, PMA exposure resulted in an increase in PKC activity, which in the cell line VACO411 could be inhibited by the PKC inhibitor, bisindolylmaleimide (Fig. 4B). The increase in PKC activity was substantially less in the MutY-proficient cell lines. The increase in PKC activity was accompanied by an approximate 2-fold increase in A-8-oxoG cleavage activities in the four MutY-defective cell lines (Fig. 4C), which similar to the increase in PKC activity, decreased significantly upon preincubation of VACO411 with bisindolylmaleimide (Fig. 4D). Similar to the lack of increase in PKC activity, there was little if any elevation in the A-8-oxoG cleavage activity in the two MutY-
proficient cell lines. MutY immunodepletion of the extracts from PMA-treated cell lines (Fig. 4C) decreased the activity to less than 10% of the activity observed after PMA treatment, and thus, it appears that the PMA-stimulated increase in cleavage activity in the MutY-defective cell lines probably involves PKC and is likely attributable to effects on human MutY.

**Human MutY Is Phosphorylated in the Cell**—Since the data suggest that MutY is directly phosphorylated by the action of PKC (Fig. 3, A and B), we investigated whether human MutY is directly phosphorylated after treatment with PMA. Fig. 5, A and B, shows, by Western blotting, that after PMA treatment of VACO411, SW837, and SW480/vector, the level of MutY protein was up-regulated 2–3-fold. There was no expression of MutY in the MutY-defective cell line SW480/AS-mutY, either with or without PMA treatment.

After PMA treatment of VACO411 and immunoprecipitation with the MutY antibody, a novel phosphoserine protein band appeared when probed with the anti-phosphoserine antibody, whereas the same antibody did not detect a band in the untreated VACO411 immunoprecipitates, suggesting that serine phosphorylation of MutY in VACO411 may be defective (Fig. 6; compare lanes 1 with lanes 2, first panel). Using the anti-phosphoserine and anti-phosphothreonine antibodies, protein bands were detected in SW837 and SW480/vector MutY immunoprecipitates both before and after PMA treatment (Fig. 6, compare lanes 1 with lanes 2 and compare lanes 5 with lanes 6, second and third panels). It is interesting to note that MutY...
Threonine residues in VACO411 were phosphorylated in the untreated cells, similar to the results observed in the in vitro study. Since the increase in MutY protein, after PMA treatment, appears to have no effect on the phosphorylation status of MutY in the MutY-proficient cell lines, after PMA treatment, it is conceivable that threonine may also be phosphorylated in response to PMA (although clearly at lower levels than the phosphorylation of serine). No bands were evident either before or after treatment of any of the cell lines when probed with the anti-phosphotyrosine antibody (lanes 3 and 4), and as expected, in the control cell line SW480/AS-mutY, no bands were detected in these immunoprecipitated samples with or without PMA, suggesting that the phospho-protein bands detected were specifically MutY protein (fourth panel). When one considers the direct increase in functional activity of antibody-purified MutY and the demonstration of direct MutY phosphorylation by PKC and after PMA treatment, we conclude that the MutY protein is likely regulated by phosphorylation in the cell.

Increased Cleavage of A8-oxoG Correlates with Decreased Levels of Elevated 8-oxoG in PMA-treated Cell Lines—Background levels of oxidative DNA damage detected in normal tissue arise through the production of endogenous reactive oxygen species, a byproduct of normal cell metabolism. Given that oxidative DNA damage can be produced endogenously, defense mechanisms such as MutY are required. The absence of these mechanisms should result in a steady increase in oxidative DNA damage since the production of the damage is unaltered but the repair mechanism is absent. In this sense, the 8-oxoG levels attributed to MutY may be thought of as a separate component, where equilibrium between production and removal (MutY) determines the baseline level. Thus, when MutY is defective, the basal level would be expected to rise.

We reported previously that these four MutY-defective cell lines possess elevated levels of 8-oxoG in their genomic DNA and hypothesized that this was a consequence of defective A8-oxoG repair. Since A8-oxoG cleavage is increased in cell extracts after PMA treatment, we hypothesized that PMA treatment may also correct the elevated 8-oxoG levels found in these CRC cell lines. Fig. 7 shows that isolated genomic DNA from the same four MutY-defective cell lines contained 2–3-fold lower levels of 8-oxoG after PMA treatment, correlating well with the 2–fold increase in repair activity (Fig. 4D). In the two MutY-proficient cell lines, no changes in the baseline levels of 8-oxoG were observed with or without PMA treatment.

Identification of Putative Phosphorylation Sites on MutY—Analysis of the amino acid sequence of human MutY using PROSITE and PHOSPHOBASE, version 2.0, identified six serine residues as potential sites of PKC phosphorylation (Fig. 8A). Fig. 8B shows that, with the exception of serine 9 and serine 85, the serine residues are highly conserved among different mammalian MutY proteins. Serines 9 and 504 are present in or around the potential binding sites for PCNA, proliferating cell nuclear antigen.

![Possible serine phosphorylation sites on human MutY](image)
Phosphorylation Up-regulates 8-oxoG Repair

RPA and proliferating cell nuclear antigen, and phosphorylation may therefore influence binding of the these proteins, as is seen with the interactions of RPA with xeroderma pigmentosum A and DNA protein kinase (8). Phosphorylation of threonine and tyrosine residues, however, in other conditions and by other kinases, cannot be explicitly excluded. Examination of which of the potential serine sites identified in Fig. 8 are nine and tyrosine residues, however, in other conditions and by PKC, suggesting that the that effects were a direct effect of PKC phosphorylation. A 8-oxoG cleavage is also increased by PMA treatment in the MutY-defective cell lines, but not in the MutY-proficient cell lines, and this can be inhibited by the PKC inhibitor bisindolylmaleimide. Furthermore, these changes correlate with phosphorylated MutY protein at serine residues in the same MutY-defective cells. In PMA-treated MutY-defective cells, this also correlates with a decrease in the elevated genomic 8-oxoG levels, whereas in the MutY-proficient cells, the baseline levels of 8-oxoG remain unchanged. Finally, up-regulation of MutY was shown to occur at the protein level.

The exact physiological role of MutY phosphorylation still has to be fully explained. As well as directly increasing the MutY repair activity, it is possible that phosphorylation regulates the interaction of MutY with its binding partners APE1, proliferating cell nuclear antigen, RPA, and MSH6 (22, 23), as is the case with the interactions of both p21 and DNA Ligase I with proliferating cell nuclear antigen (24). The phosphorylation status of MutY may also control organelle translocation as observed for the MutSα (Mut S homologs 2 and 6) protein (17) since the increased levels of A 8-oxoG repair could reduce the pool of free MutY in either the nucleus or the mitochondria and could provide the signal for increased organelle transport. Furthermore, it is known that oxidants and oxidative stress can stimulate PKC activity by reacting with its regulatory domain (25), suggesting that human MutY could be phosphorylated in response to DNA damage.

The results from this study suggest that defective repair of A 8-oxoG may be the result of defective phosphorylation. Several laboratories have documented changes in PKC activity and PKC isoform expression in intestinal neoplasms (14). Furthermore, decreased levels of PKC activity have also been observed in preneoplastic colonic mucosa and in colonic adenomas (26–28), suggesting that alterations in PKC expression and activities may occur early in the multistage process of colon carcinogenesis. Of the 10 or so PKC isoforms, decreased levels of PKC isoforms α, β, ε, η, δ, and ϵ in human and rodent colonic tumors have been reported, with the loss of α, β, and ε reported to be an early event during intestinal carcinogenesis (25, 26, 29, 30). As well as PKC, it cannot be excluded that MutY may be phosphorylated by other kinases.

In this report, we have demonstrated that MutY can be phosphorylated and that when it is, this enhances its adenine cleavage activity and decreases elevated 8-oxoG levels in MutY-defective cells. Uncovering the biochemical controls of MutY regulation will be an important step toward a comprehensive understanding of the mechanisms involved in cellular defense against oxidative DNA damage.

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