Aberrant repair initiated by the adenine-DNA glycosylase does not play a role in UV-induced mutagenesis in Escherichia coli

Caroline Zutterling¹, Aibek Mursalimov², Ibtissam Talhaoui³, Zhanat Koshenov², Zhiger Akishev⁴, Amangeldy K. Bissenbaev⁴, Gerard Mazon³, Nicolas E. Geacintov³, Didier Gasparutto⁶, Regina Groisman¹, Dmitry O. Zharkov⁷,⁸, Bakhyt T. Matkarimov² and Murat Saparbaev¹

¹ Groupe «Réparation de l’ADN», Equipe Labellisée par la Ligue Nationale Contre le Cancer, CNRS UMR8200, Université Paris-Sud, Gustave Roussy Cancer Campus, Villejuif, France
² National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan
³ CNRS UMR 8200—Laboratoire «Stabilité Génétique et Oncogénèse», Université Paris Sud (Paris XI), Gustave Roussy Cancer Campus, Villejuif, France
⁴ Department of Molecular Biology and Genetics, al-Farabi Kazakh National University, Faculty of Biology, Almaty, Kazakhstan
⁵ Chemistry Department, New York University, New York City, NY, USA
⁶ CEA, CNRS, INAC, SyMMES, Université Grenoble Alpes, Grenoble, France
⁷ SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia
⁸ Novosibirsk State University, Novosibirsk, Russia

ABSTRACT

Background: DNA repair is essential to counteract damage to DNA induced by endo- and exogenous factors, to maintain genome stability. However, challenges to the faithful discrimination between damaged and non-damaged DNA strands do exist, such as mismatched pairs between two regular bases resulting from spontaneous deamination of 5-methylcytosine or DNA polymerase errors during replication. To counteract these mutagenic threats to genome stability, cells evolved the mismatch-specific DNA glycosylases that can recognize and remove regular DNA bases in the mismatched DNA duplexes. The Escherichia coli adenine-DNA glycosylase (MutY/MicA) protects cells against oxidative stress-induced mutagenesis by removing adenine which is mispaired with 7,8-dihydro-8-oxoguanine (8oxoG) in the base excision repair pathway. However, MutY does not discriminate between template and newly synthesized DNA strands. Therefore the ability to remove A from 8oxoG•A mispair, which is generated via misincorporation of an 8-oxo-2′-deoxyguanosine-5′-triphosphate precursor during DNA replication and in which A is the template base, can induce A•T→C•G transversions. Furthermore, it has been demonstrated that human MUTYH, homologous to the bacterial MutY, might be involved in the aberrant processing of ultraviolet (UV) induced DNA damage.

Methods: Here, we investigated the role of MutY in UV-induced mutagenesis in E. coli. MutY was probed on DNA duplexes containing cyclobutane pyrimidine dimers (CPD) and pyrimidine (6–4) pyrimidine photoproduct (6–4PP). UV irradiation of E. coli induces Save Our Souls (SOS) response characterized by increased production of DNA repair enzymes and mutagenesis. To study the role of
MutY in vivo, the mutation frequencies to rifampicin-resistant (RifR) after UV irradiation of wild type and mutant E. coli strains were measured.

**Results:** We demonstrated that MutY does not excise Adenine when it is paired with CPD and 6–4PP adducts in duplex DNA. At the same time, MutY excises Adenine in A•G and A•8oxoG mispairs. Interestingly, E. coli mutY strains, which have elevated spontaneous mutation rate, exhibited low mutational induction after UV exposure as compared to MutY-proficient strains. However, sequence analysis of RifR mutants revealed that the frequencies of C→T transitions dramatically increased after UV irradiation in both MutY-proficient and -deficient E. coli strains.

**Discussion:** These findings indicate that the bacterial MutY is not involved in the aberrant DNA repair of UV-induced DNA damage.

**Subjects**  Biochemistry, Cell Biology, Genetics, Molecular Biology, Oncology

**Keywords**  Adenine-DNA glycosylase, Nucleotide excision repair, UV-induced mutagenesis, Cyclobutane pyrimidine dimer, Base excision repair, Aberrant DNA repair, Escherichia coli, Pyrimidine (6–4) pyrimidone photoproduct

**INTRODUCTION**

In all cellular organisms DNA composition is limited to only four nucleotides C, G, A and T, except for DNA methylation, which is appeared as a protection mechanism against foreign DNA or as a gene regulatory system. It is noteworthy that this strict evolutionary constraint also enables DNA repair systems to distinguish between damaged and normal bases. DNA glycosylases recognize and excise modified bases among vast majority of normal bases via the base excision repair (BER) pathway. Nevertheless, a mispair composed of two normal bases occurring due to spontaneous deamination of 5-methylcytosine to thymine and DNA polymerase errors during replication presents a challenging puzzle to repair systems.

Hydroxyl radicals (OH•) preferentially react with C₈ atom of purines to generate 7,8-dihydro-8-oxoguanine (8oxoG) in DNA and 8-oxo-2′-deoxyguanosine-5′-triphosphate (8oxodGTP) in nucleotide pool. 8oxoG is a highly mutagenic DNA adduct considered as a major oxidative DNA lesion in aerobic organisms. To counteract the genotoxic effects of oxidized guanines, organisms have evolved multiple overlapping DNA repair and error-avoiding mechanisms. The majority of oxidative DNA base lesions including 8oxoG are removed in the BER pathway (Barnes & Lindahl, 2004). BER is initiated by a DNA glycosylase which cleaves the N-glycosydic bond between the abnormal base and the deoxyribose, generating either an abasic site or single-stranded break in DNA. In E. coli, the system referred as GO pathway, which consist of three enzymes: the bi-functional 8oxoG-DNA glycosylase/AP lyase Fpg/MutM which excises the 8oxoG opposite cytosine (Tchou et al., 1991), MutY which excises adenine misincorporated across unrepaired 8oxoG in DNA template (Michaels et al., 1992) and finally MutT which cleanses cellular dNTPs pool by hydrolyzing 8oxodGTP precursors, and thus avoiding its incorporation into DNA (Maki & Sekiguchi, 1992). The crystal structure and mutagenesis studies revealed that MutY cleaves the N-glycosydic bond through a hydrolytic mechanism...
requiring Asp138, followed by an inefficient $\beta$-elimination reaction which is structurally and chemically uncoupled from the initial glycosydic bond cleavage (Guan et al., 1998; Manuel et al., 2004). Nevertheless, consistent with the established mechanism of action of bi-functional DNA glycosylases/AP lyases, MutY generates a Schiff base intermediate between the nucleophilic lysine and C1′ of the AP site (Sun et al., 1995). Multiple evidence demonstrates that the GO pathway prevents mutations induced by oxidation of guanines in DNA and nucleotide pool in many different species. For example, the E. coli fpg mutY double mutant exhibits an extreme mutator phenotype with 10,000-fold increase in G·C $\rightarrow$ T·A transversions that can be reversed by plasmids carrying the Fpg gene (Duwat et al., 1995). At the same time, E. coli mutT also shows a strong mutator phenotype, characterized by the specific increase in $\text{A·T} \rightarrow \text{C·G}$ transversions (Yanofsky, Cox & Horn, 1966). Intriguingly, the presence of mutY mutation lowers the frequency of $\text{A·T} \rightarrow \text{C·G}$ spontaneous mutations in both E. coli wild-type (WT) and mutT strains, suggesting that MutY can act in mutagenic post-replication repair pathway by excising correct A in the template DNA strand when it is opposite a misincorporated 8oxodGTP residue (Fowler et al., 2003).

MYH (MUTYH) is a human homologue of the E. coli MutY protein, which displays very similar DNA substrate specificities to the bacterial counterpart. Mutations in MUTYH gene are associated with certain types of familial colorectal tumors without a germ-line mutation in the APC gene and they also confer a spontaneous mutator phenotype in human and mice cell lines (Al-Tassan et al., 2002). Previously, Vrouwe et al., (2011) have shown that exposure of the non-cycling NER-deficient XP-C and XP-A human fibroblasts to ultraviolet (UV) radiation resulted in the generation and accumulation of single-strand DNA breaks 24 h after treatment, which in turn activated ATR-dependent DNA damage response. Intriguingly, the formation of single-strand DNA breaks at damage sites and DNA repair synthesis initiated by these breaks did not lead to removal of UV adducts in XP fibroblasts. However, recently Mazouzi et al., (2017) revealed that the presence of MUTYH protein in NER-deficient cells results in an increased sensitivity to UV light. The authors proposed that MUTYH may inhibit the alternative NER-independent repair of UV-induced DNA lesions in XP cells. Nevertheless, it might be also possible that human adenine-DNA glycosylase targets A opposite UV adducts and cause aberrant futile repair of the non-damaged DNA strand. Thus, one could imagine that the severe phenotype of XP patients might be due to a DNA glycosylase-initiated aberrant repair of UV-induced DNA lesions in human cells that would lead to an extremely mutagenic scenario.

Ultraviolet radiation generates two most common DNA lesions: the cyclobutane pyrimidine dimer (CPD) and the pyrimidine (6–4) pyrimidone photoproduct [(6–4) PhotoProduct; 6–4PP]. Both photoproducts are cytotoxic (block DNA replication and transcription) and mutagenic, while CPDs are several times more frequent than 6–4PPs (Douki et al., 2000). A hallmark of UV mutagenesis is the high frequency of C$\rightarrow$T transitions at dipyrimidine sites in DNA, possibly due to the extremely high deamination rate of cytosine residues within CPD sites in DNA (Peng & Shaw, 1996). UV radiation greatly induces mutagenesis in E. coli, however mutation in the lexA, recA and umuD(C)
abolish UV mutagenesis implying the existence of error-prone DNA repair mechanisms. Following the pioneering studies of Witkin and Radman’s hypothesis on SOS repair, work by the numerous laboratories have established and characterized to a great detail the \textit{E. coli} SOS response system. According to the widely accepted model, following DNA damage, RecA becomes activated in the presence of single-stranded DNA regions and mediates LexA proteolytic cleavage which in turn leads to the increased expression of more than 40 genes including \textit{recA}, \textit{uvrA} and \textit{umuDC}. Genetic and biochemical evidence suggests that SOS mutagenesis is largely the result of the action of error-prone translesion DNA polymerases, such as Pol V (encoded by the umuDC operon), which have the ability to insert nucleotides opposite various DNA lesions and thus enabling the lesion bypass and restart of stalled DNA replication forks by replicative DNA polymerases (Patel et al., 2010).

In our previous studies, we have established the existence of the aberrant repair pathway, initiated by the human mismatch-specific thymine-DNA glycosylase (TDG), which can target the non-damaged DNA strand and excise thymine when it is paired with a damaged adenine residue in DNA duplex (Talhaoui et al., 2014). In vitro reconstitution of BER with duplex DNA containing hypoxanthine opposite T (Hx\textbullet{}T pair) and TDG resulted in the incorporation of cytosine opposite Hx. Based on the mutagenic properties of mismatch-specific DNA glycosylases we proposed that the aberrant BER pathway initiated by MutY in \textit{E. coli} and TDG and MUTYH in mammalian cells toward unrepaired DNA lesions may lead to the mutation fixation in absence of DNA replication (Talhaoui et al., 2017). Here, we characterized in vitro the DNA glycosylase activity of \textit{E. coli} MutY protein toward short oligonucleotide duplexes containing UV-DNA adducts. In addition, we analyzed mutation rates and spectra in \textit{E. coli} strains lacking MutY after exposure to short-wavelength UV radiation. The role of MutY-catalyzed DNA glycosylase activity in the UV-induced mutagenesis is discussed.

\textbf{METHODS SUMMARY}

\textbf{Bacterial strains, plasmids and enzymes}

All bacterial genetics procedures were performed as described (Miller, 1972); these included preparation of media and mutagenesis. The rich medium was the LB broth, supplemented when required with either 100 μg⋅mL\(^{-1}\) ampicillin, or 50 μg⋅mL\(^{-1}\) kanamycin, or 100 μg⋅mL\(^{-1}\) rifampicin. \textit{E. coli} cells were transformed by DNA electroporation as described (Dower, Miller & Ragsdale, 1988).

The \textit{E. coli} K12 strains used in this study are listed in Table 1. Original Miller’s strain CC104, generously provided by Dr J.H. Miller (University of California, USA), is derivative of P90C[\textit{ara}, Δ(\textit{lac proB})\textit{XIII}] carrying a \textit{lacZ} allele on an \textit{F’}(\textit{lacI}, \textit{Z proB}\textsuperscript{+}) plasmid and, therefore, exhibiting the Lac\textsuperscript{−} phenotype (Cupps & Miller, 1989). Strains AB1157 and CC104 (wild type for DNA repair), BH200 carrying mutation in the \textit{UvrA} gene, BH980 in the \textit{MicA} (also referred as \textit{MutY}) gene, BH1070 and BH1220 containing double mutation \textit{uvrA6 micA} were from laboratory stock. Strain AK146 carrying mutation in the \textit{UvrA} gene was generously provided by Dr. A. Kuzminov (University of Illinois,
Champaign, IL, USA). Strains were grown overnight at 37 °C 200 rpm. Cultures were then plated (stripped) on LB-agar plates and incubated overnight to get single colonies.

The plasmid pJWT21-4 encoding the *E. coli* MutY/MicA protein was generously provided by Dr. P. Radicella (CEA, Fontenay-aux-Roses, France). Site-directed mutation aspartic acid 138/asparagine (D138N), within the MutY coding sequence in pJWT21-4 were generated by the QuikChange site-directed mutagenesis kit according to the manufacturer’s instructions (Stratagene Europe, Amsterdam, Netherlands). Following oligonucleotide primers were used to generate MutY-D138N mutant: forward primer, d(CACTTTCCGATTCTCAACGGTAACGTCAAACG) and reverse primer, d(CGTTTGACGTTACCGTTGAGAATCGGAAAGTG).

UV-damage endonuclease (UVDE) from *Saccharomyces pombe* and T4 endonuclease V (T4-PDG) were purchased from Trevigen (Gaithersburg, MD, USA) and New England Biolabs France SAS (Evry), respectively. The AP endonucleases from human (APE1) ([Gelin et al., 2010](#)) and *Mycobacterium tuberculosis* (MtbXthA) ([Abeldenov et al., 2015](#)) and uracil-DNA glycosylase from *E. coli* (UNG) ([Scaramozzino et al., 2003](#)) are from laboratory stock. The *E. coli* MutY protein was purified from *E. coli* B834(DE3) strain harbouring pET13a-MutY plasmid as described ([Zharkov et al., 2000](#)).

Preparation of plasmid DNA substrate. 70 µL of pBlueScript SK(+) plasmid DNA (0.1 µg·mL⁻¹) in TE buffer was placed in 0.2 mL quartz cuvette and irradiated with 254 nm germicidal UV at a dose of 1,000 J·m⁻² at 4 °C.

### Oligonucleotides and repair assay

Oligonucleotides used in the present study are presented in the 5′→3′ direction. The 24 mer oligonucleotides containing the CPD or 6–4PP adduct: d(CTTCTTGCGAAGATCGTGAAAGCAGTCAAGCAACGGTAACGTCAAACG) and reverse primer, d(CGTTTGACGTTACCGTTGAGAATCGGAAAGTG).

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| Table 1 Bacterial strains. |
|-----------------------------|
| **Strain** | **Genotype** | **Source of derivation** |
| AB1157 | thr-1 leu-6 proA2 his-4 argE3 thi-1 lacYI galK2 ara-14 xyl-5 mtl-1 tss-33 rpsL31 supE44 | Laboratory stock |
| BH200 | as AB1157 but uvrA::Tn10 | Laboratory stock |
| AK146 | as AB1157 but uvrA6 | A. Kuzminov (University of Illinois, USA). |
| BH1220 | as AB1157 but uvrA6 micA::KnR | Laboratory stock |
| BH1070 | as GC4468 F' ΔlacI169 rpsL but uvrA6 micA::KnR | Laboratory stock |
| CC104 | P90C [araΔ(lacproB)23] carrying an F'lac-Z-proB episome (G•C→T•A) | J.H. Miller (University of California, USA). |
| BH980 | as CC104 but micA::KnR | Laboratory stock |
30 mer d(GGAGGAAGAAGTCNNAGAAGAGACCT) where NN is either AA, AG, GA and GG dinucleotide opposite UV lesion; and modified 30 mer d(AGGTCTCTTC TTCTTYGCACTTTCTCTCC) and 24 mer d(CTTCTTCGAAGYGGAGCTCTCT) or d(AGAGAGCTCYYCTTGCAAGAAG) and where Y is either T, U, G or 8oxoG.

The oligonucleotides were 5'-end labelled with [γ-32P]-ATP (PerkinElmer, Villebon-sur-Yvette, France) and then annealed with the complementary strands as described (Gelin et al., 2010). The standard reaction mixture (20 μL) for DNA repair assays contained five nM of [32P]-labelled duplex oligonucleotide or one μg of plasmid DNA, 20 mM Tris–HCl (pH 8.0), 100 mM NaCl, one mM EDTA, one mM DTT, 100 μg·mL⁻¹ BSA and 100 nM MutY for 30 min at 37 °C, unless otherwise stated. The AP sites left after excision of damaged bases in synthetic oligonucleotide substrates were cleaved by incubation of the reaction mixture with light piperidine (10% (v/v) piperidine at 37 °C for 30 min and then neutralized by 0.1M HCl. Otherwise the plasmid DNA was incubated with 100 nM APE1 in DNA glycosylase buffer for 30 min at 37 °C. The samples were desalted using Sephadex G25 column (Amersham Biosciences, Little Chalfont, UK) equilibrated in 7.5M urea and the cleavage products were separated by electrophoresis in denaturing 20% (w/v) polyacrylamide gels (7 M Urea, 0.5 × TBE, 42 °C). A Fuji Phosphor Screen was exposed to the gels and then scanned with laser scanner Typhoon FLA 9500 (GE Healthcare Europe GmbH, Velizy-Villacoublay, France) and resulting digital images were analyzed using Image Gauge V3.12 software. Reaction with pBlueScript SK(+) plasmid was stopped by adding five μL of 0.25% bromophenol blue, 50% glycerol and 10 mM EDTA and the products were analyzed by 0.8% agarose gel electrophoresis (0.5X TBE).

**UV mutagenesis**

Strains were grown overnight at 37 °C 200 rpm and then streaked on LB-agar plates and incubated overnight (for 16 h) to obtain single colonies. Single colonies for each strain were grown in 10 mL of LB overnight at 37 °C and 200 rpm then culture was centrifuged and washed with Phosphate-buffered saline (PBS). Cells were collected by centrifugation and pellets were resuspended in the same volume of PBS and were exposed to standard germicidal UV lamp emitting at 254 nm. For this, two mL of cell suspension were irradiated in tissue culture dish with a different dose of UV irradiation at 254 nm. In general, to obtain mutation induction UV light was used at a dose that produced less than 10% survival. All procedures were performed in the dark to prevent photo-reversal of UV lesions and aliquots were taken to plate on LB plates to measure the survival. After irradiation, one mL of culture was diluted in two mL of fresh LB and incubated overnight at 37 °C 200 rpm. In the following day 0.1 mL of diluted and non-diluted overnight culture were plated on LB-agar plates containing or not 100 μg·mL⁻¹ rifampicin which were incubated overnight at 37 °C. After incubation survived colonies were counted and data were analyzed.

**Sequencing of mutations in the E. coli rpoB gene from RifR clones**

The E. coli chromosomal DNA from rifampicin-resistant (RifR) clones was isolated as described (Dale & Greenaway, 1984). The DNA sequencing of the rpoB gene was performed as described (Garibyan et al., 2003). Briefly, the DNA fragment containing the
main cluster II of the \( rpoB \) was obtained by PCR amplification using genomic DNA with the following oligonucleotide primers: d(CGTCGTATCCGTTCCGTTGG) and d(TTCACCCGGATAACATCTCGTC). The PCR DNA fragment was purified using the QIAquick PCR Puriﬁcation Kit Qiagen kit. The purified PCR products were then sequenced with following primer d(CGTGTAGAGCGTGCGGTGAAA) using GATC service (Eurofins Genomics Company, Ebersberg, Germany). Absolute majority of the mutations (>95%) resulting in Rif\( ^R \) clones in the present study were localized in cluster II.

**RESULTS**

**Construction and characterization of oligonucleotide duplexes containing UV-induced DNA adducts**

To examine DNA substrate specificity of mismatch-specific DNA glycosylases we have constructed short 24 and 30 mer duplex DNA oligonucleotides containing single CPD and 6–4PP adducts at positions 13–14 in 24 mer and positions 15–16 in 30 mer. For this, 5’-[\( ^{32}P \)]-labelled modified oligonucleotides were hybridized to regular complementary strands containing two Adenine residues opposite the thymine dimers: CPD and 6–4PP. The resulting duplexes containing CPD and 6–4PP adducts were referred as AA•T=T and AA•T-T duplexes, where T=T denotes CPD and T-T denotes 6–4PP, respectively. The presence of UV-DNA adducts in the duplex DNA oligonucleotides was conﬁrmed by their incubation in the presence of T4-PDG, which is bi-functional DNA glycosylase/AP lyase that speciﬁcally cleaves DNA duplexes containing CPD, but not that of 6–4PP adducts, and *Schizosaccharomyces pombe* UVDE, which recognizes both CPD and 6–4PP in duplex DNA and cleaves phosphodiester bond 5’ to the lesion by hydrolytic mechanism. To determine the length of cleavage products we generated size markers by incubation of the 5’-[\( ^{32}P \)]-labelled 24 mer regular AA•TT duplex incubated with MtbXthA, a 3’-5’ exonuclease, to generate size markers. For details see materials and Methods.

**Figure 1** Analysis of the cleavage products generated by T4-PDG and UVDE enzymes. T4-PDG and UVDE enzymes were acted upon 5’-[\( ^{32}P \)]-labelled 24 and 30 mer duplex oligonucleotides containing CPD and 6–4PP adducts. Lanes 1, 7 and 10, control non-treated oligonucleotides; lanes 2–3 and 8–9, 24 mer AA•T=T and AA•T-T duplexes incubated either with T4-PDG or UVDE; lanes 11 and 12, 30 mer AA•T=T duplex incubated with T4-PDG and UVDE, respectively; lanes 4–6, 24 mer regular AA•TT duplex incubated with MtbXthA, a 3’-5’ exonuclease, to generate size markers. For details see materials and Methods.

Full-size [DOI: 10.7717/peerj.6029/fig-1]
As expected, incubation of 24 and 30 mer AA•T=T and AA•T-T duplexes with T4-PDG and UVDE resulted in the generation of 12 mer and 14 mer cleavage products, respectively (lanes 2, 3, 9 and 11–12), indicating the presence of UV adducts at positions 12 and 14 in 24 and 30 mer DNA duplexes, respectively. It should be noted that due to difference in the mechanism of actions between DNA glycosylase and endonuclease, the 12 and 14 mer cleavage products generated by T4-PDG migrated slower than the corresponding products generated by UVDE (lanes 2 vs. 3 and 11 vs. 12). T4-PDG excises damaged base and then cleaves the remaining AP site via $\beta$-elimination to generate the slowly migrating cleavage fragments containing the 3′-phospho-$\alpha,\beta$-unsaturated aldehyde group (Dodson, Michaels & Lloyd, 1994). At the same time, UVDE generates faster migrating cleavage fragments with 3′-OH ends, which is expected from the hydrolytic mechanism of action of AP endonucleases (Hegde, Hazra & Mitra, 2008).

**E. coli MutY did not excise adenine opposite UV-induced adducts in oligonucleotide DNA duplexes**

To examine whether aberrant BER could be involved in the processing of UV-induced DNA damage, the 24 mer AA•T=T and AA•T-T duplexes, in which non-damaged DNA strand containing A opposite the lesion is radioactively labelled with 5′-[32P], were incubated in the presence of *E. coli* MutY. After reactions the resulting AP sites were cleaved by light piperidine treatment and the products were separated on the denaturing PAGE. To determine the position of cleavage site in the non-damaged strand, we generated DNA size markers using 5′-[32P]-labelled 24 mer duplexes containing either single A•G mispair at position 12, referred as AA•GT, or single A•8oxoG mispair at position 11, referred as AA•ToG (where oG denotes 8oxoG). In addition, we constructed 24 mer single-stranded oligonucleotides with single Uracil (U) residue at positions 11 or 12, referred as 24UA and 24AU, respectively. As expected, incubation of 24 mer AA•ToG duplex with MutY and 24UA with *E. coli* UNG generated 10 mer cleavage fragment (Fig. 2, lanes 6, 8 and 11), whereas incubation of 24 mer AA•GT duplex and 24AU with the same enzymes generated 11 mer cleavage fragment (Fig. 2, lanes 2 and 12, and Fig. 3, lane 8). These results indicate that MutY excises mismatched A residues opposite 8oxoG and G. Importantly, no cleavage products were observed after incubation of AA•T=T and AA•T-T duplexes, indicating that MutY failed to recognize A residues opposite CPD and 6–4PP in 24 mer duplexes (Fig. 2, lanes 4 and 10, and Fig. 3, lane 10).

Next, we examined whether MutY could excise Adenines opposite CPD in an oligonucleotide duplex with different sequence context. For this, the 30 mer AA•T=T duplex, in which the complementary non-damaged DNA strand containing A opposite the lesion is radioactively labelled with 5′-[32P], was incubated in the presence of *E. coli* MutY. We generated DNA size markers using 5′-[32P]-labelled 30 mer duplexes containing either single A•G mispair at positions 15 and 16, referred as AA•GT and AA•TG, respectively. As expected, incubation of 30 mer AA•GT and AA•TG duplexes with MutY generated 15 and 14 mer cleavage fragments, respectively (Fig. 3, lanes 2 and 4).
Again, no cleavage products were observed after incubation of AA•T=T duplex with MutY, indicating that MutY does not recognize A residues opposite CPD placed in two different sequence contexts (lane 6). In addition, we measured MutY activity on the mismatched 24 and 30 mer oligonucleotide duplexes containing CPD opposite four possible bipurine dinucleotides: GG, AG, GA and AA. The results showed no cleavage products after incubation of GG•T=T, AG•T=T, GA•T=T and AA•T=T duplexes with MutY.
MutY (Fig. S1), indicating that the adenine-DNA glycosylase does not recognize A or G residues opposite CPD in the mismatched DNA duplexes.

Still, one cannot exclude that MutY may act on Adenines opposite other bipyrimidine photoproducts such as C=T, T=C and C=C. To examine this, we incubated heavily UV-irradiated covalently closed circular plasmid DNA (ccc) with MutY, APE1 and T4 PDG. Upon cleavage at the site of damage by a DNA repair enzyme, the ccc form is converted either to an open circular form or to a linear double-stranded fragment and these three forms can be separated and quantified by electrophoresis in agarose gel (Ishchenko et al., 2003). The results showed that while T4 PDG cleaves 97% of the UV-irradiated plasmid, MutY (both in the absence and in the presence of APE1) cleaves only tiny fraction of the plasmid (Fig. S2). These results further substantiate the fact that MutY has very little or no activity toward UV adducts and Adenines on non-damaged strand in UV-irradiated DNA.

UV-induced RifS → RifR mutations in MutY and NER-deficient E. coli mutants

Genetic and biochemical studies of E. coli have established that both the RecA-dependent recombination and NER pathway participate in the removal of UV-induced DNA damage. Subsequently, it was demonstrated that UV-DNA adducts can be bypassed by UmuD′C-RecA-ATP complex in vitro with high efficiency (Jiang et al., 2009). Importantly, in the absence of functional NER system, the frequency of UV-induced mutagenesis for a given dose increases further because of the persistence of unrepaired UV adduct in DNA which in turn stimulate mutations via UmuDC-dependent pathway. To examine a potential role of the MutY-catalyzed DNA glycosylase activity in UV-induced mutagenesis, we assessed the sensitivity and mutagenesis of the E. coli MutY/MicA (micA) and NER (uvrA6) deficient strains exposed to 254 nm UV light. Previously, we hypothesized that the aberrant BER pathway induces mutation in replication-independent manner (Talhaoui et al., 2014). Furthermore, it has been demonstrated that UV-induced DNA lesions can undergo mutation fixation by the NER-induced mutagenesis (NERiM) in DNA replication-independent manner (Janel-Bintz et al., 2017). Therefore, to favor the detection of mutations occurring in a DNA replication-independent manner, we measured UV-induced mutagenesis in stationary phase cultures of the E. coli WT CC104 and AB1157 (WT), MutY-deficient BH980 (micA::KnR), NER-deficient AK146 (uvrA6) and double NER/MutY-deficient BH1220 (uvrA6 micA) strains. Data from a typical experiment are shown in Table 2, exposure of the CC104 WT strain to high doses of UV (180 J·m⁻²) resulted in 64-fold increase in the appearance of RifR colonies as compared to that of non-irradiated strain. At the same time, BH980 strain, isogenic to CC104, but micA exhibited only 11-fold increase. Similarly, exposure of AK146 strain to low dose of UV (10 J·m⁻²) resulted in 81-fold increase of RifR → RifR mutations frequencies as compared to the non-irradiated control (Table 2). Intriguingly, exposure of NER/MutY-deficient BH1220 strain to low dose of UV (10 J·m⁻²) showed only weak 3.9-fold increase in frequency of UV-induced RifR colonies as compared to the non-irradiated control. It should be stressed, that MutY-deficient strains (micA) exhibited 11- to 80-fold increase in spontaneous mutation rates as compared to MutY-proficient strains (Table 2). Combining data from several independent experiments in Fig. 4 demonstrated that WT and NER-deficient E. coli
Figure 4 Graphic representation of the UV-induced increase in mutation frequencies in *E. coli* cells. NER-proficient strains were exposed to dose 100–180 J·m⁻² UV and NER deficient strains to doses 10 J·m⁻² UV, only. Data from at least three experiments were used for statistical analysis.

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Table 2 Frequencies of UV induced Rif⁰ → Rif⁰ mutation in *E. coli* WT versus *micA* strains.

| *E. coli* strains | Dose J·m⁻² | Survival (%) | No UV LB 10⁷ cells/mL | Rif⁰ 1 mL | UV LB 10⁷ cells/mL | Rif⁰ 1 mL | No UV Rif⁰/LB (10⁻⁷) | UV Rif⁰/LB (10⁻⁷) | Fold increase of Rif⁰ mutants |
|------------------|-----------|--------------|-----------------------|----------|-------------------|----------|------------------|------------------|-----------------------------|
| CC104 (WT)       | 180 J·m⁻² | 5.1          | 3,600                 | 210      | 200               | 7,670    | 0.058            | 3.73             | 64                          |
| BH980 (*micA::Kan⁰*) | 180 J·m⁻² | 12.0         | 200                   | 129      | 87                | 640      | 0.645            | 7.37             | 11.4                        |
| AB1157 (WT)      | 100 J·m⁻² | 0.1          | 320                   | 3.5      | 140               | 476      | 0.01             | 3.4              | 311                         |
| AK146 (*uvrA6*)  | 10 J·m⁻²  | 5.2          | 255                   | 5        | 19.5              | 31       | 0.0196           | 1.59             | 81                          |
| BH1220 (*uvrA6* *micA::Kan⁰*) | 10 J·m⁻² | 1.0          | 300                   | 246      | 35                | 112.5    | 0.82             | 3.21             | 3.9                          |

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strains exhibited 45- to 220-fold increase in the UV-induced mutation frequencies, as
compared to moderate 2.3- and 13-fold increase for MutY/NER and MutY-deficient strains,
respectively. Taken together, these results suggest that in the absence of the MutY protein,
bacterial cells exhibited low to moderate induction of the UV-induced Rif\textsuperscript{S} → Rif\textsuperscript{R}
mutations frequencies.

**Molecular spectra of spontaneous and UV-induced Rif\textsuperscript{S} → Rif\textsuperscript{R} mutations in E. coli WT and micA strains**

To further examine whether MutY participates in the aberrant processing of the
unrepaired UV lesions in vivo, we measured UV-induced mutagenesis in the
NER/MutY-deficient *E. coli* BH1220 and BH1070 (*uvrA6 micA*) strains harbouring vector
pJWT21-4 coding either for MutY-WT or for catalytically inactive mutant MutY-D138N.
It should be noted that the BH1220 and BH1070 strains expressing MutY-WT
exhibited very low rate of spontaneous mutations as compared to the same strains
expressing mutant MutY-D138N. As expected, exposure of the BH1220 and BH1070
strains with MutY-WT to UV (10 J·m\textsuperscript{-2}) resulted in more than 100-fold increase in
Rif\textsuperscript{S} → Rif\textsuperscript{R} mutation frequencies (Fig. 5). In contrast, for the same dose of UV, these same
strains but containing MutY-D138N mutant exhibited only three to sevenfold increase
in the UV-induced mutations (Fig. 5). It is well established that the major type of
mutations induced by UV light are C→T transitions. Since the majority of spontaneous
mutations in MutY-deficient strains are due to oxidative damage to guanines in DNA,
we suggested that the majority of UV-induced mutations in these strains would be due to
cytosine-containing pyrimidine dimers in DNA and not due to guanine oxidation.

To examine changes in the mutation spectra in WT and DNA repair-deficient *E. coli*
strains after UV exposure, we performed DNA sequencing of RpoB gene from Rif\textsuperscript{R} clones
obtained before and after UV exposure of the cells. As expected, analysis of the
spontaneous and UV-induced mutation spectra in the *E. coli* WT, *uvrA6* and *uvrA6 micA*
strains revealed that all strains exhibit dramatic increase in the frequency of C•G→T•A
transition: from 12.6% to 81% in WT and from 2.6–6.5% to 64–76% in *uvrA6 micA*
strains (Table 3). It should be noted that BH1070 and BH1220 strains, as compared to the
MutY-proficient strains, exhibited moderate increase in the overall mutation frequencies
after UV exposure, possibly because of the masking effect of high spontaneous
mutation rate in non-exposed cells which is primarily due to G•C→T•A transversions
(which make >80% of all mutations). Remarkably, UV irradiation dramatically changes
the mutation spectra in these two *uvrA6 micA* strains with majority of mutations being
C•G→T•A transitions (which make 64–76% of all UV-induced mutations after UV
exposure (Table 3). Using these mutation spectra, we calculated the relative increase in the
frequency of C•G→T•A transitions after UV irradiation. The results revealed that
NER/MutY-deficient BH1220 and BH1070 strains exhibited dramatic 86- and 239-fold
increase, in the frequency of C•G→T•A transitions after UV exposure, although the
overall increase in Rif\textsuperscript{S} → Rif\textsuperscript{R} mutation frequencies were only 3.9- to 4.5-fold (Table 3).

Of note, the mutational signatures in the control non-irradiated strains varied
depending on the genetic background. For example, of the most frequent base substitution
in non-irradiated AB1157 (WT) was T>A>C>G transition followed by A>T>C>G transversion (Table 3). At the same time, C>G>A>T transitions were prevalent in the non-irradiated BH200 (uvrA::Tn10) and AK146 (uvrA6) strains.

**Structural features counteracting the aberrant DNA substrate specificity of MutY**

The reported opposite-base specificity of MutY includes naturally occurring bases 8oxoG, G, C (Radicella, Clark & Fox, 1988; Tsai-Wu, Liu & Lu, 1992), 8-oxoadenine (Bulychev et al., 1996) as well as artificial base analogs 8-oxohypoxanthine, 8-oxonebularine, 8-methoxyguanine, 8-thioguanine, 7-methyl-8-oxoguanine and 8-bromoguanine.
The structure of MutY from *Geobacillus stearothermophilus* (Bst-MutY) bound to DNA containing an A:8oxoG mispair reveals intrahelical 8oxoG forming specific hydrogen bonds with a conserved Ser residue, O_8[8oxoG]:::N[S308] and N7[8oxoG]:::Oc[S308] (Fromme et al., 2004; Lee & Verdine, 2009). However, this arrangement of hydrogen bonds is not possible with G and many other bases opposite the excised adenine. It has been suggested that for DNA glycosylases excising canonical bases from non-canonical base pairs, such as MutY, the decisive factor is selective destabilization of a non-canonical base pair and stabilization of the everted natural base in the enzyme-substrate complex (Talhaoui et al., 2017).

In order to rationalize the lack of activity of MutY on A opposite CPD, we have analyzed the structures of all available MutY-DNA complexes (Fromme et al., 2004; Lee & Verdine, 2009; Wang, Chakravarthy & Verdine, 2017; Wang, Lee & Verdine, 2015) and all DNA molecules containing a CPD (Biertumpfel et al., 2010; Fischer et al., 2011; Horikoshi et al., 2016; Li et al., 2004; McAteer et al., 1998; Park et al., 2002; Silverstein et al., 2010; Vasquez-Del Carpio et al., 2011; Vassylyev et al., 1995). By covalently linking two adjacent residues, a CPD places strong constraints on the geometry of the damaged nucleotides, keeping them closer than in regular B-DNA (C1′–C1′ distance 3.85 ± 0.29 Å in a CPD vs. 5.17 ± 0.45 Å in B-DNA). On the other hand, intrusion of an aromatic residue (Tyr88 in Bst-MutY) 5′ of 8oxoG stretches DNA in this region (C1′–C1′ distance

| E. coli strains | Base substitution | Mutation spectra in control cells (%) | Mutation spectra in UV irradiated cells (%) | Fold increase of C-G→T+A transitions after UV irradiation | Fold increase of Rif^R mutants after UV irradiation |
|----------------|------------------|--------------------------------------|---------------------------------------------|-------------------------------------------------------|-----------------------------------------------|
| AB1157 (WT)    | C→T & G→A        | 12.6                                 | 81.3                                        | 768                                                   | 140                                           |
|                | T→C & A→G        | 38.4                                 | 3.1                                         |                                                       |                                               |
|                | A→G & C→T        | 30.8                                 | –                                           |                                                       |                                               |
|                | A→T & T→A        | 7.6                                  | 12.5                                        |                                                       |                                               |
|                | G→T & C→A        | 3.8                                  | 3.1                                         |                                                       |                                               |
| BH200 (uvrA::Tn10) | C→T & G→A    | 40.0                                 | 92.0                                        | 224                                                   | 98                                            |
|                | T→C & A→G        | 20                                   | –                                           |                                                       |                                               |
|                | A→T & T→A        | 20                                   | –                                           |                                                       |                                               |
|                | G→T & C→A        | –                                    | 3.8                                         |                                                       |                                               |
| AK146 (uvrA6)  | C→T & G→A        | 60.0                                 | 75.0                                        | 218                                                   | 224                                           |
|                | T→C & A→G        | 15.0                                 | –                                           |                                                       |                                               |
|                | G→T & C→A        | 15.0                                 | 12.5                                        |                                                       |                                               |
|                | A→T & T→A        | 10.0                                 | 12.5                                        |                                                       |                                               |
| BH1220 (uvrA6 micA::Kan^R) | C→T & G→A | 6.5                                  | 63.6                                        | 86                                                     | 3.9                                           |
|                | G→T & C→A        | 84.0                                 | 27.3                                        |                                                       |                                               |
| BH1070 (uvrA6 micA::Kan^R) | C→T & G→A | 2.6                                  | 76.2                                        | 239                                                    | 4.5                                           |
|                | G→T & C→A        | 87.2                                 | 7.1                                         |                                                       |                                               |

(Fromme et al., 1996; Manlove et al., 2017). The structure of MutY from *Geobacillus stearothermophilus* (Bst-MutY) bound to DNA containing an A:8oxoG mispair reveals intrahelical 8oxoG forming specific hydrogen bonds with a conserved Ser residue, O_8[8oxoG] ... N[S308] and N7[8oxoG] ... Oy[S308] (Fromme et al., 2004; Lee & Verdine, 2009). However, this arrangement of hydrogen bonds is not possible with G and many other bases opposite the excised adenine. It has been suggested that for DNA glycosylases excising canonical bases from non-canonical base pairs, such as MutY, the decisive factor is selective destabilization of a non-canonical base pair and stabilization of the everted natural base in the enzyme-substrate complex (Talhaoui et al., 2017).

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7.22 ± 0.61 Å) and slightly compresses it 3′ of 8oxoG (4.50 ± 0.23 Å). Thus, a covalently bound CPD is incompatible with the geometry requirements to the non-cleaved strand in a MutY-DNA complex targeting either 5′- or 3′-adenine in the AA dinucleotide opposite a CPD (Fig. 6).

**DISCUSSION**

Recently, we showed that human TDG can target non-damaged DNA strand to remove mismatched T opposite deaminated/oxidized adenine residues in duplex DNA in TpG/CpA* context (where A* is a damaged adenine residue). This aberrant excision of a normal base initiates repair synthesis that uses damaged DNA template leading to T→C mutation fixation in the absence of DNA replication. This finding points to a possible role of other mismatch-specific DNA glycosylases which do not discriminate damaged versus non-damaged DNA strand when excising regular DNA bases such as bacterial MutY and human MUTYH proteins. Indeed, it was shown that *E. coli* MutY participates in mutagenic post-replicative excision of regular A opposite a misincorporated 8oxoG residue which results in the increased A•T→C•G transversion rates in both *E. coli* WT and *mutT* strains (Fowler et al., 2003). It has been also demonstrated that MUTYH is involved in aberrant processing of UV lesions and interferes with NER machinery (Mazouzi et al., 2017).

Based on these observations we speculated that MutY/MUTYH similar to TDG/MBD4 may initiate aberrant excision of Adenines opposite damaged Thymine residues, for example...
CPD and 6–4PP adducts, which in turn may lead to futile repair and activation of the DNA damage response in the UV-exposed cells.

In the present study, we report that the *E. coli* MutY protein cannot excise A residues opposite UV-induced CPD and 6–4PP adducts in the duplex oligonucleotide under experimental conditions used. Importantly, we observed efficient MutY-catalyzed excision of mismatched A residues when opposite G and 8oxoG residues in duplex DNA with the same sequence contexts used for UV lesions. These biochemical results suggest that MutY and perhaps its human homologue MUTYH do not participate in UV-induced mutagenesis in bacterial and human cells, respectively. The inability of MutY to excise A from opposite a CPD seems to be due, at least in part, to a severe conformational restrictions inflicted by the covalent linkage between two pyrimidines: the distance between two thymines in a dimer is less than is required to fit into MutY active center and cannot be widened (Fig. 6). Thus, the pre-catalytic MutY-DNA complex with a CPD would be destabilized, and the efficiency of the reaction significantly compromised.

To clear out the possible role of MutY in the aberrant mutagenic repair, we examined whether UV-induced mutagenesis in *E. coli* depends on the presence of this mismatch-specific DNA glycosylase. We observed that after UV exposure the MutY-deficient *E. coli* strains exhibited lower induction of Rif<sup>S</sup>→Rif<sup>R</sup> mutations as compared to MutY-proficient strains (Table 2 and Fig. 4). It should be stressed that under normal conditions *E. coli* micA strains exhibit a 100-fold increase in the spontaneous mutation rates as compared to MutY/MicA-proficient strains (*Nghiem et al., 1988*). Actually, this spontaneous mutator phenotype of *E. coli* micA strains might conceal the mutagenic effect of UV light, which, under the experimental conditions used, induces mutations at similar rates. Thus, we have analyzed mutation spectra in *E. coli* strains before and after UV exposure to see whether the types of mutations changes in MutY-deficient cells. The DNA sequence analysis of Rif<sup>R</sup> clones showed that the NER/MutY-deficient BH1220 and BH1070 strains exhibited dramatic 86- and 239-fold increase in the frequency of C<sup>G</sup>→T<sup>A</sup> transitions after UV exposure, respectively, although the overall increase in Rif<sup>S</sup>→Rif<sup>R</sup> mutation frequencies was only 3.9- and 4.5-fold, respectively (Table 3). These results indicate that the mutation spectra in MutY/MicA-proficient and -deficient cells change in similar manner after UV exposure. It appears that MutY does not play a significant role in the induction of C<sup>G</sup>→T<sup>A</sup> transitions after UV exposure in vivo. Nevertheless, recently, it has been demonstrated that the repair of UV-induced DNA lesions turns out to be mutagenic in non-dividing cells under certain circumstances. Indeed, *Janel-Bintz et al., (2017)* have demonstrated that in *E. coli* cells the removal of closely spaced UV lesions in the NER pathway can induce mutations which are not dependent on DNA replication. Interestingly, NERiM is functional in stationary phase cells and requires DNA polymerases IV and II. In line with these observations, it has been shown that active Pol V complex (UmuD<sup>2</sup>C-RecA-ATP) formed after UV irradiation does not co-localize with replicative DNA polymerase III complexes (*Robinson et al., 2015*).

Although in the present study we did not observe aberrant repair activities of MutY in *E. coli* cells, the accumulated evidence shows that mammalian homologues of MutY are involved in the aberrant and futile DNA repair. Depletion of MUTYH by acetohexamide...
in XP cells promotes an alternative repair of UV-induced lesions and thereby increases cells survival (Mazouzi et al., 2017). Under enhanced oxidative stress and in the absence of OGG1, the MUTYH-mediated BER mechanism might become engaged in futile and cytotoxic repair steps (Nakabeppu, 2014; Sheng et al., 2012). Also, it has been demonstrated that MUTYH induces the persistent accumulation of SSBs in the nascent DNA strand which in turn promotes MLH1/PARP1-dependent human cell death (Oka et al., 2014), retinal inflammation and degeneration in a mouse model of Retinis pigmentosa (Nakatake et al., 2016). A possible role of the MUTYH-initiated aberrant BER in DNA damage response was suggested by the study of the mechanisms of neurodegeneration caused by 3-nitropropionic acid (3NP) (Sheng et al., 2012). The authors showed that 3NP induced oxidative stress results in the accumulation of 8oxoG and SSBs in mitochondrial DNA of neurons. SSBs accumulation and neurodegeneration were alleviated in mutant mice lacking MUTYH. However, OGG1 and MTH1, an 8oxo-dGTP hydrolase, offered protection, suggesting that aberrant repair of the misincorporated adenine opposite 8oxoG in DNA by MUTYH leads to SSB accumulation, which in turn triggers mitochondrial impairment and retrograde signalling to the nucleus of neurons.

CONCLUSION
In the present study we further characterized the biochemical and genetic properties of the well-known bacterial MutY DNA glycosylase. The data demonstrate that bacterial MutY does not recognize A opposite damaged T residues such as pyrimidine dimers in duplex DNA and is not involved in UV-induced mutagenesis in E. coli. Based on these observations, we propose that the role of MUTYH, a human homologue of MutY, in the increased genotoxicity of UV damage in XP cells might not be due to its aberrant activity toward A residues in the non-damaged DNA strand. Although we did not find clear evidence for the involvement of MutY-like DNA glycosylases in the aberrant mutagenic repair, we cannot exclude that the non-dividing cells such as reversibly growth-arrested dormant hematopoietic stem cells or terminally differentiated neurons may be prone to the aberrant excision of regular DNA bases in damaged DNA duplexes by the mono-functional mismatch-specific DNA glycosylases. Additional studies are required to investigate whether MutY and MUTYH act on different DNA substrates with damaged Thymine residues.

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Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Caroline Zutterling performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper.
- Aibek Mursalimov performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper.
- Ibtissam Talhaoui performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, DNA sequencing analysis to identify mutations.
- Zhanat Koshenov performed the experiments, analyzed the data.
- Zhiger Aksheev performed the experiments, analyzed the data.
- Amangeldy K. Bissenbaev conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper.
Gerard Mazon conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools.

Nicolas E. Geacintov conceived and designed the experiments, contributed reagents/materials/analysis tools, oligonucleotides containing UV lesions.

Didier Gasparutto conceived and designed the experiments, contributed reagents/materials/analysis tools, oligonucleotides containing base modifications.

Regina Groisman conceived and designed the experiments, analyzed the data.

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**Data Availability**

The following information was supplied regarding data availability:

The raw data is available in the [Supplemental Information](http://dx.doi.org/10.7717/peerj.6029#supplemental-information).

**Supplemental Information**

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**REFERENCES**

Abeldenov S, Talhaoui I, Zharkov DO, Ishchenko AA, Ramanculov E, Saparbaev M, Khassenov B. 2015. Characterization of DNA substrate specificities of apurinic/apyrimidinic endonucleases from *Mycobacterium tuberculosis*. *DNA Repair* 33:1–16 DOI 10.1016/j.dnarep.2015.05.007.

Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, Hodges AK, Davies DR, David SS, Sampson JR, Cheadle JP. 2002. Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. *Nature Genetics* 30(2):227–232 DOI 10.1038/ng828.

Barnes DE, Lindahl T. 2004. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annual Review of Genetics* 38(1):445–476 DOI 10.1146/annurev.genet.38.072902.092448.

Biertumpfel C, Zhao Y, Kondo Y, Ramon-Maiques S, Gregory M, Lee JY, Masutani C, Lehmann AR, Hanaoa F, Yang W. 2010. Structure and mechanism of human DNA polymerase eta. *Nature* 465(7301):1044–1048 DOI 10.1038/nature09196.

Bulychev NV, Varaprasad CV, Dorman G, Miller JH, Eisenberg M, Grollman AP, Johnson F. 1996. Substrate specificity of *Escherichia coli* MutY protein. *Biochemistry* 35(40):13147–13156.

Cupples CG, Miller JH. 1989. A set of lacZ mutations in *Escherichia coli* that allow rapid detection of each of the six base substitutions. *Proceedings of the National Academy of Sciences of the United States of America* 86(14):5345–5349 DOI 10.1073/pnas.86.14.5345.

Dale JW, Greenaway PJ. 1984. Preparation of chromosomal DNA from *E. coli*. In: Walker JM, ed. *Methods in molecular biology*. New Jersey: Humana Press, 197–200.
Dodson ML, Michaels ML, Lloyd RS. 1994. Unified catalytic mechanism for DNA glycosylases. *Journal of Biological Chemistry* 269(52):32709–32712.

Douki T, Court M, Sauvaigo S, Odin F, Cadet J. 2000. Formation of the main UV-induced thymine dimeric lesions within isolated and cellular DNA as measured by high performance liquid chromatography-tandem mass spectrometry. *Journal of Biological Chemistry* 275(16):11678–11685 DOI 10.1074/jbc.275.16.11678.

Dower WJ, Miller JF, Ragsdale CW. 1988. High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Research* 16(13):6127–6145 DOI 10.1093/nar/16.13.6127.

Duwat P, De Oliveira R, Ehrlich SD, Boiteux S. 1995. Repair of oxidative DNA damage in gram-positive bacteria: the Lactococcus lactis Fpg protein. *Microbiology* 141(2):411–417 DOI 10.1099/13500872-141-2-411.

Fischer ES, Scrima A, Bohm K, Matsumoto S, Fatty M, Yasuda T, Cavadini S, Wakasugi M, Hanaoka F, Iwai S, Sugawara K, Thoma NH. 2011. The molecular basis of CRL4DDB2/CSA ubiquitin ligase architecture, targeting, and activation. *Cell* 147(5):1024–1039 DOI 10.1016/j.cell.2011.10.035.

Fowler RG, White SJ, Koyama C, Moore SC, Dunn RL, Schaaper RM. 2003. Interactions among the *Escherichia coli* mutT, mutM, and mutY damage prevention pathways. *DNA Repair* 2(2):159–173 DOI 10.1016/s1568-7864(02)00193-3.

Fromme JC, Banerjee A, Huang SJ, Verdine GL. 2004. Structural basis for removal of adenine mispaired with 8-oxoguanine by MutY adenine DNA glycosylase. *Nature* 427(6975):652–656 DOI 10.1038/nature02306.

Garibyan L, Huang T, Kim M, Wolff E, Nguyen A, Nguyen T, Diep A, Hu K, Iverson A, Yang H, Miller JH. 2003. Use of the rpoB gene to determine the specificity of base substitution mutations on the *Escherichia coli* chromosome. *DNA Repair* 2(5):593–608 DOI 10.1016/s1568-7864(03)00024-7.

Gelin A, Redrejo-Rodriguez M, Laval J, Fedorova OS, Saparbaev M, Ishchenko AA. 2010. Genetic and biochemical characterization of human AP endonuclease 1 mutants deficient in nucleotide incision repair activity. *PLOS ONE* 5(8):e12241 DOI 10.1371/journal.pone.0012241.

Guan Y, Manuel RC, Arvai AS, Parikh SS, Mol CD, Miller JH, Lloyd S, Tainer JA. 1998. MutY catalytic core, mutant and bound adenine structures define specificity for DNA repair enzyme superfamily. *Nature Structural Biology* 5(12):1058–1064 DOI 10.1038/4168.

Hegde ML, Hazra TK, Mitra S. 2008. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. *Cell Research* 18(1):27–47 DOI 10.1038/cr.2008.8.

Horikoshi N, Tachiwana H, Kagawa W, Osakabe A, Matsumoto S, Iwai S, Sugasawa K, Kurumizaka H. 2016. Crystal structure of the nucleosome containing ultraviolet light-induced cyclobutane pyrimidine dimer. *Biochemical and Biophysical Research Communications* 471(1):117–122 DOI 10.1016/j.bbrc.2016.01.170.

Ishchenko AA, Sanz G, Privezentzev CV, Maksimenko AV, Saparbaev M. 2003. Characterisation of new substrate specificities of *Escherichia coli* and *Saccharomyces cerevisiae* AP endonucleases. *Nucleic Acids Research* 31(21):6344–6353 DOI 10.1093/nar/gkg812.

Janel-Bintz R, Napolitano RL, Isogawa A, Fujii S, Fuchs RP. 2017. Processing closely spaced lesions during Nucleotide Excision Repair triggers mutagenesis in *E.coli*. *PLOS Genetics* 13(7):e1006881 DOI 10.1371/journal.pgen.1006881.

Jiang Q, Karata K, Woodgate R, Cox MM, Goodman MF. 2009. The active form of DNA polymerase V is UmuD'(2)C-RecA-ATP. *Nature* 460(7253):359–363 DOI 10.1038/nature08178.
Lee S, Verdine GL. 2009. Atomic substitution reveals the structural basis for substrate adenine recognition and removal by adenine DNA glycosylase. *Proceedings of the National Academy of Sciences of the United States of America* 106(44):18497–18502 DOI 10.1073/pnas.0902908106.

Li Y, Dutta S, Double S, Bdour HM, Taylor JS, Ellenberger T. 2004. Nucleotide insertion opposite a cis-syn thymine dimer by a replicative DNA polymerase from bacteriophage T7. *Nature Structural & Molecular Biology* 11(8):784–790 DOI 10.1038/nsmb792.

Maki H, Sekiguchi M. 1992. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature* 355(6357):273–275 DOI 10.1038/355273a0.

Manlove AH, McKibbin PL, Doyle EL, Majumdar C, Hamm ML, David SS. 2017. Structure-activity relationships reveal key features of 8-oxoguanine: a mismatch detection by the MutY glycosylase. *ACS Chemical Biology* 12(9):2335–2344 DOI 10.1021/acschembio.7b00389.

Manuel RC, Hitomi K, Arvai AS, House PG, Kurtz AJ, Dodson ML, McCullough AK, Tainer JA, Lloyd RS. 2004. Reaction intermediates in the catalytic mechanism of *Escherichia coli* MutY DNA glycosylase. *Journal of Biological Chemistry* 279(45):46930–46939.

Mazouzi A, Battistini F, Moser SC, Ferreira da Silva J, Wiedner M, Owusu M, Lardeau CH, Ringler A, Weil B, Neesen J, Orozco M, Kubicek S, Loizou JI. 2017. Repair of UV-Induced DNA damage independent of nucleotide excision repair is masked by MUTYH. *Molecular Cell* 68(4):797–807.e797 DOI 10.1016/j.molcel.2017.10.021.

McAteer K, Jing Y, Kao J, Taylor JS, Kennedy MA. 1998. Solution-state structure of a DNA dodecamer duplex containing a Cis-syn thymine cyclobutane dimer, the major UV photoproduct of DNA. *Journal of Molecular Biology* 282(5):1013–1032 DOI 10.1006/jmbi.1998.2062.

Michaels ML, Tchou J, Grollman AP, Miller JH. 1992. A repair system for 8-oxo-7,8-dihydrodeoxyguanine. *Biochemistry* 31(45):10964–10968 DOI 10.1021/bi00160a004.

Miller JH. 1972. *A short course in bacterial genetics. A laboratory manual and handbook for Escherichia coli and related bacteria*. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Nakabeppu Y. 2014. Cellular levels of 8-oxoguanine in either DNA or the nucleotide pool play pivotal roles in carcinogenesis and survival of cancer cells. *International Journal of Molecular Sciences* 15(7):2541–2557 DOI 10.3390/ijms15072543.

Nakatake S, Murakami Y, Ikeda Y, Morioka N, Tachibana T, Fujiwara K, Yoshida N, Notomi S, Hisatomi T, Yoshida S, Ishibashi T, Nakabeppu Y, Sonoda KH. 2016. MUTYH promotes oxidative microglial activation and inherited retinal degeneration. *JCI Insight* 1(15):e87781 DOI 10.1172/jci.insight.87781.

Nghiem Y, Cabrera M, Cupples CG, Miller JH. 1988. The mutY gene: a mutator locus in *Escherichia coli* that generates G.C—T.A transversions. *Proceedings of the National Academy of Sciences of the United States of America* 85(8):2709–2713 DOI 10.1073/pnas.85.8.2709.

Oka S, Leon J, Tsuchimoto D, Sakumi K, Nakabeppu Y. 2014. MUTYH, an adenine DNA glycosylase, mediates p53 tumor suppression via PARP-dependent cell death. *Oncogene* 3(10):e121 DOI 10.1038/oncsis.2014.35.

Park H, Zhang K, Ren Y, Nadji S, Sinha N, Taylor JS, Kang C. 2002. Crystal structure of a DNA decamer containing a cis-syn thymine dimer. *Proceedings of the National Academy of Sciences of the United States of America* 99(25):15965–15970 DOI 10.1073/pnas.242422699.

Patel M, Jiang Q, Woodgate R, Cox MM, Goodman MF. 2010. A new model for SOS-induced mutagenesis: how RecA protein activates DNA polymerase V. *Critical Reviews in Biochemistry and Molecular Biology* 45(3):171–184 DOI 10.3109/10409238.2010.480968.
Peng W, Shaw BR. 1996. Accelerated deamination of cytosine residues in UV-induced cyclobutane pyrimidine dimers leads to CC→TT transitions. *Biochemistry* 35(31):10172–10181 DOI 10.1021/bi960001x.

Radicella JP, Clark EA, Fox MS. 1988. Some mismatch repair activities in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* 85(24):9674–9678 DOI 10.1073/pnas.85.24.9674.

Robinson A, McDonald JP, Caldas VEA, Patel M, Wood EA, Punter CM, Ghodke H, Cox MM, Woodgate R, Goodman MF, Van Oijen AM. 2015. Regulation of mutagenic DNA polymerase V activation in space and time. *PLOS Genetics* 11(8):e1005482 DOI 10.1371/journal.pgen.1005482.

Scaramozzino N, Sanz G, Crance JM, Saparbaev M, Drillien R, Laval J, Kavli B, Garin D. 2003. Characterisation of the substrate specificity of homogeneous vaccinia virus uracil-DNA glycosylase. *Nucleic Acids Research* 31(16):4950–4957 DOI 10.1093/nar/gkg672.

Sheng Z, Oka S, Tsuchimoto D, Abolhassani N, Nomaru H, Sakumi K, Yamada H, Nakabeppu Y. 2012. 8-Oxoguanine causes neurodegeneration during MUTYH-mediated DNA base excision repair. *Journal of Clinical Investigation* 122(12):4344–4361 DOI 10.1172/JCI65053.

Silverstein TD, Johnson RE, Jain R, Prakash L, Prakash S, Aggarwal AK. 2010. Structural basis for the suppression of skin cancers by DNA polymerase eta. *Nature* 465(7301):1039–1043 DOI 10.1038/nature09104.

Smith CA, Taylor JS. 1993. Preparation and characterization of a set of deoxyoligonucleotide 49-mers containing site-specific cis-syn, trans-syn-I, (6-4), and Dewar photoproducts of thymidylyl(3′→5′)-thymidine. *Journal of Biological Chemistry* 268:11143–11151.

Sun B, Latham KA, Dodson ML, Lloyd RS. 1995. Studies on the catalytic mechanism of five DNA glycosylases. Probing for enzyme-DNA imino intermediates. *Journal of Biological Chemistry* 270(33):19501–19508 DOI 10.1074/jbc.270.33.19501.

Talhaoui I, Couve S, Gros I, Ishchenko AA, Matkarimov B, Saparbaev MK. 2014. Aberrant repair initiated by mismatch-specific thymine-DNA glycosylases provides a mechanism for the mutational bias observed in CpG islands. *Nucleic Acids Research* 42(10):6300–6313 DOI 10.1093/nar/gku246.

Talhaoui I, Matkarimov BT, Tchenio T, Zharkov DO, Saparbaev MK. 2017. Aberrant base excision repair pathway of oxidatively damaged DNA: implications for degenerative diseases. *Free Free Radical Biology and Medicine* 107:266–277 DOI 10.1016/j.freeradbiomed.2016.11.040.

Tchou J, Kasai H, Shibutani S, Chung MH, Laval J, Grollman AP, Nishimura S. 1991. 8-oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proceedings of the National Academy of Sciences of the United States of America* 88(11):4690–4694 DOI 10.1073/pnas.88.11.4690.

Tsai-Wu JJ, Liu HF, Lu AL. 1992. *Escherichia coli* MutY protein has both N-glycosylase and apurinic/apyrimidinic endonuclease activities on A.C and A.G mispairs. *Proceedings of the National Academy of Sciences of the United States of America* 89(18):8779–8783 DOI 10.1073/pnas.89.18.8779.

Vasquez-Del Carpio R, Silverstein TD, Lone S, Johnson RE, Prakash L, Prakash S, Aggarwal AK. 2011. Role of human DNA polymerase kappa in extension opposite from a cis-syn thymine dimer. *Journal of Molecular Biology* 408(2):252–261 DOI 10.1016/j.jmb.2011.02.042.

Vassylev DG, Kashiwagi T, Mikami Y, Ariyoshi M, Iwai S, Ohtsuka E, Morikawa K. 1995. Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA
substrate: structural basis for damaged DNA recognition. *Cell* 83(5):773–782 DOI 10.1016/0092-8674(95)90190-6.

Vrouwe MG, Pines A, Overmeer RM, Hanada K, Mullenders LHF. 2011. UV-induced photolesions elicit ATR-kinase-dependent signaling in non-cycling cells through nucleotide excision repair-dependent and -independent pathways. *Journal of Cell Science* 124(3):435–446 DOI 10.1242/jcs.075325.

Wang L, Chakravarthy S, Verdine GL. 2017. Structural Basis for the Lesion-scanning Mechanism of the MutY DNA Glycosylase. *Journal of Biological Chemistry* 292(12):5007–5017 DOI 10.1074/jbc.M116.757039.

Wang L, Lee SJ, Verdine GL. 2015. Structural basis for avoidance of promutagenic DNA repair by MutY adenine DNA glycosylase. *Journal of Biological Chemistry* 290(28):17096–17105 DOI 10.1074/jbc.M115.657866.

Yanošky C, Cox EC, Horn V. 1966. The unusual mutagenic specificity of an *E. coli* mutator gene. *Proceedings of the National Academy of Sciences of the United States of America* 55(2):274–281 DOI 10.1073/pnas.55.2.274.

Zharkov DO, Gilboa R, Yagil I, Kycia JH, Gerchman SE, Shoham G, Grollman AP. 2000. Role for lysine 142 in the excision of adenine from A: Gmispairs by MutY DNA glycosylase of *Escherichia coli*. *Biochemistry* 39(48):14768–14778 DOI 10.1021/bi001538k.