Oxidative dealkylation DNA repair mediated by the mononuclear non-heme iron AlkB proteins

Yukiko Mishina and **Chuan He**[*]
Department of Chemistry, The University of Chicago, 5735 South Ellis Avenue, Chicago, IL 60637, USA

Abstract

DNA can be damaged by various intracellular and environmental alkylating agents to produce alkylation base lesions. These base damages, if not repaired promptly, may cause genetic changes that lead to diseases such as cancer. Recently, it was discovered that some of the alkylation DNA base damage can be directly removed by a family of proteins called the AlkB proteins that utilize a mononuclear non-heme iron(II) and α-ketoglutarate as cofactor and cosubstrate. These proteins activate dioxygen and perform an unprecedented oxidative deal-kylation of the alkyl adducts on DNA heteroatoms. This review summarizes the discovery of this activity and the recent research advances in studying this unique DNA repair pathway. The focus is placed on the chemical mechanism and function of these proteins.

Keywords

DNA repair; AlkB; ABH; Direct repair; Mononuclear non-heme iron; Dioxygenase; Oxidative dealkylation; Demethylation

1. Direct repair of alkylation DNA damage

Cellular DNA is subject to nonenzymatic alkylation (methylation) by environmental or chemical mutagens, resulting in adducts that are toxic and mutagenic [1–6]. Organisms have evolved a variety of mechanisms to repair these mutagenic damages. *Escherichia coli* (*E. coli*) responds to this threat by activating an adaptive responsive pathway, mediated by the *E. coli* Ada protein [7]. Upon receiving a methyl group from the damaged DNA, Ada turns into a transcriptional activator and activates its own expression and that of the *alk*B gene and two other genes, *alk*A and *aid*B (Fig. 1(a)) [4,7,8]. The upregulated Ada is a bifunctional protein, which uses a N-terminal Cys38 residue to remove a methyl group from S_p-methyl-phosphotriester and a C-terminal Cys321 residue to remove a methyl adduct from O^6-methylguanaine (Fig. 1(b)) [9–11]. Both repair functions are irreversible and represent rare examples of direct repair of DNA damage. AlkA is a glycosylase that cleaves methylated bases from DNA and performs the first step of the well-known base excision repair of base lesions [12–14]. The precise function of AidB is still unknown. The function of the last member of this adaptive response, AlkB, also remained unknown until recently despite extensive research efforts.

2. *E. coli* AlkB

Since the first genetic study in 1983 isolating the *E. coli* mutant with specific sensitivity to the alkylating agent methylmethane sulfonate, MMS [15], the activity of AlkB was undisclosed

*Corresponding author. Tel.: +1 773 702 5061; fax: +1 773 702 0805. *E-mail address:* chuanhe@uchicago.edu (C. He).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

for almost 20 years. The research during this time included the finding of the resistance of AlkB to the treatment of the S_N2 type alkylating agents [15,16], which suggested its involvement in protecting bacteria from the lethal effects of alkylation damage [15–18]. Results from a sequence alignment study of protein fold and sequence homology on AlkB [19] placed AlkB into the α-ketoglutarate (αKG)- and Fe^{II}-dependent dioxygenase family, a group of proteins that utilize Fe^{II} to activate dioxygen and perform oxidation of various substrates.

The Fe^{II}/αKG-dependent dioxygenase superfamily is widespread from bacteria to humans [19] and is the largest known non-heme iron protein family [20–23]. It represents a wide diversity of enzymes that catalyze the hydroxylation of unactivated C–H groups of a variety of substrates by coupling reductive activation of dioxygen with a decarbox-ylation of αKG, the co-substrate, to succinate. In the event of oxidation one of the oxygen atoms from O_2 is incorporated into the succinate and the other as a hydroxyl in the product [24]. This group of enzymes is known for their importance in many environmental, pharmacological, and medical fields, and many of these reactions have not been successfully performed in the laboratory, especially ones involving the functionalization of inert C–H bonds. The reaction utilizes the mononuclear non-heme iron center, which is coordinated by a conserved His_2/Asp or the less abundant His_2/Glu motif to which dioxygen and αKG can also bind. Besides this rather simple, yet important motif, the members of this family have little sequence homology.

How can a DNA repair protein belong to an iron-containing dioxygenase family? DNA alkylation occurs due to reactions involving both S_N1 and S_N2 alkylating agents [25], and the susceptibility of each potential alkylation site on the bases or backbone varies on these reagents [2,6,25,26]. AlkB is responsible for repairing the damage caused by the S_N2 type methylating agents such as MMS and methyl halides. These can react with single-stranded DNA (ssDNA) to generate large portions of N^1-methyladenine (1-meA) and N^3-methylcytosine (3-meC) [2, 6,25,26] and they are the main methylation lesions formed [27–29]. These methyl adducts could be removed by two chemical mechanisms: (i) a direct displacement of the methyl group by a nucleophilic Cys residue or an activated water, which is similar to the mechanism used by the Ada proteins; or (ii) oxidation of the methyl group to give a hydroxyl intermediate, which decomposes in water to afford the repaired base and formaldehyde. In 2002, two groups simultaneously confirmed the second mechanism. They detected the formation of formaldehyde and showed that the AlkB protein utilizes a previously unknown oxidative demethylation pathway to repair 1-meA and 3-meC DNA damage (Fig. 1(c)) [30,31]. Thus, in the *ada* operon both Ada and AlkB use the direct repair pathways to remove methylation DNA damage. However, completely different mechanisms are adopted by the two proteins; Ada only performs stoichiometric repair, whereas AlkB catalyzes the oxidative removal of the methyl adducts (Fig. 1).

The N^1-adenine and N^3-cytosine positions are normally involved in hydrogen bonding and inaccessible in dsDNA. However, they are quite nucleophilic and vulnerable to attack by alkylating agents when exposed in ssDNA. Once alkylation has occurred on either of these two sites, the damaged bases can no longer form Watson–Crick base pairs. These non-coding lesions probably arise in vivo at DNA replication forks and transcription bubbles where they block the progression of DNA and RNA polymerases [32]. Thus, repair of these lesions is critical, as failure to repair these lesions in DNA leads to cell death in both *E. coli* and human cells [16].

3. AlkB homologs

Homologs of AlkB have been discovered based on sequence and fold similarity, and it is now known that the *alk*B gene is conserved from bacteria to human [19,33–35]. The homologs are not expressed equally in all human tissue types and may account for the discrepancies in

function [36]. The first human homolog, now called ABH1, is 52% similar and 23% identical to the *E. coli* AlkB, but ABH1 along with ABH4, ABH6, and ABH7 did not show any activity on any of the known substrates for AlkB. Two other human homologs, ABH2 and ABH3 could complement the *E. coli alk*B mutant phenotype [37,38] and they, as well as two mouse homologs mAbh2 and mAbh3, were shown to function like AlkB [36]. The activities of ABH5 and ABH8 have not been evaluated so far. All homologs share the essential motifs and residues for enzymatic activity, consisting of a HXD motif, a single H, as well as a RXXXXXR motif where the X's within the two arginines are alternating hydrophobic and polar residues (Fig. 2) [19,33–35]. The two histidines and the aspartic acid are proposed ligands to the active site Fe^{II}, whereas the first arginine is predicted to be involved in binding to the αKG [19,37] and these residues are highly conserved in the αKG-dependent oxygenases. This motif occurs as $H_{131}XD_{133}...H_{187}$ for AlkB. These three ligands at the active site of AlkB were confirmed through chemical cross-linking studies, where each of these residues in AlkB, His_{131}, Asp_{133}, and His_{187}, were mutated to a cysteine, and the mutant proteins were able to cross-link with DNA containing a modified thiol-tethered cytosine [39,40]. The cross-linking was inhibited by the addition of metal, as the cross-linking site was the Fe^{II} binding site [40].

4. The mononuclear iron center

The mononuclear Fe^{II} center of AlkB with bound αKG has been confirmed both with the overexpression and isolation of the native protein directly from *E. coli* [41] and by the addition of excess Fe^{II} and αKG to apo-AlkB [30–32,38,42–46]. Studies of the metal center has lead to an observation of an UV-vis band at 560 nm for the native protein [41]. This chromophore was assigned as the Fe^{II} to αKG charge transfer (MLCT) band, and is a spectroscopic characteristic of the Fe^{II}/αKG-dependent dioxygenases [47].

A slight change of the MLCT band was observed when an excess amount of DNA was added to the native AlkB. Further study of the protein and its DNA mixture by X-ray absorption spectroscopy provides evidence that the native AlkB binds DNA [41]. AlkB appears to have a five-coordinate Fe^{II} center in the absence of DNA, which becomes six-coordinate with the addition of ssDNA, suggesting a DNA-binding-induced geometry change of the mononuclear Fe^{II} center. Continued spectroscopic characterization of this iron center would be important to reveal properties of the Fe center in this family of novel proteins.

5. Repair mechanism

Addition of O_2 saturated H_2O to iron-loaded AlkB led to the development of a visible chromophore centered at 595 nm (ε_{590} 960 $M^{-1}cm^{-1}$) [45]. This peak was attributed to the LMCT transition of OH-Trp coordinated to Fe^{III}. The hydroxylation of the Trp side chain of AlkB, identified to be Trp_{178}, suggests formation of an oxidative intermediate in AlkB [45]. This behavior is consistent with other members of the non-heme Fe^{II}/αKG-dependent family [48–50]. The mechanism for the reactions for these enzymes, including AlkB, is proposed to use an $Fe^{IV}=O$ intermediate as the reactive oxidant. The proposed first step involves a reaction between the active site Fe^{II} and O_2 to give a superoxo anion (O_2^-) bound to Fe^{III}. Subsequently, the nucleophilic superoxide attacks at the α-keto carbon of an iron-bound αKG to give a bridged peroxotype intermediate. This bridged intermediate then undergoes a concerted decarboxylation of αKG and a heterolytic cleavage of the O–O bond to form the high-valent $Fe^{IV}=O$ intermediate [20–22]. This key active species is speculated to be used by enzymes in this family to hydroxylate C–H bonds of various substrates depending on the function of each protein. In AlkB this species hydroxylates the methyl adduct to give an unstable intermediate that decomposes in water to afford the final repaired product (Fig. 3). It should be noted that there have only been suggestions, as in the case of AlkB, and no concrete verification of the

intermediates in the proposed mechanism, including the $Fe^{IV}=O$ species, for most members of this family of enzymes.

The presence of the $Fe^{IV}=O$ species has been revealed very recently for the enzyme Taurine/ αKG dioxygenase, *Tau*D. *Tau*D catalyzes the hydroxylation of taurine to form sulfite, allowing the acquisition of sulfur from organic sulfonates for bacteria. *Tau*D has been a preferred enzyme for study in this family due to its simplicity of substrate and its ease with which it can be prepared in large quantity, as well as the characterization of its crystal structure. The high spin Fe^{IV} complex of *Tau*D was attained and characterized with kinetic and spectroscopic techniques, including absorption spectrum, Mossbauer, EXAFS and EPR experiments [51– 53]. Model compounds also provide strong evidence for the existence of $Fe^{IV}=O$ in proteins. Discrete mononuclear $Fe^{IV}=O$ species have been synthesized and characterized recently [54– 57]. These complexes serve as models for the intermediate species that may be generated in mononuclear non-heme iron-containing enzymes. In addition, ab initio calculations of non-heme intermediates support the likeliness of high spin $Fe^{IV}=O$ species [58].

Enzymes in the Fe^{II}/αKG-dependent dioxygenase family can catalyze the reaction in which the substrate oxidation can be uncoupled from aKG decarboxylation, both with or without substrates [21,59]. This uncoupled turnover of αKG results in the decomposition of aKG into succinate and CO_2 and usually leads to enzyme deactivation. Furthermore, this decomposition of aKG may account for the oxidation of the Fe^{II} center to form the inactive Fe^{III}. This oxidation process can be reversed with addition of ascorbate [31,44] as it is considered to act as an electron source in the uncoupled reaction [21]. These uncoupled conversions possibly occur due to incorrect orientation of substrate in the active site, such that the substrates are never appropriately oxidized [59].

The uncoupled reaction, leading to irreversible modifications of AlkB [45], was demonstrated as αKG decomposition was found to be partially uncoupled from DNA repair, accounting for the observed stimulation of AlkB activity in the presence of ascorbate [31,44]. The uncoupled αKG turnover was also stimulated by binding of the small molecules 1-meA, 1-methyldeoxyadenosine (1-me(dA)), 3-meC and 3-methyldeoxycytidine (3-me(dC)), but they were not repaired by AlkB [44]. Unmethylated nucleosides did not stimulate αKG turnover, indicating that the presence of a modification in the substrate is important in initiating oxidation of αKG [44].

6. Substrates

AlkB repairs various modifications as shown by the survival of alkylated bacteriophage in an *E. coli alk*B mutant [32,37,46,60,61]. Mainly, AlkB is known for the repair of 1-meA and 3-meC. ABH2, ABH3, mAbh2, and mAbh3 repair these substrates, as well [36]. Ethyl and propyl DNA adducts at the N^1-adenine and N^3-cytosine also activate the repair function of AlkB [32,37,46,60,61]. The release of formaldehyde in the repair of methylated bases supports the mechanism of repair shown in Fig. 3, and studies have confirmed that 1-ethyladenine is repaired by AlkB to form adenine [37]. The C-1 C–H group rather than the C-2 C–H group of the ethyl adduct is oxidized, forming acetaldehyde as the released product along with the repaired base [37].

S_N2 alkylating epoxides can cause hydroxyalkylated DNA lesions. For instance, ethylene oxide and propylene oxide can form 1-hydroxyalkyladenine and 3-hydroxyalkylcytosine. To create such lesions on DNA, iodoethanol, a reagent forming the same product as ethylene oxide, and propylene oxide was used to form hydroxyethyl and hydroxypropyl adducts at the N^1-position of adenine. These lesions, too, were found to be repaired by AlkB [46]. Furthermore, methylations on the N^3-position of thymine (3-meT) as well as the N^1-position of guanine (1-meG) are also repaired by AlkB [32,42,61]. Human homologs ABH2 and ABH3 are able to

demethylate 3-meT and 1-meG lesions in DNA oligonucleotides. A study on the mutagenicity, cytotoxicity and repair of 3-meT and 1-meG in wild type and *alkB* mutant strains of *E. coli* further indicates that these two lesions are indeed AlkB substrates [61].

3-meT is a minor product when ssDNA or dsDNA is treated with both S_N2 (MMS and dimethylsulfate, DMS) and S_N1 (methylnitrosourea, MNU) agents at neutral pH, constituting up to 0.8% of the total alkylated products. The yield of 3-meT is increased when DNA is treated with the S_N1 agent under alkaline conditions or when free thy-midine is exposed to DMS at high pH [32]. 3me-T has good chemical stability, and the 3-position is normally involved in base pairing, thus, like 1-meA and 3-meC, is thought to be a non-coding lesion. Both 3-meT and 3-ethylthymine in synthetic polymers completely block DNA synthesis by *E. coli* DNA polymerase I or the Klenow fragment of this enzyme [32]. The physiological importance of these lesions and their repair remains to be unveiled.

Some conclusions may be drawn about the critical features of efficient repair by comparison of 3-meT and 1-meG with 1-meA and 3-meC. Repair of 3-meT by both AlkB and ABH3 was optimal at pH 6 (range of 5.5–6.5 for ABH3) but inefficient [32]. At physiological pH, 3-meT is more slowly repaired than the major lesion found in ssDNA, 3-meC. 1-meG is a major lesion generated when tRNA is treated with MeI but is also repaired slowly compared to 1-meA or 3-meC [42]. The 3-meC and 1-meA are drawn as positively charged in solution; however, they could also exist as the neutral 6-imino (for 1-meA) or 4-imino (for 3-meC) forms, respectively, after isomerization and release of one proton from the exocyclic amino groups [62,63]. The imino nitrogen could serve as a hydrogen bond acceptor or a ligand to the active site metal, which may facilitate recognition of these two substrates. The presence of either a positive charge or an imino group may be critical features for efficient recognition or repair by AlkB. These may also account for the differences in the optimal pH values for repair of these lesions (7.5–8 for 1-meA and 3-meC). Nonetheless, damage of 1-meA and 3-meC, in particular, are thought to be the physiological substrates of AlkB. The base lesions 3-meT and 1-meG, may be recognized differently by the enzymes [42] as they did not stimulate the uncoupled AlkB-mediated decarboxylation of αKG.

7. Oxidative repair of exocyclic DNA adducts

If a high valent iron-oxo species is generated in AlkB upon exposure to dioxygen, this species may oxidize other DNA adducts that block Watson–Crick base pairing. Exo-cyclic DNA adducts such as 1,N^6-ethenoadenine (εA), 3,N^4-ethenocytosine (εC), and 1,N^2-ethenoguanine (εG) are produced when electrophilic vinyl chloride (VC) metabolites, chloroethylene oxide (CEO) or chloroacetaldehyde (CAA) react with adenine, cytosine, and guanine residues in DNA [28], respectively. The etheno bridges completely block normal Watson–Crick base pairing and are cytotoxic and potentially mutagenic [27]. These DNA adducts can arise endogeneously under oxidative stress in human cells, in particular when DNA is exposed to side products of lipid peroxidation [64–66]. The accumulation of these DNA lesions has been linked to aging and various diseases. It is known that the adaptive response pathway in *E. coli* renders the microbe resistant to induction of mutation by CAA [67,68], a hint that exocyclic DNA adducts are processed by the induced proteins.

Indeed two recent reports demonstrated that *E. coli* AlkB can repair εA and perhaps εC lesions in DNA [69,70]. Direct repair of εA in DNA by AlkB seems to be efficient both in vitro and in vivo. An epoxide intermediate was trapped and identified by MALDI-TOF mass spectrometry when a single-stranded 16 mer DNA containing a damaged base εA was used as the substrate [70]. This result supports a repair mechanism proposed in Fig. 4: the putative Fe^{IV}=O species can epoxidize the exocyclic double bond of the damaged base to give the epoxide, which is further hydrolyzed in water to afford the repaired base and glyoxal.

Production of glyoxal was detected after the repair process in both reports [69,70]. Although turnovers could be observed within minutes for the repair of εA by *E. coli* AlkB [69], this protein is more active in repairing N^1-methyladenine than εA [70]. The repair efficiency of εC is quite low [70], and its physiological relevance is unclear. ABH3, a human homolog of AlkB, exhibits the similar repair activity towards εA in DNA as *E. coli* AlkB [69]; however, the low reaction efficiency observed in vitro requires careful evaluation of this function in the future.

The exocyclic DNA adducts were known to be repaired through base excision pathways in the past [71–76]. Why does *E. coli* or perhaps other organisms possess an additional mechanism to remove these lesions? One possibility is that the AlkB proteins are used mostly for fixing damage occurred on ssDNA and RNA. Systematic comparison of the repair activity of AlkB with glycosylases would help provide further insight and reveal biological significance of this discovery in the future.

8. Repair of RNA damage

DNA damage is not the only damaged substrate repaired by AlkB. The N^1-position of adenine and N^3-position of cytosine in most RNAs are exposed in a similar way as they are in ssDNA. In fact, 1-meA and 3-meC occur naturally in transfer RNA in both prokaryotes and eukaryotes [25,77]. Repair of these lesions in RNA is required for correct RNA folding to allow efficient and accurate translation. AlkB and its human homolog ABH3 were found to effectively repair 1-meA and 3-meC lesions on RNA, a potentially important defense function for cells against alkylation damage [38,77]. AlkB, ABH2 and ABH3 are all able to demethylate 3meT and 1-meG lesions introduced by chemical methylation on tRNA as well [32,42,61]. This unique feature of the AlkB proteins suggests that these proteins may also play important roles in viruses, where RNA encodes genetic information. It will be very interesting to study the AlkB homologs in different viruses.

9. A new paradigm?

The discovery of the unique oxidative dealkylation repair of DNA/RNA alkylation damage by the AlkB proteins opens a new paradigm in DNA repair research and bioinorganic chemistry. It adds an important new function to those of the non-heme mononuclear iron proteins that activate dioxygen and perform oxidation catalysis. Further studies to reveal spectroscopic properties and the mechanisms of the AlkB proteins would greatly facilitate our understanding of these enzymes. More importantly, the discovery has also identified a new DNA repair function in human cells that may have biological significance.

At least eight homologs of AlkB exist in the human genome based on sequence alignment [34]. The functions of two of them, ABH2 and ABH3, as well as two mouse homologs mAbh2 and mAbh3 have been characterized, although the physiological roles of these proteins and other homologs are unclear. These AlkB proteins can also mediate repair of RNA damage, which is quite unique. Despite the similarities of the metal binding residues and motifs, the characterized homologs do show different properties. Although the first human homolog ABH1 was initially thought to behave similarly to AlkB, the purified gene product showed no activity [37–39]. ABH2 and ABH3, together with the mouse homologs mAbh2 and mAbh3, all show repair activities for both 1-meA and 3-meC with the same cofactors as AlkB [36]. ABH2 and ABH3 require longer polynucleotides compared to AlkB as they have low or no activity with trimer substrates, whereas AlkB shows activity on trimers that is competitive with that of longer DNA [46]. ABH2 and ABH3, although optimized for pH and cofactor requirements, had only 0.7% and 2%, respectively, of AlkB's activity when assayed with 1-meA in poly(dA) [46]. Furthermore, ABH2 prefers 1-meA while ABH3 prefers 3-meC [37, 38]. ABH2 and mAbh2 prefer dsDNA while ABH3 and mAbh3 are more like AlkB; they prefer

ssDNA and even RNA [32,36,38,39,43]. These differences in the homologs could certainly be due to their different sub-cellular localizations in human cells, or these proteins may have unique cellular roles that await to be elucidated. A future focus will certainly be to elucidate the functions and physiological roles of these human homologs.

Alkylation chemotherapies are widely used in clinics to treat various tumors [2,78–83]. The presence of DNA deal-kylation repair activity of the human homolog of C-Ada has been shown to confer resistance to some of these therapies. Whether the AlkB homologs play similar roles in the resistance is still unclear and will surely be scrutinized in the future. Perhaps inhibitors for the human homologs of AlkB could be developed to enhance the therapeutic effects of the alkylation anticancer reagents.

The function of AlkB also reveals a novel mechanism for dealkylation of DNA damage. In principle, the same mechanism can be utilized to repair other alkylation damage on heteroatoms of DNA as those shown in Fig. 5. The AlkB homologs in viruses or in human cells may use the oxidative dealkylation mechanism to remove these other alkylation adducts. It is also possible that alkylated/methylated protein residues can be dealkylated through the similar pathway catalyzed by non-heme iron proteins. The discovery of the function of AlkB may only be a beginning of uncovering a new type of dealkylation/demethylation pathway in biology.

References

1. Sedgwick B, Lindahl T. Oncogene 2002;21:8886–8894. [PubMed: 12483506]

2. Sedgwick B. Nature Rev. Mol. Cell Biol 2004;5:148–157. [PubMed: 15040447]

3. Rydberg B, Lindahl T. EMBO. J 1982;1:211–216. [PubMed: 7188181]

4. Lindahl T, Sedgewick B, Sekiguchi M, Nakabeppu Y. Annu. Rev. Biochem 1988;57:133–157. [PubMed: 3052269]

5. Barrows LR, Magee PN. Carcinogenesis 1982;3:349–351. [PubMed: 7083475]

6. Drablos F, Feyzi E, Aas PA, Vaagbo CB, Kavli B, Bratlie MS, Pena-Diaz J, Otterlei M, Slupphaug G, Krokan HE. DNA Repair (Amst) 2004;3:1389–1407. [PubMed: 15380096]

7. Teo I, Sedgwick B, Kilpatrick MW, McCarthy TV, Lindahl T. Cell 1986;45:315–324. [PubMed: 3009022]

8. Sakumi K, Sekiguchi M. J. Mol. Biol 1989;205:373–385. [PubMed: 2648001]

9. Sedgwick B, Robins P, Totty N, Lindahl T. J. Biol. Chem 1988;263:4430–4433. [PubMed: 3162236]

10. He C, Hus J-C, Sun LJ, Zhou P, Norman DPG, Dötsch V, Gross JD, Lane WS, Wagner G, Verdine GL. Mol. Cell 2005;20:117–129. [PubMed: 16209950]

11. Demple B, Sedgwick B, Robin P, Totty N, Waterfield MD, Lindahl T. Proc. Natl. Acad. Sci. USA 1985;82:2688–2692. [PubMed: 3887409]

12. Larson K, Sahm J, Shenkar P, Strauss B. Mutat. Res 1985;150:77–84. [PubMed: 4000169]

13. Labahn J, Scharer OD, Long A, Ezaz-Nikpay K, Verdine GL, Ellenberger TE. Cell 1996;86:321–329. [PubMed: 8706136]

14. Wyatt MD, Allan JM, Lau AY, Ellenberger TE, Samson LD. BioEssays 1999;21:668–676. [PubMed: 10440863]

15. Kataoka H, Yamamoto Y, Sekiguchi M. J. Bacteriol 1983;153:1301–1307. [PubMed: 6337994]

16. Chen BJ, Carroll P, Samson L. J. Bacteriol 1994;176:6255–6261. [PubMed: 7928996]

17. Kataoka H, Sekiguchi M. Mol. Gen. Genet 1985;198:263–269. [PubMed: 3884973]

18. Kondo H, Nakabeppu Y, Kataoka H, Kuhara S, Kawabata S, Sekiguchi M. J. Biol. Chem 1986;261:15772–15777. [PubMed: 3536913]

19. Aravind L, Koonin EV. Genome Biol 2001;2RESEARCH0007

20. Schofield CJ, Zhang Z. Curr. Opin. Struct. Biol 1999;9:722–731. [PubMed: 10607676]

21. Solomon EI, Brunold TC, Davis MI, Kemsley JN, Lee S-K, Lehnert N, Neese F, Skulan AJ, Yang Y-S, Zhou J. Chem. Rev 2000;100:235–349. [PubMed: 11749238]

22. Que LJ. Nature Struct. Biol 2000;7:182–184. [PubMed: 10700270]

23. Costas M, Mehn MP, Jensen MP, Que L Jr. Chem. Rev 2004;104:939–986. [PubMed: 14871146]

24. Welford RWD, Kirkpatrick JM, McNeill LA, Puri M, Oldham NJ, Schofield CJ. FEBS. Lett 2005;579:5170–5174. [PubMed: 16153644]

25. Singer, B.; Grünberger, D. Molecular Biology of Mutagens and Carcinogens. New york: Plenum Press; 1983.

26. Shooter KV, Howse R, Shah SA, Lawley PD. Biochem. J 1974;137:303–312. [PubMed: 4363111]

27. Revich GG, Beattie KL. Carciogenesis 1986;7:1569–1576.

28. Barbin A, Bartsch H, Leconte P, Radman M. Nucleic Acids Res 1981;9:375–387. [PubMed: 7010314]

29. Bodell WJ, Singer B. Biochemistry 1979;18:2860–2863. [PubMed: 476059]

30. Falnes PO, Johansen RF, Seeberg E. Nature 2002;419:178–182. [PubMed: 12226668]

31. Trewick SC, Henshaw TF, Hausinger RP, Lindahl T, Sedgwick B. Nature 2002;419:174–178. [PubMed: 12226667]

32. Koivisto P, Robins P, Lindahl T, Sedgwick B. J. Biol. Chem 2004;279:40470–40474. [PubMed: 15269201]

33. Wei YF, Carter KC, Wang RP, Shell BK. Nucleic Acids Res 1996;24:931–937. [PubMed: 8600462]

34. Kurowski MA, Bhagwat AS, Papaj G, Bujnicki JM. BMC Genomics 2003;4:48. [PubMed: 14667252]

35. Colombi D, Gomes SL. J. Bacteriol 1997;179:3139–3145. [PubMed: 9150207]

36. Lee D-H, Jin S-G, Cai S, Chen Y, Pfeifer GP, O'Connor TR. J. Biol. Chem 2005;280:39448–39459. [PubMed: 16174769]

37. Duncan T, Trewick SC, Koivisto P, Bates PA, Lindahl T, Sedgwick B. Proc. Natl. Acad. Sci. USA 2002;99:16660–16665. [PubMed: 12486230]

38. Aas PA, Otterlei M, Falnes PO, Vagbo CB, Skorpen F, Akbari M, Sundheim O, Bjoras M, Slupphaug G, Seeberg E, Krokan HE. Nature 2003;421:859–863. [PubMed: 12594517]

39. Mishina Y, Lee CH, He C. Nucleic Acids Res 2004;32:1548–1554. [PubMed: 15004242]

40. Mishina Y, He C. J. Am. Chem. Soc 2003;125:8730–8731. [PubMed: 12862460]

41. Mishina Y, Chen LX, He C. J. Am. Chem. Soc 2004;126:16930–16936. [PubMed: 15612731]

42. Falnes PO. Nucleic Acids Res 2004;32:6260–6267. [PubMed: 15576352]

43. Falnes PO, Bjoras M, Aas PA, Sundheim O, Seeberg E. Nucleic Acids Res 2004;32:3456–3461. [PubMed: 15229293]

44. Welford RW, Schlemminger I, McNeill LA, Hewitson KS, Schofield CJ. J. Biol. Chem 2003;278:10157–10161. [PubMed: 12517755]

45. Henshaw TF, Feig M, Hausinger RP. J. Inorg. Biochem 2004;98:856–861. [PubMed: 15134932]

46. Koivisto P, Duncan T, Lindahl T, Sedgwick B. J. Biol. Chem 2003;278:44348–44354. [PubMed: 12944387]

47. Hausinger RP. Crit. Rev. Biochem. Mol. Biol 2004;39:21–68. [PubMed: 15121720]

48. Ryle MJ, Koehntop KD, Liu A, Que L Jr, Hausinger RP. Proc. Natl. Acad. Sci. USA 2003;100:3790–3795. [PubMed: 12642663]

49. Ryle MJ, Liu A, Muthukumaran RB, Ho RYN, Koehntop KD, McCracken J, Que L Jr, Hausinger RP. Biochemistry 2003;42:1854–1862. [PubMed: 12590572]

50. Liu A, Ho RYN, Que L Jr, Ryle MJ, Phinney BS, Hausinger RP. J. Am. Chem. Soc 2001;123:5126–5127. [PubMed: 11457355]

51. Price JC, Barr EW, Glass TE, Krebs C, Bollinger JM Jr. J. Am. Chem. Soc 2003;125:13008–13009. [PubMed: 14570457]

52. Price JC, Barr EW, Tirupati B, Bollinger JM Jr, Krebs C. Biochemistry 2003;42:7497–7508. [PubMed: 12809506]

53. Riggs-Gelasco PJ, Price JC, Guyer RB, Brehm JH, Barr EW, Bollinger JM Jr, Krebs C. J. Am. Chem. Soc 2004;126:8108–8109. [PubMed: 15225039]

54. Grapperhaus CA, Mienert B, Bill E, Weyhermuller T, Wieghardt K. Inorg. Chem 2000;39:5306–5317. [PubMed: 11187471]

55. Kaizer J, Costas M, Que L Jr. Angew. Chem. Int. Ed. Engl 2003;42:3671–3673. [PubMed: 12916044]

56. Lim MH, Rohde JU, Stubna A, Bukowski MR, Costas M, Ho RY, Munck E, Nam W, Que L Jr. Proc. Natl. Acad. Sci. USA 2003;100:3665–3670. [PubMed: 12644707]

57. Rohde JU, In JH, Lim MH, Brennessel WW, Bukowski MR, Stubna A, Munck E, Nam W, Que L Jr. Science 2003;299:1037–1039. [PubMed: 12586936]

58. Ghosh A, Tangen E, Ryeng H, Taylor PR. Eur. J. Inorg. Chem 2004;2004:4555–4560.

59. Lee H-J, Lloyd MD, Harlos K, Clifton IJ, Baldwin JE, Schofield CJ. J. Mol. Biol 2001;308:937–948. [PubMed: 11352583]

60. Dinglay S, Gold B, Sedgwick B. Mutat. Res 1998;407:109–116. [PubMed: 9637239]

61. Delaney JC, Essigmann JM. Proc. Natl. Acad. Sci. USA 2004;101:14051–14056. [PubMed: 15381779]

62. Veldhuyzen WF, Shallop AJ, Jones RA, Rokita SE. J. Am. Chem. Soc 2001;123:11126–11132. [PubMed: 11697955]

63. Roitzsch M, Lippert B. J. Am. Chem. Soc 2004;126:2421. [PubMed: 14982449]

64. Pollack M, Oe T, Lee SH, Silva Elipe MV, Arison BH, Blair IA. Chem. Res. Toxicol 2003;16:893–900. [PubMed: 12870892]

65. Nair J, Barbin A, Guichard Y, Bartsch H. Carcinogenesis 1995;16:613–617. [PubMed: 7697821]

66. Chung FL, Chen HJ, Nath RG. Carcinogenesis 1996;17:2105–2111. [PubMed: 8895475]

67. Mroczkowska MM, Kolasa IK, Kusmierek JT. Mutagenesis 1993;8:341–348. [PubMed: 8377654]

68. Borys E, Mroczkowska-Slupska MM, Kusmierek JT. Mutagenesis 1994;9:407–410. [PubMed: 7837973]

69. Mishina Y, Yang C-G, He C. J. Am. Chem. Soc 2005;127:14594–14595. [PubMed: 16231911]

70. Delaney JC, Smeester L, Wong C, Frick LE, Taghizadeh K, Wishnok JS, Drennan CL, Samson LD, Essigmann JM. Nat. Struct. Mol. Biol 2005;12:855–860. [PubMed: 16200073]

71. Asaeda A, Ide H, Asagoshi K, Matsuyama S, Tano K, Murakami A, Takamori Y, Kubo K. Biochemistry 2000;39:1959–1965. [PubMed: 10684645]

72. O'Connor TR, Laval J. Biochem. Biophys. Res. Commun 1991;176:1170–1177. [PubMed: 1645538]

73. Lau AY, Wyatt MD, Glassner BJ, Samson LD, Ellenberger T. Proc. Natl. Acad. Sci. USA 2000;97:13573–13578. [PubMed: 11106395]

74. Dosanjh MK, Roy R, Mitra S, Singer B. Biochemistry 1994;33:1624–1628. [PubMed: 8110764]

75. Roy R, Kennel SJ, Mitra S. Carcinogenesis 1996;17:2177–2182. [PubMed: 8895486]

76. Saparbaev M, Laval J. Proc. Natl. Acad. Sci. USA 1998;95:8508–8513. [PubMed: 9671708]

77. Ougland R, Zhang CM, Liiv A, Johansen RF, Seeberg E, Hou YM, Remme J, Falnes PO. Mol. Cell 2004;16:107–116. [PubMed: 15469826]

78. Vaughan P, Lindahl T, Sedgwick B. Mutat. Res 1993;293:249–257. [PubMed: 7679475]

79. Taverna P, Sedgwick B. J. Bacteriol 1996;178:5105–5111. [PubMed: 8752326]

80. Goldman R, Shields PG. J. Nutr 2003;133:965S–973S. [PubMed: 12612183]

81. Hecht SS. Mutat. Res 1999;424:127–142. [PubMed: 10064856]

82. Hurley LH. Nature Rev. Cancer 2002;2:188–200. [PubMed: 11990855]

83. Rajski SR, Williams RM. Chem. Rev 1998;98:2723–2795. [PubMed: 11848977]
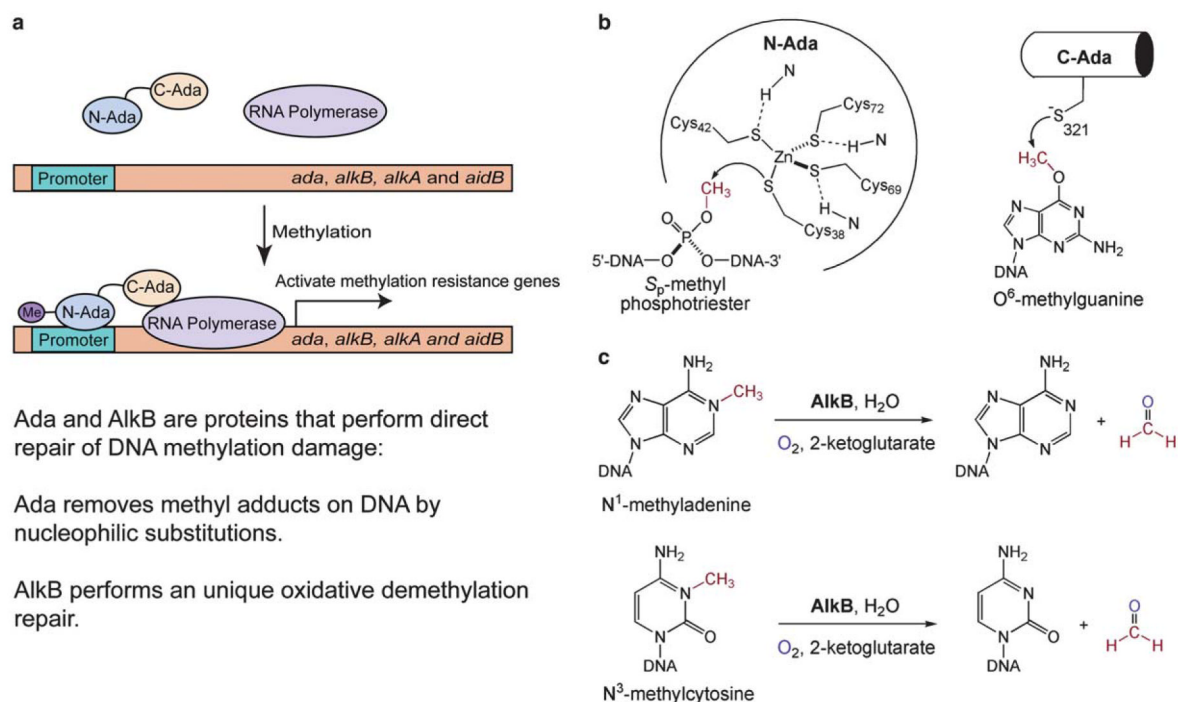
Fig. 1.
Schematic representations of the direct repair of methylation DNA damage in *E. coli* by proteins in the *ada* operon. (a) *E. coli* Ada is a transcriptional activator that senses methylation challenge and activates its own expression and three other genes, *alk*B, *alk*A and *aid*B. AlkA is a glycosylase that cleaves methylated bases from DNA. The function of AidB is still unknown. Both Ada and AlkB perform direct repair of methylation (alkylation) DNA damage. (b) Ada uses Cys residues to nucleophilically remove methyl groups from DNA backbone and bases. (c) AlkB catalyzes an oxidative dealkylation repair of DNA base lesions.

```
AlkB     RYAPGAKLSLHQDKDEPDLR--APIVSVSLGLPAIFQFGGLKRND--------------- 163
mAbh2    YKDGCDHIGEHRD-DERELAPGSPIASVSFGACRDFIFRH-KDSRG------KRPRRT-V 189
mAbh3    YRDEKDSVDWHSD-DEPSLGSCPVIASLSFGATRTFEMRK-KPPPE------ENGDYTYV 232
ABH1     YYRLDSTLGIHVD--RSELDHSKPLLSFSFGQSAIFLLGGLQRD---------------E 188
ABH2     YKDGCDHIGEHRD-DERELAPGSPIASVSFGACRDFVFRH-KDSRG------KSPSRR-V 211
ABH3     YRNEKDSVDWHSD-DEPSLGRCPIIASLSFGATRTFEMRK-KPPPE------ENGDYTYV 232
ABH4     CPERGSAIDPHLD-DAWLWG--ERLVSLNLLSPTVLSMCR-EAPGSL--LLCSAPSAAPE 212
ABH5     DYQPGGCIVSHVD---PIHIFERPIVSVSFFSDSALCFGCKFQFKP---------IRVSE 241
ABH6     QYLPGEGIMPHED-GPLYYP---TVSTISLGSHTVLDFYEPRRPEDD--DPTEQPRPPPR 88
ABH7     DLEARGYIKPHVD-SIKFCG--ATIAGLSLLSPSVMRLVH-TQE-------------PG 152
ABH8*    QYEPGQGIPAHID---THSAFEDEIVSLSLGSEIVMDFKHPDG---------------- 267


AlkB     PLKRLLLEHG----------------DVVVWGGESRLFY-HGIQPLKAG---------- 195
mAbh2    EVVRLQLAHG----------------SLLMMNPPTNTHWYHSLPIR------------ 219
mAbh3    ERVKIPLDHG----------------TLLIMEGATQADWQHRVPKE------------ 262
ABH1     APPPMFMHSG----------------DIMIMSGFSRLLN-HAVPRVLPNPEGEGLPHCL 230
ABH2     AVVRLPLAHG----------------SLLMMNHPTNTHWYHSLPVR------------ 241
ABH3     ERVKIPLDHG----------------TLLIMEGATQADWQHRVPKE------------ 262
ABH4     ALVDSVIAPSRSVLCQEVEVAIPLPARSLLVLTGAARHQWKHAIHR------------- 258
ABH5     PVLSLPVRRG----------------SVTVLSGYAADEITHCIRPQ------------ 271
ABH6     PTTSLLLEPR----------------SLLVLRGPAYTRLLHGIAAARVDALDAASSPPN 131
ABH7     EWLELLLEPG----------------SLYILRGSARYDFSHEILRDEESFFGER----- 190
ABH8*    TAVPVMLPRR----------------SLLVMTGESRYLWTHGITCRKFDTVQASENHKS 310


AlkB     ----------------------FHPLTTDCRYNLTFRQAGKKE---- 216
mAbh2    -------------------------KRVLAPRVNLTFRKILPTKK--- 239
mAbh3    ------------------------YHSRQPRVNLTFRTVYPDPRGAP 285
ABH1     EAPLPAVLPRDSMVEPCSMEDWQVCASYLKTARVNMTVRQVLATDQNFP 279
ABH2     -------------------------KKVLAPRVNLTFRKILLTKK--- 261
ABH3     ------------------------YHSREPRVNLTFRTVYPDPRGAP 285
ABH4     ------------------------RHIEARVCVTFRELSAEFGPGG 281
ABH5     ------------------------DIKERRAVIILRKTRLDAPRLE 293
ABH6     AAACP-------------SARPGACLVRGTRVSLTIRRVPRVLRAGL 165
ABH7     ------------------------RIPRGRRISVICRSLPEGMGPGE 213
ABH8*    --------------GIITSDVGDLTLSKRGLRTSFTFRKVRQTPCNCS 344
```

Fig. 2.
Sequence alignment of the AlkB family of proteins. ABH1-7 are human homologs; ABH8* is the sequence from *M. fascicularis*; and mAbh2 and mAbh3 are mouse homologs. The conserved residues are highlighted in yellow (ligands for the active site iron) and blue (two conserved Arg residues). The RXXXXXR region is a characteristic of the AlkB sequence. The first Arg residue is thought to recognize α-ketoglutarate. The role of the second Arg residue is unclear.
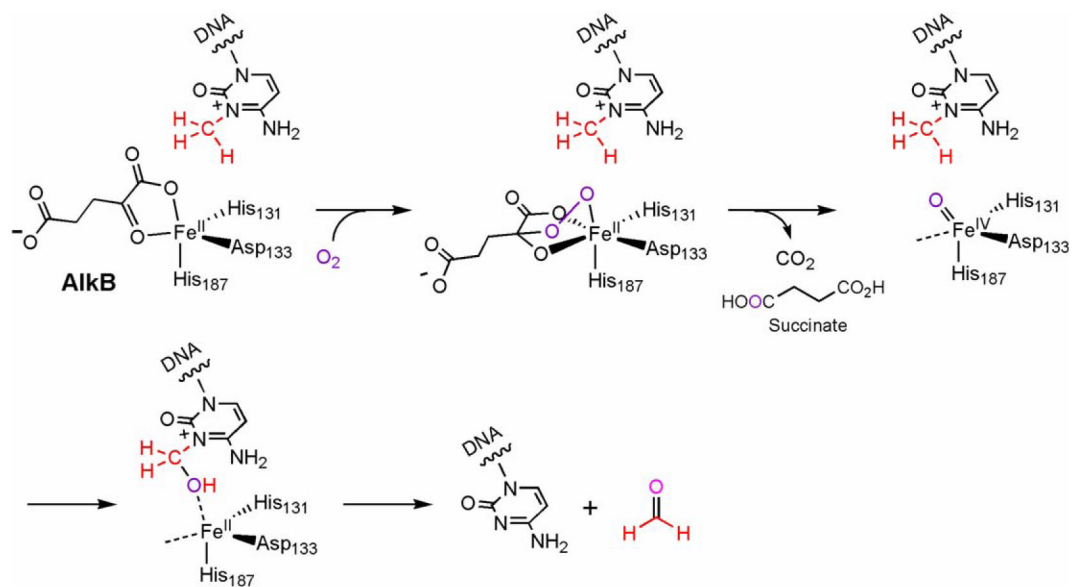
Fig. 3.
A proposed oxidative repair mechanism of *E. coli* AlkB. A putative $Fe^{IV}=O$ intermediate may form and hydroxylate the methyl lesion to give an intermediate that decomposes in water to afford the repaired base and formaldehyde.
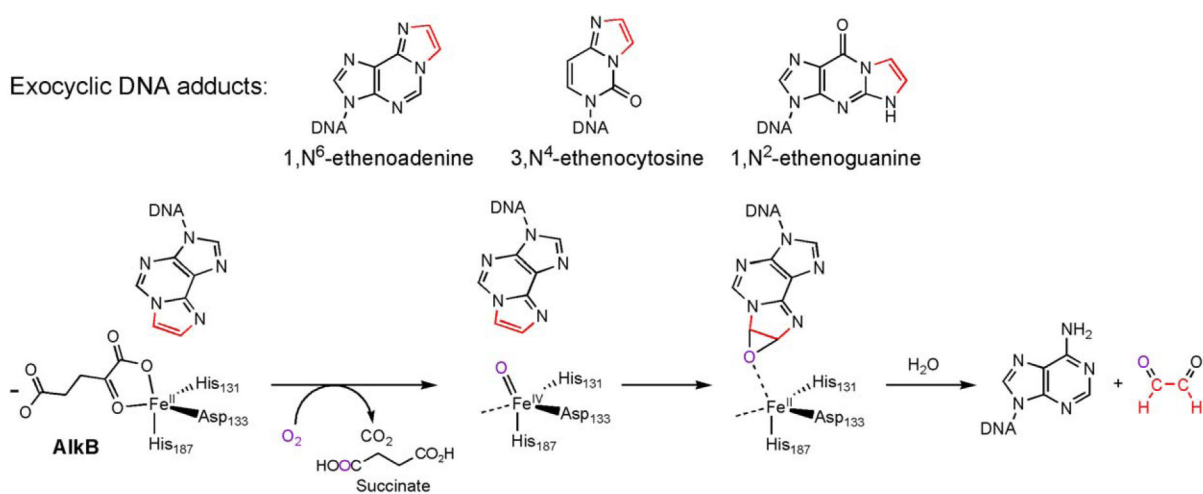
Fig. 4.
The AlkB proteins can directly repair exocyclic DNA adducts such as 1,N^6-ethenoadenine. An epoxide intermediate was observed during the course of the repair, suggesting an oxidative repair mechanism.

DNA/RNA alkylations that are known to be repaired by the AlkB type proteins:

N^1-methyladenine   N^3-methylcytosine   N^3-methylthymine   N^1-methylguanine   1,N^6-ethenoadenine
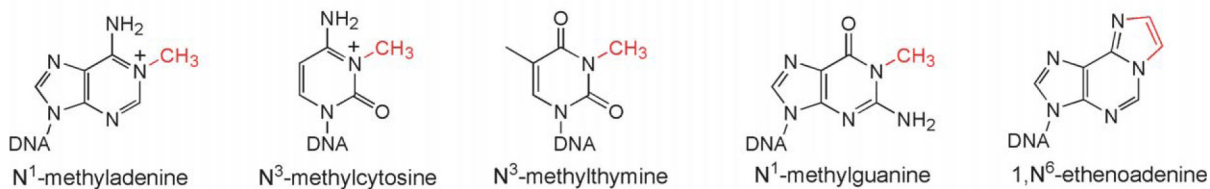
DNA/RNA methylations that may also be repaired through the oxidative pathway:
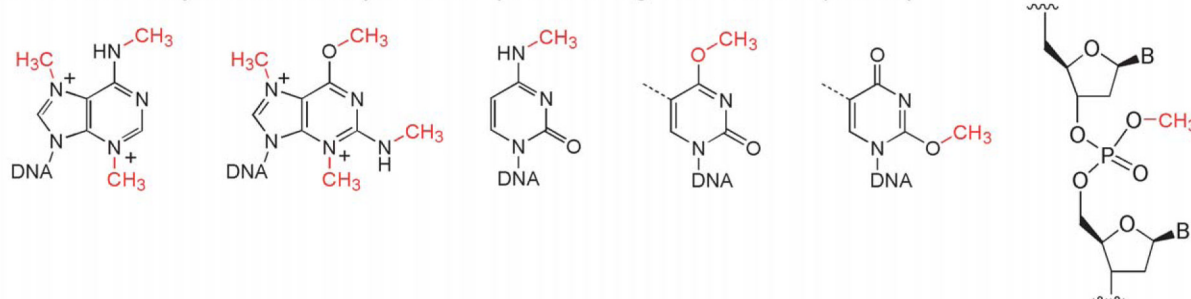
Fig. 5.
DNA/RNA alkylation damages that are known or could be repaired by the AlkB type proteins.