

Bacterial DNA repair genes and their eukaryotic homologues: 3. AlkB dioxygenase and Ada methyltransferase in the direct repair of alkylated DNA[★]

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Environmental and endogenous alkylating agents generate cytotoxic and mutagenic lesions in DNA. Exposure of prokaryotic cells to sublethal doses of DNA alkylating agents induces so called adaptive response (Ada response) involving the expression of a set of genes which allows the cells to tolerate the toxic and mutagenic action of such agents. The Ada response includes the expression of four genes: *ada*, *alkA*, *alkB*, and *aidB*. The product of *ada* gene, Ada protein, is an activator of transcription of all four genes. DNA bases damaged by alkylation are removed by distinct strategies. The most toxic lesion 3meA is removed by specific DNA glycosylase initiating base excising repair. The toxic and mutagenic O⁶meG is repaired directly by methyltransferases. 1meA and 3meC are corrected by AlkB DNA dioxygenase. The mechanisms of action of *E. coli* AlkB dioxygenase and its human homologs ABH2 and ABH3 are described in more details.

Keywords: alkylating agents, adaptive response, DNA repair, AlkB

INTRODUCTION

The DNA of all living organisms is constantly exposed to a number of exogenous (chemicals, radiation) and endogenous (side products of cellular metabolism) agents causing a variety of harmful lesions. To maintain DNA integrity, cells possess several defense systems that act at different levels to repair the damage (for more details see accompanying reviews by Arczewska & Kusmierek, 2007; Maddukuri *et al.*, 2007; Krwawicz *et al.*, 2007; Nowosielska *et al.*, 2007). Some lesions cause cell cycle arrest allowing DNA repair prior to replication. Based on their cellular conse-

quences, damaged bases may be cytotoxic, mutagenic or both. Cytotoxic lesions block replication leading to cell death while mutagenic lesions are miscoding and cause mutations in newly synthesized DNA. Mutations resulting from miscoding are thought to be a major mechanism of carcinogenesis. Cytotoxic lesions, when bypassed by low fidelity polymerase are a source of mutations and become mutagenic.

Alkylating agents are potentially cytotoxic and mutagenic chemicals whose action induces several DNA repair systems in the cell. The cellular recovery from alkylation damage involves adaptive response, a part of which is induction of AlkB protein.

[★]This paper is dedicated to our mentors: David Shugar, Celina Janion, Irena Pietrzykowska, Zofia Zarębska and Daniela Barszcz, scientists from the Institute of Biochemistry and Biophysics Polish Academy of Sciences, who contributed to a great extent to the development in the field of DNA damage, mutagenesis and repair.

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Abbreviations: 3meA, 3-methyladenine; AGT, O⁶meG-DNA methyltransferase; BCNU, *N,N'*-bis[2-chloroethyl]-*N*-nitrosourea (carmustine); CCNU, *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (lomustine); HTH, helix-turn-helix; MeI, methyl iodide; MMR, mismatch repair system; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; NNAL, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; NNN, *N'*-nitrosornicotine; O⁶meG, O⁶methylguanine; SAM, *S*-adenosylmethionine.

ALKYLATING AGENTS

The alkylating agents comprise a group of mutagens and carcinogens that modify DNA by alkylation. It is now evident that some alkylating agents are not only widespread in the environment but are also produced intracellularly (endogenously) as byproducts of normal metabolism. Alkyl base lesions can arrest replication, interrupt transcription, or signal the activation of cell-cycle checkpoints or apoptosis. In mammals they could be involved in carcinogenesis, neurodegenerative disease and aging.

Alkylating agents can introduce methyl or ethyl groups at all of the available nitrogen and oxygen atoms in DNA bases, producing a number of lesions (Fig. 1). The majority of evidence indicates that among the 11 identified base modification two, 3-methyladenine (3meA) and O⁶-methylguanine (O⁶meG), are mainly responsible for the biological effects of alkylation agents (Singer, 1976). Contribution of various lesions depends on the type of agent, its reaction mechanism and the secondary structure of the DNA target. Based on the reaction mechanism used, alkylating agents can be divided into two subgroups (Fig. 2). The S_N1 reagents, such as *N*-methyl-*N*-nitrosourea (MNU) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) use a monomolecular mechanism, while the S_N2 ones, that include methyl methanesulfonate (MMS) and methyl iodide (MeI), act by a bimolecular mechanism. The S_N1 type agents introduce alkyl adducts both at N and O atoms; in the case of the S_N2 subgroup, N-alkylation prevails. The major base modifications introduced in doubled stranded DNA by methylating agents are: 7meG, 3meA, O⁶meG, while 1meA, 3meC, 7meA, and O⁴meT represent minor modifications. In single stranded DNA 1meA and 3meC are more frequent than in dsDNA. The most mutagenic adduct introduced into DNA by methylating agents is O⁶meG. This modification mispairs during replication with thymine leading to GC→AT transition in dsDNA. N1 in adenine and N3 in cytosine are involved in Watson–Crick base pairing and in this way are protected from modifications (Falnes & Rognes, 2003).

As already mentioned alkylating agents are present in the environment but also occur endogenously in all cells. One of the central molecules in metabolism is *S*-adenosylmethionine (SAM), a principal biological methyl donor, and precursor of aminopropyl groups utilized in polyamine biosynthesis, and, in the liver, glutathione through its conversion to cysteine (Lu, 2000). Since methylation plays a critical role in cellular processes such as gene expression and membrane fluidity, any alterations in SAM concentration may affect cell functioning. Abnormalities in SAM metabolism are especially connected

with liver diseases, some neurological disorders and spontaneous carcinogenesis (Lutz, 1990).

SAM can spontaneously methylate DNA. It acts by an S_N2 mechanism and generates mainly 7meG and 3meA and, to a lesser extent, O⁶meG. Its reactivity, however, is about 2000-fold weaker than that of MMS (Rydberg & Lindahl, 1982), nevertheless it is capable of acting as a carcinogen *in vivo* (Barrows & Magee, 1982). Apart from SAM, lipid peroxidation and amine nitrosation comprise other internal sources of alkyl group. Several *N*-alkyl-*N*-nitrosocompounds (e.g. methyl nitrosourea) are generated by nitrosation of glycine and its derivatives (Shuker & Margison, 1997; Garcia-Santos Mdel *et al.*, 2001). In *Escherichia coli* strains mutated in both known genes encoding O⁶-alkylguanine-DNA alkyltransferase an elevated level of spontaneous mutations is observed.

The most abundant environmental alkylating agents are chloromethane and other halocarbons. Methyl chloride (MeCl) is generated in many terrestrial environments by abiotic conversion of chloride. In plants, pectin is a donor of methyl group (Hamilton *et al.*, 2003). In some algae and fungi living in a saline environment MeCl is also generated as a product of chloride detoxification (Sedgwick & Vaughan, 1991). Bromomethane occurs naturally in the ocean. Halohydrocarbons are also generated by anthropogenic sources and contribute to stratospheric ozone depletion (Goodwin *et al.*, 2005).

The *N*-nitroso compounds formed in tobacco smoke are recognized as one of the most important environmental alkylating agents influencing health of the persons exposed. The most carcinogenic among

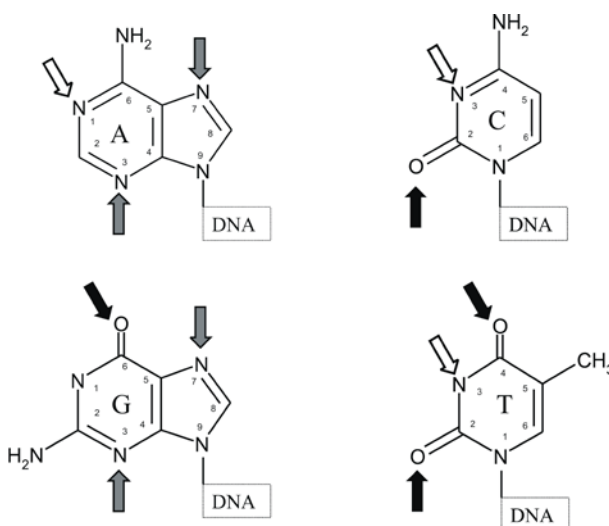


Figure 1. Possible sites of alkylation of DNA bases.

Grey arrows indicate sites alkylated by most of the agents; black arrows – sites that are most frequent alkylated by S_N1 agents; white arrows – sites alkylated by S_N2 agents in single-stranded DNA.

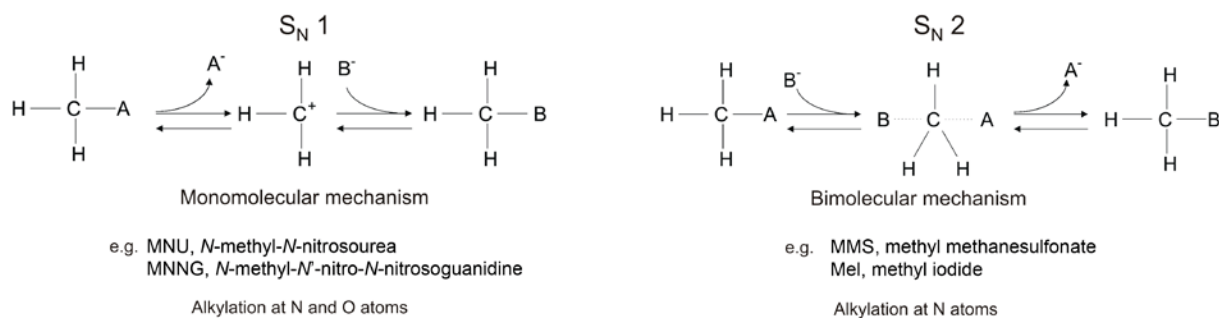


Figure 2. Two types of nucleophilic substitutions – S_N1 and S_N2.

Electron-rich nucleophile (B) attacks a molecule and replaces a departing group (A). During DNA base alkylation the source of the nucleophile is the base and the leaving anion is generated by the alkylating agent.

the tobacco-specific nitrosamines are 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL), and *N*'-nitrosornicotine (NNN) (Hecht, 2002).

One of the tobacco alkaloids, myosamine, is also present in a variety of foodstuffs, including fruits, vegetables and even milk. It is easily nitrosated to DNA-alkylating compounds, including NNN (Drablos *et al.*, 2004). Myosamine has been detected in maize, rice, wheat flour, millet, potato, and milk, also in cocoa, popcorn, tomato, carrot, pineapple, kiwi, and apples but not in other vegetables and fruits such as lettuce, spinach, cucumber, onion, banana, tangerines, or grapes. This dietary myosamine could be involved in esophageal carcinogenesis (Tyroller *et al.*, 2002).

Taking under consideration the cytotoxic effects of many alkylating agents, they have been considered as anti-cancer drugs (Chaney & Sancar, 1996, Hurley, 2002). In therapy mainly methylating (e.g., temozolomide and streptosotocin) and chloroethylating agents (e.g., chloroethylnitrosoureas, CENUs: carmustine, lomustine and fotemustine) are used. The most important target of their activity is the O⁶ position of guanine but they introduce alkyl adducts at other positions also. CENUs show a wide spectrum of activity. They have been used in Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and melanoma and because of their ability to cross the blood-brain barrier, also in the treatment of primary brain tumors (Chaney & Sancar, 1996).

The repair of damage to DNA caused by alkylating agents involves at least four different mechanisms: (i) lesions can be repaired directly by methyltransferases or oxidative demethylases; (ii) with the use of base excision repair initiated by DNA glycosylases; (iii) by mismatch repair system; and (iv) by nucleotide excision repair. Within the systems protecting cells against the action of alkylating agents in *E. coli* there is the adaptive response to DNA damage.

The strong conservation of the system indicates the evolutionary importance of alkylated-DNA repair.

Ada PROTEIN

To overcome the mutagenic and cytotoxic effects caused by alkylating agents, cells possess a mechanism of adaptation induced after exposure to small doses of such reagents. This adaptive response to alkylating agents (Ada response) has been most extensively studied in *E. coli*. It is widely accepted that induction of Ada response results in an increased expression of four genes: *ada*, *alkB*, *alkA* and *aidB* (Lindahl *et al.*, 1988). A key component of this response is the Ada protein, a multifunctional molecule showing O⁴meT and O⁶meG methyltransferase activity and functioning as an activator of transcription of its own gene as well as those encoding AlkA, AlkB, and AidB (Lindahl *et al.*, 1988; Teo *et al.*, 1984) (Fig. 3). The product of the *alkA* gene shows a DNA glycosylase activity with broad substrate specificity (for review see J. Krwawicz, this issue). The AidB protein has been supposed to take part in the degradation of endogenous alkylating agents (Landini *et al.*, 1994). It shows some homology to acyl-CoA oxidases and those containing flavins (Landini *et al.*, 1994; Rohankhedkar *et al.*, 2006). Recent observations suggest that AidB may bind to double-stranded DNA and take part in its dealkylation (Rohankhedkar *et al.*, 2006). However, to determine the precise function of AidB further investigations are necessary.

The 39 kDa Ada protein is composed of two major domains, a 19 kDa C-terminal (C-Ada19) and a 20 kDa N-terminal (N-Ada20) one, linked by a hinge region susceptible to proteolytic cleavage. These domains can function independently. C-Ada19 transfers methyl adducts from O⁶meG and O⁴meG onto its Cys-321 residue, whereas N-Ada20 demethylates S_p-diastereoisomers of methylphosphotriesters

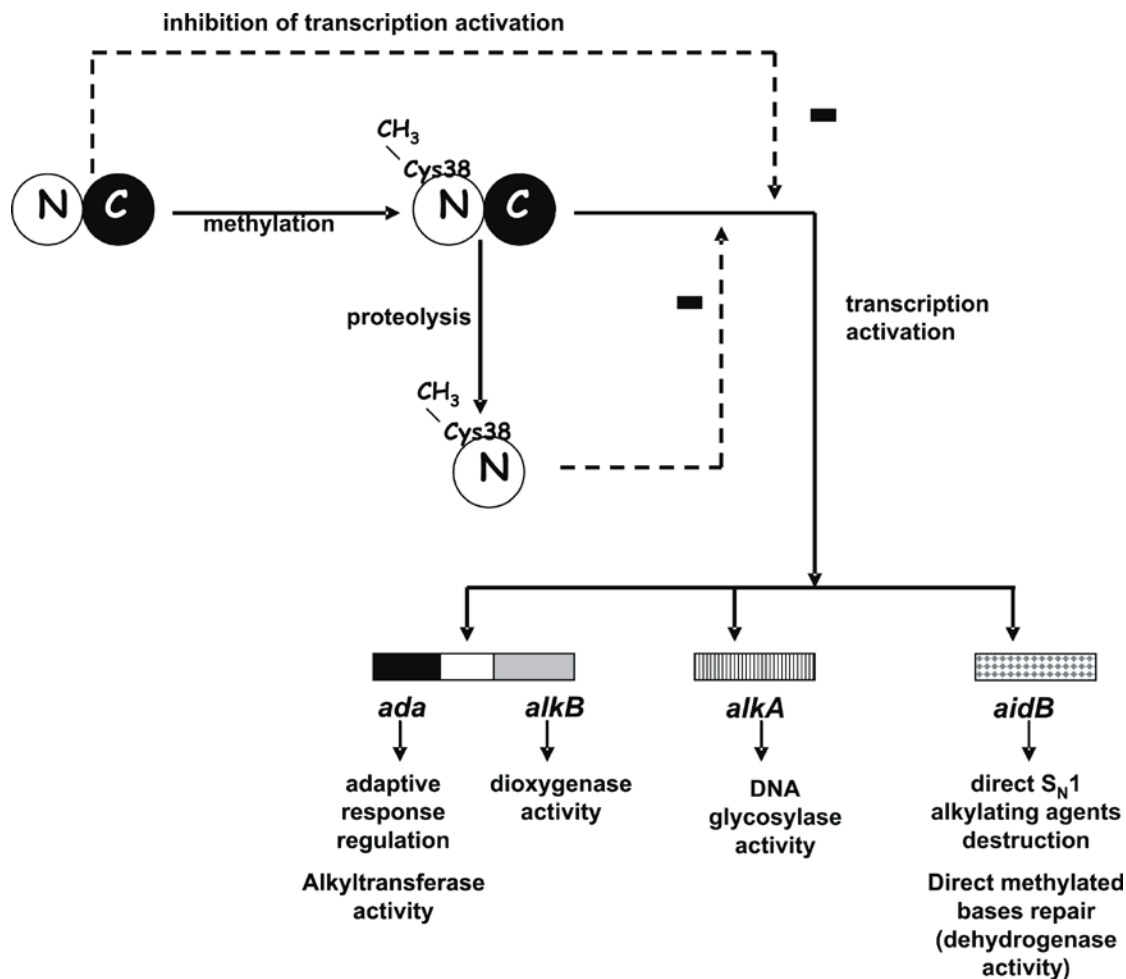


Figure 3. Adaptive response regulation.

Ada protein is activated as a transcription factor by methylation of its Cys-38 that occurs by repair of methylphosphotriesters or by direct protein methylation. (N) – N-terminal part of Ada protein; (C) – C-terminal part of Ada protein.

by methyl transfer onto Cys-38 (Kondo *et al.*, 1986; Sedgwick *et al.*, 1988; Lindahl *et al.*, 1988; Moore *et al.*, 1994; He & Verdine, 2002; He *et al.*, 2003).

The N-terminal domain possesses a high-affinity Zn^{2+} binding site. The metal ion is probably coordinated by the motif Cys- X_3 -Cys- X_{26} -Cys- X_2 -Cys. Cysteines building this motif are Cys38 (methyl acceptor), Cys42, Cys69 and Cys72. The existence of a (Cys)₄ zinc-binding element suggests a structural and evolutionary relation of *E. coli* Ada protein to some Zn-finger transcription factors of higher organisms (Myers *et al.*, 1992).

The 39 kDa Ada protein activates transcription of the Ada regulon genes in two different ways (Fig. 3). In the case of the *ada-alkB* operon and *aidB* promoter, for transcription activation N-Ada20 domain interacts with the α subunit of RNA polymerase and C-Ada19 with δ subunit. Although these interactions are independent, both are necessary for transcription activation. For activation of *alkA* gene,

the N-Ada20 domain interacts with the α and δ subunits of RNA polymerase, however, only the N-terminal domain of Ada is required and methylation of Cys-38 is probably not necessary. Cys-38 can be methylated by SN_2 agents directly, which may be an alternative but less effective way of Ada activation (Landini & Volkert, 2000).

C-Ada19 repairs O⁶meG and O⁴meT by transferring methyl moiety onto its own Cys-321 similarly as N-Ada20 onto its Cys-38. Methylation of Cys-321 is irreversible so the action of the enzyme is suicidal. Crystal structure studies have revealed two structurally distinct regions within C-Ada19. The N-terminal region shows similarity to RNase H structure, whereas the C-terminus forms predominantly α -helices and connecting loops (Katayanagi *et al.*, 1990; Yang *et al.*, 1990). Three helices within this region bear a strong resemblance to the helix-turn-helix (HTH) motif. The putative DNA binding helix is far away from the active Cys-321 so probably the

HTH motif is involved in general binding to DNA. Surprisingly, the active site thiol of the Cys residue in the Pro-Cys-His-Arg active site is buried in the structure of C-Ada19. A conformational change, therefore, is indispensable for this cysteine to carry out a nucleophilic attack on the target methyl group (Moore *et al.*, 1994). One model suggests a rotation of the most of C-terminal helix exposing a DNA-binding surface of the protein, making Cys-321 available to the substrate. On the other hand, the second helix of the HTH motif implicated in DNA binding contains a conserved RAV(A, G) sequence called "arginine-finger" that promotes base flipping of the substrate nucleotide from DNA (Daniels *et al.*, 2000, Daniels & Tainer, 2000; Wibley *et al.*, 2000).

The Ada 39 kDa protein alkylation is irreversible, so the question concerning the mechanism of the Ada response termination remains open. It has been postulated that the activated Ada protein is simply diluted by cell division after withdrawal of alkylating agents (Lindahl *et al.*, 1988). A second hypothesis indicates a possible role of the 20 kDa N-terminal product of Ada protein proteolysis in inhibition of the Ada-regulated gene transcription (Teo *et al.*, 1984; Lindahl *et al.*, 1988). Yet another possibility is that unmethylated Ada, when all repairable methylphosphotriesters have been repaired, shuts off the adaptive response by accumulation in the cell and competition with the methylated protein for binding to the regulated genes (Saget & Walker, 1994).

Under normal conditions, there are about two molecules of Ada protein per *E. coli* cell. Induction of the Ada response increases the level of Ada to about 3000 molecules per cell. The observation that some alkyltransferase activity is still present in an *E. coli* mutant with an *ada* gene deletion suggests the presence of another alkyltransferase in *E. coli* (Rebeck *et al.*, 1988; Shevell *et al.*, 1988). Indeed, the second alkyltransferase has been identified as the constitutively expressed Ogt protein, product of the *ogt* gene. The 19 kDa Ogt protein repairs O⁶meG and O⁴meT. It shows a similar activity as the C-Ada19 domain. The amino-acid sequence also shows some homology to the region containing Cys-321 (Potter *et al.*, 1987).

AGT activity has been detected in many organisms (e.g., the yeast *Saccharomyces cerevisiae*, the filamentous fungus *Aspergillus nidulans*, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*) and also in fish and mammalian cells (Friedberg, 2006).

In human cells the alkyltransferase activity is the product of the *MGMT* gene (Harris *et al.*, 1983; Kataoka *et al.*, 1986). The 21.7 kDa *MGMT* protein is built of amino-acid sequences very similar to those of both *E. coli* alkyltransferases, Ada and Ogt. In contrast to the bacterial enzymes it mainly repairs

O⁶meG, whereas removal of the alkyl adduct from O⁴meT is much slower and significantly less effective (Brennand & Margison, 1986; Koike *et al.*, 1990). The preferential repair of O⁶meG is profitable for eukaryotic cells since in experimental animals treated with alkylating carcinogens this lesion is involved in tumor stimulation. Alkylating agents producing a small amount of O⁶meG are weak carcinogens.

Expression of *MGMT* prevents cancer development associated with exposure to alkylating agents. Many animal studies have shown that overexpression of *MGMT* gene suppresses alkylation-induced carcinogenesis, e.g. MNU-induced thymic lymphomas, MNNG-induced skin cancer, dimethylnitrosamine-induced liver tumors (Dumenco *et al.*, 1993; Nakatsuru *et al.*, 1993; Becker *et al.*, 1996). Also, a low AGT activity predisposes to alkylation-induced tumorigenesis especially leading to lymphomas and breast, lung and colon cancers. These data suggest that human tissues with a low AGT level are more sensitive to carcinogenesis developed by environmental agents.

On the other hand alkylating agents are often used in cancer treatment. Many of them attack the O⁶ position of guanine. There are two major groups of such agents, chloroethylating and methylating ones. Carmustine (BCNU) and lomustine (CCNU) belong to the first group. They generate N¹-O⁶-ethanoguanine leading then to a crosslink with the opposite-strand cytosine (Tong *et al.*, 1982). The G-C inter-strand crosslink leads to replication inhibition and induction of p53 and p21, proteins acting against carcinogenesis through positive regulation of apoptosis (Gerson, 2002).

The group of methylating agents is represented by, e.g., procarbazine, temozolomide, streptozotocin, dacarbazine. During replication they form O⁶meG causing incorrect incorporation of thymine into the newly synthesized strand. This mismatch is recognized by the mismatch repair system (MMR) and, especially when there is another O⁶meG in the template DNA strand, their repair may lead to single and double strand breaks, sister chromatid exchanges, and even chromosomal aberrations. Since MMR preferentially repairs the newly synthesized strand, the alkyl lesion in the template strand may remain and start the whole MMR process again, thus effectively inducing apoptosis (Hickman & Samson, 1999).

Dysfunction of the MMR system makes cells more tolerant of lesions in DNA caused by methylating agents. However, the most important factor determining the resistance of tumor cells to alkylating anti-cancer drugs is AGT alkyltransferase. There is a strong correlation between *MGMT* gene expression and the cell survival of treatment with O⁶-alkylating agents (Gerson, 2002).

In many cases modulation of AGT activity seems to be of great importance for anti-cancer therapy. Efforts to improve effectiveness of the therapy include the sequential administration of alkylating agent (depleting MGMT) or MGMT inhibitors, e.g. *O*⁶-benzylguanine or *O*⁶-(4-bromothienyl) guanine (Middleton *et al.*, 2000). On the other hand, bone marrow cells show high sensitivity to alkylating anti-cancer drugs especially when these are used in combination with MGMT inhibitors. The solution of this problem connected with the use of alkylating agents in anti-cancer chemotherapy is perceived in the protection of bone marrow cells by selective expression of MGMT (Gerson, 2002).

AlkB DIOXYGENASE AND ITS HUMAN HOMOLOGS

The *alkB* gene is part of the *ada* regulon inducible during the adaptive response. Despite the isolation of an *E. coli* strain defective in *alkB* function as early as 1983, the nature and function of the *alkB* gene were unknown for many years (Kataoka *et al.*, 1983). First, it was discovered that the *alkB* gene product, AlkB protein, acts alone and when expressed in human cells it is able to confer resistance of them to alkylating agents (Chen *et al.*, 1994). Later it was

found that human homolog of the bacterial gene gives partial resistance of the *E. coli alkB*⁻ mutant to the methylating agent MMS (Wei *et al.*, 1996).

The observation that single-stranded phages are more sensitive to certain alkylating agents while growing in *E. coli alkB*⁻ cells has suggested that AlkB repairs lesions generated by S_N2 methylating agents specifically in single-stranded, but not in double-stranded DNA (Dinglay *et al.*, 2000). These data suggested that 1meA and 3meC can be substrates for AlkB protein, since the ring nitrogens at these positions are protected by hydrogen bond formation in duplex DNA (Lawley & Brookes, 1963; Bodell & Singer, 1979). Also, a bioinformatic analysis of predicted protein folding has led to the insightful conclusion that AlkB is a member of the α-ketoglutarate-Fe²⁺-dependent-dioxygenase superfamily (Aravind & Koonin, 2001). These non-heme iron enzymes require Fe²⁺ as a cofactor, and α-ketoglutarate and dioxygen as co-substrates (Fig. 4). They catalyze the hydroxylation of a hydrocarbon bond in the substrate coupled to the oxidative decarboxylation of α-ketoglutarate producing succinate and CO₂. One atom of oxygen from O₂ is incorporated as a hydroxyl in the substrate, the other in the carboxylate group of succinate. It is accepted that dioxygenases catalyze a variety of reactions (hydroxylations, ring desaturation, and oxidative ring closures) among

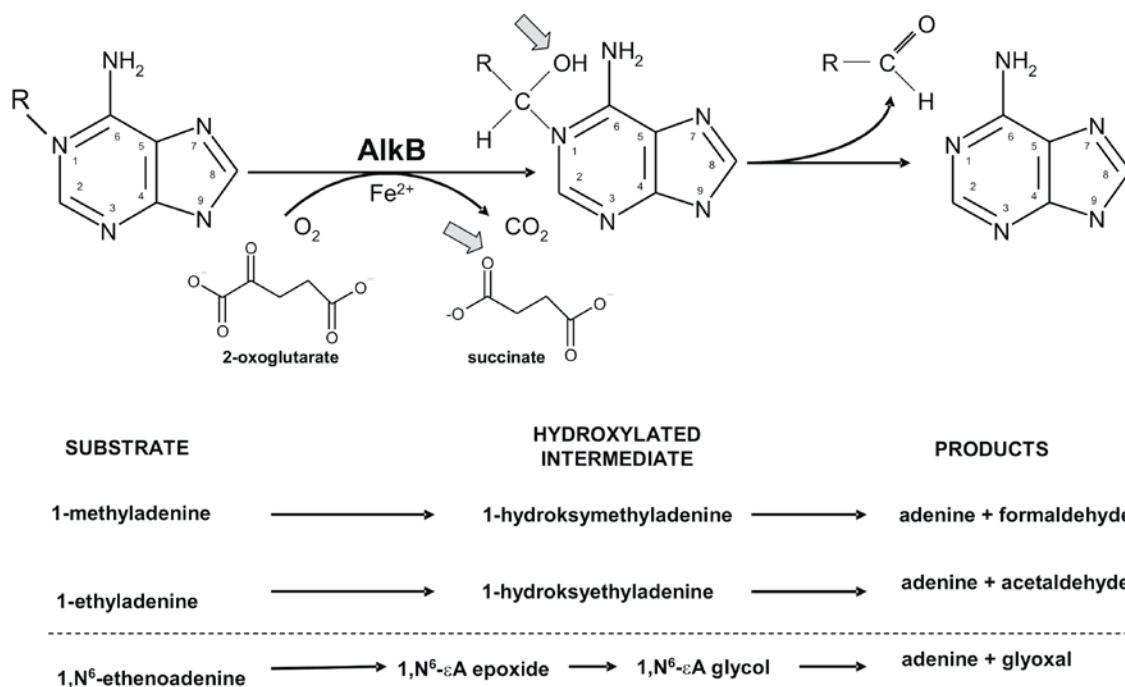


Figure 4. Mechanism of alkylated DNA repair by AlkB protein.

Grey arrows indicate oxygen atoms originated from oxygen molecule. The lower part of the figure shows examples of possible substrates, intermediates and products of AlkB-catalyzed reaction.

which participation in the oxidation of proline in collagen, the biosynthesis of some antibiotics, and biodegradation of various ring compounds are of general importance (Aravind & Koonin, 2001).

In 2002 two independent groups confirmed experimentally the participation of AlkB in DNA repair (Falnes *et al.*, 2002; Trewick *et al.*, 2002). It has been found that AlkB using the dioxygenase mechanism, when supplied with α -ketoglutarate and Fe^{2+} , reverts 1meA and 3meC in DNA directly to adenine and cytosine with the release of the oxidized methyl group as formaldehyde. The intermediate products are 1-hydroxymethyladenine and 3-hydroxymethylcytosine, both expected to be unstable and to decompose releasing formaldehyde.

Further studies have shown that trinucleotides and even methylated dAMP are recognized by AlkB as substrates. Also 1-methyldeoxyguanosine and 3-methylthymine are repaired by this *E. coli* protein. However, the repair of these lesions is significantly slower than that of 1meA and 3meC, suggesting that *in vivo* it is inefficient. An ability of AlkB to remove larger adducts like ethyl, hydroxyethyl, propyl, and hydroxypropyl has also been observed (Koivisto *et al.*, 2003).

Recent studies have shown that apart from alkylated substrates, AlkB also restores adenine and cytosine from ϵ adenine and 1, N^6 -ethenocytosine. In this case the protein acts according to a distinct chemical mechanism of direct reversal, namely, epoxidation of the double bond of the etheno adduct resulting in the release of glyoxal and the undamaged base (Delaney *et al.*, 2005; Mishina *et al.*, 2005). Recent findings of the Essigmann's group indicate the ability of AlkB to repair ethanoadenine (Frick *et al.*, 2007). Ethano adducts are formed in DNA by the anticancer chemotherapeutic agent BCNU.

The first human homolog of the *E. coli* AlkB protein, hABH1 was found in 1996 (Wei *et al.*, 1996). Two others, hABH2 and hABH3, were identified by computer analysis and functional complementation (Duncan *et al.*, 2002; Aas *et al.*, 2003). An *in silico* analysis found another five *alkB* homologs, hABH4-hABH8 (Kurowski *et al.*, 2003). Although all these proteins share the motifs and residues essential for enzymatic activity (the HXD motif, single H, and the RXXXXXR motif) until now only hABH2 and hABH3 have shown a similar activity as bacterial AlkB protein. The mentioned motifs occur as follows: $\text{H}_{131}\text{XD}_{133}\dots\text{H}_{187}\dots\text{R}_{204}\text{YNLTFR}_{210}$. The two histidines and the aspartic acid are proposed to form the active site for interaction with Fe^{II} , whereas the first arginine is involved in the binding of the C-5 carboxylate of 2-oxoglutarate. These residues are highly conserved in the α -KG-dependent oxygenases (Wei *et al.*, 1996; Aas *et al.*, 2003; Kurowski *et al.*, 2003).

Three proteins, human ABH2 and ABH3 and bacterial AlkB differ with respect to substrate specificity. In contrast to AlkB and ABH3, ABH2 preferentially acts on double-stranded DNA and more effectively repair 1meA, whereas 3meC is a preferable substrate for ABH3 (Duncan *et al.*, 2002; Falnes *et al.*, 2004).

Both AlkB and ABH3 remove 1meA and 3meC from RNA as well. In an *in vitro* experimental system AlkB repairs different types of alkylated RNA including mRNA, tRNA, rRNA and viral tRNA. AlkB as well as ABH3 have been shown to reverse the effect of MMS treatment (chemical methylation of mRNA blocks translation) on luciferase mRNA. The proteins reactivate also methylated tRNA^{Phe} (Ougland *et al.*, 2004). ABH3 acts with similar efficiency on DNA and RNA, whereas AlkB preferentially repairs DNA. This may indicate a greater importance of the repair of the more stable eukaryotic mRNA in comparison with the repair of prokaryotic mRNA (Aas *et al.*, 2003). On the other hand, many tRNAs and rRNAs require 1meA and 3meC for correct folding and activity. The question remains then, whether and how the AlkB-related enzymes distinguish between the proper and aberrant methylation, and what is their role in these RNAs' modification under normal conditions. Another important question is whether a slow demethylation of tRNA and rRNA by enzymes of the AlkB family may be tolerated by the cell and restored by RNA methyltransferases (Ougland *et al.*, 2004).

The subcellular localization of hABH2 and hABH3 has been examined by transfection experiments in HeLa cells. In non-S-phase cells hABH2 is homogeneously distributed in the nucleoplasm, with a slightly higher concentration in nucleoli. In S phase it relocates to replication foci and colocalizes with PCNA, suggesting a role in the repair of newly synthesized DNA. hABH3 has been observed both in the nucleoplasm and cytoplasm and possibly is involved in mRNA repair (Aas *et al.*, 2003).

ABH2 and ABH3 are not expressed equally in all human tissues. A high level of ABH2 mRNAs was observed in non proliferating liver tissue whereas the expression of hABH3 was high in the heart, liver, prostate and testis (Sedgwick *et al.*, 2007). The ABH3 expression is elevated in prostate carcinomas and has been reported as prostate cancer antigen-1 (PCA-1). ABH3/PCA-1 has been proposed as a diagnostic marker of prostate cancer and suggested as possible therapeutic target (Konishi *et al.*, 2005). Nevertheless, recent studies indicate a greater importance of hABH2 as a defense against toxic alkylation damage to DNA (Ringvoll *et al.*, 2006). The ability of AlkB-like proteins to repair some lesions generated by anticancer chemotherapeutics (e.g. BCNU) may indicate their role in cancer therapy.

ALKYLATED DNA REPAIR IN CELLS DEFICIENT IN AlkB PROTEIN

Damage to *alkB* greatly increases the sensitivity of bacteria to the cytotoxic action of MMS and diminishes the ability of reactivation of MMS-treated single-stranded phage DNA. It also markedly increases the level of MMS-induced *argE3*→Arg⁺ reversion in *E. coli argE3 alkB*⁻, but only a slight elevation of *lac*⁻→Lac⁺ reversion in *E. coli F'lacZ*⁻ (Δ *lacZ* CC101–CC106) strains has been observed (Yamamoto *et al.*, 1978; Kataoka *et al.*, 1983; Dingley *et al.*, 2000; Nieminuszczy *et al.*, 2006a; 2006b). A deficiency in AlkB protein followed by an inability to repair 1meA/3meC in DNA greatly increases the mutagenic potency of MMS. Interestingly, the resultant mutations are *umuDC*-dependent (Nieminuszczy *et al.*, 2006b).

MMS, apart of the Ada response, induces the SOS system that increases expression of over 40 genes involved in DNA recombination, repair, replication and mutagenesis (Fernandez De Henestrosa *et al.*, 2000; Courcelle *et al.*, 2001; Janion, 2001). Among these genes are *umuD* and *umuC*, whose products are required for translesion synthesis. UmuC and posttranslationally modified UmuD (UmuD') form the UmuD'₂-UmuC complex described as low fidelity DNA polymerase V (Pol V) (Fuchs *et al.*, 2004; Goodman & Woodgate, 2000; Reuven *et al.*, 1999). The 3meA residues introduced by alkylating agents that arrest DNA replication are also an induction signal for the SOS response (Costa de Oliveira *et al.*, 1987). Induction of the SOS system and expression of Pol V is a prerequisite for 60–70% of MMS-induced mutations occurring in wild type *E. coli*. These mutations are AT→TA transversions, whereas for the remaining 30–40% O⁶meG residues are probably the premutagenic lesions that induce *umuDC*-independent GC→AT transitions (Grzesiuk & Janion, 1994).

In *alkB* mutants the fraction of MMS-induced *umuDC*-dependent mutations is even higher than in the wild type and equals 95–98% of Arg⁺ revertants in an *E. coli argE3 alkB*⁻ strain (Nieminuszczy *et al.*, 2006b). In such strains mutations are formed from 3meC residues and are *umuDC*-dependent. Of the two premutagenic lesions O⁶meG and 3meC, only the latter requires Pol V-directed translesion synthesis to express mutations. Not long ago it has been shown that Pol V activity increases the level of mutations induced by 3meC and 1meA (Delaney & Essigmann, 2004). Mutations specific for *E. coli alkB*⁻ strains are due to GC→AT, GC→TA, and AT→TA base substitutions, and -1G and -2CG frameshifts (Dingley *et al.*, 2000; Nieminuszczy *et al.*, 2006a, 2006b). Concluding, the MMS-induced mutagenesis in *E. coli* cells depends on the balance between the repair of the methylated bases in DNA provided by Ada response and the ex-

pression of mutations due to Pol V activity induced as part of the SOS response.

In summary, finding the functions of bacterial AlkB and mammalian ABH2 and ABH3 proteins gives a new insight into the defense mechanisms protecting cells against mutagenic and cytotoxic effects of alkylation. The roles of ABH1 and ABH4-8 proteins remain unsolved.

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