The Escherichia coli AlkB protein, and two human homologs ABH2 and ABH3, directly demethylate 1-methyladenine and 3-methylcytosine in DNA. They couple Fe(II)-dependent oxidative demethylation of these damaged bases to decarboxylation of α-ketoglutarate. Here, we have determined the kinetic parameters for AlkB oxidation of 1-methyladenine in poly(dA), short oligodeoxyribonucleotides, nucleotides, and nucleoside triphosphates. Methylated poly(dA) was the preferred AlkB substrate of those tested. The oligodeoxytriphosphate structure was clearly not essential for AlkB to repair 1-methyladenine effectively, but a nucleotide 5′ phosphoty group was required. Covariantly, 1-me-dAMP(5′) was identified as the minimal effective AlkB substrate. The nucleoside triphosphate, 1-me-dATP, was insufficiently but actively demethylated by AlkB; a reaction with 1-me-dATP was even slower. E. coli DNA polymerase I Klenow fragment could employ 1-me-dATP as a precursor for DNA synthesis in vitro, suggesting that demethylation of alkylated deoxyribonucleoside triphosphates by AlkB could have biological significance. Although the human enzymes, ABH2 and ABH3, demethylated 1-methyladenine residues in poly(dA), they were inefficient with shorter substrates. Thus, ABH3 had very low activity on the trimer, d(Tp1meApT), whereas no activity was detected with ABH2. AlkB is known to repair methyl and ethyl adducts in DNA; to extend this substrate range, AlkB was shown to reduce the toxic effects of DNA damaging agents that generate hydroxyethyl, propyl, and hydroxypropyl adducts.

Alkylating agents occur in the environment and arise endogenously during cellular metabolism. They damage DNA at multiple sites, and the lesions generated may result in mutagenesis and cell death. The importance of preventing these adverse effects is highlighted by the variety of DNA repair mechanisms that have evolved to remove alkylated bases from DNA. These repair functions are conserved from bacteria to DNA repair enzymes. In this paper, we have examined the ability of E. coli AlkB, and human ABH2 and ABH3 to repair 1-meA in oligodeoxyribonucleotides, nucleotides, and nucleoside triphosphates. Substrate characteristics that influence the efficiency of repair have been defined, and the minimal substrate range in which AlkB has been determined. To extend the known substrate range of AlkB to other alkyl adducts induced by environmental agents, we have also examined
ined the ability of AlkB to counteract the adverse effects of small epoxides and larger alkyl halides.

**EXPERIMENTAL PROCEDURES**

**Materials**—[14C]MeI (58 mCi/mmol), [γ-32P]ATP (3000 Ci/mmol), and poly(dA) (average chain length 310 residues) were obtained from Amersham Biosciences. Oligonucleotides were prepared on an Applied Biosystems 394 synthesizer by the Cancer Research UK oligonucleotide synthesis facility. Other reagents were from Sigma-Aldrich.

**Preparation of Substrates**—[14C]MeI-treated poly(dA) was prepared as described previously (5, 7). Non-radioactive substrates were made by repeat cycles of depurination, oligonucleotides, and methylating with methylating a nucleoside in 100 mM ammonium acetate, pH 6.5, to a 10-fold molar excess of dimethylsulfate (DMS) every 2–3 h for 24 h at 20 °C. The pH of the reaction was maintained between 4 and 7 by adding NaOH when necessary (16). Products methylated at N1-adenine were purified by reverse phase HPLC using a 2 × 150 mm Phenomenex Luna C-18 (2) column and Beckman System Gold. The flow rate was 0.2 mL/min, and a linear gradient of 1–46% MeOH in 50 mM ammonium acetate, pH 6.5, was applied over 45 min. Products were monitored at A260. In all cases, the methylated product eluted before the non-methylated material and was clearly resolved. Highly purified 1-me-dA, free of dA, was prepared by collecting the 1-me-dA peak fractions and repeating the purification. Products were quantitated by the purified methylated products, a sample was depurinated in 0.1 M HCl at 95 °C for 5 min, and the released 1-me-dA measured by reverse phase HPLC and A260 readings. A linear gradient of 1–6% MeOH was applied between 0 and 5 min followed by 6–26% MeOH between 5 and 10 min.

**Enzyme Kinetics**—E. coli B AlkB protein with an amino-terminal His6 tag was purified as previously described (5, 13). The His6 tag is separated by a thrombin cleavage site from the initial methionine of the AlkB protein (MGSSHHHHHHSSLYPR | GSIL M. . . . ). The His6 tag was removed using thrombin Cleavlease kit (Sigma). His-tagged AlkB was incubated with thrombin-agarose for 20 h. After centrifugation, nickel-nitritotriacetic acid agarose was added to the supernatant, to 4°C, to bind the released tag and any uncleaved protein. After several washings, the column was loaded on a 450 μL column of nickel-nitritotriacetic acid agarose (Qiagen), the resin washed with 10 column volumes of lysis buffer lacking Nonidet P-40 but containing 10 mM imidazole, and bound proteins eluted using lysis buffer lacking Nonidet P-40 but containing 40 mM imidazole. Protein-containing fractions were analyzed using an Agilent Bioanalyzer, and purity was calculated to be 95% for ABH2 and 100% for ABH3.

**Survival of Alkylated M13 Phage**—M13 single-stranded DNA phage were treated with various alkylating agents. Ethyl iodide (EtI) and propyl iodide (PrI) were diluted 2-fold in dimethylsulphoxide and added to 100 μL of phage suspension to the desired final concentration. The temperatures and times of treatment with the different agents are indicated in Fig. 3. Survival of alkylated M13 transfected into E. coli K12 AB1157/F′ (parent strain) and BS87/F′ (alkB::117 mutant) was assayed as previously described (13).

**RESULTS**

**Demethylation of 1-meA in Oligonucleotides and Mononucleotides by AlkB**—One aim was to determine whether AlkB could repair 1-meA in short oligonucleotides and mononucleotides. To prepare the various substrates, oligomers d(TpApT), d(TpA), and d(ApT) and adenine nucleotides were heavily methylated by long exposures to DMS, such that 70–80% of the adenine residues in the starting materials were converted to 1-meA. The methylated products were purified by reverse phase HPLC. His6-tagged AlkB protein was incubated with purified substrates in standard assay conditions (5). Fig. 1A shows representative data for purification of d(TpImeApT). This methylated trimer was directly reverted to unmodified d(TpApT) by AlkB and also inefficiently by the human ABH3 protein (Fig. 1B and see below under “Weak Activity of Human AlkB Homologs on Oligo- and Mononucleotide Substrates”). Furthermore, AlkB demethylated the dimer, d(TpImeA), and interestingly, also 1-me-dAMP and 1-me-dATP (Table I).

To determine the relative efficiency of repair of 1-meA in different contexts, AlkB was assayed using increasing concentrations of the various substrates, and the Km and kcat values calculated from Hanes plots of the data. All experiments were performed at least twice with consistent results, and standard deviations are given for experiments repeated three or four times.

In **Vitro DNA Synthesis**—An oligonucleotide primer (42-mer), 5'-GTTTTCCTGCTGTCGTTGCTCCGGTTCTCCTGTGTTGGC-3', and the template (77-mer), 5'-AGCAAGAATCCTGCAAGATGCAGATGCGAACCTGCAGGAAACGAGAAGACAGGACAGACAGGCACACGATGGAGATGACACGATGGG-3', were purified by urea-PAGE. The primer was end-labeled with [γ-32P]ATP using T4 polynucleotide kinase and purified through a 45-50 Sephadex spin column. The primer and template were mixed in stoichiometric amounts in 25 mM Tris-HCl, pH 7.5, 50 mM NaCl, and annealed by heating to 95 °C for 5 min followed by cooling slowly to 20 °C. Primerextension reactions were performed using commercially available DNA polymerases (New England Biolabs, Amersham-Biosciences, and Trevigen) in the supplied buffers. Briefly, one unit of polymerase was added to a 5 or 10 μL reaction mixture containing 10 μM of each deoxynucleoside triphosphate and 50 nM annealed substrate. Reactions proceeded for 30 min at 37 °C. The reaction products were ethanol precipitated, resuspended in 5 μL of formamide, heated at 95 °C for 1 min, and analyzed by denaturing urea-PAGE. Radiolabeled products were detected by phosphoimaging of the dried gel.

**Construction of Vectors and Infectious Baculovirus for Expression of ABH2 and ABH3 in S9 Cells**—Recombinant baculovirus genomes harboring the ABH2 or ABH3 open reading frames were constructed using Gateway technology (Invitrogen). Cloning of ABH2 and ABH3 into the entry vector pDONR221 has been described elsewhere (7). Open reading frames were shuttled from pDONR221 to pDEST10 using LR clonase reactions. These constructs were transformed into E. coli strain DH10Bac (Bac-to-Bac, Invitrogen), and bacteria harboring bacmid DNA containing ABH2 or ABH3 selected using tetracycline, carbenicillin, gentamycin, and blue-white colony screening. Single white colonies were re-streaked to confirm their phenotype. Recombinant bacmid DNA was isolated and transfected into S9 insect cells according to the manufacturer’s instructions. Tissue culture supernatants containing infectious baculovirus were harvested 3 days post-transfection to generate an initial viral stock.

**Survival of His6-tagged ABH2 and ABH3 from Baculovirus-infected S9 Cells**—Sample insect cells were infected with recombinant baculovirus at a multiplicity of infection of 5, and grown for 3 days when expressing ABH2 and for 4 days when expressing ABH3. Cell pellets were resuspended in five pellet volumes of lysis buffer containing 25 mM Tris-HCl, pH 8, 150 mM NaCl, 0.1% Nonidet P-40, and 1 mM imidazole. After 20 min on ice, cellular debris was pelleted by centrifugation at 14,000 × g at 4 °C. Cell extracts were loaded onto a 50-μL packed volume of nickel-nitritotriacetic acid agarose (Qiagen), the resin washed with 10 column volumes of lysis buffer lacking Nonidet P-40 but containing 10 mM imidazole, and bound proteins eluted using lysis buffer lacking Nonidet P-40 but containing 40 mM imidazole. Protein-containing fractions were analyzed using an Agilent Bioanalyzer, and purity was calculated to be 95% for ABH2 and 100% for ABH3.
which may reflect the absence of a phosphate residue 5’ to 1-meA in this dimer. In agreement with this notion, AlkB demethylated the phosphorolyated 1-me-dAMP much more efficiently than 3’-phosphorylated 1-me-dAMP. Furthermore, 5’ phosphorylation of d(1meApT) using polynucleotide kinase greatly improved its susceptibility to demethylation by AlkB (data not shown). Thus, a phosphate residue 5’ to the lesion greatly increased the affinity of AlkB for 1-meA. AlkB had slight but significant activity on 1-methyldeoxyadenosine; using a 10-fold excess of substrate over enzyme, about 2% of the methylated deoxynucleoside was converted to the unmethylated form in 1 min (data not shown).

1-me-dATP was actively demethylated by AlkB, but the catalytic efficiency was lower than for 1-me-dAMP(5’). This observation of slow correction of the methylated deoxynucleoside triphosphate is nevertheless of interest because the intracellular pools of nucleoside triphosphates are larger than those of mononucleotides, so they represent a correspondingly larger target for alkylation. The ribonucleoside triphosphate, 1-me-ATP, could also be demethylated by AlkB, but even less efficiently than 1-me-dATP (Table I).

To check whether the His6 tag influenced AlkB activity, the tag was removed by thrombin cleavage. The untagged protein had the same $K_m$ values as the tagged protein for activity on both methylated poly(dA) and d(Tp1meApT), however the $k_{cat}$ values were 2- to 3-fold higher (data not shown). The relative activity of AlkB on the different substrates was unaffected by the histidine-tag.

### Competition between Low Molecular Weight and Polymer Substrates

AlkB protein binds to single-stranded DNA, and slightly more efficiently to the methylated form (13). The enzyme proceeds along single-stranded polymer substrates in a processive manner, checking every base residue for potential damage by insertion into the substrate binding site (18). The mode of enzyme-substrate interaction with low-molecular weight substrates cannot be processive. To verify that repair of polymers is the most efficient, competition experiments using high- and low-molecular weight substrates were performed. To check that repair of polymers is the most efficient, competition experiments using high- and low-molecular weight substrates were performed. To check that repair of polymers is the most efficient, competition experiments using high- and low-molecular weight substrates were performed. To check that repair of polymers is the most efficient, competition experiments using high- and low-molecular weight substrates were performed. To check that repair of polymers is the most efficient, competition experiments using high- and low-molecular weight substrates were performed.
residues were modified. Addition of the competitors resulted in a decreased rate of release of 14C-formaldehyde. However, a 5- to 20-fold excess of the low-molecular weight substrates was required to achieve a 2-fold decrease in the rate of reaction with the polymer substrate, the trinucleotide being a better competitor than the mononucleotide (Fig. 3). These results confirm the preference of AlkB for a polydeoxynucleotide substrate.

**Incorporation of 1-me-dAMP Residues by the Klenow Fragment of E. coli DNA Polymerase I**—Demethylation of 1-me-dATP by AlkB suggests a possible role for AlkB in removing this damaged nucleoside triphosphate from the DNA precursor pool. This would only be of biological significance if 1-me-dATP can be used as a substrate by DNA polymerases. A previous report suggested that 1-me-dATP was utilized during in vitro DNA synthesis by T4 DNA polymerase; however, a mixture of 1-me-dATP and 3-me-dATP (ratio 7:1) was used in those experiments (19). To verify that 1-me-dATP can be used as a DNA precursor, 1-me-dATP was purified and incorporation of 1-me-dAMP by E. coli polymerase I Klenow fragment during in vitro DNA synthesis examined. The 1-me-dATP was purified free from dATP and 3-me-dATP by repeated HPLC fractionations (Fig. 4A), and purity checked by depurination and HPLC analysis (Fig. 4B). The 1-me-dATP preparation was estimated to be more than 99.5% pure. A 5'-32P-end-labeled oligonucleotide primer (42-mer) was annealed to a 75-mer template (Fig. 4C) and extended by the Klenow fragment in the presence of various combinations of deoxyribonucleoside triphosphates (Fig. 4D). In the presence of 1-me-dATP or dideoxyATP (ddATP), Klenow fragment (with 3'→5' exonuclease activity) added a single residue to the primer terminus opposite a thymine base in the template. The incorporated 1-me-dAMP could be extended further by the polymerase when dGTP, dCTP, and dTTP were present. Thus, 1-me-dAMP was both incorporated and extended by Klenow polymerase. Using a 3' exonuclease-deficient form of the Klenow fragment, weak bands indicating slow insertion and/or extension of 1-me-dAMP were evident at each template thymine residue (data not shown). When an equimolar amount of dATP was also added, the bands indicating pausing of the polymerase were no longer evident. Thus, 1-me-dATP appeared to be less readily employed for DNA synthesis than dATP. In contrast to the Klenow fragment, neither T7 Sequenase with low 3'→5' exonuclease activity nor human DNA polymerase β incorporated a 1-me-dAMP residue at the primer terminus opposite thymine (data not shown).

**Weak Activity of Human AlkB Homologs on Oligo- and Mononucleotide Substrates**—Two human homologs, ABH2 and ABH3, of the E. coli AlkB protein were previously identified (7, 8). Both enzymes have the same cofactor requirements as AlkB, and repair 1-meA and 3-meC in DNA. The assay conditions of ABH2 and ABH3 were previously optimized with regard to pH and cofactor requirements. Nevertheless, activities of the recombinant proteins in vitro were low, being only 0.7 and 2% of AlkB activity, respectively, when assaying repair of 1-meA in poly(dA).

**Fig. 3. Competition between low molecular weight and polymer substrates for AlkB.** Reaction mixtures (100 μl) containing 0.7 pmols AlkB, 14C-methylated poly(dA) (with 50 pmols 1-meA), and an excess of nonradioactive d(Tp1meA-T) (Δ) or 1-me-dAMP (●) were incubated at 37°C for 1 min. The released radioactivity was monitored by scintillation counting.

**Fig. 4. Incorporation of 1-me-dAMP residues into DNA by DNA polymerase I Klenow fragment.** A, 1-me-dATP was prepared by methylation of dATP with DMS, and purified free from unmethylated dATP and 3-me-dATP by repeated HPLC fractionations. B, purity of 1-me-dATP examined by depurination of an aliquot and monitoring for 1-meA, 3-meA, and adenine by HPLC. C, a 5'-32P-end-labeled oligonucleotide primer was annealed to a template containing several thymine residues. D, the primer was extended by Klenow polymerase in the presence of various dNTPs added in equimolar amounts, and the products analyzed by urea-PAGE. The dNTPs added are indicated above each lane of the gel. Lane 1 contains 32P-end-labeled primer and template as markers.
enhances their activities, the ABH2 and ABH3 open reading frames were subcloned into baculovirus expression vectors. ABH2 and ABH3 purified from insect cells were, respectively, 50- and 5-fold more active than when purified from E. coli, their activities being 33 and 10%, respectively, of AlkB activity on methylated poly(dA). Gel purified protein bands were analyzed by peptide mass fingerprinting, but no post-translational modifications were detected (data not shown). The enhanced activity of ABH2 and ABH3 purified from insect cells may result from improved folding of the overexpressed proteins. To determine whether the human proteins were active on oligo- and mononucleotide substrates, ABH2 and ABH3 purified from insect cells were assayed with the trimer d(Tp1meApT) and 1-me-dATP. ABH3 was found to demethylate d(Tp1meApT), but the activity was very low (Fig. 1B). Activity of ABH2 on d(Tp1meApT) was not detectable, nor of either ABH2 or ABH3 on 1-me-dATP (data not shown).

**Effect of AlkB on Larger Alkyl Adducts in DNA—AlkB mutants are defective in reactivation of M13 phage treated with S_{\text{N}}2 methylating and ethylating agents.** This observation provided evidence that AlkB repairs both methyl and ethyl adducts generated in single-stranded DNA (7, 13). We have used the same experimental approach as an indication of whether AlkB can repair other types of alkylation damage including larger alkyl and hydroxyalkyl bases. In this way, the known substrate range of AlkB may be extended. M13 phage was treated with EtI, PrI, or hydroxyalkylating agents, and phage survival assayed in an *alkB* mutant and its parent strain. The survival of EtI-treated M13 phage was examined previously (7), but is included here for comparison with the other alkylating agents. The reactivation of both EtI and PrI-treated M13 phage was defective in the *alkB* mutant indicating that AlkB repairs both ethylated and propylated DNA bases (Fig. 5). Hydroxyalkylated DNA lesions, including 1-hydroxyalkyladenine and 3-hydroxyalkylcytosine, are induced by the S_{\text{N}}2 alkylating epoxides, such as ethylene oxide and propylene oxide (20). Due to the volatility and consequent difficulty of handling ethylene oxide, we used iodoethanol as a model compound to induce hydroxethyl DNA lesions (21), and propylene oxide to generate hydroxypropyl adducts (Fig. 4). After treatment with iodoethanol or propylene oxide, M13 reactivation was lower in the *alkB* mutant than in the parent strain, indicating that AlkB repairs both hydroxethyl and hydroxypropyl base adducts (7).

**DISCUSSION**

Substrate determinants for AlkB activity have been investigated by examining the ability of this enzyme to demethylate 1-meA residues in various polynucleotides, oligonucleotides, and mononucleotides. The favored substrate of those examined was methylated poly(dA). Nevertheless, 1-meA residues within the trimer d(Tp1meApT), and also, strikingly in the mononucleotide, 1-me-dAMP(5'), were efficiently repaired. We conclude that these low molecular weight substrates contain all the structural determinants essential for AlkB to bind to its substrate and correctly position 1-meA in its active site. Thus, a polynucleotide backbone with phosphodiester linkages is not crucial for binding or demethylation activity, but may result in optimal orientation of 1-meA for oxidation. By contrast, poor substrates were those lacking a phosphate 5' to the lesion, including the dimer d(1-meA-pT) and 1-me-dAMP(3'). Similarly, *E. coli* uracil-DNA glycosylase requires the presence of a 5' phosphate to excise a 5' terminal uracil residue from a minimal trinucleotide substrate (22). In a previous study (23), the methylated deoxynucleosides, 1-methyldeoxyadenosine and 3-methyldeoxycytidine, were not detectably demethylated by AlkB, although they were recognized by the AlkB active site and stimulated AlkB catalysis of an uncoupled reaction of \(\alpha\)-ketoglutarate with dioxygen. Presumably, the methyl group on these deoxynucleosides is positioned incorrectly in the active site and fails to react efficiently with the oxidizing species. Here, we observed very slow demethylation of 1-methyldeoxyadenosine by AlkB. Contacts with a 5' phosphate on the substrate may be crucial to correctly position the methyl group for oxidation. We have therefore identified 1-me-dAMP(5') as the minimal effective AlkB substrate. The identification of d(Tp1meApT) and 1-me-dAMP(5') as low molecular weight but effective substrates of AlkB containing a single defined adduct may be of relevance in future investigations of the three-dimensional structure of the AlkB protein. Analysis of the conforma-
tion of AlkB bound to its substrate will be necessary to confirm and extend the present data on substrate recognition. In this regard, we have observed by competition experiments that several transition metals, including Co$^{2+}$ and Mn$^{2+}$, apparently can replace Fe(II) at the metal binding site of the protein, but such complexes are enzymatically inactive.

DNA polymerase I Klenow fragment was shown to incorporate and extend 1-me-dAMP residues using a 1-me-dATP precursor during DNA synthesis in vitro, although pause sites indicated that incorporation and/or extension was inefficient. The extent to which insertion and extension could occur in vivo would depend on the DNA polymerases involved, whether they can incorporate non-hydrogen bonding 1-me-dAMP residues and extend from the distortion imposed by 1-meA at the primer terminus. In this respect, it may be of interest that human DNA polymerase β could not use 1-me-dATP in our in vitro assays. A possible explanation for this difference is the ability of the Klenow fragment, but not polymerase β, to utilize precursors that are unable to form hydrogen bonds with the template (24). Demethylation of 1-me-dATP by AlkB could prevent its use for DNA synthesis in vivo by Pol I in E. coli. Other enzymes that act on damaged DNA precursors are the E. coli MutT and Dtt proteins and their human counterparts, which dephosphorylate 8-oxodGTP and dUTP to prevent incorporation of these mutagenic and toxic nucleotides into DNA (25, 26). Because AlkB mutants of E. coli do not show a markedly increased mutation frequency on treatment with a methylating agent (13), misincorporation of 1-me-dAMP residues appears to be a less threat to genomic integrity than misincorporation of 8-oxodGMP and dUMP residues during DNA synthesis.

The AlkB enzyme could demethylate both 1-me-dATP and 1-me-ATP, but the ribonucleoside triphosphate was a poor substrate (Table I). It has been shown previously that several DNA repair enzymes acting by direct damage reversal can also repair altered RNA. Thus, ultraviolet light-irradiated tobacco mosaic virus RNA is corrected by a host photoreactivating enzyme in plant leaf cells, catalyzing light-dependent cleavage of uracil dimers to regenerate biological activity of the viral RNA (27). The extensively investigated DNA photolyase from E. coli cleaves cyclobutane pyrimidine dimers not only in DNA, but also in RNA, and “therefore might be considered an RNA photolyase as well” (28). Moreover, the O$^6$-methylguanine-DNA methyltransferase, which counteracts DNA alkylation damage, can directly demethylate O$^6$-methylguanine residues in RNA (29, 30). These early results on RNA repair have failed to elicit much interest, because the reactions are much slower and less efficient than those with damaged DNA. Aas et al. (8) reported recently that E. coli AlkB and human ABH3, but not ABH2, can demethylate 1-meA and 3-meC residues in RNA. These reactions only proceed at slow rates, about 10-fold more slowly than the reaction of AlkB and human ABH3, but not ABH2, in DNA with 1-meA and 3-meC residues in RNA. These reactions only proceed at slow rates, about 10-fold more slowly than the reaction of AlkB and human ABH3, but not ABH2, in DNA with 1-meA and 3-meC residues in RNA. The reaction with RNA by the AlkB and ABH3 enzymes could have an adverse effect in vivo, because 1-meA and 3-meC are naturally occurring bases in tRNA molecules, essential for correct folding (31, 32). The problem of putative cytotoxic RNA destruction by AlkB and ABH3 could be circumvented if demethylation events occur at slow rates, so that the RNA can be immediately remethylated by the modifying enzyme. Strategies to avoid in vivo depletion of active tRNA molecules may include maintaining AlkB protein at a low constitutive level in E. coli, and compartmentalization of ABH3 in eukaryotic cells to the cell nucleus. The previously described localization of green fluorescent protein-tagged ABH3 to the cytoplasm as well as the nucleus could result from overexpression (7, 8). It remains an interesting possibility that primary transcripts (hnRNA), the main form of single-stranded RNA in cell nuclei, could be a relevant target for toxic alkylating agents resulting in impairment of splicing reactions and mRNA processing in the absence of ABH3. At present, however, it is unclear if the slow RNA repair reactions catalyzed by AlkB and ABH3 are of relevance only in regard to host-cell reactivation of viral RNA, and if the slow repair of 1-me-ATP by AlkB is of any biological significance.

By examining the survival of alkylated bacteriophage in an E. coli alkB mutant, we have extended the AlkB substrate range to include methyl, ethyl, propyl, hydroxethyl, and hydroxypropyl DNA adducts. The methylated and ethylated DNA bases that are repaired are 1-alkyladenine and 3-alkylcytosine (5, 7), and this is most likely also the case for the propyl and hydroxyalkyl adducts. 1-Ethyladenine is repaired less efficiently than 1-meA. The difference in M13 survival in the alkB mutant and its parent strain was much smaller after treatment with the hydroxyalkylating agents than after treatment with EtI or PrI. Repair of hydroxyalkyl lesions by AlkB may therefore be even less efficient than that of ethyl and propyl adducts, or alternatively other toxic hydroxylalkyl lesions may be formed that are not substrates of AlkB. DNA glycosylases and DNA methyltransferases that repair methyl adducts also often repair larger alkyl adducts with a lower efficiency (33, 34).

By analogy with 3mea-DNA glycosylases and O$^6$me-G-DNA methyltransferases, we propose that AlkB and the human proteins ABH2 and ABH3 are referred to as 1-meA-DNA dioxygenases. All these DNA repair enzymes act on several different DNA lesions, but their names relate to the major substrate identified in initial characterizations. 3-MeC in DNA is another important substrate of AlkB and its homologs, so the repair function could also be called a 1-meA/3meC-DNA dioxygenase, or a 1-alkyladenine-DNA dioxygenase, when required.

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