3-Methyladenine DNA glycosylases: structure, function, and biological importance

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Summary

The genome continuously suffers damage due to its reactivity with chemical and physical agents. Finding such damage in genomes (that can be several million to several billion nucleotide base pairs in size) is a seemingly daunting task. 3-Methyladenine DNA glycosylases can initiate the base excision repair (BER) of an extraordinarily wide range of substrate bases. The advantage of such broad substrate recognition is that these enzymes provide resistance to a wide variety of DNA damaging agents; however, under certain circumstances, the eclectic nature of these enzymes can confer some biological disadvantages. Solving the X-ray crystal structures of two 3-methyladenine DNA glycosylases, and creating cells and animals altered for this activity, contributes to our understanding of their enzyme mechanism and how such enzymes influence the biological response of organisms to several different types of DNA damage. *BioEssays* 21:668–676, 1999.

Introduction

DNA carries life's genetic information encoded in the arrangement of bases along the length of the DNA molecule. Each DNA base has a distinct chemical structure that is appropriately interpreted by DNA-processing enzymes. Unfortunately, these bases are also chemically reactive, and the inevitable base modifications produce a variety of biological outcomes, depending on how a cell recognizes and responds to the modification. Cellular DNA repair mechanisms target these inappropriate DNA structures, and play a vital role in maintaining genomic integrity.

Base excision repair (BER) is one of several DNA repair pathways that maintain genomic integrity, and a wide variety of damaged bases induced by endogenous metabolites and exogenous agents are purged from the genome by BER. DNA glycosylases initiate BER by recognizing and removing improper or modified bases; there are at least six different classes of DNA glycosylase for the excision of alkylated bases, deaminated bases, oxidized bases, and mismatched bases, among others. DNA glycosylases cleave the *N*-glycosylic bond between the target base and the deoxyribose to produce an abasic site. The abasic site is then processed by one of two pathways, termed short-patch and long-patch BER.(1) In the first, a 5'-apurinic/apyrimidinic (AP) endonuclease or an AP lyase generates a DNA strand break at the abasic site and the abasic terminus is removed by deoxyribophosphatase or diesterase activity (Fig. 1).(1) In mammalian

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Abbreviations: BER, base excision repair; 3-MeA, 3-methyladenine; AAG (also known as ANPG, MPG), human 3-methyladenine DNA glycosylase; Aag, mouse 3-methyladenine DNA glycosylase; MAG, *S. cerevisiae* 3-methyladenine DNA glycosylase; AlkA, *E. coli* 3-methyladenine DNA glycosylase II; polβ, DNA polymerase β; dRpase, deoxyribophosphodiesterase; MMS, methyl methanesulfonate; MNNG, 1-methyl-3-nitro-1-nitroso-guanidine; BCNU, 1,3-bis (2-chloroethyl)-1-nitrosourea; MMC, mitomycin C; S_N2, bimolecular nucleophilic substitution (In this case, the incoming nucleophile is proposed to be an activated water and the leaving group is the N9-nitrogen of the base.); π clouds, electron density in the π bond orbitals above and below planar aromatic rings; HhH, helix-hairpin-helix motif.

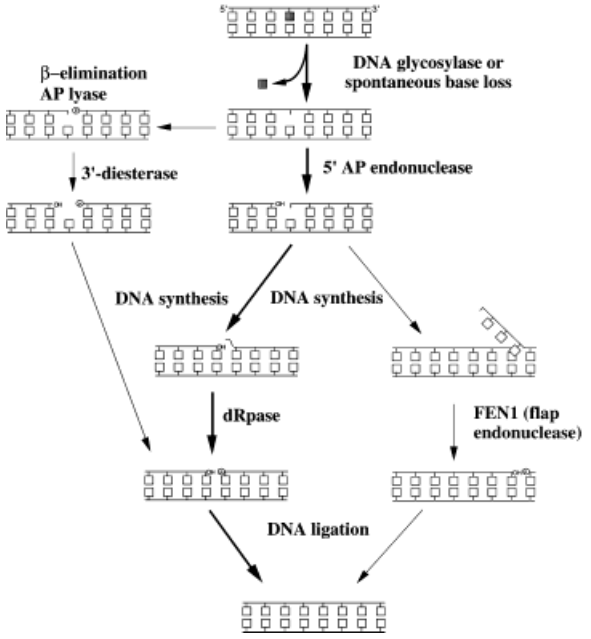

Figure 1. General scheme for base excision repair. The pathway in *bold arrows* represents the polβ-dependent pathway or ''short-patch'' base excision repair (BER) in which a single nucleotide gap is filled. DNA glycosylase activity or spontaneous base loss breaks the *N*-glycosylic bond between the base and the deoxyribose. 5'-AP endonuclease activity cleaves the deoxyribose and produces a strand break. DNA synthesis replaces the lost nucleotide(s). The 3'-diesterase, deoxyribophosphodiesterase (dRpase), and flap endonuclease activities modify the DNA ends to facilitate the DNA synthesis and DNA ligation steps. DNA ligation restores the phosphodiester bond to seal the nick. The right branch after AP endonuclease activity represents the PCNA-dependent ''long-patch'' BER. The left branch after DNA glycosylase activity represents the processing of a nick resulting from an AP lyase activity that some DNA glycosylases possess.

cells, the single nucleotide gap is then filled by DNA polymerase β (polβ) and the remaining DNA strand break sealed by DNA ligase III, probably in complex with XRCC.[2] The long-patch BER pathway requires proliferating cell nuclear antigen (PCNA) and replication factor C (RFC), presumably for DNA synthesis by a replicative polymerase. AP endonuclease generates a strand break at the abasic site and polymerase δ or ϵ extends the 3'-OH approximately seven nucleotides, simultaneously displacing the strand containing the terminal deoxyribose phosphate. The displaced strand is removed by FEN1 (DNase IV or flap endonuclease), and the remaining nick is resealed by DNA ligase I (Fig. 1).[3–5] In addition to these two pathways, there exists an alternative way to process abasic sites. Some DNA glycosylases have an associated β-lyase activity that produces a strand break 3' to the abasic site. The resulting 3' end lacks a hydroxyl and must be removed before DNA synthesis can occur. Although the relative contribution of each branch of the BER pathway has yet to be fully elucidated, our understanding of many BER enzymes and the repair steps they catalyze has grown tremendously in recent years.[6] Here, we discuss the recent advances in our understanding of one class of DNA glycosylases that removes 3-methyladenine (3-MeA). This discussion covers the substrate range, reaction mechanism, expression, and the development of a mouse model specifically deficient in 3-MeA DNA glycosylase activity.

3-MeA DNA glycosylases excise a wide range of substrates

DNA glycosylases that remove 3-MeA DNA lesions are found in bacteria, yeast, plants, rodents, and humans. The widespread occurrence of this DNA repair activity underscores its importance.[7] All 3-MeA DNA glycosylases studied thus far share the ability to rescue 3-MeA DNA glycosylase-deficient *Escherichia coli* from killing induced by the alkylating agent methylmethane sulfonate (MMS). However, the substrate range of most 3-MeA DNA glycosylases is not limited to 3-MeA, and these enzymes act on a wide variety of damaged DNA bases, listed in Table 1. The collective substrate range for these enzymes includes the following: five methylated bases, 3-MeA, 7-methylguanine (7-MeG), 3-methylguanine (3-MeG), O^2-methylthymine (O^2-MeT), O^2-methylcytosine (O^2-MeC); more complex alkylation products such as 7-chloroethylguanine (7-CEG), 7-hydroxyethylguanine (7-HEG), 3-chloroethylguanine (3-CEG), and the bridged alkyl adducts 1, N^6-ethenoadenine (ϵA) and 3, N^2-ethenoguanine (ϵG);[8] deaminated adenine (hypoxanthine, Hx),[9] and the oxidized guanine and thymine bases 8-oxoguanine, 5-hydroxymethyl uracil, and 5-formyluracil.[10,11] The structurally diverse lesions in this catalogue may occur spontaneously in untreated organisms and some are known to have quite different biological consequences. Interestingly, the *E. coli* 3-MeA DNA glycosylase (AlkA) can remove normal guanines, adenines, cytosines, and thymines, and the *Saccharomyces cerevisiae* 3-methyladenine (MAG) and human 3-methyladenine (AAG) DNA glycosylases can remove normal guanines[12] (Table 1). The implications of this activity will be explored below. With regard to removing a diverse array of substrates, the *E. coli* Tag glycosylase appears to be the exception, as it only removes 3-MeA and, to a much lesser extent, 3-MeG. Although all the known 3-MeA glycosylases have not been tested for each substrate in the catalogue, they collectively remove a wide range of toxic, mutagenic, and innocuous bases. Therefore, BER initiated by 3-MeA DNA glycosylases may serve to limit mutagenic and clastogenic events in addition to providing protection against cytotoxicity induced by environmental and chemotherapeutic agents. However, in addition to these beneficial effects, the ability of 3-MeA DNA

TABLE 1. Known Substrates Released by 3-MeA DNA Glycosylates*

	tag	AlkA	MAG	mag1	ADPG	Aag	AAG	aMAG
3-MeA	+	+	+	+	+	+	+	+
3-MeG	+	+	+			+	+	
7-MeG	−	+	+	+	+	+	+	+
O^2-MeT	−	+						
O^2-MeC	−	+						
7-CEG		+	+					
7-HEG		+	+					
7-EthoxyG		+						
ϵA	−	+	+		+	+	+	+[b]
ϵG							+	
8-oxoG						+	+	
Hx	−	+	+		+	+	+	+[b]
A		+				+[a]		
G	−	+	+			+[a]	+	
T		+						
C		+						

*Note, the + and − designations do not specify the relative preferences of the enzymes for the different substrates, but only whether the biochemical activity was present (+) or absent (−). Abbreviations: 3-MeA, 3-methyladenine; 3-MeG, 3-methylguanine; O^2-MeT, O^2-methylthymine; O^2-MeC, O^2-methylcytosine; 7-MeG, 7-methylguanine; 7-CEG, 7-(2-chloroethyl)guanine; 7-HEG, 7-(2-hydroxyethyl)guanine; 7-EthoxyG, 7-(2-ethoxyethyl)guanine; ϵA, 1, N^6-ethenoadenine; ϵG, N^2, 3-ethenoguanine; 8-oxoG, 8-oxoguanine; Hx, hypoxanthine. Tag and AlkA from *E. coli;* MAG from *S. cerevisiae;* mag1 from *S. pombe;* ADPG from rat; Aag from mouse, AAG from humans, aMAG from *A. thaliana.*

[a]R. Hampson, M.D. Wyatt, and L. Samson, unpublished observations.

[b]M.D. Wyatt, A. Britt, and L. Samson, unpublished observations.

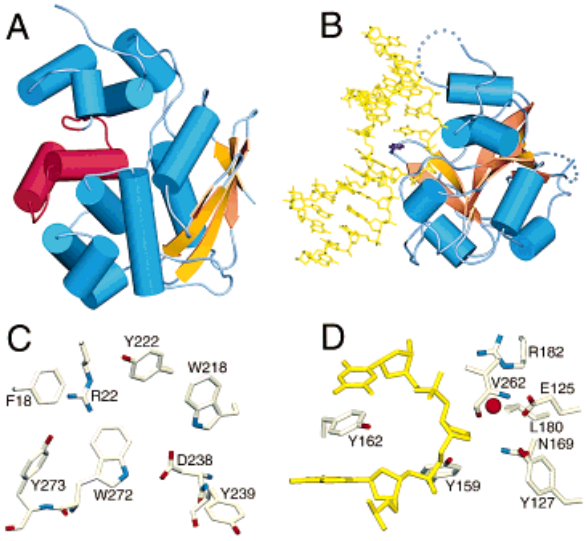

Figure 2. Crystal structures of *E. coli* AlkA and human 3-methyladenine DNA glycosylase (AAG). The fold of *E. coli* 3-methyladenine DNA glycosylase II (AlkA) (A) is very different from that of AAG (B). AlkA is made up of three domains, two α-helical domains and one mixed αβ domain, and features a helix-hairpin-helix (HhH) motif, colored red. AAG, in contrast, is made up of a single mixed α domain and lacks the HhH motif. AAG was crystallized bound to DNA (colored *yellow*) containing a mechanism-based pyrrolidine inhibitor. The pyrrolidine ring is shown flipped out of the DNA double helix, and the resulting gap in the DNA is filled by tyrosine 162, colored *purple*. The dotted segments in B indicate disordered loops in the crystal structure of AAG. The active site of AlkA (C) also differs from that of AAG (D). Both active sites, however, are rich in aromatic residues, and it has been proposed that these residues may serve to stack against a flipped-out target base (see text). In AlkA, the catalytically essential aspartate 238 is proposed to initiate glycosylic bond cleavage by activating a water molecule. In AAG, this catalytic role is assigned to glutamate 125, which is seen in the crystal structure to be well-positioned to deprotonate a water molecule (*red sphere*) that is bound near the flipped-out pyrrolidine ring. The base of a flipped-out nucleotide is likely to stack between tyrosines 127 and 159.

glycosylases to remove innocuous bases from the genome may, under certain circumstances, have detrimental consequences.

Three-dimensional structure of AlkA suggests a mechanism of action of 3-MeA DNA glycosylases

A major advance in understanding how 3-MeA DNA glycosylases operate was achieved by solving the crystal structure of the *E. coli* AlkA 3-MeA DNA glycosylase.[13,14] The 3-dimensional picture of AlkA provides possible explanations for its broad substrate range and catalytic mechanism (see Fig. 2A). AlkA has three domains bordering an adjustable cleft, which is thought to contact DNA substrates. The enzyme is thought to flip the nucleotide out of the DNA helix and into an active site lined with amino acids that interact with the target base.[13,14] Substrate recognition is likely mediated by the amino acids lining the active site, and glycosylic bond cleavage appears to be initiated by the activation of a water molecule by the catalytically essential aspartate 238 residue[13,14] (see Fig. 2C). The proposed active site pocket is lined with hydrophobic tryptophan and tyrosine residues that have planar and electron-rich conjugated rings, and these are positioned to stack against the target base. Conjugated (or aromatic) rings are unusually stable because the bonding orbitals overlap favorably and the resulting electron delocalization over each of the atoms above and below the plane provides a ring of electron density, as π clouds. The target bases for AlkA contain diverse modifications; for instance, the addition of an alkyl group onto different positions of adenine (N3) or guanine (N7 or N3), or purines with a bridged alkene (ϵA). Although the substrates differ in their chemical structures, they share the trait of being electron poor. The π clouds presumably interact more strongly with an electron poor base than a normal base. In other words, electron-rich amino acids may be the sensors for electron-deficient AlkA substrates.

Berdal et al.[12] proposed a model for 3-MeA DNA glycosylase mechanism of action based on the activation energy for spontaneous base release. It was shown that AlkA removes guanine, adenine, thymine, and cytosine at low but significant rates. The relative rates of excision of the normal bases by AlkA correlate with their susceptibility to acid-catalyzed base release, i.e., guanine has the highest spontaneous release rate and is also released at the highest relative rate by AlkA. Good substrates such as 3-MeA and 7-MeG have unstable glycosylic bonds in comparison to adenine and guanine, and, therefore, the activation energy for release of 3-MeA and 7-MeG should be lower than that for adenine and guanine. The authors suggest that 3-MeA DNA glycosylases simply lower the activation energy for spontaneous base release and that the specificity of these glycosylases is derived from the inherent instability of the substrates' glycosylic bond.[12] The model is interesting, but it may not apply for all 3-MeA DNA glycosylases. For instance, Dosanjh et al.[15] show that human AAG removes ϵA at least 10 times faster than 3-MeA, whereas AlkA and MAG appear to remove ϵA and Hx at least 100 times slower than the rat or human enzymes.[9,16] These findings suggest that other factors, in addition to the chemical instability of the glycosylic bond, contribute to the substrate preferences of 3-MeA DNA glycosylases.

AlkA shares a common Helix-hairpin-Helix motif with other DNA glycosylases

Somewhat surprisingly, the AlkA structure proved similar to another *E. coli* DNA glycosylase, endonuclease III, that excises a completely different set of damaged bases.[17] Although the amino acids of AlkA and Endo III are quite different, and each enzyme has a domain that the other lacks, the proteins adopt similar folds around their active sites.[13] However, the amino acids lining their active sites are different (hydrophilic residues line the endonuclease III active site), which presumably explains the different substrate specificities of AlkA and Endo III. Furthermore, the three-dimensional structures and sequence alignment reveal a Helix-hairpin-Helix (HhH) motif common to DNA glycosylases with very different substrate ranges, prompting the hypothesis that DNA glycosylases from many different species belong to a HhH "super family."[18,19] The structural similarity of the conserved region suggests a common progenitor with glycosylase function that diverged to accommodate different substrates and biological roles through adaptive replacements around the active site.[19] However, the HhH glycosylase super family does not include all 3-MeA DNA glycosylases. Specifically, the *Arabidopsis thaliana*, mouse, rat, and human 3-MeA DNA glycosylases do not share significant sequence identity with the HhH super family, and, as detailed below, the three-dimensional shape of the human 3-MeA DNA glycosylase AAG is very different from that of AlkA.

Three-dimensional structure of human AAG complexed to DNA: a nucleotide flipper with a new look

The crystal structure of human AAG in complex with DNA containing a modified abasic site has revealed an intriguing and novel protein: DNA structure.[20] The N-terminally truncated AAG protein consists of a single mixed α/β domain that does not appear to share similar folds or shape to any other known protein (Fig. 2). AAG displays a relatively flat DNA binding surface with a protruding hairpin that intercalates into the DNA by means of the minor groove, thus displacing the nucleotide of the target base into the active site pocket. Tyrosine 162 occupies the site in the DNA helix that the flipped out abasic nucleotide once occupied. Despite the global structural differences between AlkA and AAG, there are some interesting similarities. As was found with AlkA, the active site of AAG is lined with aromatic amino acids, including two tyrosines between which an electron-deficient base could stack. Glutamate 125 is well placed to deprotonate a water molecule that is bound near the pyrrolidine ring of the modified abasic site and in the center of a hydrogen bonding network (Fig. 2D). Recall that an aspartate is hypothesized to deprotonate water for glycosylic bond attack in AlkA.[13,14] The AAG structure strongly supports a mechanism of glycosylic bond cleavage in which an activated water is the attacking nucleophile of an bimolecular nucleophilic substitution (S_N2)-type displacement reaction that releases the base.

In addition to demonstrating that AAG uses a nucleotide flipping mechanism for substrate recognition, the crystal structure offers suggestive evidence for a model of how the enzyme finds its substrates in DNA. Processive or scanning models have been proposed for the T4 endoV pyrimidine dimer DNA glycosylase and the human and rat uracil DNA glycosylases,[21–23] but the exact mechanism of substrate recognition remains undetermined. For 3-MeA DNA glycosylases that efficiently excise bases with major or minor groove modifications, this prospect appears to be particularly challenging. Verdine and Bruner hypothesized that glycosylases might move along DNA by flipping, or attempting to flip, successive nucleotides out of the DNA helix and into the enzyme active site where substrate recognition occurs.[24] The energetic cost of flipping a nucleotide would be compensated for by flipping the previous nucleotide back into the DNA helix. In the crystal structure of AAG, tyrosines 162 and 165 and methionine 164 form a rigid structure that might be responsible for distorting the minor groove and flipping nucleotides. Methionine 164 and tyrosine 162 appear to push against the sugar and base opposite the target base, which has the effect of destabilizing the stacking interaction with its 5'-adjacent base. Lau et al. propose that this destabilizing effect favors progression in the direction of the destabilization, and lessens the energetic cost of flipping out the next

nucleotide.[20] The crystal structure suggests that human AAG locates target bases by processing along the helix and flipping each nucleotide out of the helix. This is a daunting and provocative hypothesis worthy of further exploration.

The structures of AlkA and AAG, despite their differences, strongly support a common nucleotide flipping mechanism for substrate recognition and excision. It should be noted that human uracil DNA glycosylase (UDG) flips uracil nucleotides out of the DNA helix and into an active site pocket[25]; however, UDG does not contain the HhH motif. Studying the differences and similarities between glycosylases should highlight key aspects of substrate recognition by DNA glycosylases. Moreover, identification of glycosylase mutants with altered substrate specificities could have broader biological implications. As an example, site-directed mutants of human uracil DNA glycosylase, with an increased ability to excise thymine and cytosine, have been shown to confer mild mutator phenotypes in *E. coli*.[26] Wild-type or mutant DNA glycosylases that remove normal DNA bases at an appreciable level could have a profound influence on spontaneous mutation rates. The excessive production of abasic sites, which are potentially mutagenic, can lead to increased mutation, if the rest of the BER pathway is unable to efficiently complete repair.[26–28] The consequences of such imbalances in BER are explored in a later section.

3-Methyladenine DNA glycosylases protect against the toxic, clastogenic, and mutagenic effects of DNA alkylation damage

BER initiated by 3-MeA DNA glycosylases provides protection against the toxic effects of numerous agents, presumably because these enzymes have a relatively broad substrate range (Table 1). For example, the murine 3-MeA DNA glycosylase (Aag) protects against the cell-killing effects of MMS, 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU), and mitomycin C (MMC).[29] AlkA protects against the toxic effects of mechlorethamine, a DNA cross-linking nitrogen mustard,[30] and MAG protects against toxicity by chloroethyl nitrosourea (CNU) in *S. cerevisiae*.[31] These alkylating agents interact with DNA to produce many structurally diverse lesions, which leads us to pose the following questions: (1) what is a toxic lesion; (2) how is toxicity mediated; and (3) how does 3-MeA DNA glycosylase activity provide protection? For MMS-induced 3-MeA lesions, toxicity is likely to result from inhibition of DNA polymerization. 3-MeA lesions are known to block in vitro DNA synthesis by bacterial and viral DNA polymerases.[32] Furthermore, the specific introduction of 3-MeA lesions into the genome of Aag-deficient murine cells was shown to inhibit in vivo DNA replication and, thus, the progression of cells through S-phase.[33] An explanation for the inhibitory effect of 3-MeA on DNA synthesis comes from X-ray crystallographic studies. For several DNA polymerases, interactions occur between polymerase residues and the N3 position of purines and O^2 position of pyrimidines in the template strand.[34–36] Indeed, for mammalian DNA polymerase β and T7 DNA polymerase, these interactions are crucial for catalytic activity.[34,37] Thus, 3-methylguanine and O^2-methylpyrimidine, which are also substrates for certain 3-MeA DNA glycosylases (Table 1), might have biological effects similar to 3-MeA.

MMC, BCNU, CNU, and the nitrogen mustards, in addition to inducing mono-adducts in DNA, are each capable of inducing DNA interstrand cross-links. Interstrand cross-links, if left unrepaired, are particularly toxic because they prevent strand separation during DNA replication or chromosome segregation. 3-MeA DNA glycosylase activity may ameliorate the toxicity of these agents by the repair of monolesions that are precursors for subsequent cross-link formation; alternatively the enzyme may initiate repair at the site of a cross-link. AlkA has been shown to create abasic sites in DNA modified by nitrogen mustard,[30] but whether these were formed at cross-links, at their precursors, or at other lesions is not known. It remains to be determined how the mouse 3-methyladenine DNA glycosylase (Aag) protects against BCNU and mitomycin C–induced toxicity. For BCNU, the similarity of the BCNU-induced 1, O^6-ethanoguanine lesion (the immediate precursor to the major class of BCNU-induced interstrand cross-link[38] and the 1, N^6-ethenoadenine lesion (Table 1), which Aag excises efficiently,[39] suggests that Aag may provide protection against BCNU by means of repair of the 1, O^6-ethanoguanine lesion.

DNA damage may induce toxicity by signaling a programmed cell death pathway, at least in cells derived from multicellular organisms. Indeed, 3-MeA and lesions induced by MMC and BCNU appear to signal apoptosis in a murine cell model, and Aag null cells show enhanced apoptosis in response to MMC and BCNU, and in response to the specific introduction of 3-MeA into the genome.[33,40] Alkyl lesions have also been shown to actively signal an S-phase checkpoint arrest in yeast.[41] However, it remains to be determined how these lesions signal apoptosis or activate cell-cycle checkpoints and whether the inhibition of replication, transcription, or mitosis is required for such signaling.

In addition to protecting against cytotoxicity, the Aag enzyme protects against other deleterious effects of DNA alkylating agents, including clastogenicity.[29] However, with the exception of 3-MeA, which has been shown to induce both sister chromatid exchange and chromosome aberration,[33] the identities of the clastogenic lesions repaired by Aag are not known, nor is it known how they destabilize chromosomes. 3-Methyladenine DNA glycosylases also have excision activity for some mutagenic lesions. For example, hypoxanthine, 1, N^6-ethenoadenine, 8-oxoguanine, 5-formyluracil, and 5-hydroxyuracil are all mutagenic DNA lesions, and each lesion is thought to be formed endogenously. Surprisingly, the loss of 3-methyladenine DNA glycosylase activity

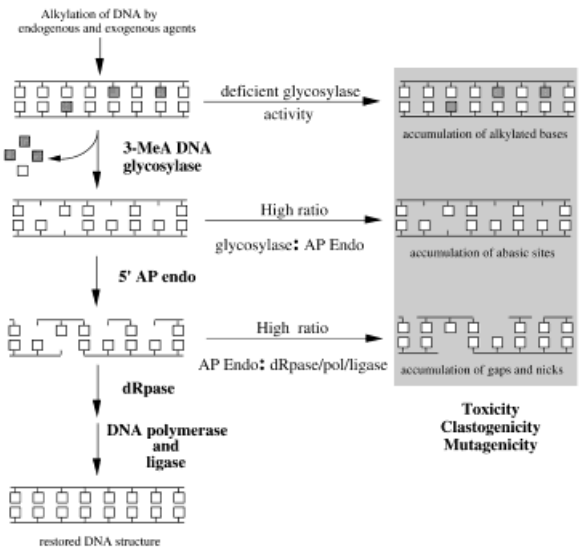

Figure 3. Potential imbalance of base excision repair. The descending pathway on the left indicates the normal steps of base excision repair. The *arrows* that lead to the right-hand column indicate potential imbalances that might lead to toxicity, clastogenicity, and mutagenicity.

has little or no effect on the rate of spontaneous mutation in *S. cerevisiae* and *E. coli*.[27,42]

Biological consequences of inappropriate 3-MeA DNA glycosylase expression

Although 3-MeA DNA glycosylase activity and the BER pathway can protect against the deleterious effects of DNA alkyl lesions, inappropriate expression of this activity may have detrimental consequences. For example, induced expression of AAG in CHO cells not only failed to protect, but actually sensitized cells to sister chromatid exchange and chromosome aberrations caused by MMS and 1-methyl-3-nitro-1-nitroso-guanidine (MNNG).[43,44] These observations led to the hypothesis that an imbalance in DNA BER can cause the accumulation of downstream BER intermediates (either abasic sites or single-strand breaks), which are responsible for the increase in clastogenic events (Fig. 3). A similar effect has been observed for *E. coli* strains with a defective *xthA* gene, which encodes the major AP endonuclease. *XthA* mutants are more sensitive to MNNG-induced mutagenesis than wild-type cells, but this sensitivity is abolished in the *xthA alkA* double mutant,[45] again implicating DNA glycosylase-generated abasic sites as the mutagenic lesion.

This hypothesis may be extended to include other deleterious biological endpoints. For example, *S. cerevisiae* deficient in the APN1 AP-endonuclease have an increased spontaneous mutation rate compared with wild type.[27,46] Furthermore, this effect could be suppressed by a deficiency in the *S. cerevisiae* MAG 3-MeA DNA glycosylase, or exaggerated by increased expression of MAG.[27,28,47] These and other results implicate the abasic site as the responsible mutagenic lesion, but it remains to be determined which endogenous DNA base lesions are converted to abasic sites. 3-MeA is unlikely to contribute significantly to this effect because, unlike MAG, the expression of the 3-MeA specific enzyme Tag in *APN1* deleted *S. cerevisiae* does not significantly increase spontaneous mutation.[28] Whatever the responsible lesion(s), they must be less mutagenic than the abasic site, otherwise elevated spontaneous mutation would not be seen after their conversion to abasic sites. It is quite possible that the excision of normal DNA bases may contribute to elevating spontaneous mutation, at least in *S. cerevisiae*.[12,28]

These results demonstrate that the activities of the BER pathway must be balanced for optimal protection against the biological consequences of damaged DNA bases, underscoring that, until the last step of BER has been completed (i.e., ligation), damage is still present in the genome. Thus, an elevation in 3-MeA DNA glycosylase activity may only confer protection under certain circumstances: (1) when 3-MeA DNA glycosylase activity is initially rate limiting to the BER pathway; or (2) when the DNA base lesions excised by 3-MeA DNA glycosylase are more toxic/clastogenic/mutagenic than either the abasic sites or the single-strand breaks that may accumulate during BER (Fig. 3). Indeed, several reports in the literature cite the protective effects of increased 3-MeA DNA glycosylase activity. For example, expression of Tag in CHO cells protects against MMS and MNNG.[48,49] Elevated Tag expression in *E. coli* also protects against MMS and MNNG, whereas elevated AlkA expression sensitizes *E. coli* to these agents.[50] Protection by Tag, which almost exclusively repairs 3-MeA, may be due to such glycosylase activity initially being rate limiting, or because the highly toxic 3-MeA lesions are converted to less toxic abasic sites. Elevated expression of AlkA, which excises 7-MeG and normal bases in addition to 3-MeA, may sensitize cells to DNA-damaging agents, because it converts these innocuous bases into more toxic abasic sites. Thus, whether 3-MeA DNA glycosylase activity protects or sensitizes cells to the toxic effects of DNA alkyl lesions may depend not only on the balance of the various BER components, but also on the particular 3-MeA DNA glycosylase and the toxic potential of the DNA lesions it excises.

Cellular regulation of 3-MeA DNA glycosylase activity

Clearly, the expression of 3-MeA DNA glycosylase activity relative to other BER components can have a profound effect on the efficiency of this pathway in protecting against the deleterious effects of DNA alkyl lesions. This leads us to question how 3-MeA DNA glycosylase activity is expressed in different cell types, tissues, and organisms, how it is regu-

lated under various environmental conditions and how its activity compares to that of other BER enzymes.

In mammals, the level of 3-MeA DNA glycosylase activity can vary enormously among tissue types,[39,51] and even among mouse strains and animals of different ages.[51,52] In plants, transcription of the *Arabidopsis thaliana* 3-MeA DNA glycosylase gene *aMAG* was elevated in rapidly dividing tissues.[53] Thus, tissue differences may reflect cell division status (and hence DNA replication status). Four different sequences of the human AAG 3-MeA DNA glycosylase DNA have been reported by independent groups.[54–57] Although some differences might result from sequencing errors, others may represent true polymorphisms in *AAG*, potentially encoding AAG glycosylases with different activities or substrate specificities. A limited amount of evidence suggests that a bimodal distribution of 3-MeA DNA glycosylase activity exists among humans, which suggests that a common AAG polymorphism may indeed exist.[58] Altered 3-MeA DNA glycosylases could also arise from alternatively spliced mRNA transcripts; the existence of alternatively spliced 3-MeA DNA glycosylase transcripts has been demonstrated in human tissues,[59] although their biological significance remains to be determined.

The expression of 3-MeA DNA glycosylase activity may vary in response to changing environmental conditions, although this is not a universal feature of all 3-MeA DNA glycosylases. In *E. coli*, Tag is constitutively expressed independent of environmental conditions, whereas AlkA expression can be induced as part of the adaptive response to alkylating agents.[60,61] *S. cerevisiae MAG* is also induced by alkylating agents, but the *S. pombe mag1* gene appears to be expressed constitutively.[62] Some mammalian 3-MeA DNA glycosylases are also induced in response to DNA damage.[58,63–65] In the rat, induction occurs in response to many types of DNA damaging agents, and such regulation, therefore, is different from the adaptive response of *E. coli* that is specific for alkylating agents.[65,66]

The evolution of different regulatory mechanisms for 3-MeA DNA glycosylase activity may have been driven by a need to maintain an appropriate balance between BER intermediates, as discussed earlier. Tag, which almost exclusively excises 3-MeAs, may be expressed constitutively, because it can prevent the accumulation of highly toxic 3-MeA lesions without overloading the BER pathway with abasic sites by means of removal of the more abundant 7-MeG lesions and normal bases. AlkA, in contrast, excises a much broader range of lesions, and its expression, therefore, may be tightly regulated to prevent overloading the cell's repair machinery for abasic sites. Regulation of AlkA expression may also be required to limit excision of normal unmodified bases by this glycosylase, a property not shared by Tag.[12]

Development of a mouse model null for 3-MeA DNA glycosylase

During the past few years, several groups set out to engineer BER-deficient mice by targeted homologous recombination. Although mice that are heterozygous for null mutations in the DNA polymerase β, *APE* AP endonuclease, *XRCC1,* and *LIG1* DNA ligase genes are normal, homozygous mutations in each gene creates a strong embryonic lethal phenotype.[67] It was uncertain, therefore, whether or not 3-MeA DNA glycosylase-deficient mice (i.e., *Aag* null mice) would be viable. However, viable *Aag* null mice that completely lack 3-MeA, hypoxanthine, and εA DNA glycosylase activities were recently engineered by two independent groups.[39,68] To date, the *Aag* null mice appear to be perfectly healthy and do not display an obvious phenotype. However, cells derived from the null mice are sensitive to alkylating agents, and the null mice are also expected to show an alkylation-sensitive phenotype. With the generation of numerous other DNA repair-deficient strains of mice, it will now be possible to assess the relative contributions of DNA repair methyltransferase, nucleotide excision repair, mismatch repair, recombination repair, and base excision repair to protecting animals against the numerous deleterious effects of alkylating agents. It should be possible to determine whether the relative roles of each DNA repair pathway differs among tissues and among strains of mice. The *Aag* null mice can also be used for the expression of human AAG DNA glycosylase in its various polymorphic forms, effectively humanizing the mouse and allowing the determination of human AAG function in a whole animal setting. Moreover, information about enzyme mechanism, gained from X-ray crystallographic analysis of the human AAG enzyme,[20] should allow one to predict which polymorphic variants of AAG are likely to be biologically significant. The lessons learned from studying knockout and transgenic mice might enable us to understand and predict the influence of each DNA repair pathway in protecting human beings against the effects of the various alkylating agents present inside cells, present in the environment, and used in the clinic as chemotherapeutic agents.

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