1,N^6-Ethenoadenine: From molecular to biological consequences

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ABSTRACT

Genomic DNA is chemically reactive and therefore susceptible to damage by many exogenous and endogenous sources. Lesions produced from these damaging events can have various mutagenic and genotoxic consequences. This Perspective follows the journey of one particular lesion, 1,N^6-ethenoadenine (εA), from its formation to replication, repair, and its role in cancerous tissues and inflammatory diseases. εA is generated by reaction of adenine (A) with vinyl chloride or lipid peroxidation products. We present the miscoding properties of εA with an emphasis on how bacterial and mammalian cells can process lesions differently, leading to varied mutational spectra. But with information from these assays, we can better understand how the miscoding properties of εA lead to biological consequences and how genomic stability can be maintained via DNA repair mechanisms. We discuss how base excision repair (BER) and direct reversal repair (DRR) can minimize the biological consequences of εA lesions. Kinetic parameters of glycosylases and AlkB family enzymes are described, along with a discussion of the relative contributions of the BER and DRR pathways in the repair of εA. Because eukaryotic DNA is

packaged in chromatin, we also discuss the impact of this packaging on BER and DRR, specifically in regards to repair of εA. Studying DNA lesions like εA in this context, from origin to biological implications, can provide crucial information to better understand prevention of mutagenesis and cancer.

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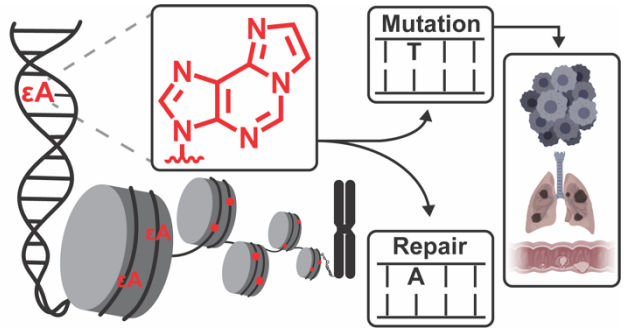

1. INTRODUCTION

Genomic DNA is constantly subjected to endogenous and exogenous damaging agents, and the resulting damage can lead to a variety of consequences including mutagenesis, cancer, neurological disorders, autoimmune disease, and aging.[1-3] Modified DNA nucleobases, referred to here as lesions, are formed by a variety of mechanisms with deamination, oxidation, and alkylation being especially prominent.[4] For example, 1,N^6-ethenoadenine (εA, Figure 1), which is the focus of this Perspective, is generated by reaction of vinyl chloride (VC) or lipid peroxidation (LPO) products with adenine (A).[5, 6] The lesion is so named because of two extra carbons attached to A in an exocyclic arrangement. Upon replication by a DNA polymerase, the εA lesion is mutagenic in human cells resulting in primarily A→T transversions as well as A→G and A→C mutations.[5, 7] These εA-induced mutations have been shown to be associated with p53 mutation hotspots, cancerous tissues, and inflammatory diseases (Figure 1).

Fortunately, cells have DNA repair processes such as base excision repair (BER) and direct reversal repair (DRR) to combat these lesions. BER involves the excision and replacement of the modified nucleobase through cleavage of the glycosidic bond by a glycosylase, followed by filling the resulting "hole" by downstream enzymes.[8] BER works on a wide range of lesions created by deamination, oxidation, and alkylation, including εA.[9] The DRR pathway works on a much smaller subset of lesions, including some alkylated lesions, by directly converting the lesion to the canonical nucleobase, for example εA to A.[10, 11]

This Perspective outlines the mutagenic and genotoxic effects of εA, as well as the DNA repair mechanisms capable of mitigating these effects at the cellular and organismal level. We also

consider the hierarchy of DNA packaging in human cells and how chromatin structure may affect these repair processes and biological consequences.

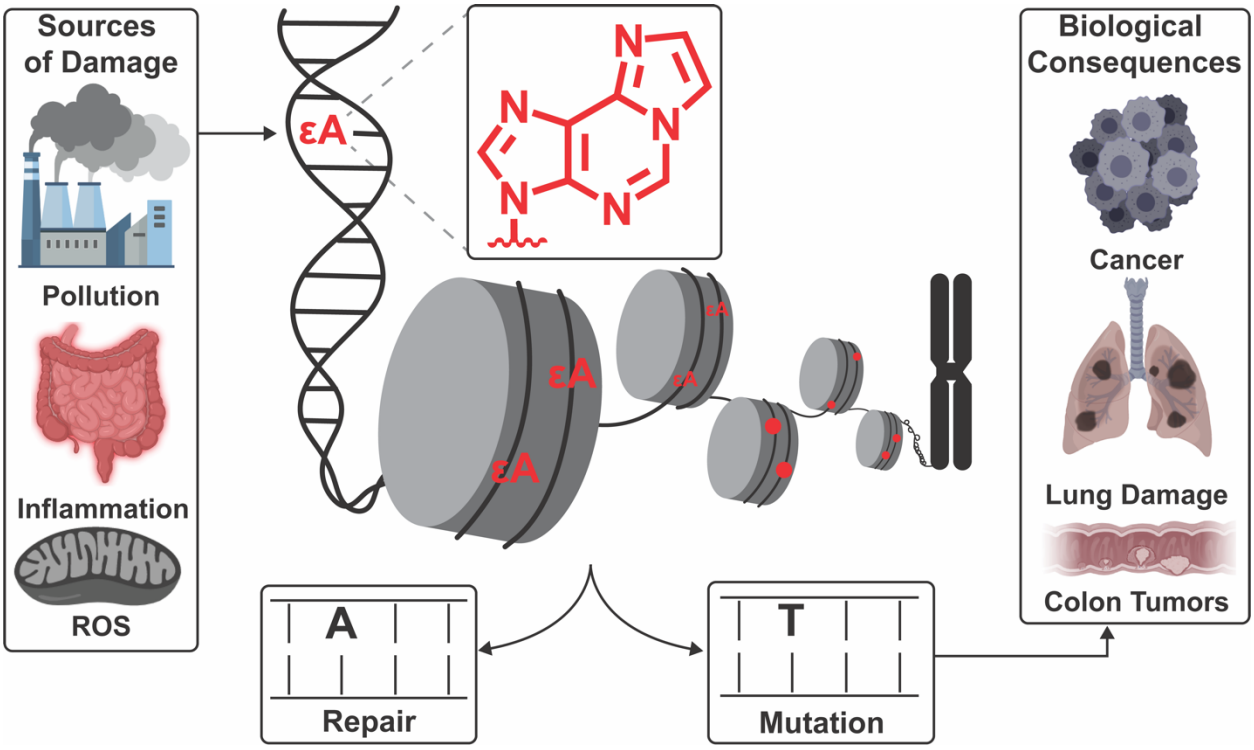

Figure 1. Formation and consequences of the DNA lesion 1,N^6-ethenoadenine (εA). εA is generated by the industrial pollutant vinyl chloride and lipid peroxidation byproducts associated with inflammation and metabolism. εA can be repaired or replicated to cause mutations and biological consequences related to cancer.

2. FORMATION OF εA LESION

The primary damaging agents responsible for formation of εA are aldehyde byproducts of LPO and the industrial pollutant VC.[12, 13] LPO is an oxidative stress response that generates byproducts known to cause DNA damage.[12, 14] Endogenous reactive oxygen and nitrogen species (RONS) degrade phospholipids to create reactive aldehydes such as 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA).[15] These aldehyde byproducts of LPO react with DNA nucleobases to

generate lesions like εA. Indeed, etheno DNA lesions have been shown to arise endogenously in both rodents and humans[16, 17] and are efficiently induced in chronically inflamed human tissues.[6]

VC is a known procarcinogen activated by cytochrome P450 to create the metabolites chloroethylene oxide (CEO) and chloroacetaldehyde (CAA).[5, 13, 18] These metabolites react with DNA to form exocyclic etheno adducts like εA.[18] Etheno lesions have been identified in rodents and humans exposed to VC and its metabolites.[6] Notably, VC has also been shown to cause tumors in individuals whose occupation results in chronic exposure.[19]

The etheno ring of εA interferes with formation of hydrogen bonds on its Watson-Crick face. NMR and molecular modeling of oligomer duplexes revealed that εA does not hydrogen bond to T when both are in the *anti* conformation but, instead, the bases adopt a non-planar alignment.[20] However, in similar experiments hydrogen bonding was observed between an εA:G base pair,[21] with εA and G in *syn* and *anti* conformations, respectively. X-ray crystal structures of ternary complexes with a DNA polymerase and dATP or dGTP have shown that εA remains in the preferred *anti* conformation while the incoming purine adopts a staggered orientation.[22] These data provide a molecular basis for the mutational and genotoxic properties observed for εA.

εA is also an inherently fluorescent molecule with a fluorescence maximum at 415 nm.[23, 24] As a result, it has been used as a tool for determining nucleic acid structure,[25, 26] dynamics of DNA binding and nucleotide flipping,[27] and to monitor biochemical processes.[28]

3. MUTATIONAL AND GENOTOXIC PROPERTIES OF ALKYLATED ADENINE

The downstream effects of DNA lesions can be described in two ways. A lesion is genotoxic when it inhibits replication by blocking DNA polymerase activity. A lesion is mutagenic when

replication by a DNA polymerase occurs, but the base pairing is incorrect, meaning that the lesion is miscoding.

3.1 Mutational and genotoxic properties of εA *in vitro*

In vitro replication assays have been crucial to our understanding of the molecular basis for εA-induced biological consequences. In fact, early studies with εA were the first to show that a known human carcinogen, in this case VC, can form lesions that are miscoding during replication, linking molecular-level observations to cancer.[26] *In vitro* assays examining replication of εA by *E. coli* DNA polymerase I (Pol I) demonstrated its miscoding properties.[26] Homopolymers of poly(dA) were exposed to CEO or CAA to generate templates for replication assays. Upon exposure to increasing concentrations of CEO or CAA, inhibition of and errors in DNA replication both increased. Specifically, replication of the etheno poly(dA) template was inhibited 100-fold. When replication did occur, εA:G pairs increased by several hundred-fold compared to εA:T.

In other work, synthetically-prepared εA templates as well as CAA-modified templates were used to examine replication of lesions by Pol I.[29] Replication of the synthetic templates resulted in misincorporation of dGTP at a rate of 1 in 500 lesions.[29] This rate of misincorporation is lower than reported previously[26], suggesting the previously observed high levels of dGTP misincorporation were due to high concentrations of CAA exposure. Indeed, poly(dA) treated with CAA under conditions producing comparable levels of εA as the synthesized template behaved in the same manner with a low level of misincorporation of dGTP observed.[29]

Using extracts of HeLa cells, two-dimensional gel electrophoresis of a double-stranded M13 bacteriophage genome containing a site-specifically incorporated εA established that the lesion is genotoxic with significant inhibition of replication fork movement.[30] εA was weakly mutagenic

when replicated by human polymerases and induced all three base substitutions in approximately equal frequency.[5, 7] The frequency of A→T mutations increased 5-fold in extracts of XPV cells, which lack the translesion DNA polymerase η (Pol η), suggesting that the replication-blocking lesion εA requires a Y-family polymerase for translesion synthesis.[30]

Further studies probed the efficiency and fidelity of translesion synthesis of εA by Pol η,[22] which was reported to be 100-fold more active than Pol κ in replication past εA.[31] Steady-state kinetic analysis of nucleotide incorporation opposite εA, and LC-MS/MS analysis of replication products, revealed that Pol η preferentially pairs dATP (3.7-fold) and dGTP (2.5-fold), instead of dTTP, with εA.[22] Additionally, -1 frameshifts were observed suggesting pairing of the newly incorporated purine and polymerase slippage before further replication. X-ray crystal structures of Pol η ternary complexes revealed incoming non-hydrolyzable dATP (dAMPNPP) or dGTP (dGMPNPP) analogs not paired with, but instead in a staggered configuration relative to εA, thus opposite a 5'-T in the template, explaining the propensity for the observed frameshifts.[22] In these ternary complexes with Pol η the lesion remains in the preferred *anti* conformation and the incoming purine adopts a staggered orientation (Figure 2).

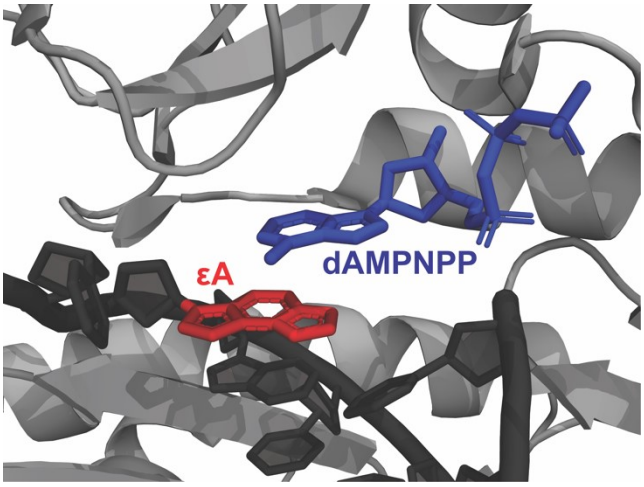

Figure 2. Crystal structure of ternary complex of Pol η (gray) with εA lesion (red) and incoming dAMPNPP (blue) in a staggered configuration (PDB: 5DG8).

3.2 Mutational and genotoxic properties of εA *in vivo*

To evaluate its mutagenic and genotoxic properties *in vivo,* εA was site-specifically incorporated into the single-stranded genome of an M13 bacteriophage and transfected into *E. coli*.[32] The lesion is genotoxic in *E. coli* reducing survivability by 65%. Mutation frequency was determined by exploiting the placement of εA in an in-frame TAG amber codon. Wild-type phage would exhibit a light blue plaque color phenotype whereas any targeted mutation at the second base of the amber codon would restore the Lac+ phenotype and generate dark blue plaques on IPTG/X-gal containing medium. DNA sequencing analysis identified the specific type of mutations. Using these techniques, εA was found to be very weakly mutagenic with only 0.1% of survivors exhibiting predominately A→G transition mutations. When a double-stranded version of the M13 genome was used in similar experiments, the genotoxicity and mutagenicity of εA was eliminated, suggesting the presence of effective repair mechanisms in *E.coli*.[32]

Over a decade later, other work used a similar single-stranded M13 bacteriophage genome with a site-specifically incorporated εA. Lesion genotoxicity and mutagenicity were determined using the competitive replication of adduct bypass (CRAB)[33] and restriction endonuclease and postlabeling (REAP)[33] assays, respectively.[10] εA was found to be a nontoxic lesion when replicated in wild-type *E. coli*, having a level of bypass comparable to controls. In striking contrast, when the same viral genome was replicated in *E. coli* deficient in AlkB-mediated repair, the lesion revealed itself to be extremely toxic, as it was bypassed only ~5% as well as the control. This vast difference in lesion tolerance between wild-type and AlkB-deficient *E. coli* is suggestive of a role for AlkB in

mitigating lesion toxicity through repair of εA (*vide infra*). Indeed, AlkB is now known to be an α-ketoglutarate/Fe(II)-dependent DRR enzyme responsible for repair of alkyl DNA lesions. Although toxicity can be partially overcome by SOS bypass polymerases,[10] AlkB was identified as the main mechanism for avoiding εA-induced genotoxicity in the single-stranded bacteriophage genome. In terms of its miscoding potential, εA was negligibly mutagenic (<0.5%) in wild-type *E. coli*,[10] consistent with other studies done in AlkB-proficient *E. coli*.[7, 32, 34, 35] However, εA was 35% mutagenic in AlkB-deficient cells, yielding 25% A→T, 5% A→G and 5% A→C mutations.

In other work, using a single-stranded pMS2 shuttle vector, the genotoxicity and mutagenicity of εA was compared in five strains of *E.coli* and simian kidney cells (COS7).[35] The lesion was neither genotoxic nor mutagenic in the bacteria with only εA:T base pairings observed. In contrast, when replicated in the mammalian cells, εA caused a mutation frequency of 70% with predominantly A→G transitions, indicating that εA:C base pairings are most common in these cells. These results are consistent with earlier reports using human cells with a shuttle plasmid modified with CAA, where A→G transitions were the most prominent among mutations observed at A:T base pairs.[36]

Studies have also examined the replication of εA in human cells. The lesion was incorporated at the second position of 5′-CAA corresponding to codon 61 of the *ras* oncogene, in both single- and double-stranded pSBK vectors.[7] When replicated in HeLa cells, εA directs the misincorporation of dATP, leading to A→T transversions. These results are also consistent with others demonstrating A→T transversions in p53 and *ras* genes.[19, 37] Similar to earlier work, εA in the pSBK vectors was not miscoding when replicated in *E. coli* even in the presence of induced SOS functions,[7] further highlighting the need for mutational assays in human cells. **4. REPAIR OF εA**

The BER and DRR pathways are responsible for the repair of εA to A. As seen in Figure 3a, repair of εA restores A:T base pairing. Both BER and DRR are described in detail below, followed by a discussion of the relative contributions of these two repair pathways in minimizing the genotoxicity and mutagenicity of εA in bacterial and mammalian cells.

4.1 BER of εA

A glycosylase is responsible for initiating BER by recognizing and excising the lesion via cleavage of the *N*-glycosidic bond, generating an apurinic/apyrimidic (AP) site (Figure 3b).[8] AP Endonuclease 1 (APE1) incises the sugar-phosphate backbone creating a nick with 3′-OH and 5′-deoxyribose phosphate (dRP) termini. Polymerase β (Pol β) then removes the dRP group and incorporates a dNTP. Finally, ligase seals the backbone nick to complete the repair.

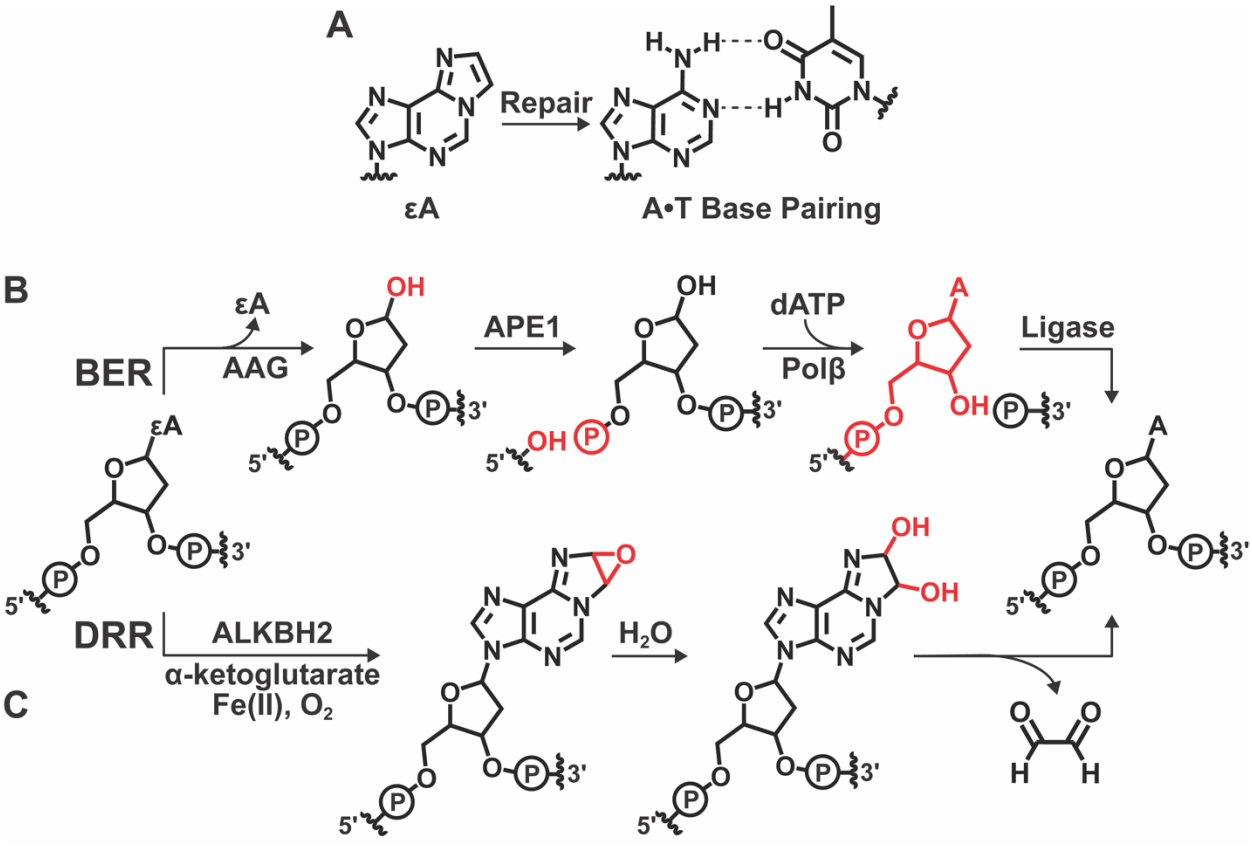

Figure 3. Repair mechanisms for εA. (A) Repair of εA to A restores hydrogen bonding between an A:T base pair. (B) Mechanism of base excision repair (BER) of εA initiated by the glycosylase AAG. (C) Mechanism of direct reversal repair (DRR) of εA initiated by ALKBH2. The region of DNA altered in each step is shown in red.

The *E. coli* AlkA glycosylase is part of the adaptive response that protects the bacterial genome against alkylation damage.[38] In the early 1990s it was demonstrated that human and rodent cells also have DNA binding proteins that possess an εA glycosylase activity, later identified as alkyladenine DNA glycosylase (AAG).[39-41] AAG is the only mammalian glycosylase known to initiate BER on alkylated lesions including 7-methylguanine (7-meG), 3-methyladenine, and εA.[42-44] (We wish to note that in the literature AAG is also referred to as *N*-methyl purine DNA glycosylase (MPG) and alkylpurine-DNA-*N*-glycosylase (APNG); in this Perspective we will use AAG). Our focus here will be on describing the activity of the human glycosylase AAG and the reader is referred to other sources regarding AlkA and its contributions to the adaptive response in bacteria.[38, 45]

AAG works on double-stranded DNA to excise εA via a base-flipping mechanism[27, 43] but only requires contact with the lesion-containing strand.[46] (It has been shown that AAG can excise εA in single-stranded DNA, but with much lower levels of activity.[43]) Base flipping of εA into the AAG active site is favorable and occurs rapidly with an equilibrium constant of ~1,300 and rate of 160 min^{-1}.[27] Base flipping of εA is thermodynamically favored because the lesion lacks hydrogen bonding with T but the extrahelical lesion forms a hydrogen bond with the backbone amide of His136 to form a stable complex. X-ray co-crystal structures revealed that when εA is flipped out

(shown in red in Figure 4a), Tyr162 fills the resulting space by intercalation into the DNA helix (shown in green in Figure 4a).[42, 46] The *N*-glycosidic bond that attaches εA to the sugar-phosphate backbone is then cleaved at a rate of 0.04 min^{-1}, which is the rate-limiting step of AAG.[27] Finally, the εA nucleobase and abasic DNA product are released.

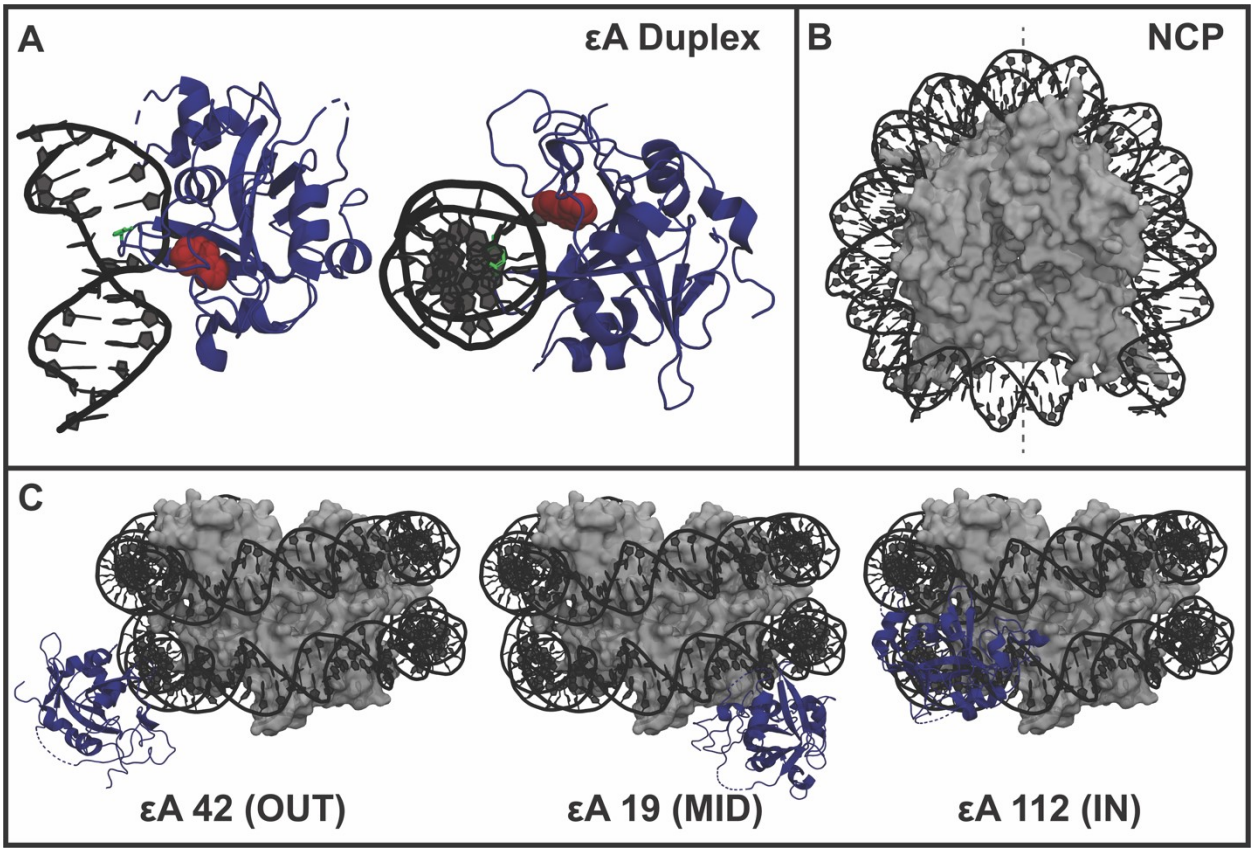

Figure 4. AAG binding to duplex DNA and NCP structures. (A) Co-crystal structure of AAG (blue) bound to εA-containing oligomer duplex (PDB: 1EWN) (left: side view, right: view down helical axis of DNA). εA (red) is flipped into the active site of AAG. Intercalation of the Tyr162 side chain in place of εA, is shown in green. (B) X-ray crystal structure of an NCP (PDB: 3LZ0). Histone protein core is gray, DNA is black, and the dyad axis is shown as a dotted line. (C) Docking analysis of AAG (PDB: 1EWN) with NCP (PDB: 3LZ0) at εA sites (left to right) 42, 19, and 112 with rotational positioning of OUT, MID, and IN, respectively.

BER is responsible for the repair of ~10,000 lesions per cell per day.[1] In order to effectively search for lesions, glycosylases are thought to diffuse along DNA by either sliding and/or hopping. This process, known as processivity, allows glycosylases to efficiently search for and identify their target lesions.[47] AAG has been shown to utilize hopping to more effectively search for multiple εA lesions in a single binding encounter.[47, 48] Furthermore, when AAG encounters a protein bound tightly to the DNA, it can microscopically dissociate or "hop" to search both duplex strands and find two damage sites.[48] The *Eco*RI endonuclease dimer was used as a representative roadblock between two εA sites and AAG was able to bypass it ~50% of the time.[48] Hopping allows AAG to effectively search for DNA damage and circumvent tightly bound proteins, which may be relevant to repair of DNA packaged in chromatin. In cells AAG has also been shown to bind to chromatin in complex with RNA Pol II and its activity can be coupled with the transcription elongation process.[49]

4.2 DRR of εA

In addition to BER, εA is also repaired via DRR by the AlkB family of dioxygenases.[10, 50] This family of enzymes use α-ketoglutarate and Fe(II) to directly repair εA to A by oxidative dealkylation (Figure 3c).[51-54] The prototypic and homonymous family member is an *E. coli* enzyme which, along with the AlkA glycosylase, is part of the bacterial adaptive response to alkylation damage. Analysis by mass spectrometry (MS) confirmed the direct reversal mechanism.[10, 55] Incubation of an εA oligomer duplex with AlkB caused a loss of 24 Da, consistent with removal of the two carbon etheno bridge and conversion to A. Two intermediates were also observed: a species that was 16 Da heavier than the εA oligomer duplex, consistent with the epoxide intermediate, and a species was 34 Da heavier, consistent with the glycol intermediate. Notably,

it has also been proposed that DRR is mediated by a zwitterionic intermediate, which cannot be distinguished from the epoxide intermediate by MS alone.[56] Based on quantum mechanical/molecular mechanical (QM/MM) calculations it has been proposed that the "epoxide" intermediate is an aldehyde resulting from rearrangement of the zwitterionic intermediate.[56]

A total of nine mammalian homologs of AlkB exist (ALKBH1-8, FTO), but only a subset functions in DNA repair.[57] Beyond DNA repair, functions of the AlkB family include protein demethylation (ALKBH1, ALKBH4), RNA demethylation (ALKBH5), and tRNA modification (ALKBH8).[57] ALKBH2 and ALKBH3 are the only homologs known to facilitate DNA repair.[50, 57-59] ALKBH2 is responsible for direct repair of εA to A in genomic DNA.[57] Also referred to as the "housekeeping homolog",[60] ALKBH2 acts on double-stranded DNA and flips εA into its active site, much like AAG.[61-63] But in contrast to AAG, ALKBH2 requires contact with both strands of the duplex.[61] ALKBH3 facilitates DNA repair on single-stranded DNA and RNA substrates.[57, 58]

Interestingly, AlkB, ALKBH2, and ALKBH3 have been shown to be inhibited by the oncometabolites D- and L-2-hydroxyglutarate,[64] which are found at abnormally high concentrations in some tumors, Cu(II) ion which is dysregulated in Wilson's disease,[65] and hydrolyzable tannins that are found in many natural products.[66]

Recent transient kinetic studies revealed *E.* coli AlkB is much faster than previously reported.[67] A DNA-glycosylase coupled assay was developed to evaluate AlkB kinetics in both single-stranded and double-stranded DNA. Previous reports of k_{cat} values for repair of εA in single-stranded DNA ranged from 0.001-0.03 s^{-1},[10, 11] but with this rapid detection method, k_{max} was found to be 0.75 s^{-1}. Maximal rate constants are similar, within 2-fold difference, for both single and double-

stranded substrates. While this work was conducted using *E. coli* AlkB, the methodology could also be applied to a kinetic analysis of the human homologs involved in DNA repair, ALKBH2 and ALKBH3.

4.3 Repair in nucleosome core particles

While the BER and DRR pathways are well understood for DNA oligomer substrates, it is important to consider how DNA packaging affects these repair processes.[68, 69] The fundamental unit of packaging in eukaryotic cells is the nucleosome core particle (NCP), comprised of double-stranded DNA wrapped around a histone octamer core.[70] The NCP contains a dyad axis, which is a 2-fold axis of pseudo-symmetry (Figure 4b).[71] Nucleobase positions in an NCP are described translationally, relative to the dyad axis, and rotationally, relative to the histone core. Nucleobases that face outward from the histone core are more solution accessible relative to those that face inward. NCPs also undergo transient unwrapping where the DNA entry-exit regions spontaneously and transiently dissociate from the histone core.[72] Different translational positions experience different degrees of nucleosome dynamics and therefore can affect access to otherwise occluded nucleobases in the NCP.

Biochemical studies of excision of a site-specific εA from NCPs by AAG showed that lesion excision is correlated with rotational positioning.[73] AAG activity was determined using strongly-positioned NCPs with lesions positioned near the dyad axis and with varying rotational positions including outward towards solution (OUT), inward towards the histone core (IN), and a rotational position in between (MID). εA^{OUT} was excised from NCPs to the greatest extent, εA^{IN} was not excised, and removal of εA^{MID} was at an intermediate level. Single-turnover kinetic analyses revealed that excision of εA from the NCP is much slower than from the same double-stranded

DNA control not bound to histones. Shown in Figure 4c are models of AAG bound to an NCP at three representative εA positions, (OUT, MID, IN) to highlight how AAG interacts with different rotational positions. Notably, for εA^{IN} there is severe steric clash between the glycosylase and histone core, consistent with a lack of activity at such a site. These observations support the idea that rotational positioning affects the physical accessibility of lesions to a glycosylase.

In other experiments with strongly-positioned NCPs, a global approach to examining repair by AAG was used. A population of NCPs was prepared with εA lesions at 49 locations, with a variety of rotational and translational positions.[74] Under reaction conditions in which all εA lesions were fully excised from double-stranded DNA controls, 30/49 lesion sites had less than 30% excision. The level of εA excision by AAG generally correspond to solution accessibility, with some exceptions. Repair is suppressed in the dyad region, even at OUT sites. This lower level of repair may be due to altered periodicity[75, 76] or reduced dynamics. Meanwhile, some IN and MID sites at the entry-exit DNA regions experience higher levels of repair than expected, further emphasizing the effect of translational positioning on glycosylase activity.[72, 74] High levels of excision of IN positions was seen mostly at the DNA end known to preferentially unwrap from the histone core.[77]

The population of lesions not repaired is due to a structural impediment and/or conformation derived from the histone octamer which renders the lesion inaccessible to AAG activity. In the cell, repair of these lesions might require chromatin remodelers, coupling with RNA Pol II, and/or additional factors. Previous kinetic characterizations of human glycosylases OGG1 and UNG2 acting on site-specific lesion NCP systems revealed multiphasic kinetics for removal of lesions in some positions, which was attributed to conformation changes in the NCP.[78, 79] Considering AAG's

slower rate of glycosylase activity compared with OGG1 and UNG2, the monophasic behavior observed for AAG[74] suggests that analogous conformational changes cannot be resolved on this timescale.

These results obtained with NCPs are consistent with *in vivo* evidence for excision of alkylation damage in yeast by Mag1, which is the yeast homolog of AAG. The alkylation damage 7-meG in strongly-positioned NCPs in yeast was repaired to a lesser extent at the dyad region compared to the DNA entry-exit regions.[80] Mag1-deficient strains had high and comparable levels of unrepaired lesions throughout the NCPs, indicating that these observations are due to differential repair and not because of a bias in formation of damage.

The efficiency of repair initiated by AAG is also dependent on its searching ability *in vivo*.[81] AAG mutants showed relative positive linear correlations between cell survivability and kinetic parameters like catalytic specificity (k_{cat}/K_M), fraction processivity (F_p), and efficiency of excision. Specifically, AAG mutants were expressed in yeast deficient in Mag1 and exposed to alkylating agents to evaluate how AAG searches chromatin. Mutants with poor searching ability or processivity showed decreased levels of excision. The mutations did not affect catalysis of glycosidic bond cleavage. These studies concluded that repair of alkylation damage in chromatin is governed by the searching ability of AAG.[81]

5. RELATIVE CONTRIBUTIONS OF BER AND DRR

AlkA and AlkB have almost identical overall repair efficiencies for εA in double-stranded DNA, with comparable $k_{max}/K_{½}$ values. However, to achieve this repair efficiency, AlkB couples a high k_{max} (which reflects chemistry) with relatively weak lesion recognition, whereas AlkA has an almost 400-fold lower k_{max} value but a much higher substrate affinity.[67]

The relative contributions of AlkA and AlkB to *E. coli* survival upon CAA challenge has also been examined.[10] In this experimental model, the majority of etheno lesions would presumably be in double-stranded DNA. A deficiency in either repair protein rendered cells more sensitive to CAA, with loss of AlkA conferring a more severe phenotype than loss of AlkB.[10]

To compare how BER and DRR are affected by DNA packaging, the processes were compared in a population of NCPs with globally incorporated εA lesions.[82] The ability of AAG to excise εA from NCPs was compared to the ability of ALKBH2 to directly repair the lesion. While AAG activity generally correlated with rotational positioning of a lesion in the NCPs,[73, 74] the same correlation was not observed for ALKBH2. Furthermore, while only AAG has, at some sites, full activity on εA in NCPs, ALKBH2 is better at repairing occluded εA lesions that are poorly excised by AAG. Modelling of AAG and ALKBH2 docked at OUT sites in an NCP provided insight into binding of these two enzymes.[82] Steric interactions between the histone core and the long loop of ALKBH2, which is known to play an essential role in substrate binding, modulates binding to the NCP. This comparison emphasizes how the BER and DRR pathways may work together to repair εA lesions yet certain sites in the NCP are still not readily repaired.[82]

6. BIOLOGICAL IMPLICATIONS

In diseases like ulcerative colitis and irritable bowel disease, where chronic inflammation is a major side effect, LPO is increased. Increased amounts of gastric lesions lead to gastric cancer after exposure to *Helicobacter pylori*. Mouse models mimicking these diseases help understand how DNA repair can protect against colon carcinogenesis.[83] Mice were exposed to dextran sulfate sodium (DSS) to cause chronic inflammation and generate global DNA damage. AAG knockout mice accumulated damage suggesting that DNA repair is involved in preventing colon cancer.

AAG repair was shown to prevent epithelial damage in the colon and reduce the severity of DSS-induced colon tumors.[83]

Repair of εA lesions was observed in lung tissue extracts and blood leukocytes from cancer and healthy patients. Comparing normal and cancerous lung tissue, there was no difference in εA levels.[84] This result may be due to higher levels of repair needed to maintain lesion levels in cancer lung tissue. The authors verified that the levels of repair were not significantly affected by age, sex, or smoking habits. However, in leukocytes of cancer patients, significantly lower levels of εA repair lead to higher levels of lesions than in healthy individuals.[84] Repair levels were found to be especially low in lung adenocarcinoma leukocytes compared to lung squamous cell carcinoma. From these results the authors concluded that LPO has a more significant impact on lung adenocarcinoma and a lack of enzyme repair activity on εA may be a risk factor for this disease.[84]

Accumulation of εA lesions has also been correlated with chronic ethanol consumption.[85] εA is generated in liver cells over-expressing cytochrome P4502E1 (CYP2E1) when incubated with ethanol. Additionally, in liver biopsies εA lesions correlated significantly with CYP2E1; such a correlation was also found in the esophageal- and colorectal mucosa of alcoholics. The level of εA lesions is also increased in liver biopsies from patients with non-alcoholic steatohepatitis (NASH). In various animal models with fatty liver, CYP2E1 is induced and high levels of εA are observed, which are further elevated by alcohol consumption. Children with NASH have elevated levels of εA and these lesions may contribute to hepatocellular cancer development later in life. These data implicate εA as a driving force for malignant disease progression.

To determine the effects of DNA sequence context, levels of εA repair by AAG were observed in mutation hotspots of the p53 tumor suppressor gene.[86] These results were compared to non-hotspot locations in hepatocytes and endothelial cells. In-cell and *in vitro* experiments showed that low AAG turnover causes decreased repair at mutation hotspots. Specifically, at hotspots, AAG product dissociation rate was about 5-12 times lower than at non-hotspots.[86] These experiments further suggest the importance of BER in the maintenance of genomic stability and prevention of cancer.

While BER and DRR generally modulate DNA damage to prevent biological consequences, these processes can also lead to adverse effects. Alkylating agents used as chemotherapeutics induce a range of DNA lesions meant to be cytotoxic to cancer cells.[87] When repair pathways respond to this damage, alkylation sensitivity has been observed from the cellular to organismal level.[87-89] Specifically, AAG has been shown to promote alkylation-induced tissue damage and whole animal lethality[90] as well as ischemia reperfusion injury[91] in mice. In both experiments, wild-type mice showed the respective effects of alkylation sensitivity compared to $AAG^{-/-}$ mice. It is thought that an upregulation of glycosylase activity in response to the alkylation damage, if not complemented by activity of the downstream enzymes of BER, leads to an increase in AP sites, double-strand breaks, and is therefore toxic to the cell.[90, 91] These consequences of alkylation-induced damage highlight the disadvantage of toxicity to non-cancerous tissue and the importance of balance between alkylation damage and repair for it to remain an effective treatment.

7. CONCLUSIONS

Biochemical and cell-based assays highlighted that εA is only weakly mutagenic in repair-proficient *E. coli*, but is significantly genotoxic and mutagenic in mammalian and human cells. In

the absence of repair of εA, mutational hotspots and cancerous tissues are prominent. BER and DRR work to alleviate the effects of εA damage but are affected by chromatin packaging. εA serves as a representative of many types of nucleobase damage. Future studies can elucidate the balance between replication and repair of lesions to better understand prevention of disease and other biological consequences.

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BIOGRAPHIES

Katelyn Rioux received her B.S. degree in Chemistry with a concentration in Biochemistry in 2018 from Worcester State University. At WSU, she conducted computational research with Prof. Eihab Jaber on the contribution of hydrogen bonding to the stability of guanine tetramers as well as analytical research with Prof. Kathleen Murphy on quantifying pharmaceutical pollutants in wastewater developed for the classroom laboratory. She is currently a Ph.D. candidate working under the supervision of Prof. Sarah Delaney in the

Department of Chemistry at Brown University. Her current research focuses on the effects of chromatin packaging on base excision repair.

 **Sarah Delaney** received her B.A. in Chemistry from Middlebury College and conducted research with Prof. Sunhee Choi on the mechanism of action of anti-cancer analogs of cisplatin. She completed her Ph.D. at the California Institute of Technology, working in the laboratory of Prof. Jacqueline Barton on the ability of the DNA to serve as a medium for charge transfer reactions. Prof. Delaney was a Damon Runyon postdoctoral fellow in the laboratory of John Essigmann at MIT where she studied the mutagenicity and toxicity of a variety of oxidized guanine lesions. She is currently a Professor of Chemistry at Brown University where she also serves as the Director of Graduate Studies. Research in her laboratory is focused on establishing a chemically-logical roadmap to understand how DNA damage relates to genetic change and human disease. In recognition of her research accomplishments she was awarded an Outstanding New Environmental Scientist (ONES) Award from NIH/NIEHS. Attesting to her skills and commitment to mentoring and training the next generation of scientists, Prof. Delaney was awarded the Philip J. Bray Award for Excellence in Teaching in the Physical Sciences and the Graduate Student Mentoring and Advising Award, both from Brown University. In addition to researching the biochemistry of DNA damage she has an interest in cooking and how chemistry

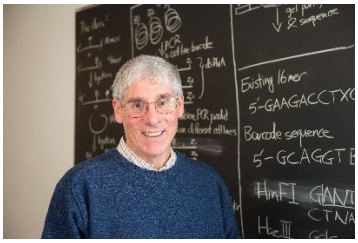
influences food. She teaches courses on *Chemical Biology*, *Organic Chemistry*, and a broad interest course entitled *Kitchen Chemistry*.

DEDICATION

I (S.D.) vividly remember the first time I met John Essigmann. I had traveled to MIT to interview for a postdoctoral position in his laboratory. As he welcomed me into his office, I noticed the shelves of three-ring binders labeled with the names of various biochemistry courses he had taught over the years. He pulled one down and flipped through a few colorful pages revealing a stunning collection of intricately drawn chemical structures, biochemical pathways, and bullet points. As we sat down at a table in his office, I saw that he had laid out an oversized sketch pad, the type used by artists, and a collection of colored markers. I quickly recognized the oversized pieces of paper that had been carefully folded into the binders lining the walls. As John and I discussed ongoing research in the lab, and ideas for new directions, he masterfully translated our conversation into structures and graphics on the pages of the sketch pad. He effortlessly rattled off references to papers that bolstered his hypotheses and peppered me with questions. But rather than feeling like I was being questioned in an interview, it felt like a discussion with a colleague who wanted to work together to push the frontiers of science forward. I came to John's lab as a chemist having studied the ability of DNA to mediate charge transfer reactions with Jackie Barton at Caltech, and it was my goal as a postdoc to learn some "biology". For me, that meant examining the mutagenicity and toxicity of oxidized guanine lesions in *E. coli*. I learned so much from John about toxicology and the repercussions of DNA damage, but I learned much more than the science. John is an outstanding teacher, a tremendous

storyteller, and one of the most effective communicators of science I have met. In his laboratory John brought together a talented group of folks from a wide variety of scientific backgrounds – synthetic chemists, biochemists, toxicologists, mathematicians, and animal biologists. And while he is one of the leaders in chemical toxicology, he was always sure to give credit to his lab members and acknowledge the work of others in the field. As members of the lab, John gave us all so much freedom and independence to pursue the science that interested us most. I remember him saying "It's my job to get the money and your job to do the science." I am thankful for the time he spent with me brainstorming for and writing grant proposals. To this day, I still return to a proposal we wrote together when I need a reminder of excellent grantsmanship. I am grateful for my time in John's lab, which greatly influenced my career and the way I strive to run my own research lab, and for John's continued generosity and mentorship in the ensuing years.

BIOGRAPHY OF MENTOR



John Essigmann is currently the William R. (1956) and Betsy P. Leitch Professor in Residence of Chemistry in the Department of Chemistry at MIT and Professor of Toxicology and Biological Engineering in the Department of Biological Engineering at MIT. He served as the Associate Head of the Department of Chemistry until 2012, a role in which he was responsible for graduate and undergraduate education, and from 2012 to 2019 he was the Director of the

MIT Center for Environmental Health Sciences. He also serves as the longstanding Housemaster of Simmons Hall, an undergraduate dormitory at MIT.

Research in the Essigmann group uses their ability to chemically synthesize oligonucleotides containing DNA lesions formed by environmental toxins and chemotherapeutic drugs. The group developed ways to introduce these oligonucleotides into the genomes of viruses, which are then replicated inside cells. For many environmental carcinogens and drugs, the group has defined the type and amount of mutations induced and the genetic requirements for these mutational changes. The group has also applied similar techniques to elucidate the mechanism by which DNA damage caused by anticancer drugs cause cell death.

Prof. Essigmann has received numerous awards in recognition of his research accomplishments, excellence in teaching, and mentorship. At MIT, he twice received the Graduate Student Council Teaching Award. He also received the School of Science Teaching Prize for Excellence in Undergraduate Education and was appointed a Margaret MacVicar Faculty Fellow for a ten-year term. He is the recipient of both the National Cancer Institute Outstanding Investigator Award and MERIT Award, the Arthur C. Smith Award, the Mutation Research Award for Scientific Excellence, and a Susan B. Komen Breast Cancer Foundation Award. Prof. Essigmann was the recipient of the Princess Chulabhorn Gold Medal award from Thailand for his research on problems affecting the developing world and for a teaching program he and Prof. Ram Sasisekharan helped create in Thailand. For over two decades Prof. Essigmann taught in Thailand through the Asian Institute of Technology, Mahidol University, and the Chulabhorn Graduate Institute. Prof. Essigmann was also awarded a Martin Luther King, Jr. Leadership Award from MIT for his work with and support of underrepresented populations. He has served on many

government-sponsored research review groups, most recently the Council of the National Institute of Environmental Health Sciences.

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