A Chemical Genetics Analysis of the Roles of Bypass Polymerase DinB and DNA Repair Protein AlkB in Processing $N^2$-Alkylguanine Lesions In Vivo

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Introduction

The genome is vulnerable to damage from exogenous and endogenous chemical reactions, including alkylation, oxidation, and deamination [1,2]. Not surprisingly, several different lesion tolerance and repair pathways have evolved to deal with these types of DNA damage. DNA adduct bypass by translesion synthesis (TLS) polymerases allows for genome replication in the presence of DNA damage, while canonical DNA repair pathways, which include direct repair, base-excision repair, nucleotide-excision repair, non-homologous end joining and homologous recombination remove such damage prior to replication. A holistic approach for addressing the impact of such variables on lesion toxicity and mutagenicity is shown in Figure 1.

The present work explored the in vivo consequences (replication efficiency and fidelity) and genetic requirements (presence or absence of bypass polymerases or DNA repair enzymes) of four $N^2$-guanine DNA alkyl adducts: $N^2$-furfurylguanine (FF), 2-tetrahydrofuran-2-yl-methylguanine (HF), 2-methylguanine (m2G), and 2-ethylguanine (e2G) (Figure 1A). Previously, we have shown that the direct reversal DNA repair enzyme AlkB can repair these lesions in vitro [3]; the present study explored the relevance of AlkB repair of these lesions in living cells. Additionally, we have shown that both DinB and pol k are capable of bypassing the FF lesion in vitro [4]; however, it remained unknown whether the same

Abstract

DinB, the E. coli translesion synthesis polymerase, has been shown to bypass several $N^2$-alkylguanine adducts in vitro, including $N^2$-furfurylguanine, the structural analog of the DNA adduct formed by the antibacterial agent nitrofurazone. Recently, it was demonstrated that the Fe(II)- and α-ketoglutarate-dependent dioxygenase AlkB, a DNA repair enzyme, can dealkylate in vitro a series of $N^2$-alkylguanines, including $N^2$-furfurylguanine. The present study explored, head to head, the in vivo relative contributions of these two DNA maintenance pathways (replicative bypass vs. repair) as they processed a series of structurally varied, biologically relevant $N^2$-alkylguanine lesions: $N^2$-furfurylguanine (FF), 2-tetrahydrofuran-2-yl-methylguanine (HF), 2-methylguanine, and 2-ethylguanine. Each lesion was chemically synthesized and incorporated site-specifically into an M13 bacteriophage genome, which was then replicated in E. coli cells deficient or proficient for DinB and AlkB (4 strains in total). Biochemical tools were employed to analyze the relative replication efficiencies of the phage (a measure of the bypass efficiency of each lesion) and the base composition at the lesion site after replication (a measure of the mutagenesis profile of each lesion). The main findings were: 1) Among the lesions studied, the bulky FF and HF lesions proved to be strong replication blocks when introduced site-specifically on a single-stranded vector in DinB deficient cells. This toxic effect disappeared in the strains expressing physiological levels of DinB. 2) AlkB is known to repair $N^2$-alkylguanine lesions in vitro; however, the presence of AlkB showed no relief from the replication blocks induced by FF and HF in vivo. 3) The mutagenic properties of the entire series of $N^2$-alkylguanines adducts were investigated in vivo for the first time. None of the adducts were mutagenic under the conditions evaluated, regardless of the DinB or AlkB cellular status. Taken together, the data indicated that the cellular pathway to combat bulky $N^2$-alkylguanine DNA adducts was DinB-dependent lesion bypass.

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were true in vivo. Given these previous observations, both AlkB and DinB were selected as genetic variables for our in vivo chemical genetics study.

All of the $N^2$-alkylguanine lesions in this study are important biomarkers or structural mimics of exposure to known mutagens or carcinogens. The m2G adduct, the smallest alkyl adduct in the series, is a mimic of the imino or hydroxymethyl adducts formed by the reaction $N^2$-amino group of guanine with formaldehyde [5,6]. Classified by the International Agency for Research on Cancer (IARC) as a human carcinogen [7], formaldehyde is an ubiquitous pollutant in vehicle exhaust and cigarette smoke and a common endogenous metabolism byproduct [7]. The m2G adduct can also form when cellular DNA is exposed to exogenous [5] or endogenous [8] methylating agents. The e2G DNA adduct is a well-established biomarker of exposure to acetaldehyde [9–14]. Acetaldehyde, classified as an animal carcinogen, and as a possible human carcinogen (group 2B) by IARC [15], is both an exogenous pollutant in cigarette smoke [16,17] and an endogenous metabolite of ethanol [18–20]. The FF lesion is a mimic of the $N^2$-guanine adduct of nitrofurazone (NFZ) [21], a potent antibacterial agent commonly used for treating serious skin conditions (burns, grafts) [22,23]. NFZ reduction metabolites have been shown to be mutagenic and carcinogenic in rodent models [22,24] and to cause free radical damage, strand breaks, and $N^2$-dG adducts in DNA [25–27]. HF, the saturated analog of FF is included here to study the effect of aromaticity on bypass and repair of a bulky $N^2$-alkylguanine.

In Escherichia coli, the dinB gene encodes the Y family DNA polymerase pol IV (DinB) [28,29], which is one of three TLS polymerases that is part of the SOS pathway [30]. The dinB gene was first identified as one of the damage inducible genes in E. coli [31–34], and it is the only Y-family DNA polymerase that is conserved across all domains of life (bacteria, eukaryotes, and archaea) [35], a result of selective constraints imposed on the encoding gene [36]. It is also present at a relatively high intracellular concentration of 250 molecules per cell, more than that of DNA pol III (10–30 molecules/cell) and on par with the level of the $\beta$-processivity clamp [37,38]. Upon SOS induction, the concentration of DinB escalates to 2500 molecules per cell [39]. DinB is implicated in both the insertion and extension steps in the bypass of lesions that block replicative polymerases [40]. It may also have a role in alleviating the cytotoxicity of alkyl DNA adducts as demonstrated by Bjedov et al., who showed that DinB is essential for the survival of AalkA Aag cells exposed to the...
alkylating agent methyl methanesulfonate [38]. In vitro experiments have shown that DinB can perform DNA synthesis, with efficiency and accuracy, across a variety of base modifications [29], such as FF [4] and N2-(1-carboxyethyl)-2'-deoxyguanosine (N2-CEdG) [41]. In vivo bypass is observed for site-specifically placed benzo[a]pyrene (BaP) lesions [42–45], and for lesions induced by chemical treatment of cells with 4-nitroquinoline-1-oxide (4-NQO) and NEZ [4] as well as incorporation of reactive oxygen species-derived dNTPs [46,47]. The function of DinB (and also its human homolog pol ε) is of particular importance as cells are exposed to alkylating agents from both endogenous and exogenous sources, including cancer, inflammation and chemotherapy [2].

The AlkB enzyme is an Fe(II)- and 2-ketoglutarate-dependent dioxygenase that repairs DNA alkyl lesions by a direct reversal of damage mechanism as part of the adaptive response in E. coli [48,49]. Different homologs of AlkB exist in prokaryotic and eukaryotic species; nine such homologs exist in mammalian cells [48,49]. Different homologs of AlkB exist in prokaryotic and E. coli damage mechanism as part of the adaptive response in PLOS ONE | www.plosone.org 3 April 2014 | Volume 9 | Issue 4 | e94716

DNA [54–56]. In the case of the AlkB is much more efficient at repairing lesions in single-stranded repair many of these lesions in a double-stranded DNA context, current study (m2G, e2G, FF and HF) [3]. Although AlkB can including cancer, inflammation and chemotherapy [2].

Oligonucleotides

All unmodified oligonucleotides and primers were obtained from Integrated DNA Technologies (IDT, Coralville, IA) unless specified otherwise. The lesion-containing 16mer oligonucleotides of the sequence 5’-GAAGACCTXGGCCTGC3’ (where X denotes an N2-alkylguanine lesion or controls) were synthesized using phosphoramidite solid-phase methods described before [3,4,56]. A convertible nucleoside, 2-fluoro-O2-[(trimethylsilyl)-2’-deoxyinosine (ChemGenes, Wilmington, MA) was initially incorporated at the X position (Figure 1A). After hydrolysis from the resin and deprotection with 0.1 M NaOH for 8 h at 25°C, the oligonucleotides were desalted (SepPak, Millipore) and lyophilized. The 2-fluoro atom was then substituted with an amine group in parallel reactions with 2-fold molar excess of methyl-amine, ethyl-amine, furfurylamine and tetrahydrofurfuryl amine to yield m2G, e2G, FF and HF, respectively. The reactions were carried out in DMISO, in the presence of N,N-diisopropylhydrazine (5X molar excess) at 60°C for 12 h. Finally, the trimethylsilyl group was removed with by treatment with an excess solution of 3% acetic acid at room temperature for 4 h. The deprotected oligonucleotides were purified by reversed-phase HPLC using an analytical column (Varian Microsorb-MV 100-5 C18 250 × 4.6 mm) at a flow of 1 ml/min and a gradient of 0 to 50% B over 60 min (A: 100 mM triethylammonium acetate; B: 100% acetonitrile).

Sixteen-mer oligonucleotides with the above sequence but with X = G, A, T, or C, were used as controls. Scaffold oligonucleotides (5’-GTTCTTCCACTGATACATGTTATAC 3’ and 5’-AAAAGCGCGGCCAGTGATTTGACGG 3’) were used to ligate the 16mers into the M13 vector. The 19-mer of the sequence 5’-GAAGACCTTGTAGGGCAGG 3’ was used to construct the “+3 competitor” for the CRAB assay.

DinB status of the constructed cell lines was confirmed using the upstream primer 5’-GATATGTGCTGTGCAAAAAATGCG 5’ and the downstream primer 5’-GGCTGGCACTTAAAGAGATATCCTGTGGGG 3’. The M13 progeny DNA was amplified in the CRAB/REAP assays using the following: 5’-YCACTATGAGACCATTGCTTCACTGGAGAC 3’ (CRAB/REAP forward primer), 5’-YCAAGGTTTGCCATTCACTGAGACCTTTGCTGA 3’ (CRAB reverse primer) and 5’-YTGCAAAACGGGCTGCCGTGGAACGAGCCGAGC 3’ (REAP reverse primer).

Materials and Methods

Cell strains

All the E. coli strains used in this work contain the F’ episome, which enables infection by M13 phage. GW5100 strain was used for large scale preparation of M13 phage DNA; SCS110 (JM110, endA1) was used for amplification of progeny phage post-electroporation; NR9050 strain was used for double agar plating with X-gal for blue-clear detection of plaques. HK81 (as AB1157, but nalA) and HK82 (as AB1157, but nalA alkB22, AlkB-deficient) were the DinB”AlkB” and DinB’AlkB” strains used in the study. P1 vi phage transduction was used to create HK83 (as HK81, but dinB-deficient) and HK84 (as HK82, but dinB-deficient). Briefly, recipient cells (HK81 and HK82) from 1 ml of overnight saturated cultures were resuspended in 500 μL LB containing 10 mM MgSO4 and 5 mM CaCl2. Approximately 100 μl of these solutions were mixed with 0, 25, 50, or 100 μL of P1 lysate containing a chloramphenicol resistance gene (cam) flanked by fit sequences designed for insertion at the dinB site. After 30 min incubation at 30°C, 100 μl of 1 M sodium citrate was added to stop the P1 infection. Following an additional incubation at 30°C for 1 h to allow expression of the chloramphenicol gene, the cells were plated on LB + chloramphenicol (10 μg/ml) plates. After an overnight incubation at 37°C, colonies were obtained, replated on LB + chloramphenicol plates and genotype confirmed by PCR.

Enzymes and chemicals

All restriction enzymes, T4 DNA Ligase, T4 DNA polymerase and their enzyme reaction buffers were from New England Biolabs. Shrimp alkaline phosphatase (SAP) was from Roche. P1 nuclease, 3-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal) and isopropyl b-D-1-thiogalactopyranoside (IPTG) were from Sigma Aldrich. T4 Poly nucleotide kinase was from Affymetrix. Sephadex G-50 Fine resin was from Amersham Biosciences. Hydroxylapatite resin, 19:1 acrylamide-bisacrylamide solution, and N,N,N,N-tetra-methyl-ethylenediamine (TEMED) were from Bio-Rad. Phenol:chloroform:isoamyl alcohol (25:24:1; pH 8) was from Invitrogen. [32P-γ-ATP] was from Perkin Elmer. Non-radioactive ATP was from GE Healthcare Lifesciences.
DinB Bypasses FF and HF Lesions

Results

Lesion bypass and mutagenesis assays

The relative bypass of each lesion was measured using the CRAB assay; mutational analysis was performed by using the REAP assay [57]. Briefly, the constructed viral genomes were first normalized using an established protocol [57]. Each lesion-containing genome was then mixed with the “+3” competitor genome in a 75:25 ratio (ratio empirically determined, see Supporting Methods S1 in File S1) and then electroporated into E. coli strains of all combinations of AlkB and DinB proficiency and deficiency. After 6 h incubation at 37°C, the progeny phage were isolated and amplified by infecting SCs110 wild-type cells, to dilute out any lesion-containing genomes that did not electroporate and replicate in cells. Single-stranded M13 DNA was then isolated from the amplified progeny, using the M13 Qiaprep columns (Qiagen). The region of interest was then PCR amplified using the CRAB primers for the lesion bypass assay, or the REAP primers for the mutagenesis assay. The PCR products were subsequently digested with BbsI, HaeIII and radiolabeled to yield a 21-mer DNA fragment that contains at its 5’ end the specific site that initially contained the lesion of interest. The “+3” competitor genome was only amplified by the CRAB primers and yielded a 21-mer fragment. To quantitate the lesion bypass, the ratio between the intensities of the 18-mer and 21-mer fragments was determined and normalized to the ratio of the same bands for the unmodified “G” control, considered 100% bypass. To analyze the mutagenicity of a lesion, the radiolabeled 18-mer band was cut out from the gel and digested to single nucleotide monophosphates with nuclease P1. The nucleotides were then separated on PEI-TLC plates using a saturated solution of ammonium phosphate (pH = 5.8), and the radioactive signals quantitated using phosphorimagery. An approximately equimolar of GATC control genome mixture, which yielded four distinct TLC spots corresponding to the four normal nucleotides, was used as a mixture of standards. The detailed protocols for the CRAB and REAP assays are included in the Supporting Methods S1 in File S1.

Results

DinB Bypasses FF and HF Lesions In Vivo

The CRAB assay is a quantitative tool used to determine to what extent a given lesion blocks DNA replication in vivo (Figure 1B) [57]. In essence, a lesion-bearing genome is mixed with a nonlesion competitor genome in a specific input ratio and replicated in E. coli cells of a given repair/bypass genetic background. The output ratio of progeny phage from the lesion-bearing genome with respect to the nonlesion competitor genome indicates the relative amount of replication from the lesion-bearing genome. A decrease in the lesion:competitor output ratio signifies a lesion-induced replication block (i.e., lower bypass efficiency). The competitor genome, lacking a replication-inhibiting lesion, acts as an internal standard in this competitive assay. The CRAB assay was performed on the N²-dG lesions (m2G, e2G, FF, and HF) in E. coli strains that capture all possible combinations of DinB and AlkB proficiency/deficiency (a total of 4 strains). The genomes of m3C, a good substrate for AlkB both in vivo and in vitro [53], and unmodified “G” were used as controls.

The results of the CRAB assay for toxicity are summarized in Figure 2 and Table S1 in File S1. To tease out the relative contribution of each genetic variable (DinB and AlkB) to the bypass efficiency of N²-dG lesions, data were graphed in pairs in which one of the variables was kept constant. Based on the previously reported in vitro findings, it was hypothesized that both DinB and AlkB might act on N²-dG lesions; therefore, the most relevant pair wise comparisons are those in which one of these enzymes is knocked out. To understand the effect of DinB on the in vivo bypass of N²-dG lesions (independent of AlkB), the two AlkB⁻ strains were compared (AlkB⁻ DinB⁺ vs. AlkB⁻ DinB⁻) (Figure 2A). To understand the effect of AlkB (independent of DinB), the two DinB⁻ strains were compared (AlkB⁺ DinB⁻ vs. AlkB⁺ DinB⁺).

In the absence of DinB, the bulky lesions FF and HF are strong blocks to replication, with measured bypass efficiencies of only 36% and 40%, respectively (Figure 2A). However, the presence of DinB more than doubled the bypass efficiencies of FF and HF to ∼99% (p-value = 0.0006) and ∼87% (p-value = 0.0015), respectively (Figure 2A), thus greatly relieving the replication block. By contrast, the presence of AlkB did not change the bypass efficiencies of FF and HF; no significant difference in bypass was observed between the two DinB⁻ strains (Figure 2B). This finding was unexpected, given that AlkB is biochemically competent to repair the FF and HF lesions in vitro [59]. Possible reasons for AlkB’s lack of effect on the bypass efficiencies of FF and HF are included in the discussion section.

The simple-alkyl lesions m2G and e2G were not significant replication blocks in the double mutant strain (AlkB⁻ DinB⁺); the presence of DinB (Figure 2A), AlkB (Figure 2B) or both (Table S1 in File S1) did not significantly change the relative bypass of these lesions. From the point of view of this assay, these two modified guanines behave like a normal guanine, being virtually invisible to the replication machinery.

Consistent with previously published data [53], the m3C lesion was a good control for AlkB activity. In AlkB⁻ strains, m3C was a very strong block to replication (relative bypass ∼10%). The presence of DinB did not alleviate the toxicity of m3C (Figure 2A). However, in AlkB⁺ strains, the relative bypass of m3C jumped to ∼100%, consistent with the expectation that this lesion is efficiently repaired by AlkB, before being encountered by replicating polymerases.

The N²-dG Lesions are Not Significantly Mutagenic in any Cell Strain

The REAP assay determines the mutation frequency and mutation type after the lesion of interest has been processed by the intracellular replication machinery [57]. The REAP assay was performed on all of the N²-dG lesions, the control m3C, unmodified G, and an approximately equimolar mixture of genomes carrying unmodified ‘G,’ ‘A,’ ‘T,’ and ‘C’ bases at the lesion site (denoted as GATC, Figure 3).
One could not predict a priori whether or not these lesions would be mutagenic in vivo, but the results clearly show that none of the N2-alkylguanine lesions are significantly mutagenic in the presence or absence of DinB or AlkB (Figure 3 and Table S2 in File S1). HF and FF show mutation frequencies of ~1% regardless of DinB status, which is essentially the same as the mutation frequency we detected for the control genome having a normal G at the lesion site. m2G and e2G also show a small non-G signal of 1 to 4% in all cell strains (Table S2 in File S1), but this mutation frequency is not statistically different from the control genome baseline.

Discussion

The Effect of DinB on N2-alkylguanine Adducts

Figure 2. Bypass efficiency of m2G, e2G, FF, and HF as a function of DinB status (A) or AlkB status (B). M13 genomes containing the four N2-alkylguanine lesions were constructed and normalized to one another before being combined with a competitor genome; genomes containing m3C and undamaged G were used as controls. Each mixture was transformed into the E. coli cell strains indicated at the top of each graph in triplicate, and bypass efficiencies were calculated by using the signal from the undamaged G genome mixture as 100% bypass; error bars represent one standard deviation. For the FF and HF lesions, the significance of the difference between two populations was tested using the Student’s two-tailed t test. (** indicates p-value <0.001, N.S. indicates not significant). All bypass data are summarized in Table S1 in File S1.
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Figure 3. Mutagenesis of m2G, e2G, FF, and HF in the four strains of E. coli cells lacking or expressing DinB or AlkB. Each panel (A to D) corresponds to the E.coli strain indicated at the top of the graph. Genomes containing m3C, undamaged G, and an approximately equimolar mixture of unmodified G/A/T/C bases at the site of inquiry (denoted as GATC) were used as controls. Genomes containing the lesions of interest were transfected into E. coli in triplicate. The percentage of G, A, T, and C at the lesion site reveals the mutagenicity of the lesions, with error bars representing one standard deviation. All mutagenesis data are summarized in Table S2 in File S1.
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polymerase DinB efficiently bypasses the FF adduct (Figure 1A), a homolog of the major adduct formed by the reaction of NFZ with guanine [4]. Catalytically, DinB is about 15-fold more proficient at inserting a cytosine opposite the FF adduct than opposite undamaged guanine [4]. Additionally, due to its increased affinity for dCTP, DinB is 25-fold more efficient at extending beyond a cytosine opposite the FF lesion than opposite guanine [40]. The current study is the first in vivo quantitative analysis of the mutagenic and toxic properties of the FF lesion and its saturated homolog HF, as a function of the DinB genotype of the cell. The key findings of this study are: 1) FF and HF are strong blocks to replication when introduced in DinB cells on a single-stranded vector; 2) The replication inhibition caused by FF and HF is substantially alleviated by the presence of DinB in vivo, further supporting the role of DinB in N2-alkylguanine lesion bypass observed previously in vitro [4]; 3) The lesion bypass occurs in an error-free manner, as the correct base (cytosine) is always inserted opposite the guanine lesions by DinB. This last finding is also in concordance with previously published in vitro bypass results [4,40]. Taken together with the in vitro data available for DinB and its homologs, the current study suggests that these Y-family polymerases bypass bulky N2-guanine adducts, such as the one formed by NFZ, in an error-free manner, in vivo. Given that NFZ is an antibiotic, DinB may be an important biochemical shield evolved for the defense of E. coli against certain types of ‘chemical warfare’ from other species. This finding is also consistent with the proposed role of DinB in transcription-coupled translesion synthesis across N2-dG lesions formed by NFZ [60]. It is worth noting that, while FF and HF are strong blocks to replication in the absence of DinB, the level of bypass detected in the DinB- cells (28 to 40%) was higher than that seen for the concurrently run positive control m3C (∼10%) or other alkyl lesions (i.e., m1G or m3T) tested previously in AlkB-negative cells [53]. One possible explanation is that there might be other bypass/repair mechanisms at play (i.e., Pol V) that assist with lesion tolerance in the absence of DinB to the extent observed in this study. While it has been proposed that nucleotide excision repair (and not TLS) might be the primary repair pathway that deals with NFZ-induced damage [61], that pathway requires a double-stranded DNA context, which is obviated by our experimental system that utilizes single-stranded M13 genomes.

In contrast to the bulky FF and HF lesions, the small N2-alkylguanine lesions m2G and e2G were neither replication blocks nor were they mutagenic in any of the E. coli cells tested. Since no replication inhibition was seen in DinB- cells, the presence of DinB did not change the bypass efficiencies of m2G and e2G; in fact, there is no evidence that DinB was actually recruited at the replication fork, when m2G or e2G lesions were encountered. This in vivo result under physiological conditions is in contrast with what has been observed for e2G in in vitro assays with other Y-family and replicative polymerases [62–64]. It could be that there is another enzyme or enzymes that preferentially and efficiently repairs or bypasses these lesions such that the supplementary role of DinB in the bypass of m2G and e2G is overshadowed beyond the detection limit of our assay.

One possible explanation for the non-toxic phenotype of FF and HF seen in DinB+ cells is that DinB can tolerate the N2-alkyldG lesions. These lesions can occupy the minor groove of DNA [63] and interfere with polymerase-minor groove interactions [66–68], should the alkyl group swivel near the N3 atom of guanine. Several B-family polymerases are known to have a conserved motif that scans the DNA minor groove for lesions and misincorporations [69], which is lacking in the Y-family DNA polymerases. It is speculated that this may be the case for the Y-family mammalian pol κ, as deduced from X-ray crystal structure studies of the catalytic core of the polymerase with a primer-template DNA and an incoming nucleotide; the structure reveals the lack of a “steric gate” in scanning the minor groove at the primer-template junction [70]. It is proposed that DinB can accommodate minor groove lesions to enable bypass with correct base pairing, even for lesions such as BaP [43], containing alkyl groups much bulkier than those used in the current study.

The FF and HF lesions, while they are strong replication blocks, are not mutagenic in any repair/bypass background; these results can be explained by the availability of a hydrogen atom at the N2 position of guanine and the possibility of free rotation around guanine’s exocyclic nitrogen-carbon bond. This free rotation can generate a guanine-like Watson-Crick hydrogen bonding pattern with cytosine. In addition, free rotation around the carbon-nitrogen bond would enable the extraneous alkyl group, irrespective of size, to swivel away from the base pairing face of guanine into the minor groove, thus alleviating steric hindrance caused by attachments to the N2 position. Similar freely rotating small alkyl modifications, such as N2-methyl adenine and N2-methylcytosine, are very well tolerated and even utilized as DNA replication biomarkers in prokaryotic cells [71]. Alternative mechanisms to explain the correct base pairing of N2-dG lesions with cytosine, such as ‘wobble’ base pairing [63], or Hoogsteen base pairing [62,72] have been proposed or observed. However, for an N2-dG lesion to pair correctly with a cytosine using either mechanism, the cytosine base has to be in its protonated form (for Hoogsteen base pairing), or its imine tautomeric form (for wobble base pairing), which is rarely observed in duplex DNA under physiological conditions.

The Effect of AlkB on N2-dG Lesions

A number of N2-dG lesions were tested as possible substrates for AlkB, in line with the theme of this study on the cellular processing of N2-guanine DNA lesions. While all the four N2-dG lesions studied are repaired by AlkB in vitro [59], the results from this study show that AlkB does not have a discernible effect on either the polymerase bypass or mutagenicity of these lesions in vivo. FF and HF are replication blocks in the absence of DinB (Figure 2A and Table S1 in File S1). While the presence of DinB alleviates the replication inhibition, AlkB has no significant effect on the bypass efficiencies of FF and HF lesions in DinB- cells (Figure 2B and Table S1 in File S1). There are two possible explanations for these experimental observations: 1) Given AlkB’s low cellular concentration (2 molecules/cell) [73], it does not effectively repair the bulky FF and HF lesions in vivo, before they are encountered by the replication machinery; or 2) AlkB does perform the initial oxidation step on these lesions, but the subsequent intermediates, (such as FF-2H and HO-HF, described in our previous paper [59]), may be long-lived and equally strong replication blocks. By contrast, the smaller N2-dG lesions (m2G and e2G) were not toxic or mutagenic in any of the four cell strains studied (Figures 2 and 3). This suggests that at least in AlkB- cells, a mechanism of tolerance of small minor groove lesions by replicative polymerases is operating; as mentioned above, these lesions may rotate into the minor groove during replication [59,70]. However, effective repair of m2G or e2G by AlkB in vivo may still occur in the AlkB- cell strains, supplementing the free rotation mechanism.

In conclusion, this work demonstrates that inside living cells, DNA adduct bypass by DinB is the mechanism of choice to overcome the deleterious consequences of bulky N2-dG adducts, such as FF and HF. While we have shown that AlkB can repair these lesions and the simpler m2G and e2G adducts in vitro, the AlkB effect on FF and HF in vivo is not significant, possibly because
the repair intermediates are also strong replication blocks, long-lived and not cleared efficiently before encountering replication machinery. Our study highlights the fact that even though multiple DNA repair or tolerance pathways can act on N2-alkylguanine DNA lesions in vivo, one pathway, lesion bypass, is the preferred mechanism for maintaining genomic integrity in vivo.

Supporting Information

File S1  Supporting Methods S1, Supporting Tables S1–S2 and Supporting References, (PDF)

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Conceived and designed the experiments: NS BIF DL JCD LEF JJF GCW JME. Performed the experiments: NS BIF DL JCD LET JME. Analyzed the data: NS BIF DL JCD JME. Contributed reagents/materials/analysis tools: LEF JFF. Wrote the paper: NS BIF DL JCD LET JME GCW JME.
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