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AP endonuclease 1 prevents the extension of a T/G mismatch by DNA polymerase β to prevent mutations in CpGs during base excision repair

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ABSTRACT

Dynamics of DNA methylation and demethylation at CpG clusters are involved in gene regulation. CpG clusters have been identified as hot spots of mutagenesis because of their susceptibility to oxidative DNA damage. Damaged Cs and Gs at CpGs can disrupt a normal DNA methylation pattern through modulation of DNA methylation and demethylation, leading to mutations and deregulation of gene expression. DNA base excision repair (BER) plays a dual role of repairing oxidative DNA damage and mediating an active DNA demethylation pathway on CpG clusters through removal of a T/G mismatch resulting from deamination of a 5mC adjacent to a guanine that can be simultaneously damaged by oxidative stress. However, it remains unknown how BER processes clustered lesions in CpGs and what are the consequences from the repair of these lesions. In this study, we examined BER of an abasic lesion next to a DNA demethylation intermediate, the T/G mismatch in a CpG dinucleotide, and its effect on the integrity of CpGs. Surprisingly, we found that the abasic lesion completely abolished the activity of thymine DNA glycosylase (TDG) for removing the mismatched T. However, we found that APE1 could still efficiently incise the abasic lesion leaving a 3-terminus mismatched T, which was subsequently extended by pol β. This in turn resulted in a C to T transition mutation. Interestingly, we also found that APE1 3′–5′ exonuclease activity efficiently removed the mismatched T, thereby preventing pol β extension of the mismatched nucleotide and the resulting mutation. Our results demonstrate a crucial role of APE1 3′–5′ exonuclease activity in combating mutations in CpG clusters caused by an intermediate of DNA demethylation during BER.

1. Introduction

Dynamics of cytosine methylation, i.e., formation of 5-methyl cytosine (5mC) and demethylation of 5mC in CpG islands (CGIs) in the mammalian genome are actively involved in the regulation of gene expression, inactivation of the X chromosome, and gene imprinting, among others [1–4]. It is estimated that only approximately 1–2% of cytosines in the mammalian genome are unmethylated [5–8], whereas 60–90% of CpGs are methylated [1,9]. The DNA methylation pattern regulates gene transcription by facilitating or blocking access of transcription factors to gene promoter or transcribed regions directly [10,11], or by modulating the recruitment of methyl CpG binding proteins [12–15], as well as by altering histone modifications and chromatin structures [16–18]. A normal DNA methylation pattern is essential for maintaining the homeostasis of gene expression and cellular function, whereas an aberrant DNA methylation pattern is associated with the onset and progression of many diseases [19–21]. It has been found that hypermethylation of CpGs on tumor suppressor genes (TSGs) [22,23] and hypomethylation of CpGs on oncogenes result in deregulation of expression of the genes leading to the development of cancer [4].

5mC can be removed by a process called DNA demethylation, which can occur either passively or actively. Passive DNA demethylation results from the failure of DNA methyltransferases to methylate a cytosine during DNA replication and cell division [2]. Passive DNA demethylation may also result from the loss or substitution of cytosines by DNA damage and repair in the context of CpGs. Active DNA demethylation is mediated by sequential enzymatic reactions that can occur independent of DNA replication and cell division [2,3,24,25]. Removal of a 5mC by active DNA demethy-

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lation is initiated by modifications of the nucleotide through several types of enzymatic reactions, including hydroxylation, deamination and oxidation that convert the 5mC into a modified base or base lesion that is recognized and cleaved by DNA glycosylases [2,3]. This subsequently leads to replacement of the 5mC through the DNA base excision repair (BER) pathway [2,3,25]. It has been shown that BER-mediated active DNA demethylation is accomplished through several pathways depending on the type of modifications of 5mC that are removed by different types of DNA glycosylases [2,3,24,26]. One of the pathways is initiated by a direct deamination of 5mC by activation-induced cytidine deaminase (AID) that converts a 5mC to a thymine, resulting in a T/G mismatch [27]. The mismatched T can be subsequently excised by thymine DNA glycosylase (TDG) [25,28–30], leaving an abasic site that is then subjected to BER. This ultimately leads to replacement of the 5mC with an unmethylated C [2]. Another BER-mediated DNA demethylation pathway is initiated by single-strand-selective monofunctional uracil-DNA glycosylase 1 (SMUG1) that removes a 5-formyluracil generated from oxidation of a 5mC by a family of enzymes called Ten Eleven Translocation (TET), a methylcytosine dioxygenase [31–33], or from oxidative DNA damage induced by hydroxyl radicals [34,35]. Thus, in mammalian cells, DNA base damage and BER are strategically used as a mechanism for both passive and active DNA demethylation [26].

While the cytosine in a CpG dinucleotide is a substrate for DNA methylation and demethylation, the neighboring 3′-guanine is a hot spot of oxidative DNA damage. As the most abundant form of oxidative DNA damage in mammalian cells, 8-oxoguanine (8-oxoG) readily accumulates in CpGs and may affect the integrity of CpGs by modulating the production and processing of DNA demethylation intermediates. It has been found that 8-oxoG can cause accumulation of T/G mismatched base pairs when it occurs adjacent to 5mC by inhibiting the removal of the mismatched T [36]. Moreover, when BER of 8-oxoG encounters active DNA demethylation, repair of the lesion may be affected by a DNA demethylation intermediate. This has been supported by a recent study from the Wilson group showing that the efficiency of removal of 8-oxoG by OGG1 was significantly reduced by an adjacent 5′-T/G mismatch, a DNA demethylation intermediate generated from deamination of 5mC in CpGs [36]. This subsequently inhibited the completion of BER. This suggests that a base lesion interferes with an essential step of BER-mediated DNA demethylation, thereby compromising the efficiency and fidelity of DNA demethylation in CpGs. These studies further suggest that BER of oxidative DNA damage in CpGs and DNA demethylation intermediates has to be properly coordinated to maintain the integrity and fidelity of CpGs. However, it remains unknown how the fidelity of CpGs may be maintained by BER in coordinating its dual function in repairing a base lesion as well as in mediating active DNA demethylation. In this study, we explored how an abasic lesion that occurs in a CpG at the 3′-side of a T/G mismatch could affect the integrity of a CpG dinucleotide by modulating the removal of the mismatched T during BER, and how BER may coordinate the removal of the base lesion and maintenance of the fidelity of CpG dinucleotides. For the first time, we have shown that the abasic lesion completely inhibited the removal of its adjacent 5′-mismatched T by TDG leading to accumulation of the mismatched nucleotide. We show that DNA polymerase β (pol β) readily tolerated the mismatched T and efficiently extended the mismatched nucleotide, allowing the sustainment of the T/G mismatch and leading to a C to T transition mutation during BER. Interestingly, we discovered that AP endonuclease 1 (APE1) 3′–5′ exonuclease efficiently removed the mismatched T, thereby preventing the mutation. Our results indicate that APE1 3′–5′ exonuclease plays a crucial role in maintaining the integrity of CpGs during BER and DNA demethylation. This demonstrates that the coordination between BER enzymes effectively removes a 3′-mismatched T, thus preventing mutations that result from BER and BER-mediated active DNA demethylation. Our study provides new insights into the molecular mechanisms underlying the roles of BER in preventing T/G mismatches and sustaining the integrity of CpGs during BER and active DNA demethylation.

2. Materials and methods

2.1. Materials

Oligonucleotides were synthesized by Integrated DNA Technologies Inc. (Coralville, IA). The radionucleotides [γ-^{32}P] ATP (6000Ci/mmol) and cordycepin 5′-triphosphate 3′-[α-^{32}P] (5000Ci/mmol) were purchased from PerkinElmer Inc. (Boston, MA). Micro Bio-Spin 6 chromatography columns were from Bio-Rad (Hercules, CA). T4 polynucleotide kinase (PNK) and terminal deoxynucleotidyl transferase *(TdT)* were from Fermentas (Glen Burnie, MD). Adenosine 5′-triphosphate (ATP) (100 mM) was from USB (Cleveland, Ohio). Purified thymine DNA glycosylase (TDG) was from Enzymax, LLC (Lexington, Kentucky). Purified APE1, pol β, flap endonuclease 1 (FEN1) and DNA ligase I (LIG I) were generous gifts from Dr. Samuel H. Wilson at the Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Science/National Institutes of Health (NIEHS), Research Triangle Park, NC. All other reagents were from Thermo Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO).

2.2. Oligonucleotide substrates

An oligonucleotide substrate containing a T/G mismatched base pair adjacent to a G/C matched base pair or a tetrahydrofuran (THF), an abasic site analog, was designed to mimic the intermediates resulting from deamination of a 5-methylcytosine by AID in a CpG dinucleotide with an undamaged G or an abasic lesion (AP site) that substituted G. Substrates containing a T/G mismatch at the upstream primer and a 5′-THF residue at the downstream primer were designed to mimic the BER intermediates containing an oxidized AP site adjacent to a T/G mismatch that is 5′-incised by APE1 opposite to a template C or T. The substrate containing an intact or preincised AP site opposite to a template C and adjacent to a C/G matched base pair was used as the control. Substrates for measuring TDG activity were constructed by annealing the strand containing a T with the template strand containing a G that was base paired with the T at a molar ratio of 1:1.5. Substrates for measuring BER enzymatic activity were constructed by annealing the upstream primer with a 3′-T that mispaired with a template G and the downstream primer with a 5′-THF residue to the template strand at a molar ratio of 1:1:2. The sequences of the oligonucleotide substrates are listed in Supplementary Table S1.

2.3. Measurement of TDG activity in removing a T/G mismatch adjacent to a THF residue, an analogue of an AP site

TDG activity for removing the T from a T/G mismatch adjacent to a THF residue was measured by incubating increasing concentrations of TDG from 50 nM to 70 nM with 25 nM substrate containing a T/G mismatch with or without the THF residue. The activity was examined at 37 °C for 30 min in 10 μl reaction buffer with 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, and 0.01% Nonidet P-40. Reactions were terminated by transferring to 95 °C for 5 min. The reaction mixture was then treated with 0.1 M NaOH and denatured at 95 °C for 10 min in buffer containing 95% formamide and 10 mM EDTA. Substrates and products were separated by 15% urea-denaturing polyacrylamide gel electrophoresis (PAGE)