DNA Repair xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

When DNA repair goes wrong: BER-generated DNA-protein crosslinks to oxidative lesions

Jason Luis Quiñones, Bruce Demple*

Stony Brook University School of Medicine, Department of Pharmacological Sciences, Stony Brook, NY, 11794, USA

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Base excision DNA repair
DNA polymerase β
AP lyase
Oxidative DNA damage
2-deoxyribonolactone
DNA-protein crosslinks

ABSTRACT

Free radicals generate an array of DNA lesions affecting all parts of the molecule. The damage to deoxyribose receives less attention than base damage, even though the former accounts for ~20% of the total. Oxidative deoxyribose fragments (e.g., 3′-phosphoglycolate esters) are removed by the Ape1 AP endonuclease and other enzymes in mammalian cells to enable DNA repair synthesis. Oxidized abasic sites are initially incised by Ape1, thus recruiting these lesions into base excision repair (BER) pathways. Lesions such as 2-deoxypentos-4-ulose can be removed by conventional (single-nucleotide) BER, which proceeds through a covalent Schiff base intermediate with DNA polymerase β (Polβ) that is resolved by hydrolysis. In contrast, the lesion 2-deoxyribonolactone (dL) must be processed by multinucleotide ("long-patch") BER: attempted repair via the single-nucleotide pathway leads to a dead-end, covalent complex with Polβ cross-linked to the DNA by an amide bond. We recently detected these stable DNA-protein crosslinks (DPC) between Polβ and dL in intact cells. The features of the DPC formation in vivo are exactly in keeping with the mechanistic properties seen in vitro: Polβ-DPC are formed by oxidative agents in line with their ability to form the dL lesion; they are not formed by non-oxidative agents; DPC formation absolutely requires the active-site lysine-72 that attacks the 5′-deoxyribose; and DPC formation depends on Ape1 to incise the dL lesion first. The Polβ-DPC are rapidly processed in vivo, the signal disappearing with a half-life of 15–30 min in both mouse and human cells. This removal is blocked by inhibiting the proteasome, which leads to the accumulation of ubiquitin associated with the Polβ-DPC. While other proteins (e.g., topoisomerases) also form DPC under these conditions, 60–70% of the trapped ubiquitin depends on Polβ. The mechanism of ubiquitin targeting to Polβ-DPC, the subsequent processing of the expected 5′-peptidyl-dL, and the biological consequences of unrepaired DPC are important to assess. Many other lyase enzymes that attack dL can also be trapped in DPC, so the processing mechanisms may apply quite broadly.

1. Introduction

The genome is under continual threat by DNA damage from a variety of endogenous and environmental sources. The most frequent endogenous DNA lesions are chemical alterations to the nitrogenous bases that include products of oxidation, alkylation and deamination reactions [1]. Many types of base damage destabilize the *N*-glycosylic bond, leading to the formation of abasic (AP) sites, and these add to the estimated 10,000 AP sites per day in each human cell arising from the hydrolytic loss of undamaged purine bases [2–4]. Many of the hydrolytic, oxidative or alkylation base lesions cause minimal structural effects on DNA, so they escape effective processing by nucleotide excision repair (*see article by Sugasawa in this issue*). Instead, the mutagenic and genotoxic threat of these lesions is offset by mechanisms such as the base excision DNA repair (BER) system [5,6] (Fig. 1).

The BER pathway can be initiated by any of the eleven DNA glycosylases in mammalian cells [7]. Each glycosylase detects a specific range of base lesions and hydrolyzes the *N*-glycosylic bond between the base and DNA deoxyribose, forming an AP site (Fig. 1, step I). These AP sites and those generated by spontaneous purine hydrolysis are then incised by an AP endonuclease, with the main activity in mammalian cells residing in the Ape1 protein [8,9]. Ape1 hydrolyzes the phosphodiester on the immediate 5′ side of the AP site to generate a single-strand break bracketed by 3′-OH and 5′-deoxyribose-5-phosphate (5′-dRp) residues (Fig. 1, step 1A, B). In

Abbreviations: Cu(OP)$_2$, 1,10 copper-*o*-phenanthroline; dL, 2-deoxyribonolactone; 5′-dRp, 5′-deoxyribose-5-phosphate; AP, apurinic/apyrimidinic (abasic); Pol&beta, DNA polymerase β DPC, DNA-protein crosslink; MEF, mouse embryonic fibroblast; Oxa, oxanine; TPZ, tirapazamine; Top1, topoisomerase 1; Top2, topoisomerase 2.

* Corresponding author.
E-mail address: bruce.demple@stonybrook.edu (B. Demple).

http://dx.doi.org/10.1016/j.dnarep.2016.05.014
1568-7864/

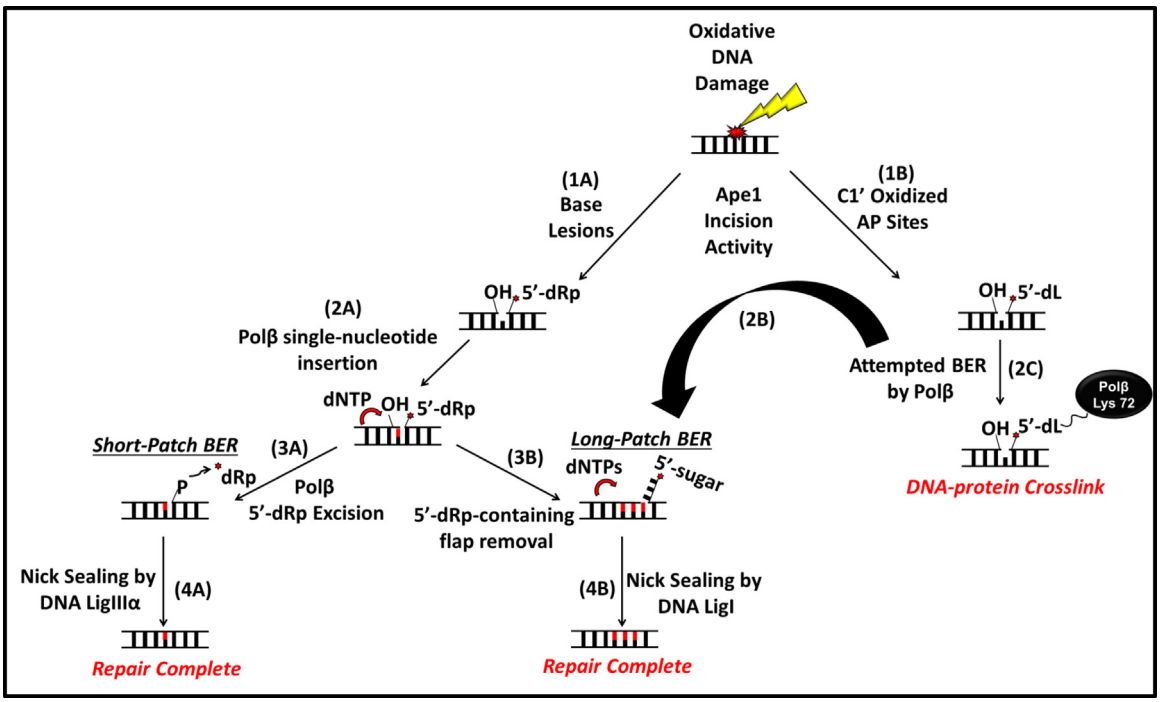

Fig. 1. BER processing of oxidative DNA damage and the formation of DPC. Following base damage and glycosylase processing, or the oxidation of DNA deoxyribose, the resulting abasic sites are cleaved by Ape1 (1A, B). The incised AP sites are then channeled through single-nucleotide ("short-patch") or multinucleotide ("long-patch") BER. In the former, following incision of the AP site, Polβ inserts a single-nucleotide to replace the damaged nucleotide (2A) and removes the 5′-dRp residue generated by Ape1 (3A). Synthesis of ≥2 nucleotides in multinucleotide BER generates a flap that prevents 5′-dRp excision and requires activities such as Fen1 (3B). Multinucleotide BER processes dL lesions effectively (2B), but in some circumstances attempted 5′-dL excision results in the formation of Polβ-DPC (2C). Short-patch BER is completed by DNA Ligase IIIα (4A) and in the case of long-patch BER, repair is completed by DNA Ligase I (4B) restoring the DNA backbone to its native condition.

"classical" BER, only the missing nucleotide is replaced by DNA polymerase β (Polβ) (Fig. 1, step 2A), and the 5′-dRp residue is excised by a 5′-dRp lyase activity in a discrete domain of Polβ (Fig. 1, step 3A) [10–12]. The result is a nicked DNA that is competent for ligation, usually by DNA ligase IIIα (Fig. 1, step 4A).

Another sub-pathway called long-patch BER (LP-BER) also exists, in which DNA repair synthesis replaces 2–10 nucleotides, Fen1 ("flap") endonuclease excises the displaced strand (Fig. 1, 3B), and DNA ligase I seals the nick [13–15] (11, step 4B). LP-BER repair synthesis can be initiated by Polβ, which may (inefficiently) insert a second nucleotide. When the 5′-dRp strand is displaced by two or more nucleotides, the 5′-dRp lyase of Polβ no longer functions, requiring Fen1 to remove the single-stranded flap. There is evidence that DNA polymerase δ and DNA polymerase ε can participate in LP-BER in a PCNA-dependent fashion, but the real contributions of the different DNA polymerases have not been established in vivo [16–19]. When LP-BER was discovered [20–22], its role was unclear, with speculation about unknown modified 5′ termini possibly requiring LP-BER. A clear case came for the major oxidative lesion 2-deoxyribonolactone (dL) (Fig. 1, step 2B), which cannot be repaired by classical single-nucleotide BER [14,23].

2. The formation of BER-mediated DNA-protein crosslinks

2.1. The formation of oxidative polβ-DPC in vitro and in vivo

Free radicals, such as the by-products of aerobic metabolism, damage all the components of DNA including the deoxyribose [24]. The latter products include strand breaks with deoxyribose fragments attached to the termini, and AP sites with oxidized sugars. An important example is dL [24], which almost 50 years ago was the first chemically defined lesion of H_2O_2 damage [25]. Other oxidative agents, such as X-rays, also form dL as one of many products [26–28], while certain agents can generate the lesion more selectively. These include some genotoxic compounds that attack C1′ of deoxyribose specifically, such as neocarzinostatin (NCS) [28–30], tirapazamine (TPZ) [31–33] and the organometallic 'chemical nuclease' 1,10-copper-*o*-phenanthroline [$(Cu(OP)_2$] [34–37]. However, the chemical instability of dL and other features prevented a systematic study of its processing for decades. Finally, three labs report generally similar methods for generating dL site-specifically in synthetic oligodeoxynucleotides [38–40] which allowed a more detailed understanding of its effects in vitro [21,41–47].

In vitro reactions with dL-oligonucleotide substrates showed that Ape1 cleaved the lesions about as well as it does a regular AP site [37]. Subsequent BER steps were stymied, however, and molecular analysis showed that Polβ had been captured in a stable, covalent complex with the DNA [23]. This study revealed a form of enzyme mechanism-based cross-linking, dependent on the lyase active-site residue lysine-72 (K72) (Fig. 1, step 2C, and Fig. 3A). In mouse embryonic fibroblast (MEF) extracts incubated with dL-DNA, Polβ-DPC were the major species detectable, dependent on the *POLB* gene [14,48,49]. Around the same time, it was reported that *Escherichia coli* endonuclease III can be trapped at (uncleaved) dL sites via that enzyme's lyase activity [46]. We later showed that mitochondrial DNA polymerase γ (Polγ), which harbors a 5′-dRp lyase similar to that of Polβ, is also trapped by 5′-dL [15]. In contrast to enzymatic AP lyases, the cross-linking of lysine-rich proteins such as histones to dL-DNA occurs much less efficiently, at a rate ~100-fold lower than that of Polβ [47]. Thus, dL in DNA constitutes a specific threat to the various lyases that act on DNA abasic sites, especially significant because dL would be generated in vivo at a rate similar to that for 8-oxoguanine [28].

The finding that dL traps DNA repair lyases in DPC suggested that cells ought to have mechanisms to avoid this hazard. Consistent with this hypothesis, we showed that dL residues incubated

Please cite this article in press as: J.L. Quiñones, B. Demple, When DNA repair goes wrong: BER-generated DNA-protein crosslinks to oxidative lesions, DNA Repair (2016), http://dx.doi.org/10.1016/j.dnarep.2016.05.014