Role of base excision repair subpathways in correcting oxidized abasic sites in DNA

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In order to ensure genetic stability, the DNA repair systems need to handle various lesions that can arise in the genome. One such type of lesion is the oxidized abasic site, which is formed when a base is removed from the DNA backbone, leaving a gap in the helix. The base excision repair (BER) system is responsible for the removal of these oxidized bases, which can be caused by both endogenous and exogenous factors. The BER process involves the initial incision of the abasic site by the Ape1 protein, followed by the synthesis of a single nucleotide by DNA polymerase β (short-patch BER) or multiple nucleotides by the long-patch BER pathway. Recent studies have shown that the Ape1-incised oxidized base can form a stable covalent crosslink with the DNA polymerase β, which can complicate the repair process.

Keywords
2-deoxyribonolactone; DNA polymerase beta; DNA-protein crosslinks; FEN1 protein; long-patch BER; oxidized abasic sites; short-patch BER

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Genetic stability is threatened by the continuous assault on cellular DNA by various reactive species of both endogenous and exogenous origins. The most common types of DNA damage are associated with DNA base alteration. A well-characterized DNA base modification is uracil, which can arise in genomic DNA by misincorporation of dUMP during DNA synthesis, or by the spontaneous deamination of cytosine in G:C base pairs to form a premutagenic lesion [1,2]. Reactive oxygen species, the products of normal cellular respiration, also generate a variety of oxidized DNA base damages, including an 8-oxoguanine that is frequently used as a biomarker for oxidative DNA damage [3,4]. Enzymatic methylation of DNA bases, predominantly cytosines, plays an important role in gene regulation, but nonenzymatic alkylation from endogenous sources forms cytotoxic and mutagenic products, such as 3-alkyladenine and O6-alkylguanine [5,6]. Metabolic by-products (such as epoxyaldehydes), produced during cellular lipid peroxidation, are exogenously-induced DNA damage. The BER system must be able to respond to these lesions and ensure the integrity of the genome.

Abbreviations
AP, apurinic/apyrimidinic; BER, base excision DNA repair; DPC, DNA-protein crosslink; dL, 2-deoxyribonolactone; 5’-dL, 5’-terminal dL-5-phosphate residues; 5’-dRp, 5’-deoxyribose-5-phosphate; MEF, mouse embryonic fibroblasts; 5-MF, 5-methylene-2-furanone; PARP-1, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; Polβ, DNA polymerase β.

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Base excision DNA repair (BER) is fundamentally important in handling diverse lesions produced as a result of the intrinsic instability of DNA or by various endogenous and exogenous reactive species. Defects in the BER process have been associated with cancer susceptibility and neurodegenerative disorders. BER funnels diverse base lesions into a common intermediate, apurinic/apyrimidinic (AP) sites. The repair of AP sites is initiated by the major human AP endonuclease, Ape1, or by AP lyase activities associated with some DNA glycosylases. Subsequent steps follow either of two distinct BER subpathways distinguished by repair DNA synthesis of either a single nucleotide (short-patch BER) or multiple nucleotides (long-patch BER). As the major repair mode for regular AP sites, the short-patch BER pathway removes the incised AP lesion, a 5’-deoxyribose-5-phosphate moiety, and replaces a single nucleotide using DNA polymerase (Polβ). However, short-patch BER may have difficulty handling some types of lesions, as shown for the C1’-oxidized abasic residue, 2-deoxyribonolactone (dL). Recent work indicates that dL is processed efficiently by Ape1, but that short-patch BER is derailed by the formation of stable covalent crosslinks between Ape1-incised dL and Polβ. The long-patch BER subpathway effectively removes dL and thereby prevents the formation of DNA-protein crosslinks. In coping with dL, the cellular choice of BER subpathway may either completely repair the lesion, or complicate the repair process by forming a protein–DNA crosslink.
These types of DNA lesions are formed by the action of various physical and chemical agents, including UV and γ-irradiation, heterocyclic N-oxides of the tirapazamine family, organometallic oxidants and the anticancer antibiotics (such as neocarzinostatin) of the ene-diyne family [18–21]. The formation of oxidized AP sites is initiated by the reaction of free radicals with the deoxyribose sugar components of DNA and subsequent chemical rearrangements that are modulated by the presence of molecular oxygen [22,23]. The earliest identified X-ray damage in DNA was a C1′-oxidized abasic lesion, 2-deoxyribonolactone (dL) [24], which is generated by initial hydrogen abstraction from the deoxyribose C1′ carbon, followed by O2 addition and base loss (Fig. 1B). Successive β- and δ-eliminations of dL residues yields a strand break with 3′- and 5′-phosphate ends and liberates 5-methylene-2-furanone (5-MF) (Fig. 1B). 5-MF has been employed as a characteristic product of dL in its detection in DNA [25,26]. As determined by comparing the release of 5-MF with concomitant DNA breakage, dL lesions may account for up to 72% of the total sugar damage in the irradiated DNA in vitro [25]. Comparison of the rate of spontaneous strand scission at dL sites to the regular (aldehyde) AP sites shows that cleavage at dL sites is 12- to 55-fold faster than at AP sites [27]. However, the immediate breakage of DNA at the dL lesion would not be expected under physiological conditions.
The half-life of dL for spontaneous cleavage under simulated physiological conditions was estimated to be 32–54 h in duplex DNA [28]. Recent understandings of the chemical properties of dL indicates that these lesions are probably subjected to cellular DNA repair or translesion DNA synthesis, rather than directly contributing to the formation of DNA strand scission.

**Short- and long-patch BER in mammalian cells**

A simplified version of BER for AP sites can be described as follows: (a) enzymatic incision of the AP site; (b) excision of the cleaved AP site at the single-strand break; (c) repair DNA synthesis; (d) ligation of the nick in DNA. In mammalian cells, the major AP endonuclease, Ape1 (also called Apex, HAP1, or Ref-1), hydrolyzes the 5' phosphodiester bond of the AP site to generate a DNA repair intermediate that contains a single strand break with 3'-hydroxyl and 5'-deoxyribose-5-phosphate (5'-dRp) termini [29,30]. Further repair is achieved through at least two distinct BER subpathways that involve different subsets of enzymes, and which result in the replacement of one nucleotide (short-patch BER), or two or more nucleotides (long-patch BER) (Fig. 2).

In mammalian short-patch BER, the major 5'-dRp excision is attributable to DNA polymerase β (Polβ). The dRp excision involves a lyase activity in the Polβ 8 kDa N-terminal domain acting through a covalent, Schiff base intermediate [31,32] (Fig. 3A). Single-nucleotide gap-filling DNA synthesis is associated with the DNA polymerase activity of Polβ, which therefore plays dual roles in short-patch BER. In an earlier study, the simplest form of short-patch BER of uracil was reconstituted in vitro by using purified human proteins, including Ung, Ape1, Polβ and DNA ligase III [33]. Similar in vitro reconstitution experiments for the repair of other base lesions or the AP site also suggested essential roles of Polβ in the short-patch BER pathway [34–36]. Involvement of Polβ in the short-patch BER of various types of DNA lesions has been demonstrated by using cell extracts from wild-type and Polβ null mouse embryonic fibroblasts (MEF) cells [37–40]. Some short-patch BER is still observed with Polβ-deficient cell extracts, however, which suggests...
that there is functional redundancy at the level of DNA polymerases to provide cells with backup systems [41–43]. Despite this possibility, Polβ is encoded by an essential gene, the deletion of which causes embryonic lethality in mice [44]. Polβ-deficient MEFs exhibit hypersensitivity to DNA alkylating agents that require BER [44]. Somewhat surprisingly, near-normal resistance could be restored in MEFs by providing only the N-terminal dRp lyase domain of Polβ [45], which suggests greater functional redundancy for BER repair polymerase activities than for dRp excision.

The long-patch BER pathway involves strand displacement repair synthesis of at least two nucleotides, with excision of the 5’-dRp residue as part of a flap oligonucleotide cleaved by the FEN1 nuclease [34,46]. The identity of the polymerases involved in the long-patch BER pathway is not yet fully understood. It has been suggested that Polβ may be responsible for the initiation of strand displacement synthesis [40,47]. In addition, the involvement of other DNA polymerases, such as Polδ and Polε, in long-patch BER has been suggested [43,48,49]. A reconstituted enzyme system was developed for long-patch BER of a reduced AP site utilizing purified Ape1, Polβ, Polδ, proliferating cell nuclear antigen (PCNA), FEN1 and DNA ligase I, where Polδ substituted for Polβ when PCNA was present in the reaction [34]. PCNA-dependent long-patch BER was also demonstrated in extracts of Polβ-deficient MEF cells, but it appeared to be heavily dependent on the use of circular DNA substrates [38,41]. During the PCNA-independent long-patch BER mode, Polβ may be the major DNA polymerase in strand displacement DNA synthesis [40]. However, comparative analysis of BER in wild-type and Polβ null cell extracts showed the occurrence of long-patch BER, even in the absence of Polβ, suggesting that various DNA polymerases provide functional redundancy in long-patch BER DNA synthesis [38,41].

Various interactions among BER proteins may alter the choice of BER subpathways. Ape1, when bound to DNA, interacts with Polβ, which also physically interacts with the scaffold protein, XRCC1 [33,50,51]. Poly(ADP-ribose) polymerase (PARP-1), the enzyme that immediately binds to the incised AP site and undergoes self-ADP-riboisylation, interacts with XRCC1 and Polβ and affects BER [51,52]. The involvement of PARP-1 can increase the overall BER rate, especially by enhancing short-patch BER, by antagonizing the action of Polβ, producing a complete block of long patch BER strand-displacement DNA synthesis [53]. Long-patch BER reactions are also well co-ordinated through protein–protein interactions between PCNA and various BER enzymes, including Polβ, Polδ/ε, FEN1 and DNA ligase I [9,54–56]. When such interactions are disrupted by p21-derived peptide that binds specifically to PCNA, the mode of AP site repair was skewed towards short-patch BER, but only in the presence of Polβ [41,57]. Recently, adenomatous polyposis coli, the tumor suppressor protein, has been implicated in preventing Polβ-mediated strand displacement synthesis by masking the domain of Polβ that interacts with PCNA, thereby decreasing long-patch BER, but not short-patch BER [58].

An additional variation of BER has been suggested, as some bifunctional DNA glycosylases are associated with AP lyase activity that can carry out the cleavage of AP sites by β-elimination. These reactions generate 3’ termini that are blocked by the lyase product, which must be removed by an enzyme, such as Ape1, to allow repair DNA synthesis (Fig. 2). In this pathway, the 3’ repair diesterase activity of Ape1 plays an important role [59], as it also does in the excision of 3’ phosphoglycolate esters generated by ionizing radiation or chemical oxidation [29,60]. More recently, human polynucleotide kinase has been implicated in the repair of 3’ phosphate damage, and its interaction with other BER proteins, including XRCC1, Polβ and DNA ligase III, has been shown [61].

In general, long-patch BER has been considered to be a minor pathway relative to the predominant short-patch BER. However, several in vitro and in vivo studies suggest a significant contribution of the long-patch BER mode in some circumstances, particularly in the repair of regular AP sites or of the damaged base lesions that become AP sites by the action of monofunctional DNA glycosylases [39,41,62,63]. As measured by an in vivo assay using a plasmid containing a single AP site in the stop codon of the gene encoding enhanced green fluorescent protein, > 80% of the repair accompanying the reversion of the stop codon occurred by long-patch BER [63]. This result is consistent with a previous observation that 70–80% of uracil-initiated BER was mediated by long-patch BER, when examined by utilizing a circular DNA substrate and cell-free extracts of MEF cells [41].

The detailed mechanism that governs the selection between the short- or long-patch BER modes remains a major unknown. Previously, it has been suggested that it is the nature of the DNA lesion that determines the type of DNA glycosylase (monofunctional versus glycosylase lyase), which, in turn, determines the selection of the repair pathway [39]. BER, initiated by bifunctional DNA glycosylases with associated AP lyase activity, is mainly mediated by the short-patch pathway because the resulting BER intermediate, containing a single nucleotide gap bracketed by a
3′-hydroxyl and a 5′-phosphate, can be readily filled in by Polβ. In contrast, DNA repair, involving a mono-functional DNA glycosylase that generates an AP site, may involve both the short- and long-patch BER pathways. In this model, the removal of 5′-dRp, which appears to be the late-limiting step in short-patch BER [64], may be critical in determining the mode of BER.

**DNA–protein crosslink formation in the short-patch BER of dL**

Chemical methods for the specific generation of dL lesions within DNA oligonucleotides have been independently developed by several laboratories [65–67]. All of these methods involve the photolysis of a stable precursor and its conversion to dL at a defined site in synthetic DNA oligonucleotides. These approaches facilitated the study of the biological fate of this key oxidative deoxyribose damage in DNA. Initial investigation of dL repair by *Escherichia coli* endonuclease III, a bifunctional DNA glycosylase associated with AP lyase activity, revealed the formation of a stable DNA–protein crosslink (DPC) with dL, which was AP lyase activity, revealed the formation of a stable DPC species was found to contain Polβ, which appears to be the late-limiting step in short-patch BER [64], may be critical in determining the mode of BER.

In an effort to determine the biological significance of such crosslink formation, a cell-free extract system was utilized to react with oligonucleotide DNA containing a site-specific dL residue [57]. Under nonrepair conditions (no added dNTPs or Mg2+), the most predominant DPC species was found to contain Polβ, because this species was not observed in the reactions with extracts of Polβ null mouse cells. As the dRp lyase activity of Polβ constitutes the major activity for removing 5′-dRp residues in mammalian cells [32,44], the results indicate that DPC formation, specific to the 5′-dLp lesion, occurs mainly through the abortive attempt of the dRp lyase activity of Polβ to remove this incised dL lesion. Polβ displays strong affinity for 5′-dRp residues at the incised AP site, while Ape1 recruits Polβ to the incised AP site and stimulates its dRp lyase activity [50,74]. Thus, this enzyme–substrate specificity may promote the interaction of Polβ with a 5′-dLp lesion at a DNA nick, thereby increasing the rate of Polβ-specific DPC formation. On the other hand, it has been recently verified that dRp lyase activity lags behind the polymerase activity in the dual functions of Polβ, while Ape1 suppresses the polymerase activity [75]. In this scenario, Ape1 may modulate Polβ to pause prior to acting at the 5′-dLp, possibly suppressing an abortive attempt to excise the lesion. Whether interactions between Ape1 and Polβ, or the involvement of other factors, stimulates or inhibits the covalent trapping of Polβ to the 5′-dLp residue, must await further analysis.

**Use of long-patch BER in the repair of dL**

The major difference found in the sequential enzymatic steps between short-patch and long-patch BER is the removal of the incised abasic residue (5′-dRp). While the dRp lyase activity of Polβ participates in the processing of this residue, an attempt to remove the 5′-dLp residue by Polβ using the same mechanism results in trapping of the repair enzyme at the lesion. In the alternative long-patch BER pathway, removal of the 5′-dRp moiety is independent of the Polβ dRp...
lyase activity and is mediated mainly by strand displacement DNA synthesis followed by FEN-1 excision. Therefore, it is not unreasonable to expect that the Ape1-incised dL residue may be repaired by the long-patch BER pathway.

Reconstitution of dL-mediated BER conducted with partial components of long-patch BER, including Ape1, Polβ and FEN-1, revealed that the formation of dL-mediated DPC was dependent on both Ape1 (for cleavage) and Polβ, but that the amount of this DPC product was markedly decreased in reactions including FEN1 and dNTPs (Fig. 4A). Repair DNA synthesis, displacing the 5'-dLp residue by Polβ alone, did not block the DPC formation, indicating that removal of dL-containing DNA fragment by FEN1 plays a key role in preventing crosslinking with the DNA substrate (Fig. 4B). This result suggests that sequential enzymatic activities in long-patch BER can effectively process the lesion and avoid dL-mediated DPC formation. This hypothesis was further supported by the demonstration of efficient processing of a 5'-dLp flap oligonucleotide by FEN1 [57], consistent with previous observations showing that the enzyme tolerates a variety of small modifications of the flap 5' terminus [76]. Investigation of dL-mediated long-patch BER was performed by utilizing circular DNA with a defined dL residue, incubated with whole-cell extracts [57]. The repair of dL was detected in both wild-type and Polβ-null MEF cell extracts, with concomitant reduction of subsequent crosslinking activity. Analysis of the patch size distribution associated with BER of site-specific lesions showed that the single-nucleotide replacement was the predominant repair patch (35% of the total) for a regular AP site in the Polβ-proficient cell extract, but this event

![Fig. 4.](image-url)

**Fig. 4.** In vitro reconstituted long-patch base excision DNA repair (BER) mediates the repair of 2-deoxyribonolactone (dL) and inhibits the formation of a dL-mediated DNA-protein crosslink (DPC). (A) A duplex 3'-32P-labeled DNA substrate, containing a site-specific dL, was incubated with different combinations of Ape1, DNA polymerase β (Polβ) and FEN1 in the presence or absence of a dNTP mix excluding dTTP. After the incubation, one-half of each reaction mixture was analyzed on a DNA sequencing gel. Ape1 converted the majority of the DNA substrate to the DNA cleavage product, while additional treatments with Polβ and FEN1 mediated further processing of the DNA only in the presence of dNTPs. The generation of the 11-mer is consistent with strand displacement DNA synthesis of seven nucleotides by the polymerase, followed by removal of the displaced DNA flap by FEN1. (B) The remainder of each reaction mixture was analyzed by SDS/PAGE. The dL-mediated DPCs with Polβ are observed with mobilities slower than those of Polβ and the free DNA. The generation of DPC was markedly reduced when the reaction allowed the combined action of repair synthesis by Polβ and flap excision by FEN1. (C) Schemes for the Ape1 incision of DNA at the 5'-side of the dL lesion (1), the strand displacement DNA synthesis of seven nucleotides by the DNA polymerase activity of Polβ (2), removal of the 5'-dLp-containing flap by FEN1, resulting in a nick on DNA (3), and DPC formation via an abortive attempt to remove the 5'-dLp residue by the dRp lyase activity of Polβ (4). The combined processes of (2) and (3) mediate removal of the dL-containing oligonucleotide fragment from the DNA substrate and prevent DPC formation with Polβ (4). Adapted from a previous publication [57].
was significantly reduced (< 10% of the total) for repair of the dL substrate. Instead, repair patches of two or more nucleotides were the predominant mode for dL with both Polβ-proficient and -deficient cell extracts. It was also confirmed that only the long-patch BER mode was mostly associated with the complete repair process, including the final DNA ligation step [57]. Therefore, at least in mammalian cell extracts, dL appears to be resistant to repair by short-patch BER, but effectively and exclusively repaired by long-patch BER, thereby preventing the formation of deleterious DPC adducts in DNA.

**Concluding remarks**

In spite of numerous efforts in defining the biological and biochemical mechanisms involved in BER, the cellular choice of the specific BER mode remains an intriguing question. A similar diversity in BER modes is also found in *E. coli* [77–79], which indicates that multiple subpathways of BER are favored by evolution for defending against various types of nonbulky damage lesions in the genetic material. Our studies of dL-mediated BER provide at least one clear rationale for the evolution of long-patch BER to handle a naturally occurring lesion. While dL residues present serious problems for cells by mediating stable DPC formation with Polβ, particularly in the course of the short-patch BER pathway, it appears that the operation of the long-patch BER pathway substantially avoids this detrimental consequence. However, under conditions of extensive oxidative stress, it seems possible that long-patch BER components may become limiting because of their participation in the repair of many other lesions, with the attendant hazards if short-patch BER increasingly attempts to handle dL lesions. On the other hand, the induction of proteins that could modulate the subpathways of BER, as shown with p21, may alter the outcome of BER operating on dL [57]. In such circumstances, Ape1-incised dL residues could remain in the DNA for longer periods, increasing the opportunity for DPC formation. Further studies of dL will provide more understanding of the BER switching mechanism that governs the short-versus long-patch BER distribution under varying circumstances of damage load and repair enzyme availability.

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