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Dynamics and diversions in base excision DNA repair of oxidized abasic lesions

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Introduction

Oxidized abasic sites: an important component of free radical damage to DNA

Research on the repair of DNA lesions formed by reactions with cellular by-products has focused particularly on oxidative damage. Evidence exists for the formation of oxidative DNA damage in cells during normal growth, but virtually all of this work has centered on the analysis of various base lesions (Dizdaroglu *et al*., 2002). Our understanding of the repair of such lesions has been enhanced by studies of repair in response to DNA damage induced in cells exposed to chemical oxidants, certain antitumor drugs, or ionizing radiation, which carry out the same or similar reactions with DNA (Dedon and Goldberg, 1992; Von Sonntag, 1987).

A long-known class of oxidative DNA lesions has not been investigated to nearly the same extent as base lesions – oxidized abasic sites (OAS). Lesions of this type include the earliest-identified X-ray damage in DNA, 2-deoxyribonolactone (dL) (Von Sonntag, 1987) (Figure 1). Other well-known OAS include 2-deoxypentos-4-ulose (KA), 3′-phosphoglycolate esters (3′-PG), and 3′-phosphates (3′-P) (Von Sonntag, 1987) (Figure 1). Much is known about the free radical reactions that initiate formation of these lesions and the pathways of their generation *in vitro* (Dedon and Goldberg, 1992; Von Sonntag, 1987). However, knowledge of the relevant repair pathways for OAS is rather scanty, as is direct *in vivo* measurement of individual OAS lesions, but some progress is in the offing (Nakamura *et al*., 2000). This review addresses some of the fundamental issues on this subject, and points to areas where important progress may be at hand.

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Formation of OAS: radiation biology and synthetic approaches

Since the 1950's it has been known that most DNA damage caused by ionizing radiation results from the generation of free radicals in the medium along an energy deposition track (Von Sonntag, 1987). These short-lived radicals react with nearby molecules, such as DNA. Most of the atoms comprising DNA are subject to attack (Von Sonntag, 1987). Although dL was the first radiation lesion specified chemically, the subsequent research effort focused largely on identifying DNA base modifications rather than deoxyribose damage. Ironically, the most frequently measured indicator of radiation damage, DNA strand breakage, results from the formation of OAS (Von Sonntag, 1987).

During the formation of OAS, the initial deoxyribose-centered radicals can undergo chemical rearrangements that are modulated by the presence of molecular oxygen (Von Sonntag, 1987). For example, dL plausibly arises from initial hydrogen abstraction from the nucleotide C1′ carbon, followed by O_2 addition and base loss (Figure 1). The same C1′ radical can alternatively undergo different subsequent reactions to form strand breaks with 3′-P residues, which have received renewed attention recently (see Mammalian polynucleotide Kinase and 3′ repair). At least two other lesions are initiated by hydrogen abstraction from the deoxyribose C4′ carbon, depending on reaction with oxygen: the abasic 2-deoxypentos-4-ulose (a C4-keto-Cl-aldehyde) without chain breakage, or a 3′-PG on one side of a strand break (Figure 1). Oxidized abasic sites may comprise as much as 20% of total DNA damage induced by ionizing radiation (Von Sonntag, 1987), which underscores their likely biological significance.

A critical feature of ionizing radiation is its ability to generate clusters of DNA damage. A single radiation track may generate up to five closely spaced hydroxyl radical pairs (Goodhead, 1994), which are then poised to attack DNA within a small region. A recent study, using various repair enzymes to detect oxidative base damage or abasic residues, indicates that clustered lesions (including OAS) can constitute 80% of complex damages, while direct double-strand breaks accounted for only about 20% of the total (Sutherland *et al*., 2000a,b). Thus, additional double-strand breaks can arise from enzymatic processing and attempted DNA repair of radiation damage, which could thereby potentiate the cytotoxic and mutagenic potential of

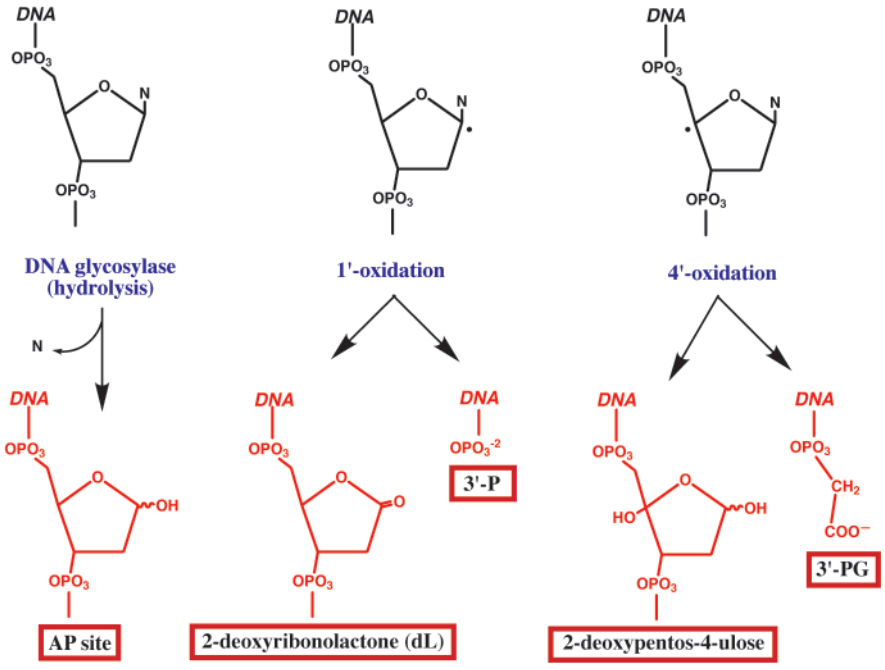

Figure 1 Oxidized abasic sites. 'Regular' abasic sites are formed by hydrolytic removal of DNA bases, either enzymatically by DNA glycosylases or through acid-catalysed depurination or depyrimidination. Shown here are two pathways for the formation of oxidized abasic sites initiated by H atom abstraction from, respectively, the 1′ or 4′ carbons of DNA deoxyribose. The initial radical can undergo various reactions, depending on the availability of molecular oxygen, which can yield either the oxidized residue in an unbroken DNA chain (dL and KA), or 3′-P and 3′-PG (through chemical rearrangements)

closely opposed DNA lesions (Blaisdell and Wallace, 2001).

Chemical oxidants and some antitumor drugs can also form OAS through hydrogen abstraction from the deoxyribose carbons. An important example of the latter is bleomycin, which exclusively attacks C4′ carbons to yield either 3′-PG or 2-deoxypentos-4-ulose (a C4-keto-Cl-aldehyde); the proportions of these lesions depend on the amount of molecular oxygen available (Dedon and Goldberg, 1992). Bleomycin is able to carry out successive attacks on the two DNA strands to generate clustered DNA damage, and the agent shows some sequence selectivity (Bennett *et al*., 1993; Dedon and Goldberg, 1992; Povirk, 1996). Some ene-diyne antitumor antibiotics, such as neocarzinostatin, attack the C1′ carbon to form dL residues and some 3′-phosphate-terminated strand breaks (Hashimoto *et al*., 2001; Povirk, 1996). Ene-diyne drugs can also attack the C5′ carbon to yield a strand break terminated by an oxidized 5′-aldehydic nucleotide, which retains the base (Dedon and Goldberg, 1992). Chemical oxidants such as Cu(II)-phenanthroline (Marshall *et al*., 1981; Meijler *et al*., 1997; Perrin *et al*., 1996; Pope *et al*., 1982; Zelenko *et al*., 1998) can generate C1′-derived OAS (dL and 3′-P), while H_2O_2 in the presence of reduced iron or copper produces a range of OAS (and damaged bases) (Dizdaroglu *et al*., 2002). However, chemical agents often have low sequence selectivity, and their utility in studying the enzymology of BER for OAS is therefore more limited than drugs such as bleomycin.

The biology of OAS

While the DNA of cells exposed to ionizing radiation or chemical oxidants harbors strand breaks that clearly arise from OAS, recent findings suggest that the formation of OAS by endogenous oxidation may also be significant. Nakamura and Swenberg (1999) reported that 5′-cleaved abasic residues were present at steady-state levels of 50 000 – 200 000 per cell in the DNA of various rat tissues and in human liver, which suggested that excision of the abasic residue following cleavage by Ape1 is the rate-limiting repair step *in vivo*. More recently, the same authors showed that a significant number of 5′-cleaved abasic sites was detected in human cells exposed to H_2O_2 (Nakamura *et al*., 2000). Since these sites persisted during a post-exposure period, it was concluded that these OAS are poorly handled by the excision step of short-patch BER. However, the exact nature of the OAS formed *in vivo* was not determined in these studies, and the estimated amount of damage per cell seems very high. It is hard to imagine how cell function could be maintained in the face of such damage levels. Additional approaches to measure endogenous OAS will be useful.

Although the mutagenic and carcinogenic effects of ionizing radiation and chemical oxidants such as H_2O_2 have been studied extensively, the complex nature of the DNA damage caused by these agents has made it generally difficult to attribute specific biological endpoints to individual types of OAS. Agents such as bleomycin, which generates a more restricted range of

DNA damage, have permitted some insights. For example, Bennett *et al.* (1993) characterized the mutagenic potential of bleomycin-induced abasic sites by transfecting drug-treated shuttle vectors into mammalian cells. They observed deletions of various sizes targeted around the damage sites, consistent with the ability of bleomycin to generate double-strand breaks from closely opposed single-strand breaks and abasic sites (Bennett *et al.*, 1993). The recent development of methods for generating 3′ PG or dL site-specifically will allow more refined analysis of the biological consequences of these OAS.

The clustering of oxidative damage might lead to special problems, such as the conversion of closely-opposed damages into double-strand break. However, the activity of DNA glycosylases such as the Fpg and Nth proteins is strongly sensitive to the presence of damage in the opposing strand (David-Cordonnier *et al.*, 2001; Harrison *et al.*, 1998). The human apurinic/apyrimidinic (AP) endonuclease Ape1 is inhibited by a second abasic residue in the opposing strand 5′ to its target AP site, but is unaffected by such lesions on the 3′ side (Chaudhry *et al.*, 1999; Strauss *et al.*, 1997; Wilson *et al.*, 1995). One interesting suggestion is that these effects would promote sequential rather than simultaneous repair of certain closely opposed lesions and so limit the formation of double-strand breaks. Nevertheless, Blaisdell and Wallace (2001) recently showed that the formation of double-strand breaks in *E. coli* by ionizing radiation depends on the presence of several DNA glycosylases for oxidative damage. Surprisingly, in the absence of these enzymes, the cells were also more radioresistant (Blaisdell & Wallace, 2001). This result suggests that enzyme specificity does not always prevent simultaneous incision at closely opposed lesions.

A paradigm for repair of OAS: 3′-PG

As noted above, oxidative DNA damage is characterized by a substantial component of direct strand breaks. As first shown for the antitumor drug bleomycin (Giloni *et al.*, 1981), the 3′ termini of oxidative strand breaks bear phosphoglycolate esters, the 2-carbon fragments resulting from deoxyribose breakage between C4′ and C3′ of the nucleotide (Figure 1). The other fragment, a base-propenal, is lost from the DNA. 3′-PG thus correspond to one-nucleotide gaps with a residual blocking fragment on one side. This observation nicely rationalized one notable feature of oxidative strand breaks: they cannot be directly rejoined by DNA ligase (Von Sonntag, 1987). Although oxidative strand breaks in genomic DNA are repaired very rapidly *in vivo*, the process must involve an excision step to remove the 3′-PG, followed by repair synthesis to replace the missing nucleotide, and finally ligation.

During the 1970's and early 1980's, several groups identified DNA exonucleases that might be involved in the repair of 3′-PG. The first clear demonstration of enzymatic removal of 3′-PG followed the identification of radiation-induced 3′-PG using high-resolution electrophoresis (Henner *et al.*, 1983). Application of this approach showed that *E. coli* exonuclease III excises 3′-PG, while DNA polymerase I was incapable of removing this lesion. At about the same time, it was reported that exonuclease III-deficient *E. coli* are acutely hypersensitive to the toxicity of H_2O_2 (Demple *et al.*, 1983). These observations prompted the hypothesis that a 'class II' (hydrolytic) AP endonuclease, exonuclease III, might have the key role in excising H_2O_2-generated 3′-PG. Indeed, it was subsequently shown that unrepaired strand breaks accumulate in the chromosomal DNA of exonuclease III-deficient cells treated with H_2O_2 (Demple *et al.*, 1986).

A key advance in addressing the biology and repair of 3′-PG came with the development of defined substrates. The first of these was an analog of 3′-PG, 3′-phosphoglycolaldehyde (3′-PGA), which could be generated in labeled form and in high yield using a series of enzymatic reactions and group-specific chemistry (Demple *et al.*, 1986). The same substrate could be differentially processed to yield 3′-P or other residues. The use of 3′-PGA substrates allowed the demonstration that exonuclease III is by far the major 3′-repair diesterase of *E. coli*, but that another class II AP endonuclease, endonuclease IV, constitutes ~5% of the total cellular activity (Demple *et al.*, 1986). Furthermore, endonuclease IV was shown to share with exonuclease III a robust 3′-phosphatase activity.

The biological function of the 3′-phosphatase activity of exonuclease III had been unclear at its discovery (Richardson *et al.*, 1964), since no intracellular enzymes were known that generate 3′-P. This question seemed to be resolved by the recognition that bacterial class II AP endonucleases harbor general functions to 'clean up' 3′ termini at oxidative strand breaks. Other 3′-repair activities also seem to exist in *E. coli*, however. With 3′-PGA excision as an assay, three additional activities were resolved in extracts of *E. coli* deficient in exonuclease III and endonuclease IV (Bernelot-Moens and Demple, 1989). These proteins have not been further characterized, and it remains unclear whether they are also class II AP endonucleases.

The use of the 3′-PGA substrate greatly facilitated the isolation and characterization of the main baker's yeast class II AP endonuclease/3′-repair diesterase enzyme, which was named Apn1 (Johnson and Demple, 1988b). The *S. cerevisiae* protein proved to be clearly homologous to *E. coli* endonuclease IV (~40% identity) (Popoff *et al.*, 1990), and Apn1 had catalytic properties essentially identical to the bacterial enzyme (Johnson and Demple, 1988a). The only significant difference identified between Apn1 and endonuclease IV was the presence in the yeast protein of a nuclear targeting signal in a short C-terminal extension (Ramotar *et al.*, 1993). Eventually, the construction of Apn1-deficient yeast confirmed the role of the enzyme in the repair of oxidative damage, presumably including the excision of 3′-PG at oxidative strand breaks (Ramotar *et al.*, 1991).

The utility of the 3′-PGA substrate extended to studies in mammalian cells to identify 3′-repair activities. That work demonstrated two independent activities distinguishable by various criteria of chromatographic properties, molecular size, and lack of immunological cross-reactivity (Chen *et al*., 1991). The major enzyme proved to be identical to the previously identified class II AP endonuclease of human cells, which served to generalize the observation that 3′-repair appears to be mediated primarily by this class of enzymes (Chen *et al*., 1991). The more minor activity has yet to be characterized at the molecular level, and others have since reported a third possible activity that was not further characterized. The eventual isolation of the cDNA and gene encoding human AP endonuclease (Demple *et al*., 1991; Robson and Hickson, 1991), given the systematic gene name *APE1*, but known variously as *APEX* (genome designation) (Seki *et al*., 1991a,b), *HAP1*, or *REF1* (Xanthoudakis *et al*., 1992), allowed tests of its function. Although an *APE1* knock-out produces embryonic lethality in (Xanthoudakis *et al*., 1994), antisense experiments showed that Ape1 makes a significant contribution to cellular resistance to oxidative damage (Ono *et al*., 1994; Walker *et al*., 1994), in line with a role in repair of 3′-PG and other oxidative lesions. More recently, the ability of Ape1 to excise 3′-PG was confirmed *in vitro* (Chaudhry *et al*., 1999; Winters *et al*., 1994), and an adaptive response has been described in which the induction of Ape1 expression plays a critical role (see Cellular modulation of BER for oxidative damage). Recently, Mitra and colleagues showed that Ape1 carries out the rate-limiting step in 3′-PG repair (Izumi *et al*., 2000).

The ability to release 3′-PG has been used to characterize enzymes isolated from a variety of other organisms, confirming a widespread requirement for repair of oxidized abasic sites. In *Schizosaccharomyces pombe* and *Neurospora crassa*, UV damage endonuclease (UVDE) possesses 3′-repair activities for AP sites nicked by AP lyase and for 3′-phosphoglycolate produced by bleomycin (Kanno *et al*., 1999). Separately, a partially purified 45 kDa protein from *S. pombe* is also reported to remove 3′-PG (Jilani and Ramotar, 2002). Still other examples of such 3′-repair activity include ZmDP2, a plant protein from maize (Betti *et al*., 2001); L1Tc from the parasite *Trypanosoma cruzi* (Olivares *et al*., 1999); and Rrp1 from *Drosophila* (Sander and Huang, 1995). Additional proteins from *S. cerevisiae*, including APN2 (Unk *et al*., 2001) and Pdel (Sander and Ramotar, 1997), are also reported to excise 3′-PG, suggesting that some redundancy is important.

Mammalian polynucleotide kinase and 3′ repair

As noted earlier, a proportion of radiation-induced strand breaks bears 3′-P termini (Henner *et al*., 1983). In some organisms, the repair of these lesions may be mediated by AP endonucleases. The bacterial AP endonucleases exonuclease III and endonuclease IV and the *S. cerevisiae* Apn1 protein all have substantial 3′-phosphate activity (Demple and Harrison, 1994) that could carry out this task. However, human Ape1 protein has a very low level of 3′-phosphatase activity (Chen *et al*., 1991), which suggests that other proteins might be involved in removing these blocking groups in mammalian cells.

A good candidate for the biologically relevant 3′-end phosphatase activity has emerged recently in the form of mammalian polynucleotide kinase (Jilani *et al*., 1999b). Bifunctionality in the polynucleotide kinase of bacteriophage T4 has been known for quite a long time, and the kinase and phosphatase functions seems to be centered in separate active sites in the prokaryotic enzyme (Soltis and Uhlenbeck, 1982). Molecular cloning of the human gene encoding polynucleotide kinase (*PNKP*) has helped establish the bifunctionality of the enzyme and point to a role for it in repairing oxidative strand breaks (Jilani *et al*., 1999a). For example, the human *PNKP* gene cross-complements some of the hypersensitivity to oxidative agents of AP endonuclease-deficient (*xth nfo)*, *E. coli* (Jilani *et al*., 1999a).

Recent studies of the role of polynucleotide kinase in repairing oxidative strand breaks reveal some specific functions in human cells. Human polynucleotide kinase interacts with Xrcc1 protein, which together with the other BER repair proteins DNA polymerase β (Polβ) and DNA ligase III forms a complex (Whitehouse *et al*., 2001). The interaction of polynucleotide kinase with Xrcc1 stimulates both the kinase and phosphatase activities (Whitehouse *et al*., 2001), which further supports a biological role for the interaction. More recently, an alternative role is indicated by the dependence of the kinase activity of polynucleotide kinase on Xrcc4 protein and the catalytic subunit of DNA-dependent protein kinase (Chappell *et al*., 2002). These observations point to a role in non-homologous end-joining, a process that corrects DNA double-strand breaks but risks the loss of some sequence information. In some instances, the lack of appropriate homology or other constraints may demand the non-homologous pathway; certainly, oxidative strand breaks cannot be rejoined directly (Von Sonntag, 1987) owing to the loss of a nucleoside during the formation of 3′-PG or 3′-P.

AP Endonuclease incision at oxidized abasic sites

Most abasic DNA lesions are likely processed by one of the central components of BER, the AP endonuclease (Demple and Harrison, 1994). The enzyme specifically hydrolyzes the phosphodiester on the 5′-side of the abasic residue to produce a 3′-hydroxyl nucleotide. This 3′-end can then support subsequent repair synthesis by DNA polymerase. The best studied AP endonucleases are *E. coli* exonuclease III and its human homolog Ape1, and also *E. coli* endonuclease IV (Mol *et al*., 2000). Less well-characterized members of this family have recently been identified in yeast

(Eth1/Apn2 protein) (Bennett, 1999; Johnson *et al*., 1998b) and mammalian cells (Ape2 protein) (Hadi *et al*., 2002; Hadi and Wilson, 2000; Tsuchimoto *et al*., 2001). A structurally distinct family of AP endonucleases is typified by *E. coli* endonuclease IV and *S. cerevisiae* Apn1 protein.

The ability of AP endonucleases to cleave different OAS has been explored to some extent. In one set of experiments using plasmid DNA, higher concentrations of exonuclease III (13-fold) or endonuclease IV (threefold) were required for cleavage at OAS damage generated by Cu(II)-phenanthroline (presumptive dL residues) compared with heat/acid-generated abasic sites (Häring *et al*., 1994). For bleomycin-induced OAS (2-deoxypento-4-ulose), exonuclease III was 400-fold less effective than at regular AP sites, while endonuclease IV was fourfold more effective (Häring *et al*., 1994).

The human endonuclease Ape1 was relatively ineffective against both dL and KA in that study (Häring *et al*., 1994). In contrast, in experiments with a duplex oligonucleotide containing just a single predominant site for bleomycin damage, human Ape1 protein incised the C4′-keto-C1′-aldehyde sites only slightly less well than regular abasic sites at the same location (Xu *et al*., 1998). A substrate with a site-specific dL residue (generated by using a photosensitive nucleotide analog) revealed that human Ape1 has the same k_{cat} for dL as for a regular AP site at the same position, and a K_m only fivefold higher (Xu *et al*., 2002). It may be that the plasmid substrates have sequence context or superhelicity effects that interfere with action by Ape1; since such effects might be quite relevant to the effectiveness of repair *in vivo*, this issue merits further study.

Initial studies using synthetic substrates showed that human Ape1 was ~100-fold less active at 3′-PGA residues than at hydrolytic AP sites, and ~1000-fold less active at 3′-P (Chen *et al*., 1991). These results are consistent with more recent studies showing that Ape1 hydrolyzed 3′-PG 25-fold more slowly than the 2-deoxypentos-4-ulose residues (Xu *et al*., 1998). A study using crude cell extracts further supports this observation by demonstrating that Ape1 is the rate-limiting enzyme for 3′-phosphoglycolate excision (Izumi *et al*., 2000). Thus, although it has been repeatedly suggested that other proteins might replace or activate Ape1 in handling 3′-terminal abasic lesions and fragments, such activity was not apparent in cell-free extracts.

The kinetic data indicate that OAS would be readily processed in the first step of BER, especially since AP endonucleases are quite abundant in most cells (Demple and Harrison, 1994). For instance, human fibroblasts are estimated to contain 200 000 – 300 000 Apel molecules per cell (Cappelli *et al*., 2001; Chen *et al*., 1991), and HeLa cells may have up to 7×10^6 Ape1 molecules per cell (Chen *et al*., 1991). It is therefore expected that incision by AP endonuclease would incise most dL or KA sites long before competing enzymes, such DNA glycosylase-associated AP lyases would have an opportunity to interact with these OAS. Further, there is evidence that several such AP lyases fail to cleave dL sites and only poorly process C4′-oxidized AP sites (Häring *et al*., 1994). This proposed predominance of AP endonuclease incision at OAS may also be consistent with a report that most endogenous abasic sites in mammalian cells were recovered in a form already cleaved on the 5′ side of the lesion (Nakamura and Swenberg, 1999).

Excision of OAS

Dual pathways of BER

Following incision by AP endonuclease, a 5′-terminal deoxyribose-phosphate group remains and must be removed prior to a final ligation step. This process is achieved differently by two sub-pathways of BER (Frosina *et al*., 1996; Klungland and Lindahl, 1997; Matsumoto *et al*., 1994). The predominant, 'short-patch' mode is limited to excision of only the terminal abasic residue. In mammalian cells, most dRp excision is attributable to Polβ (Sobol *et al*., 1996), specifically its amino-terminal 8-kilodalton domain (Matsumoto and Kim, 1995). In this process, lysine-72 performs a nucleophilic attack on the C1-carbonyl carbon of the 5′-terminal AP site, which forms a Schiff base intermediate between the enzyme and the DNA (Feng *et al*., 1998; Piersen *et al*., 1996) (Figure 2). β-elimination breaks the 3′ phosphodiester, and the enzyme-deoxyribose complex is hydrolyzed to yield the free abasic-5-phosphate residue. Considerations of enzyme kinetics suggest that DNA synthesis may precede AP excision (Srivastava *et al*., 1998), but this order of events has not been directly established and may well vary, dependent on the relative amounts of the BER enzymes and the distribution of different DNA lesions.

In the 'long-patch' mode of BER, the 5′-terminal AP site is removed as part of a short segment of DNA, which includes one to several additional nucleotides (DeMott *et al*., 1996; Dianov *et al*., 1999; Frosina *et al*., 1996). This endonucleolytic excision is performed in different organisms by structurally-related enzymes, including the N-terminal nuclease domain of DNA polymerase I in *E. coli* (Dianov and Lindahl, 1994), Rad27/RTH1 in *Saccharomyces cerevisiae* (Johnson *et al*., 1998a; Sommers *et al*., 1995), and the 'flap' endonuclease (FEN1) in mammalian cells (DeMott *et al*., 1996; Klungland and Lindahl, 1997; Prasad *et al*., 2000). The preferred substrate for these enzymes is a 5′-terminal flap structure that results from strand displacement DNA synthesis. Thus, in long-patch BER, AP excision necessarily follows repair synthesis. Polβ may be responsible for most of the strand displacement synthesis in long-patch BER (Dianov *et al*., 1999), but there is also some evidence for involvement of other DNA polymerases (Fortini *et al*., 1998). Another important component of mammalian long-patch repair is the protein PCNA (proliferating cell nuclear antigen), which assists replication by DNA polymerases δ and ε, but has also been shown to stimulate excision of the flap by FEN1 (Matsumoto *et al*., 1999; Tom *et al*., 2000). Indeed,

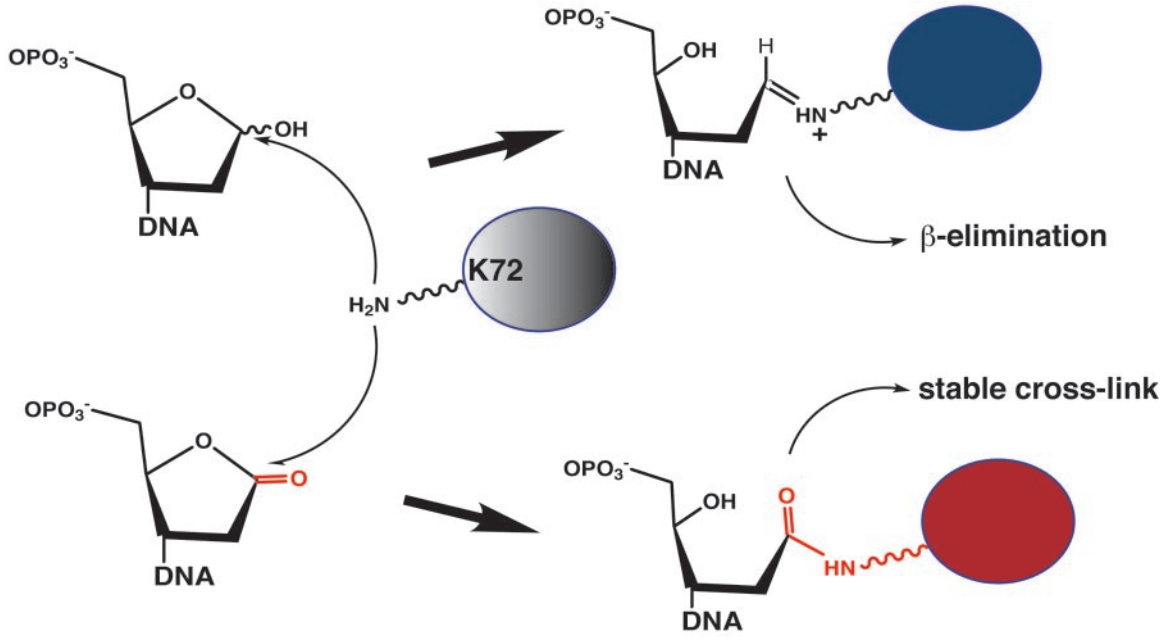

Figure 2 Trapping of Polβ during attempted excision of 5′-dL. Following incision by Ape1 or another 5′ AP endonuclease, abasic sites are removed by Polβ in a β-elimination reaction dependent on lysine-72 of the polymerase (upper pathway). In contrast, the corresponding reaction with Ape1-incised dL generates an amide linkage between Polβ and dL, which traps the polymerase in a stable, covalent protein-DNA cross-link (lower pathway)

ionizing radiation triggers induction of PCNA and repair patches even in cells defective for nucleotide excision repair (Karmakar *et al*., 2001), suggesting a role for PCNA-dependent BER in repair of oxidative DNA damage. Excision by FEN1 appears to be accurate enough, such that the products can then be directly ligated to complete the repair process (DeMott *et al*., 1996, 1998; Murante *et al*., 1995).

The ability of Polβ to excise OAS following incision by Ape1 is likely a key determinant for determining whether the short-patch or long-patch BER pathway is activated. One observation, using whole-cell extracts, indicated that a significant fraction of radiation-induced OAS might be resistant to excision by Polβ (Klungland and Lindahl, 1997). On the other hand, *in vitro* data indicate that Polβ is indeed capable of excising at least one type of OAS efficiently: bleomycin-induced C4′-oxidized AP sites were readily incised by human Ape1 and excised by Polβ (Xu *et al*., 1998).

Dangerous liaisons: Enzyme trapping by dL

An important advance recently has been the development of methods to generate dL lesions site specifically (Hwang *et al*., 1999; Jourdan *et al*., 1999; Kotera *et al*., 2000; Lenox *et al*., 2001). In these approaches, a photosensitive nucleotide analog is incorporated during oligonucleotide synthesis at a defined position, and converted photochemically to a C1′ radical. Under aerobic conditions, this radical predominantly forms dL, although some direct strand breaks bearing 3′-P are also produced (Chatgilialoglu and O'Neill, 2001). Hashimoto *et al*. (2001) reported that *E. coli* endonuclease III, a DNA glycosylase with associated AP lyase activity, formed a protein-DNA cross-link with dL dependent on the key lyase active site residue (lysine-120). That work also showed that the same cross-linked species was generated upon incubation of endonuclease III with DNA treated with neocarzinostatin, which forms dL (Hashimoto *et al*., 2001).

In contrast to endonuclease III, the Apel AP endonuclease incises dL residues rather efficiently (DeMott *et al*., 2002; Xu *et al*., 2002). In view of the amount of Ape1 present in most cell types (Cappelli *et al*., 2001; Chen *et al*., 1991), it appeared likely that most dL residues would be 5′ cleaved by the enzyme before AP lyases would have the opportunity to react. The next enzyme of BER, Polβ, would then be poised to mediate excision of the abasic residue. However, chemical considerations and the above observations with endonuclease III suggested that there would be mechanistic problems with such a reaction. Indeed, reaction of Polβ with an oligonucleotide containing an Ape1-cleaved dL residue led to the formation of a protein-DNA cross-link between the oligonucleotide and the polymerase (DeMott *et al*., 2002) (Figure 2). The formation of this cross-link depended on the lyase active site lysine-72 of Polβ (DeMott *et al*., 2002). The formation of such cross-links would clearly be a problem for cells, since the reaction would lock in a DNA strand break and introduce an unusual bulky lesion that likely presents problems for cellular repair processes. One interesting study indicates that the bacterial nucleotide excision repair system can incise

DNA containing an AP lyase trapped by chemical reduction in an unbroken DNA strand (Minko *et al*., 2002). However, the processing of a protein trapped at a DNA strand break (as is the case for dL-trapped Polβ) remains unexplored.

Cellular modulation of BER for oxidative damage

BER adaptive responses to oxidative stress

Over the past 20 years, it has become clear that all types of cells respond to oxidative stress (excess production or insuficient scavenging of reaction oxygen species) by activating sets of defense genes (Martindale and Holbrook, 2002; Pomposiello and Demple, 2002). While these adaptive response systems usually include many direct antioxidant functions, such as superoxide dismutase or catalase, individual BER enzymes may also be induced.

In *E. coli*, exposure to superoxide-generating agents (e.g., paraquat) or to nitric oxide activates the *SoxRS* regulon. This system controls the expression of >50 genes (Pomposiello *et al*., 2001), and it was found early on that the paraquat-inducible *nfo* gene (encoding endonuclease IV) is a component of the *soxRS* regulon (Chan and Weiss, 1987). In contrast, neither the genes encoding the oxidative damage-specific DNA glycosylases, such as endonuclease III, nor those for DNA polymerases are controlled by *soxRS*.

A rationale for the specific induction of endonuclease IV activity (which is increased up to $\sim$20-fold) derives from the enzyme's special role in repairing oxidative DNA damage. Although exonuclease III is present in 20 : 1 excess over endonuclease IV, *nfo* mutant cells (lacking endonuclease IV) are hypersensitive to the antitumor drug bleomycin (Cunningham *et al*., 1986). Bleomycin generates a rather restricted set of oxidative DNA lesions: only 3′-PG and KA lesions are formed, although they are distributed in clusters that give rise to a high proportion of double-strand breaks (Povirk, 1996). Since exonuclease III can act readily at 3′-PG sites (Siwek *et al*., 1988) and is present in abundance, an attractive hypothesis is that endonuclease IV is important for handling the KA lesions. Consistent with this view, the *in vitro* activity of endonuclease IV against belomycin-induced KA sites is $\sim$300-fold higher than that of exonuclease III (Häring *et al*., 1994). Moreover, chromosomal DNA isolated from bleomycin-treated *nfo*$^-$ cells contains unrepaired damage that can be detected *in vitro* by its sensitivity to cleavage by purified endonuclease IV (Levin and Demple, 1996). Direct evidence that this damage corresponds to KA lesions is still needed.

Endonuclease IV has also been shown to cleave effectively at α-nucleotides, an activity not shared by exonuclease III (Ide *et al*., 1994). Like 3′-PG and KA, α-nucleotide lesions are radiation products and probably produced by many oxidative agents (Raleigh, 1989), although they have not been reported as bleomycin products. One can therefore argue that the induction of endonuclease IV as a component of a general oxidative stress response is due to the enzyme's ability to attack diverse types of abasic or abasic-like oxidative lesions.

The expression of exonuclease III is also modulated in *E. coli*. Stationary-phase cells contain higher levels of the enzyme due to transcriptional induction of the *xth* gene under control of the RpoS gene product (Sak *et al*., 1989). RpoS controls a large number of genes, including the *katE*-encoded catalase and other functions against oxidative stress (Hengge-Aronis, 1999). Stationary-phase cells have increased resistance to oxidants such as H_2O_2, which depends on the *xth* and *katE* genes. Hence, it is likely that exonuclease III contributes particularly to repair of oxidative DNA damage in stationary-phase bacteria. The sources and nature of these damages remain to be described.

The yeast *S. cerevisiae* contains one partial homolog of exonuclease III, the Eth1/Apn2 protein (Bennett, 1999; Johnson *et al*., 1998b). This protein consists of a $\sim$350-residue N-terminal domain homologous to exonuclease III and an C-terminal extension of $\sim$160 residues unrelated to other AP endonucleases. Human cells have a counterpart to this protein that has been named Ape2 (Hadi and Wilson, 2000; Tsuchimoto *et al*., 2001). The yeast Eth1/Apn2 protein may function as a back-up activity for the main AP endonuclease Apn1 in the repair of alkylation-induced AP sites. Evidence for a supplemental role for Eth1/Apn2 in genetic stability is shown by the increases in spontaneous mutation rate upon combination of an *eth1*Δ mutation with an *apn1*Δ mutation (Bennett, 1999). Notably in the current context, the *ETH1* gene is strongly induced in cells exposed to alkylating agents (Bennett, 1999). These observations collectively indicate a specific role of Eth1/Apn2 in BER, perhaps in the repair of specific lesions or structures in damaged DNA.

Inducible resistance to oxidative stress/damage has also been described in mammalian cells, but at a rather elementary level of detail so far. Several groups (Fung *et al*., 1998; Grosch *et al*., 1998; Ramana *et al*., 1998) have shown that transcription of the *APE1* gene is induced in human or rodent cells exposed different types of oxidative agents: asbestos, H_2O_2, bleomycin, sodium hypochlorite. A role for CREB has been indicated for the induction of the *APE1* gene in murine cells (Grosch and Kaina, 1999), but the responsible transcription factors and the stress signal transduction pathways are otherwise unknown. However, cells treated to induce *APE1* express increased resistance to the cytotoxicity of bleomycin and other redox agents (Grosch *et al*., 1998; Ramana *et al*., 1998). These results indicate that the level of Ape1 (or another activity induced under the same conditions) is limiting for repair of one or more important oxidative lesions in DNA. In fact, in experiments with cell-free extracts the addition of extra Ape1 protein enhanced the repair of 3′-PG lesions, while Ape1 depletion using specific antibodies blocked such repair (Izumi *et al*., 2000). It remains to be shown that this role accounts for the increased resistance to oxidants in cells with induced levels of Ape1.

Conclusion

While the existence of OAS has been known for some time, our understanding of these lesions is only now gaining clarity. Recent evidence of high steady-state levels of 5′-cleaved abasic sites resistant to Polβ (Nakamura and Swenberg, 1999), along with their increased formation upon treatment with H_2O_2 (Nakamura *et al*., 2000), and the potential of some OAS to form repair debilitating protein-DNA cross-links (DeMott *et al*., 2002; Hashimoto *et al*., 2001) points to the likely significance of these lesions. More refined techniques for efficient, site-specific generation of different OAS lesions, especially 3′-PG and dL, will prompt further inquiry into their mutagenic effects and the pathways responsible for the avoidance of mutagenesis. Developing methods for the direct detection of specific OAS *in vivo* is a technical challenge, the solution of which will open the door to a refined understanding of the biology of OAS.

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