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A Novel Link to Base Excision Repair

David M. Wilson III* and Michael M. Seidman

Laboratory of Molecular Gerontology National Institute on Aging, National Institutes of Health, IRP
251 Bayview Blvd, Suite #100, BRC Baltimore, MD 21224 USA

Abstract

DNA interstrand crosslinks (ICLs) can arise from reactions with endogenous chemicals, such as the lipid peroxidation product malondialdehyde, or from exposure to a variety of clinical anti-cancer drugs, most notably bifunctional alkylators and platinum compounds. Because they covalently link the two strands of DNA, ICLs are absolute blocks to transcription and replication, and as a result, lethal to the cell. It is well established that proteins of nucleotide excision repair and homologous recombination are involved in ICL resolution. Recent work, coupled with a much earlier report, suggest an emerging link between proteins of the base excision repair pathway and crosslink processing.

Overview

DNA interstrand crosslinks (ICLs) consist of a covalent linkage that physically connects the two strands of DNA. As ICLs prevent strand separation, they are absolute blocks to processes such as DNA replication or RNA transcription. Consequently, ICLs are potent toxic DNA lesions that have the ability to promote either genetic instability or cell death, and as a result, contribute to disease and aging¹. Whereas prior studies have clearly revealed the contributions of components of nucleotide excision repair (NER) and homologous recombination in the resolution of ICLs, we discuss herein emerging evidence that implicates proteins of base excision repair (BER) in the response to crosslinking agents. Specifically, the *nei* endonuclease VIII-like 1 (NEIL1) DNA glycosylase and the *N*-methylpurine DNA glycosylase (MPG; we note that MPG, the approved HUGO nomenclature, is often referred to as AAG in the literature), as well as the major apurinic/apyrimidinic (AP) endonuclease (APE1), contribute to cellular resistance to crosslinking agents. Furthermore, biochemical studies suggest that NEIL1 processes intermediates in the crosslink repair pathway.

Formation of ICLs

ICLs can be formed by both endogenous and exogenous chemical agents (Figure 1). For example, in the former case, malondialdehyde, which is a product of lipid peroxidation, can create etheno adducts in DNA that can undergo rearrangement to create an ICL². In the latter case, aldehydes, such as acrolein and crotonaldehyde found in tobacco smoke or automotive exhaust, can react with DNA bases in a way that generates ICLs^{3,4}. In addition, psoralens, which are found as natural products in plants, can be activated by long-wave ultraviolet light (UVA) to form covalent monoadducts with pyrimidines or an ICL between the thymines of TA sequences in opposite strands⁵. Psoralen + UVA is widely used in the treatment of psoriasis,

*Corresponding author: wilsonda@mail.nih.gov, Ph (410) 558-8153, Fx (410) 558-8157.

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eczema, vitiligo, and cutaneous T-cell lymphoma. Finally, many of the chemotherapeutic agents used today to eradicate cancer involve the formation of DNA ICLs to induce cell killing, including bifunctional alkylating agents, such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, Carmustine) and nitrogen mustard, and the platinum compounds, such as cisplatin (cis-diamminedichloroplatinum(II)) and its derivative forms^{6,7}.

Major Repair Mechanisms for ICLs

As ICLs involve covalent linkage of the two strands of DNA, the crosslink must be “unhooked” to allow for strand separation required for either transcription of a coding region or replication of the genome. In the absence of crosslink resolution, the damage will likely result in cell death, or minimally, in chromosomal instability to permit survival. There are multiple pathways for ICL repair, variable as to the cell cycle phase and mechanism of ICL recognition, each requiring two stages to complete removal of the adduct (see⁸ for review).

In G1 phase (Figure 2a), ICL repair is dependent on the xeroderma pigmentosum group C (XP-C)–human RAD23 homolog (HR23B) protein complex, which binds helical distortions, and directs adducts into the NER pathway, a process defective in the UV sensitive, cancer-prone syndrome XP⁹. Alternatively, ICLs can be encountered by the transcriptional apparatus and enter a transcription-coupled repair pathway, again requiring NER functions¹⁰. Excision repair cross-complementing 1 (ERCC1)–XP-F-dependent incision of one strand on either side of the crosslinked base unhooks the duplex and generates an intermediate that is often depicted as a gapped structure, but likely requires further processing^{11,12}. In particular, it is believed that the incised oligonucleotide is digested by nucleases (SNM1A is a candidate¹³) to form a gapped intermediate, with the adducted base in the non-incised strand linked to at least one base from the excised oligonucleotide. This crosslink “remnant” will be present during gap-filling synthesis by lesion bypass polymerases. Recent cellular and biochemical work implicates polymerases κ , η and ζ , and the deoxycytidyl transferase REV1, in this process^{14–16}. Moreover, the bypass step can be the source of mutations, which appear to be dependent on polymerase ζ ^{17,18}. Closure of the gap restores the duplex (completion of stage I) and forces out the remnant monoadduct, which then enters a second repair cycle involving classic NER processing (stage II).

In S phase cells (Figure 2b), the encounter of a replication fork with a crosslink triggers a complex response in which the crosslink is removed and the fork reconstructed. The current view is that the stalled fork is recognized by a number of proteins (including FANCM and FAAP24, which are proteins defective in the rare inherited cancer-prone, blood disorder Fanconi anemia (FA)) followed by incision by the MUS81–EME1 nuclease on one side of the crosslink; ERCC1–XP-F makes a second incision on the other side (reviewed in^{19,20}). This series of events unhooks the crosslink, setting the stage for processing of the incised crosslinked oligonucleotide and gap repair. The FA proteins participate in lesion bypass synthesis²¹, and are required for mutagenesis²². Classic NER functions, other than the nuclease activity of the ERCC1–XP-F complex, do not play a role in the first stage of repair. Following gap filling, the crosslink remnant is excised in a second stage of repair by the classic NER pathway. Once the ICL has been completely removed, the fork can be reconstructed by homologous recombination (involving additional FA proteins, including the breast cancer-associated protein, FANCD1; also called BRCA2, among others), and replication renewed.

BER Pathway

BER is an evolutionarily conserved pathway that copes primarily with spontaneous decay products and oxidative damage that arises in DNA. In addition, the pathway corrects non-bulky base modifications, abasic (or AP) sites and single-strand breaks that are generated by environmental agents such as alkylators or ionizing radiation. In its most simplistic form, BER

involves five major steps (Figure 3): (i) release of the damaged substrate base from the intact sugar-phosphate backbone by a DNA glycosylase, (ii) cleavage of the phosphodiester linkage immediately adjacent to the abasic site product by an AP endonuclease, (iii) clean-up of the 3' and/or 5' termini by a collection of enzymes to create a strand break that harbors a 3'-hydroxyl priming group and a 5'-phosphate, (iv) replacement of the missing nucleotide by a DNA polymerase, and (v) sealing of the final nick by a DNA ligase (see^{23–26} for reviews). Defects in this pathway have been linked either directly or indirectly to cancer predisposition and neurodegenerative disease²⁷.

Participation of BER in the Response to ICLs

Typically we think of DNA glycosylases as processing simple, non-bulky base adducts. However, early findings that suggested a role for BER factors in the removal of complex DNA lesions were the observation of binding of MPG to cisplatin intrastrand diadducts²⁸, and the mild hypersensitivities of homozygous *Mpg*-deficient mouse cells to BCNU and mitomycin C (MMC)^{29,30}. At the time, it was unknown whether or not this increased sensitivity resulted from impaired removal of ICLs or one of the many precursor monoadducts. Moreover, yeast cells lacking the equivalent DNA glycosylase (*MAG1*) were shown to be hypersensitive to nitrogen mustard treatment³¹. More recently, Samson and colleagues reported that *Mpg*^{−/−} mouse embryonic stem cells are more sensitive than their wild-type counterparts to the interstrand crosslinking agent 4,5',8-trimethylpsoralen, but behave normally when challenged with angelicin, a psoralen derivative which forms mainly monoadducts and no crosslinks^{32, 33}. In addition, *Mpg*^{−/−} cells displayed an altered double-strand break response and increased apoptosis when exposed to 4,5',8-trimethylpsoralen and UVA. The lack of increased sensitivity to angelicin and the appearance of the double-strand break marker γ -H2AX (albeit with abnormal kinetics) in *Mpg*-deficient cells implies that the protein does not function in monoadduct repair or prevent the formation of ICLs. *In vitro* studies of the Samson group show that MPG has no obvious glycosylase activity on DNA substrates harboring a MMC ICL, an N2-guanine monoadduct, or a monoadduct produced by 4,5',8-trimethylpsoralen or 8-methoxypsoralen^{30,32}, suggesting a non-enzymatic, structural role for MPG in the early steps of ICL resolution (Figure 4). Moreover, MPG, at least under conditions optimal for binding cognate base damage, does not form a stable protein–DNA complex with ICL-containing DNA in electrophoretic mobility shift assays, suggesting that the protein does not play a direct role in ICL recognition.

Prompted in part by the observation that cells depleted for APE1 exhibit hypersensitivity to MMC, Saparbaev and colleagues initiated an effort to evaluate the contribution of BER to psoralen–DNA photoadducts³⁴. Strikingly, they found that siRNA depletion of APE1 resulted in a hypersensitivity of HeLa cells to 8-methoxypsoralen + UVA similar to that seen with cells depleted for FANCD2. Subsequent biochemical studies revealed that NEIL1, but not the human glycosylases MPG, NEIL2, *nth* endonuclease III-like 1 (NTH1) or 8-oxoguanine DNA-glycosylase 1 (OGG1), was able to act in concert with APE1 to initiate repair of photoactivated psoralen-induced DNA monoadducts, but not ICLs, presumably via a classic BER-type response (Figure 4). Consistently, *Neil1*-deficient cells exhibited increased sensitivity to 8-methoxypsoralen + UVA. Unfortunately, it was not determined whether *Neil1* or *Ape1* mutant cells are hypersensitive to angelicin. In a more recent report³⁵, Saparbaev and colleagues found that NEIL1 efficiently excises an unhooked ICL fragment within a three-stranded DNA structure, suggesting that the glycosylase can also operate at the later stages of ICL removal (Figure 4).

Concluding Remarks and Future Perspectives

Participation of DNA Glycosylases in ICL Resolution

Current evidence suggests that a traditional BER response involving excision of the modified base from the sugar-phosphate backbone could operate as an alternative mechanism in crosslinking agent resistance to the major protective pathways, namely NER and homologous recombination (Figure 2). Specifically, biochemical data indicate a direct role for NEIL1 glycosylase function in the removal of the initial precursor monoadducted base (i.e. before ICL formation) or the unhooked three-stranded base remnant product (i.e. prior to stage II repair) (Figure 4). As NEIL1 typically processes base modifications such as formamidopyrimidine and 5-hydroxyuracil, which involve relatively subtle chemical changes to DNA, how NEIL1 recognizes and promotes enzymatic release of such sizeable DNA lesions needs to be determined.

How might NEIL1 recognize a psoralen monoadduct? Glycosylases are generally thought to bind specifically to modified bases that exhibit increased residence in an extrahelical configuration^{36,37}. However, the suggestion that NEIL1 is active on a psoralen-T monoadduct requires additional consideration. The reaction of psoralen with thymidine destroys the aromaticity and planarity of the thymine ring, and as a result, the stacking interactions of the thymine are suppressed. NMR analysis of psoralen monoadducts, however, revealed intercalation of the psoralen attached to the thymidine, accompanied by stacking of the psoralen with the adjacent adenines³⁸. This feature actually reduced the frequency of flipping of the adducted thymidine out of the helix. Nevertheless, as pointed out by the Spielmann group^{39, 40}, the structural interactions of other bases in the vicinity of the monoadduct were perturbed. They proposed that these perturbations could be recognized by DNA damage recognition proteins. Thus, although the frequency of extrahelical presentation of a thymidine would be reduced by the psoralen monoadduct, the affinity of a repair protein, such as NEIL1, for the immediate vicinity might be increased. Consequently, the glycosylase would spend relatively more time near the monoadducted thymidine, increasing the probability of capturing the base when it does become extrahelical. Of course all of this conjecture is predicated on the ability of the glycosylase active site to accommodate the rather large base modification, a fact that will need to be verified by further studies. Alternatively, it remains a possibility that the NEIL1 glycosylase actually excises an unstable neighboring base, as opposed to the monoadduct itself, facilitating a subsequent nearby incision event.

As for an ICL, the linked bases have no opportunity to become extrahelical, and so would not be substrates for a DNA glycosylase. If, however, the ICL enters a classic repair response, then a crosslink remnant will appear as a product of the first repair cycle (Figure 2). The restoration of the duplex following repair synthesis across the crosslinked base of the intact strand might force the base, and any joining bases remaining from the incised fragment, out of the helix. If that occurred, then the extrahelical structure could be a substrate for a glycosylase such as NEIL1 or possibly MPG. Obviously, this scenario similarly requires that the thymine (in the case of psoralen) with its bulky additions be accommodated in the active site of the glycosylase^{41,42}. It would therefore be of interest to compare the kinetics of cleavage by the glycosylases on duplexes containing a "classic" base substrate (e.g. formamidopyrimidine), a psoralen monoadduct, or a psoralen-T crosslink remnant. It should be emphasized that the work on MPG suggests that this protein does not function in the excision of monoadducts (e.g. angelicin), is not involved in crosslink recognition, and contributes at some step after initial ICL formation in a non-enzymatic/structural capacity (Figure 4). Whether the DNA glycosylase activity of MPG is required for crosslinking agent resistance should be further evaluated using a catalytically-inactive MPG protein in complementation assays.

BER in the Cellular Context

When considering the recruitment and activity of glycosylases on the various crosslink substrates, it is important to note that crosslinks are the targets of other DNA repair pathways, including NER⁸. Thus, entry of the different DNA structures into NER can obscure a role for glycosylases in the repair of these adducts *in vivo*. Consequently, experiments designed to examine a role for DNA glycosylases at various stages in crosslink repair would probably be best performed in NER-deficient cells, in which a major competing repair pathway has been removed. Furthermore, when assessing the influence of a glycosylase deficiency on the sensitivity of cells to crosslinking agents, it would again be best examined in an NER-deficient background. Additionally, the nature of the crosslinking agent is an important consideration, as different agents will elicit different repair responses. For example, MMC forms crosslinks that are relatively non-distorting and thus not engaged effectively by NER⁴³. Conversely, psoralen forms more distorting crosslinks⁴⁴, which can be processed by the NER pathway^{45, 46}. As crosslinks can be “recognized” by replication forks, it will also be necessary to consider a contribution for DNA glycosylases in ICL repair in the context of a replication fork, in addition to a non-replicative setting.

Strengthening the Link to BER

Recently, using a photo-crosslinking technique, Lippard and colleagues found that XRCC1, a nuclear protein that contributes to single-strand break repair and BER, binds to cisplatin-DNA ICLs⁴⁷. This observation expands the list of BER-related factors that might participate in ICL recognition and resolution, as well as the spectrum of ICL chemical forms that could be processed by a BER-type response. How broadly BER might function in crosslinking agent resistance is presently unclear, and it would be valuable to explore the sensitivity of additional BER-deficient cell lines to various crosslinking compounds (for instance those in Figure 1). However, it is important to keep in mind that treatment with most exogenous agents, including MMC and cisplatin, induces not only a range of DNA crosslinks and monoadducts, but also oxidative stress and an assortment of associated oxidative DNA base lesions, which are known to be substrates for DNA glycosylases such as MPG (hypoxanthine and 1,N(6)-ethenoadenine) and NEIL1. As an additional strategy to evaluate the contribution of BER to ICL repair, the use of modified versions of ICLs that permit easy visualization would allow one to monitor the excision kinetics of such DNA lesions in a particular repair-deficient background⁴⁸. It will also be interesting, using technologies that employ site-specific damage-introduction and protein re-localization detection, to define with greater accuracy the molecular stages at which BER factors arrive in response to ICLs⁴⁹. Obviously, defining the molecular mechanisms of ICL repair; the BER proteins that participate in this process, either directly, as auxiliary factors, or in a backup capacity; and the extent to which BER operates in crosslinking agent resistance are important steps towards the design of more effective treatment strategies that involve clinical DNA crosslinking drugs.

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Figure 1.

Examples of DNA Crosslinking Agents. The chemical structure of the indicated agent is shown in the top row. The bottom row depicts the nature of the ICL. (i) Nitrogen mustards form a 1,3 ICL between the N7-positions of two G residues on opposite strands within a 5'-d(GNC) sequence. Mitomycin C (MMC; ii) and cisplatin (iii) generate ICLs between juxtaposed Gs of 5'-d(GC). (iv) [scf]Psoralen can produce ICLs between the T residues of 5'-d(TA) complementary sequences upon exposure to UVA. The frequency of ICLs for cisplatin is around 5–10% of the total adducts, about 5% for nitrogen mustards, less than 10% for MMC, and 20–30% for psoralen. See⁵⁰ for further details.

Figure 2.

Conventional Mechanisms for ICL Repair. Cells cope with ICLs differently depending on the stage of cell cycle and mode of ICL recognition. a) G1 phase repair. ICL recognition is mediated by XPC–HR23B dependent functions, or perhaps by a block to transcription (not shown). The ERCC1–XPF endonuclease is required for crosslink “unhooking”, in which incisions are placed on either side of the linked base on one strand of the duplex. Further processing, perhaps by the SNM1A nuclease, converts the incision product to a gapped structure that can be filled-in by a translesion DNA synthesis (TLS) polymerase. This step completes the first stage of ICL repair and produces an intact duplex with a crosslink remnant that is a substrate for classic NER⁵¹, which carries out the second and final stage of ICL repair. b) S phase repair. Recognition as a consequence of collision of the ICL with a progressing replication fork is followed by cleavages by MUS81–EME1 and ERCC1–XPF which unhook the crosslink. Additional processing as in G1 phase results in lesion bypass [with the aid of Fanconi anemia (FA) proteins] to generate a crosslink remnant. Classic NER excises and replaces the segment of DNA harboring the remnant, before the replication fork is reestablished by homologous recombination.

Figure 3.

BER Pathway. The five enzymatic steps of BER are indicated to the left, and the major proteins involved in executing these steps are shown to the right. In brief, a DNA glycosylase (e.g. MPG or NEIL1) cleaves the bond that links the substrate base (filled diamond) to the sugar phosphate backbone, creating an AP site intermediate (filled circle). The AP site is typically incised by AP endonuclease 1 (APE1) 5' to the lesion to create a strand break with a 3'-hydroxyl group and a 5'-abasic residue (deoxyribose phosphate, dRP). Polymerase β (POL β) then replaces the missing nucleotide (thicker line) and excises the 5'-dRP group. The remaining nick is sealed by a complex comprising X-ray repair cross-complementing 1 (XRCC1) and DNA ligase 3 (LIG3 α). Shown is short-patch (or single nucleotide) BER, but there are alternative BER-related responses, such as long-patch BER, which involves the incorporation of multiple nucleotides and the formation of a 5'-flap DNA intermediate that requires processing by flap-endonuclease 1 (FEN1) (see^{26,52} for further details).

Figure 4. Potential Involvement of BER Proteins in ICL Resolution. NEIL1 might initiate a classic BER response by excising (i) a psoralen monoadduct (MA) prior to the commencement of Stage I of ICL repair or (ii) the crosslink remnant (CR) formed at the completion of the first stage of ICL repair (see also Figure 2). This excision (glycosylase) activity would generate an abasic site (filled circle) that would be processed by APE1 and the remaining BER proteins (Figure 3). The precise involvement of MPG is less clear, but evidence suggests participation after ICL formation in a structural, non-enzymatic capacity.