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A role for the base excision repair enzyme NEIL3 in replication-dependent repair of interstrand DNA cross-links derived from psoralen and abasic sites

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Abstract

Interstrand DNA-DNA cross-links are highly toxic lesions that are important in medicinal chemistry, toxicology, and endogenous biology. In current models of replication-dependent repair, stalling of a replication fork activates the Fanconi anemia pathway and cross-links are “unhooked” by the action of structure-specific endonucleases such as XPF-ERCC1 that make incisions flanking the cross-link. This process generates a double-strand break, which must be subsequently repaired by homologous recombination. Recent work provided evidence for a new, incision-independent unhooking mechanism involving intrusion of a base excision repair (BER) enzyme, NEIL3, into the world of cross-link repair. The evidence suggests that the glycosylase action of NEIL3 unhooks interstrand cross-links derived from an abasic site or the psoralen derivative trioxsalen. If the incision-independent NEIL3 pathway is blocked, repair reverts to the incision-dependent route. In light of the new model invoking participation of NEIL3 in cross-link repair, we consider the possibility that various BER glycosylases or other DNA-processing enzymes might participate in the unhooking of chemically diverse interstrand DNA cross-links.

Graphical abstract

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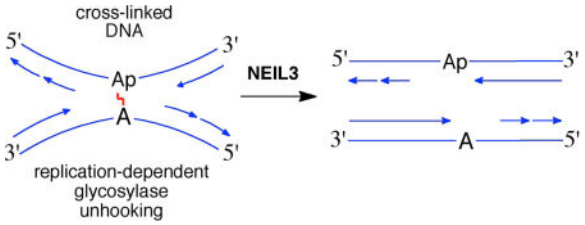
Conflict of interest

The authors declare no conflict of interest



Keywords

DNA cross-link; cross-link repair; Fanconi anemia; homologous recombination; base excision repair; XPF-ERCC1; NEIL; abasic site; psoralen

1. Introduction. DNA Interstrand cross-links in biology and medicine

Reading and replicating the genetic information encoded in the sequence of nucleobases in DNA requires cellular operations that separate the two strands of the double helix [1–3]. Interstrand cross-links introduced by bifunctional alkylating agents such as nitrogen mustard anticancer drugs[4–7] prevent strand separation and compromise critical cellular functions of duplex DNA [8, 9]. As a result, DNA cross-links are highly toxic to cells [10, 11]. A wide range of organisms, from bacteria to mammals, possess elaborate systems for the repair of interstrand cross-links [12]. Over twenty proteins are required for cross-link repair in vertebrates [12–16]. The conserved nature of such resource-intensive repair systems suggests that the formation of interstrand cross-links in cellular DNA is an inevitable fact of life. Endogenous processes or unavoidable exposure to environmental agents have the potential to generate interstrand cross-links in cellular DNA [8, 17–27], but the chemical nature of the selection pressures driving the evolution and retention of cross-link repair systems across all walks of life remains uncertain. In medicine, the repair of interstrand cross-links helps define response and resistance to drugs such as cisplatin, carmustine (BCNU), and nitrogen mustards that are commonly used in the treatment of human cancers [28–33].

2. Replication-dependent cross-link repair

When DNA replication is stalled by an interstrand cross-link, complex repair processes are activated [34]. There are excellent comprehensive reviews of replication-dependent interstrand cross-link repair [12–16] and we will summarize briefly here. Advancement of a replication fork is stalled when the Cdc45-MCM-GINS (CMG) helicase complex [2, 35, 36] encounters an interstrand cross-link. Due to the size of this complex, DNA polymerization halts between −40 and −20 nucleotides (nt) from the 3′-side of the cross-link on the leading strand (Figure 1) [34, 37]. Stalling of a replication fork leads to activation of the Fanconi anemia pathway and association of the FANCD2/FANCI heterodimer with the stalled replication fork leads to ubiquitination of this protein complex [34, 38–45]. An emerging view holds that cross-link repair is initiated when two replication forks converge at the lesion [37]. The CMG helicases then dissociate in a process that involves BRCA1-BARD1 and ubiquitin signaling [46]. Dissociation of the helicase complex [2, 35] enables DNA

polymerization to progress to the −1 position immediately preceding the cross-link on the leading strand (Figure 1). The activated FANCD2/FANCI complex recruits endonucleases that make incisions on either side of the cross-link [38, 41, 45]. In this process, the SLX4 protein serves as a scaffold that coordinates the action of several structure-specific endonucleases including XPF-ERCC1, MUS81-EME1, and SLX1 [45, 47, 48]. The involvement of XPF-ERCC1 has been clearly demonstrated[49, 50] and cells deficient in these proteins are acutely hypersensitive to interstrand cross-linking agents [44, 51–54]. The identity of other endonucleases that make incisions during cross-link repair, and the circumstances under which they become involved, remains under investigation [45, 48, 51, 52, 55–58]. Incisions by structure-specific endonucleases “unhook” the cross-link and generate a double strand break (Figure 1). The unhooked cross-link remnant may be trimmed [34] to a smaller adduct (possibly by the exonuclease activity of SNM1A) [51, 59] to facilitate lesion bypass by translesion synthesis (TLS) polymerases such as Polκ (kappa), Polη (eta), and Polν (nu) [60–63]. Extension may then be carried out by a complex containing REV1 and Polζ (zeta) [34, 64, 65]. Indeed, cells deficient in REV1 and Polζ are hypersensitive to cross-linking agents.[64, 66] It remains uncertain whether REV1/Polζ cooperate with other TLS polymerases as seen in the bypass of pyrimidine dimers.[67] Homologous recombination (HR) using the newly synthesized duplex on the leading strand as the homology repair partner is required to repair the double-strand break [68–71]. Finally, nucleotide excision repair (NER) may remove the unhooked cross-link remnant to regenerate a native DNA duplex [70]. There is much evidence supporting the general pathway shown in Figure 1; however, it is worth noting that a distinct pathway involving replication traverse of a psoralen-derived cross-link without repair has been suggested [72, 73].

3. Base excision repair DNA glycosylases and their involvement in cross-link repair

The base excision repair (BER) pathway is critical for the removal of damaged, misincorporated, and mispaired DNA bases [74–81]. BER typically begins with DNA glycosylase enzymes that recognize a damaged or mispaired nucleobase (B* in Figure 2A) and catalyze hydrolysis of the glycosidic bond holding the base to the deoxyribose backbone [82]. Various BER glycosylases remove a wide array of damaged and mispaired nucleobases [74–80]. Importantly, substrate recognition and catalysis by glycosylases typically involve “flipping out” (extrusion of) the target base from the double helix – a process that is presumably precluded for bases involved in an interstrand cross-link (Figure 2B) [81–83]. Monofunctional BER enzymes catalyze hydrolytic removal of a nucleobase leaving an intact abasic (Ap) site in the DNA (Figure 2A), while bifunctional DNA glycosylases remove the base and also catalyze a subsequent elimination (lyase) reaction that generates a strand break with a 5′-phosphoryl end group and 3′-deoxyribose phosphate sugar remnant [74, 76, 78, 79, 82, 84–86].

Over the years, there have been reports that BER proteins can interact with cross-link repair pathways [87]. For example, there is evidence that murine 3-methyladenine glycosylase (Aag) plays a role in cellular resistance to psoralen-derived cross-links [88]. However, *in*

vitro studies showed that human AAG did not bind or process a 21 base pair duplex containing a psoralen-derived cross-link, leading the authors to conclude that "Aag's role in conferring protection against psoralen-induced (cross-links) is either indirect or involves a DNA substrate that is an intermediate of the repair process" [88]. In a separate example, the glycosylase NEIL1 was shown to accumulate at psoralen-derived interstrand cross-links in cells and *inhibit* repair of these lesions [89]. *In vitro* gel electrophoretic mobility shift assays demonstrated that NEIL1 bound to a 21 base pair cross-linked duplex, with an affinity at least 4-fold greater than that for a native control duplex [89]. In another set of studies, it was found that the deletion of the BER proteins Pol β and uracil DNA glycosylase (UNG) conferred resistance to cisplatin toxicity [90]. This led to a proposal that UNGs remove uracil residues generated by deamination of cytosine residues near cisplatin cross-links, thus inhibiting subsequent repair [91]. In a final early example where BER may influence the repair of cross-links, Couvé et al. showed that NEIL1 can excise an unhooked, psoralen-derived cross-link remnant from a three-stranded structure (e.g. bottom duplex in Figure 3) [92, 93]. This type of glycosylase activity would not provide initial unhooking of the cross-link, but could provide an alternative to NER for the final "clean-up" of the psoralen cross-link remnant (last step in Figure 3).

4. Evidence for a direct role for the catalytic action of a BER glycosylase in cross-link repair

A recent study by Walter and coworkers provides evidence that the catalytic activity of a BER glycosylase enzyme can play a central role in the repair of interstrand DNA cross-links [94]. In this work, the direct catalytic action of the BER glycosylase NEIL3 was implicated in the repair of both psoralen-derived and abasic site-derived interstrand DNA cross-links in *Xenopus* egg extracts.

Psoralens are a class of plant-derived DNA cross-linking agents [95]. The planar furocoumarin system intercalates between the base pairs of duplex DNA where 300–400 nm light can induce reactions between the pi-bonds of the natural product and the 5,6-double bonds of thymine and cytosine residues to generate both covalent monoadducts and interstrand cross-links (Figure 4A) [96–99]. Interstrand cross-links arise preferentially at 5′-TA sequences[100] via sequential photoinduced [2+2] cycloadditions of thymine residues with the 4′,5′-double in the furan ring and the 3,4-double bond of the pyrone system [95, 98] (Figure 4B). DNA duplexes containing a psoralen cross-link display local unwinding at the cross-link site but, overall, retain a B-form structure [96–102]. However, the cross-link structure in duplex DNA may not be directly relevant to the X-shaped and Y-shaped structures generated at a stalled replication fork, which are the true substrates for replication-coupled repair [48].

In the *Xenopus* egg extract system of Semlow et al., the repair of a psoralen cross-link was distinct from that previously observed for a cisplatin interstrand cross-link [94]. Relatively small amounts of homologous recombination intermediates were seen and, while FANCD2 was ubiquitinated, depletion of the FANCD2/FANCI complex had a minimal effect on repair. This led the authors to consider the possibility that the psoralen cross-link might be

unhooked by the action of a BER glycosylase. This process would generate an abasic site on one strand and a modified thymine residue on the other (Figure 3). Several lines of evidence supported this supposition. Depletion of the TLS polymerase REV1 caused stalling of replication at or near the −1 position on both strands. Consistent with the proposed glycosylase-mediated generation of an abasic site on one of the strands, the repair intermediates could be digested by the enzyme apurinic endonuclease (APE) [103–105]. Furthermore, introduction of 2′-deoxy-2′-fluoroarabinofuranosyl thymine (**FdT**) residues at the cross-link site shunted repair of the cross-link to the incision-dependent (Fanconi anemia) pathway shown in Figure 1. This result strongly implicated glycosylase activity in repair of the psoralen cross-link because the electron-withdrawing effect of the 2′-fluoro substituent in FdT (Figure 2C) inhibits the action of BER glycosylases through destabilization of the oxocarbenium ion-like transition state of these enzymatic reactions [81, 82, 106, 107].

Repair of an Ap-derived cross-link was also investigated by Walter and coworkers [94]. Ap sites are ubiquitous endogenous lesions in cellular DNA [19, 103, 108] and recent work has characterized interstrand cross-links arising from the reaction of the Ap aldehyde residue with the exocyclic amino groups of adenine and guanine residues in duplex DNA (Scheme 1) [17, 109, 110]. The Ap-derived cross-links are chemically stable in duplex DNA [110, 111] and the dA-Ap cross-link was previously shown to block DNA replication by the strand-displacing polymerase ϕ29, stalling primer extension at the −1 position immediately preceding the cross-link [112]. The stability of the Ap-derived cross-links may derive from the fact that they exist as cyclic aminoglycosides rather than in the ring-opened imine form (Scheme 1) [111, 113, 114]. The ubiquitous nature of Ap sites in genomic DNA makes endogenous cellular formation of Ap-derived cross-links an intriguing possibility.

In the *Xenopus* system, approximately 20% of the dA-Ap cross-link was repaired by the FANCD2/FANCI incision-dependent pathway, while 80% of the repair proceeded via the incision-independent route [94]. As in the case of the psoralen cross-link described above, the repair intermediates could be digested by APE. Depletion of Rev1 prevented approximately half of the glycosylase-dependent repair, consistent with unhooking of the *non-native* glycosylase linkage in the cross-link to generate an Ap site on one strand and a native adenine residue on the other (Figure 5 and Scheme 1).

5. The glycosylase NEIL3 is involved in unhooking psoralen and Ap-derived interstrand cross-links

Semlow et al. provided evidence that the glycosylase NEIL3 is responsible for unhooking of the psoralen and Ap-derived cross-links in the *Xenopus* egg extracts [94]. For example, immunodepletion of NEIL3 led to a decrease in repaired products and addition of exogenous active NEIL3 enzyme to the assay reversed this effect [94].

NEIL3 has a number of properties that are consistent with its newly defined role in replication-dependent cross-link repair. The enzyme is expressed in early S and G2 phases of the cell cycle [115, 116], during DNA replication and unpublished data cited in the Semlow publication [94] notes that NEIL3 is associated with the replisome. As discussed above,

BER enzymes typically act on DNA substrate conformations in which the target base is extruded from the double helix (Figure 2B) [81–83]. Clearly, a nucleobase involved in a cross-link cannot be extruded in the typical manner. However, DNA structure at a stalled replication fork is atypical [48]. When NEIL3 unhooks cross-links at a stalled replication fork, the enzyme presumably must act upon a nucleobase that is located near the junction of a strand-separated Y-shaped or X-shaped DNA structure [2, 3, 34–36, 48]. Significantly, NEIL3 displays preferences for the removal of damaged bases from non-duplex substrates including single-stranded DNA, bubble structures, and G-quadruplexes [115, 117, 118]. There are a few examples of glycosylases that operate without extrusion of the damaged base [119–122] and it will be interesting to learn how the catalytic machinery of NEIL3 gains access to the glycosidic bond of cross-linked nucleobases in the non-canonical DNA structures generated at a stalled replication fork.

It is possible to rationalize the observation that NEIL3 has the catalytic power to deglycosylate nucleobases involved in the psoralen and Ap-derived cross-links [94]. To understand these activities it may be useful to compare the cross-link structures to previously characterized NEIL3 substrates such as FapyA, FapyG, thymine glycol, and dihydrothymine [115, 118]. The non-native glycosidic bond in the dA-Ap cross-link resembles the glycosidic bonds in the NEIL3 substrates FapyA and FapyG, while the saturated thymine rings in the psoralen cross-link resemble the known substrates thymine glycol and dihydrothymine (Figure 6). A previous observation that the related BER glycosylase NEIL1 removes psoralen monoadducts from DNA provides further support for the idea that NEIL3 has the catalytic power to deglycosylate a psoralen-thymine adduct [93].

6. Comparison of incision-dependent and incision-independent cross-link repair pathways

The evidence presented by Semlow et al. suggests that the incision-independent, glycosylase unhooking pathway involving NEIL3 represents the front-line mechanism for repair of the two cross-links studied in the *Xenopus* system [94]. If the incision-independent pathway is thwarted, for example when glycosylase-resistant FdT sugars are present at the psoralen cross-link site, the slower incision-dependent (Fanconi) pathway is engaged (in general, these repair processes take place over the course of several hours) [94].

It may be advantageous to unhook cross-links at a stalled replication fork via an incision-independent pathway. In the incision-independent glycosylase pathway, the bifunctional (lyase) property of NEIL3 [115] is evidently suppressed, thus avoiding double-strand break formation (Figures 3 and 5) [94]. In contrast, the actions of structure-specific endonucleases [45, 47] in the incision-dependent Fanconi pathway generate a double-strand break (Figure 1). Avoidance of double-strand break formation in the glycosylase-dependent unhooking mechanism eliminates the need to engage homologous recombination, a process that is quite complex in its own right and can lead to insertions and deletions in the genome [69, 123]. Both the incision-dependent (Fanconi) and incision-independent cross-link repair pathways include error-prone, potentially mutagenic steps [64, 65, 124], involving the bypass of psoralen adduct remnants [62, 63, 125, 126] and/or Ap sites [127, 128].

It may be important to mesh the new evidence implicating NEIL3 in cross-link unhooking with literature supporting the involvement of Fanconi proteins, XPF/ERCC1, and double-strand breaks in the repair of psoralen-derived cross-links [129–131]. Three possible explanations were offered in this regard [94]: i. in some clonogenic survival experiments, the number of cross-links generated by the psoralen-UV treatment may overwhelm the capacity of NEIL3, necessitating engagement of the incision-dependent Fanconi pathway, ii. some cross-links generated in chromatin may not be sterically accessible to NEIL3, thus requiring recognition and repair involving Fanconi proteins, iii. Fanconi proteins such as FANCD2 may have critical functions in cross-link repair other than unhooking. For example, FANCD2 can modulate chromatin dynamics [132] and may interact with FANCM in the reversal of stalled replication forks [133].

It is also interesting to note that *Neil3*$^{-/-}$ knockout mice have been generated [134, 135]. These mice do not display a profound phenotype, although loss of proliferating neuronal progenitors was noted after hypoxia-ischemia, leading to the suggestion that “NEIL3 exercises a highly specialized function through accurate molecular repair of DNA in rapidly proliferating cells” [134]. The lack of a severe phenotype in *Neil3*$^{-/-}$ mice, such as would be expected to arise from cross-link repair deficiencies (e.g. Fanconi anemia) [41, 44, 45, 136, 137], could be due to the strongly overlapping functions of the Fanconi anemia and NEIL3-dependent repair pathways [94]. In addition, it is possible that overlapping functions of NEIL3 and the related NEIL1 glycosylase in cross-link unhooking could blunt the effects of NEIL3 deletion. NEIL1 displays substrate specificity similar to NEIL3 in terms of the damaged nucleobases that it excises and, like NEIL3, displays the ability to deglycosylate damaged bases located in non-duplex structures (in bubbles and near nicks) [138]. Interestingly, NEIL1 is associated with the replisome and a “cowcatcher” role has been proposed, in which the enzyme carries out pre-replication removal of oxidatively-induced lesions from single-stranded regions of DNA at the replication fork [139].

7. New possibilities suggested by the role of NEIL3 in cross-link repair: considering other enzyme activities that could catalyze replication-dependent unhooking of interstrand cross-links

The participation of NEIL3 in cross-link unhooking offers the possibility of an entirely new role for BER glycosylases and other DNA-processing enzymes. There are several properties that might favor the participation of any given enzyme in cross-link unhooking. i. Physical association with the replisome or Fanconi anemia proteins such as activated FANCD2/FANCI could direct enzyme activity to a cross-linked nucleotide located at a stalled replication fork. ii. Unhooking enzymes must have catalytic activity on a cross-linked nucleotide located at the junction of Y-shaped, X-shaped, or Holliday junction (chickenfoot) structures at a stalled replication fork [2, 3, 34–36, 48, 140]. In the case of NEIL3, such activity was foreshadowed by studies demonstrating its ability to remove damaged nucleobases from non-canonical substrates such as single-stranded DNA, quadruplex DNA, and bubbles [115, 117, 118]. Borrowing terminology used to describe endonucleases such as XPF-ERCC1 [45, 47], we could say that an enzyme such as NEIL3 has “structure-specific glycosylase” activity. iii. An enzyme involved in incision-independent unhooking of a cross-

link must have the catalytic power to cleave one of the covalent bonds involved in the interstrand cross-link. Although the mechanisms by which NEIL3 gains access to the glycosidic bonds in the psoralen-derived and dA-Ap cross-links remain unknown, precedents (Figure 6 and discussion above) indicate that the enzyme possesses the fundamental chemical power to cleave the glycosidic bonds in these cross-links.

Each BER glycosylase has the catalytic power to process a limited structural group of nucleobases [74–80]. There are many structurally diverse cross-links [8, 20] and the reactivity of each particular cross-link will define its susceptibility toward enzymatic unhooking. In this regard, it is important to recognize that interstrand cross-links are not a generic, interchangeable, or homogeneous group. Differences in the chemical structure and reactivity of various cross-links are important. It is almost certain that many cross-links will be chemically resistant to the unhooking action of NEIL3. However, other glycosylases may have the catalytic power to unhook cross-links that are refractory to NEIL3. For example, AAG may act upon cross-links derived from nitrogen mustards (Figure 7A) [141–143]. This is suggested by the ability of AAG to catalyze hydrolysis of the glycosidic bonds in *N*7-alkyl-dG and *N*3-alkyl-dA monoadducts [144]. Similarly, AAG has the chemical potential to unhook the *N*1-dG attachment in a cross-link derived from the anticancer drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, Figure 7B) [145, 146], as suggested by the ability of the enzyme to deglycosylate *N*1-methyl-dG [144]. AAG typically operates on extruded nucleobases[81, 147] and it is completely unknown whether the active site of this enzyme can accommodate (and act upon) a cross-linked nucleotide at a stalled replication fork. In this context, however, it may be noteworthy that AAG is able to remove lesions located in non-duplex (i.e. single-stranded) substrates [144].

Some cross-links may not be amenable to unhooking by glycosylase enzymes. For example, there are no BER enzymes that deglycosylate platinated nucleobases generated by the antitumor agent cisplatin [74–80]. Similarly, there are no known BER glycosylases that act on N^6-alkyladenine, N^2-alkylguanine, or *N*3-cytosine residues [74–80]. However, other classes of DNA-processing enzymes conceivably could unhook cross-links containing these types of chemical attachments. For example, the iron(II) and α-ketoglutarate-dependent dioxygenase activity of ALKB enzymes can carry out the oxidative dealkylation of *N*3-alkylcytosine residues [148, 149]. This chemistry could enable unhooking of the *N*1-dG-ethyl-*N*3-dC cross-links derived from BCNU (Figure 7C). Some isoforms of human ALKB enzymes act on non-duplex substrates [148, 149].

The dioxygenase activity of ALKB enzymes also has the potential to unhook formaldehyde-derived [21] N^6-dA-CH_2-N^6-dA, N^2-dG-CH_2-N^2-dG, N^2-dG-CH_2-N^4-dC, and N^6-dA-CH_2-N^4-dC cross-links (Figure 7E). ALKB enzymes are known to catalyze the oxidative dealkylation of N^6-alkyl-dA and N^2-alkyl-dG residues [148, 149].

Enzymes with adenosine deaminase activity have the potential to unhook N^6-dA-CH_2-N^6-dA cross-links derived from formaldehyde (Figure 7D) [21]. However, it must be noted that the enzyme adenosine deaminase (ADA) has weak activity on oligomeric DNA substrates [150]. ADAR enzymes are RNA editing enzymes that deaminate adenine residues in

oligomeric RNA substrates [151]. Interestingly, Beal's group showed that ADAR-2 can deaminate a 2′-deoxyadenosine residue located at the 5′-end of an RNA substrate [152].

The deaminase activity associated with activation-induced cytidine deaminases (AID/APOBEC family of proteins) [153–155] has the potential to unhook N^4-dC-CH_2-N^6-dA cross-links derived from formaldehyde [21]. Some RNA-editing enzymes also have the potential to catalyze deamination of cytosine residues in DNA [156] and might participate in the unhooking of N^4-dC-CH_2-N^6-dA cross-links if this activity could be selectively directed at a cross-linked cytosine residue.

These selected examples (perhaps unnecessarily limited to consideration of enzymatic activities associated with known DNA-processing enzymes) serve to illustrate how diverse enzymatic activities could carry out the unhooking of various interstrand cross-links. However, we emphasize that the above discussion is highly speculative because the postulated "structure-specific" activities deviate, substantially in some cases, from the established substrate specificities of these enzymes [157].

8. Summary

Deep appreciation of various DNA repair pathways has been built upon the *in vitro* characterization of the fundamental chemical, biochemical, and structural properties of individual repair proteins [158–161]. Along these lines, it will be important to elucidate the biochemical activities of NEIL3 (and other enzymes) on cross-linked DNA substrates that resemble a stalled replication fork or transcription bubble. In addition, NEIL3 presents an excellent opportunity to elucidate the structural mechanisms by which a BER enzyme can access the glycosidic bonds in a cross-linked duplex. In addition, chemical biologists can help address the roles of other BER glycosylases and ALKB family enzymes in the replication-independent unhooking of interstrand cross-links. For instance, methods are in place for the preparation of duplex DNA substrates housing a site-specific and structurally defined interstrand cross-link [17, 110, 146, 162–174]. This will enable shuttle vector-based methods, in conjunction with genetic manipulation, for interrogating the roles of BER glycosylases and ALKB family proteins in the removal of interstrand cross-link lesions in cells [164, 175]. Likewise, genetic modulation of these DNA repair enzymes, together with measurements of interstrand cross-links in cellular DNA by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [72, 176–181], also offers the opportunity for exploring the roles in these enzymes in the unhooking and repair of interstrand cross-links. At the same time, it will be important for studies in cellular systems (and cell extracts) to establish how proteins present at a stalled replication fork choreograph the action of NEIL3 and other enzymes involved in cross-link unhooking, and to gather data from living organisms that shed light on the biological roles and potential medicinal relevance of these repair pathways.

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Abbreviations

BCNU	carmustine or 1,3-bis(2-chloroethyl)-1-nitrosourea
BER	base excision repair
CMG helicase	Cdc45-MCM-GINS helicase
NEIL	Nei-like
TLS	translesion synthesis
HR	homologous recombination
NER	nucleotide excision repair
FdT	2′-deoxy-2′-fluoroarabinofuranosyl thymine
Ap	DNA abasic site
APE	apurinic endonuclease, Fapy, formamidopyrimidine
LC-MS/MS	liquid chromatography-tandem mass spectrometry

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Highlights

- Current models for replication-dependent cross-link repair involve activation of the Fanconi anemia pathway is activated and cross-links are “unhooked” by the action of structure-specific endonucleases such as XPF-ERCC1 that make incisions flanking the cross-link.
- Recent work provides evidence for a new, incision-independent unhooking mechanism involving the intrusion of a base excision repair enzyme, NEIL3, into the world of cross-link repair.
- The evidence suggests that the glycosylase action of NEIL3 unhooks interstrand cross-links derived from an abasic site or the psoralen derivative trioxsalen.

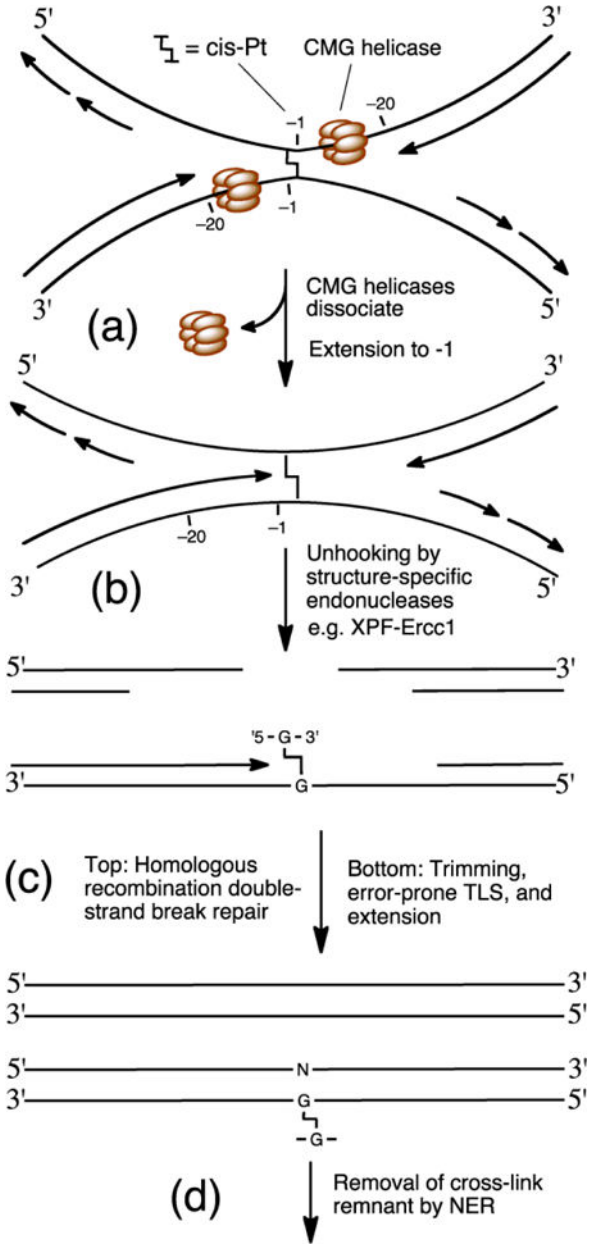


Figure 1.

Model for replication-dependent interstrand cross-link repair. (a) CMG helicase collides with cross-link and dissociates. (b) Structure-specific endonucleases unhook the cross-link. (c) Homologous recombination repairs double-strand break (top duplex). Possible nuclease trimming of the oligonucleotides flanking the adduct, TLS, and extension past the adduct remnant (bottom duplex). (d) Cross-link remnant may be removed by NER.

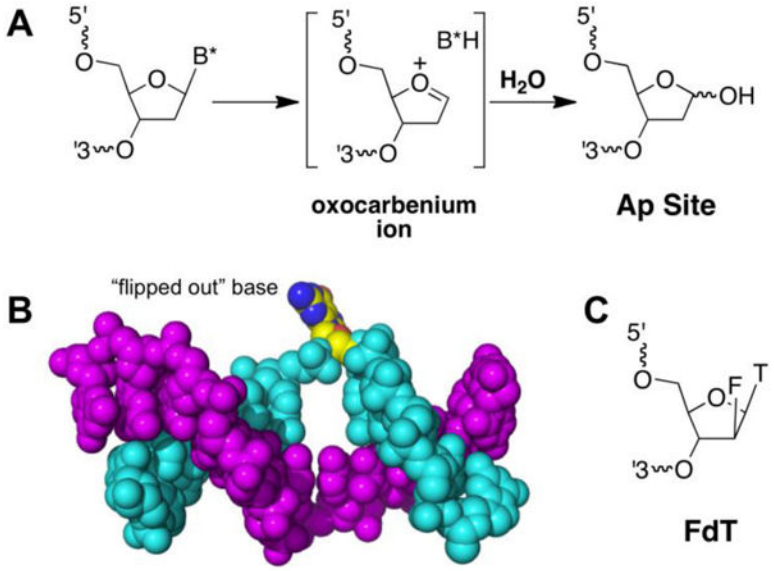


Figure 2.

Base excision repair enzymes remove damaged or mispaired nucleobases from DNA. Panel A: The mechanism of base removal by BER enzymes involves hydrolysis of the glycosidic bond. Shown here is a dissociative hydrolysis mechanism involving an oxocarbenium ion intermediate. Panel B: BER enzymes typically act on DNA substrates in which the damaged nucleobase is extruded from the double helix. The image shows the DNA substrate in a DNA-hOGG1 complex (protein structure omitted) in which the 8-oxo-G base is extruded from the duplex (image prepared from pdb entry 3ktu). Panel C: Damaged bases attached to 2′-deoxy-2′-fluoroarabinofuranosyl residues are resistant to hydrolysis by BER enzymes because the electron-withdrawing fluoro substituent on the sugar destabilizes the oxocarbenium ion-like transition state of the glycosylase reaction.

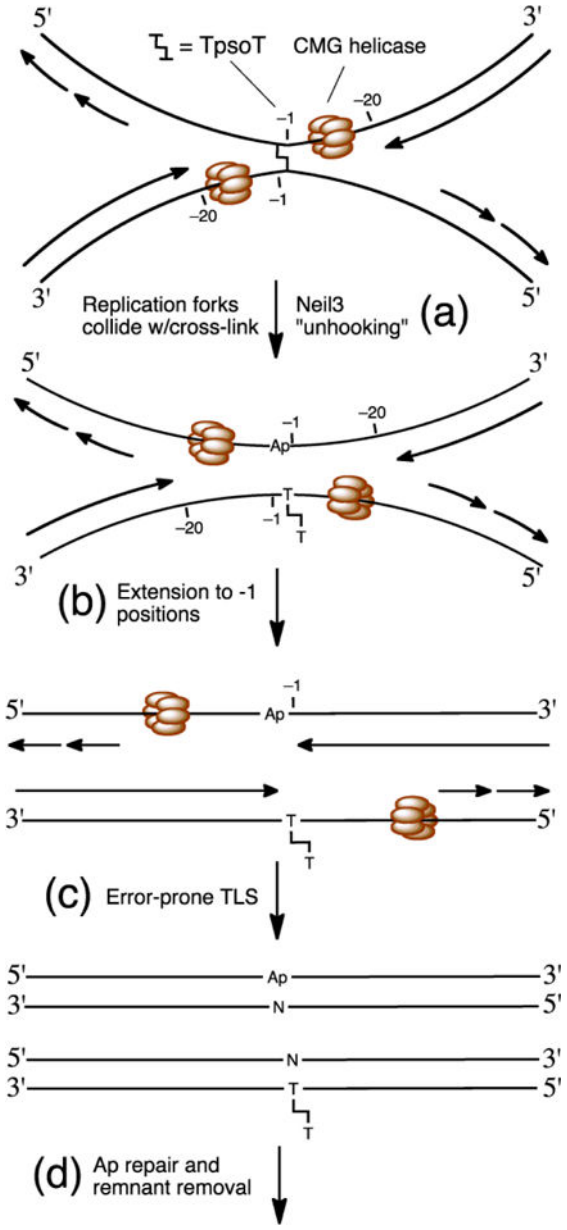


Figure 3.

Model for replication-dependent, incision-independent repair of a psoralen-derived interstrand cross-link. (a) CMG helicase stalls at cross-link. (b) NEIL3 unhooks the cross-link. (c) TLS and extension past the Ap site and psoralen adduct remnant. (d) Repair of the Ap site by a pathway involving APE, pol β, and ligase III (upper duplex) and NER or BER to remove the psoralen cross-link remnant (lower duplex).

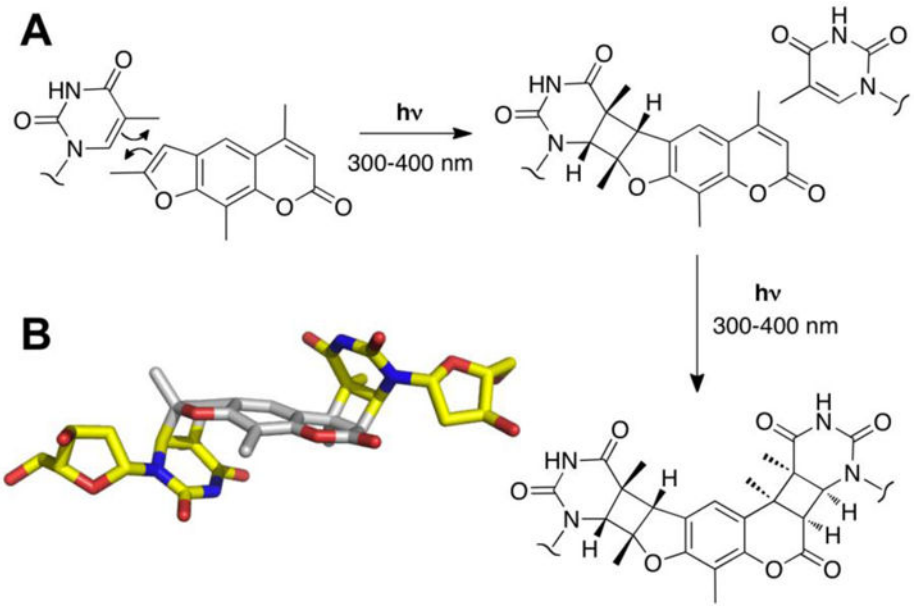


Figure 4.

Formation and structure of a psoralen-derived interstrand cross-link. Panel A: Two sequential photoinduced [2+2] cycloaddition reactions generate interstrand cross-links preferentially at 5′-TA sequences in duplex DNA. The reaction is shown for the psoralent derivative trioxsalen. Panel B: The three-dimensional structure of a psoralen-derived cross-link in duplex DNA. Thymine residues are shown with carbons colored yellow and psoralen with carbons colored in gray (image prepared by modification of pdb 204d).

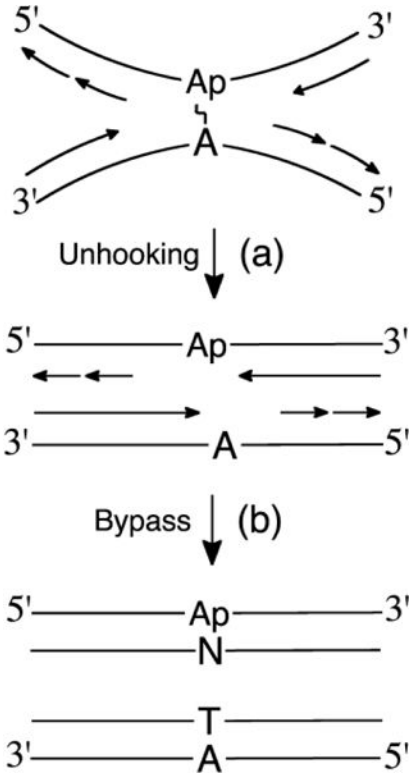


Figure 5.

Model for replication-dependent, incision-independent repair of an Ap-derived interstrand cross-link. (a) NEIL3 unhooking of the cross-link by cleavage of the non-native glycosidic bond. (b) TLS and extension past the Ap site and normal extension past the native adenine residue. Repair of the Ap-containing duplex may be completed by the BER proteins APE, pol β, and DNA ligase.

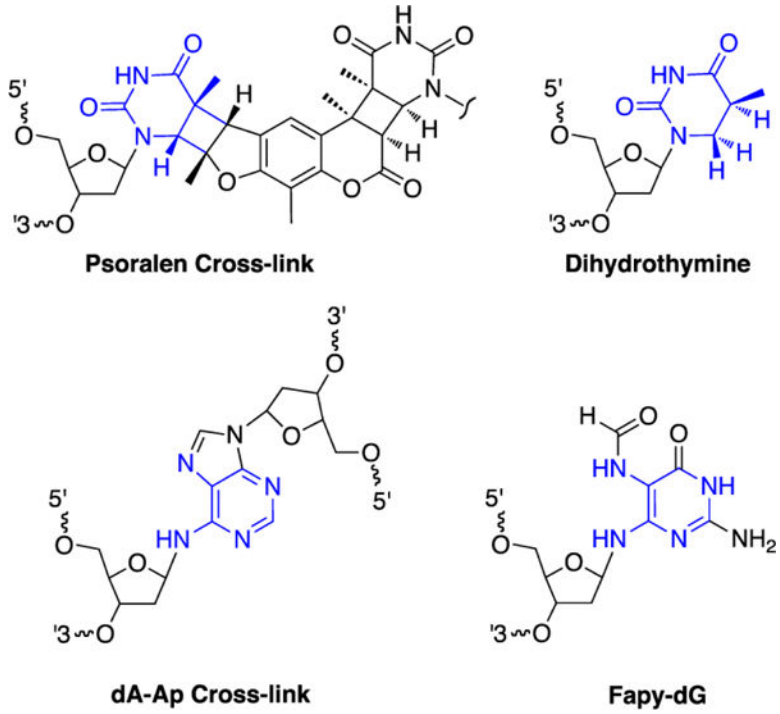


Figure 6.

Psoralen- and Ap-derived cross-links (left side) are structurally analogous to known NEIL3 substrates (right side).

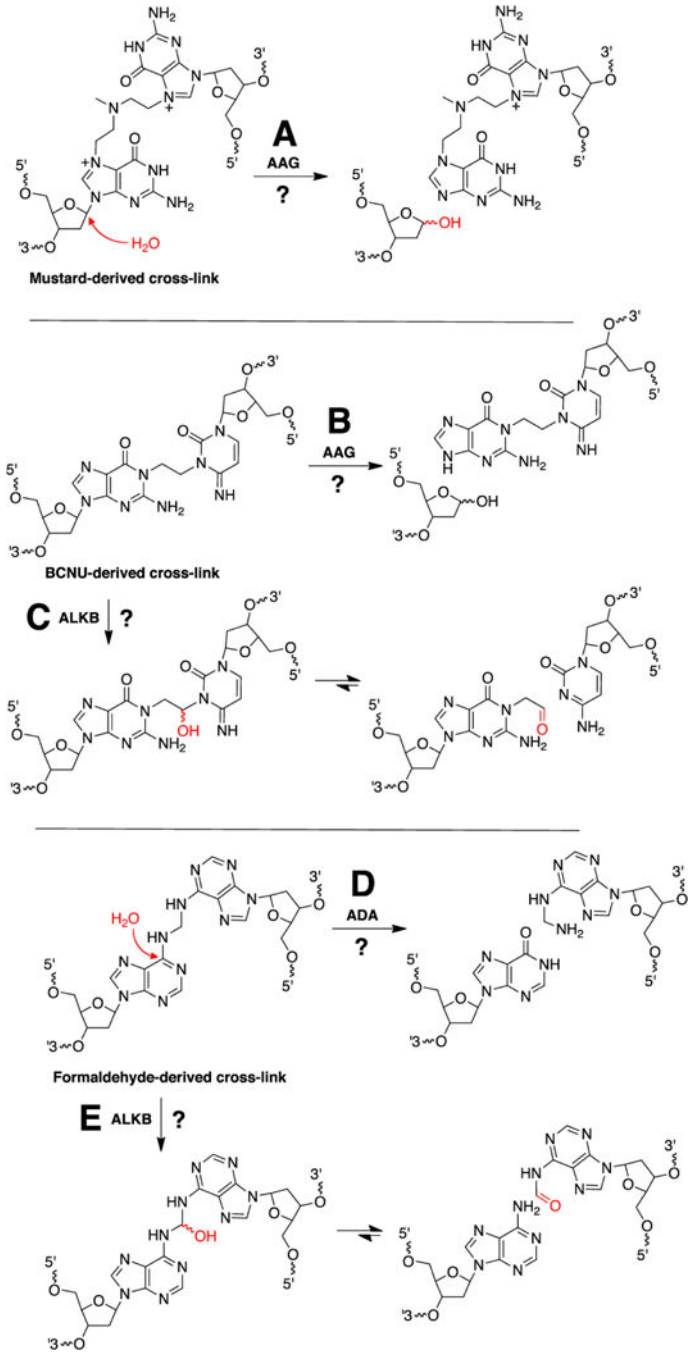
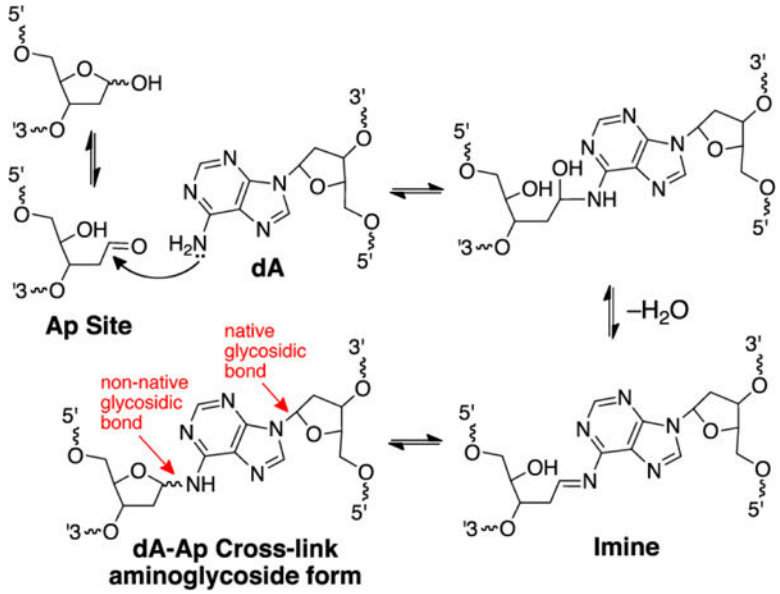


Figure 7.

Speculative mechanisms for incision-independent unhooking of structurally diverse interstrand cross-links.



Scheme 1.

Formation and structure of the dA-Ap interstrand cross-link.