Interstrand cross-links arising from strand breaks at true abasic sites in duplex DNA

Zhiyu Yang1, Nathan E. Price2, Kevin M. Johnson1, Yinsheng Wang2,* and Kent S. Gates1,3,*

1Department of Chemistry, University of Missouri, 125 Chemistry Building, Columbia, MO 65211, USA, 2Department of Chemistry, University of California, Riverside, CA 92521-0403, USA and 3Department of Biochemistry, University of Missouri, 125 Chemistry Building, Columbia, MO 65211, USA

Received March 23, 2017; Revised April 24, 2017; Editorial Decision April 25, 2017; Accepted May 02, 2017

ABSTRACT

Interstrand cross-links are exceptionally bioactive DNA lesions. Endogenous generation of interstrand cross-links in genomic DNA may contribute to aging, neurodegeneration, and cancer. Abasic (Ap) sites are common lesions in genomic DNA that readily undergo spontaneous and amine-catalyzed strand cleavage reactions that generate a 2,3-didehydro-2,3-dideoxyribose sugar remnant (3’ddR5p) at the 3’-terminus of the strand break. Interestingly, this strand scission process leaves an electrophilic α,β-unsaturated aldehyde residue embedded within the resulting nicked duplex. Here we present evidence that 3’ddR5p derivatives generated by spermine-catalyzed strand cleavage at Ap sites in duplex DNA can react with adenine residues on the opposing strand to generate a complex lesion consisting of an interstrand cross-link adjacent to a strand break. The cross-link blocks DNA replication by DNA polymerase, a highly processive polymerase enzyme that couples synthesis with strand displacement. This suggests that 3’ddR5p-derived cross-links have the potential to block critical cellular DNA transactions that require strand separation. LC-MS/MS methods developed herein provide powerful tools for studying the occurrence and properties of these cross-links in biochemical and biological systems.

INTRODUCTION

Damage to cellular DNA is unavoidable (1). A wide variety of chemical and enzymatic processes contribute to the degradation of cellular DNA, including oxidation, alkylation, misincorporation of ribonucleotides, hydrolytic deamination of the nucleobases, and hydrolysis of the glycosidic bonds to generate abasic (Ap) sites (1–8). DNA repair systems mitigate the effects of DNA damage (9–11), but some lesions inevitably evade repair with deleterious consequences. Endogenous DNA damage in mammalian cells may contribute to aging, neurodegeneration, mitochondrial dysfunction, mutagenesis and cancer (12–19).

Not all DNA lesions have equal bioactivity. Certain types of DNA damage such as double-strand breaks (20), interstrand cross-links (21–24), and clustered lesions (typically defined as more than one DNA lesion within a 10 base pair region) (25,26) have exceptionally potent biological effects even when generated at low levels in cellular DNA. To better understand the roles of endogenous DNA damage in human health and disease, it is critical to identify the exceptionally bioactive lesions that occur spontaneously in cellular DNA. Along these lines, we have identified a new, structurally complex, interstrand cross-link arising from strand breakage at Ap sites in duplex DNA.

Ap sites are ubiquitous endogenous lesions in genomic DNA that are generated by spontaneous or enzyme-catalyzed hydrolysis of the glycosidic bonds linking the nucleobases to the sugar-phosphate backbone (1,27,28). Ap sites can be converted to strand breaks via β-elimination of the 3’-phosphoryl group (Scheme 1) (1,29–33). This reaction occurs spontaneously under physiological conditions and also can be catalyzed by amino groups in DNA repair enzymes, histones, small peptides and endogenous polyamines such as spermine (Scheme 1) (1,31–48). Interestingly, this strand scission process generates an electrophilic 2,3-didehydro-2,3-dideoxyribose derivative (3’ddR5p) embedded within the DNA duplex at the 3’-terminus of the strand break (Scheme 1) (30,44,49,50).

Here, we present evidence that 3’ddR5p derivatives generated by spermine-catalyzed strand cleavage at Ap sites in duplex DNA can react with adenine residues on the opposing strand to generate a complex lesion consisting of an interstrand cross-link adjacent to a strand break.
EXPERIMENTAL

Materials and methods

Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA, USA), [γ-32P]-ATP (6000 Ci/mmol) was purchased from Perkin-Elmer, uracil DNA glycosylase (UDG) and d29 DNA polymerase were from New England Biolabs (Ipswich, MA, USA), C-18 Sep-Pak cartridges were purchased from Waters (Milford, MA, USA), and BS Poly-prep columns were obtained from BioRad (Hercules, CA, USA). Acrylamide/bis-acrylamide 19:1 (40% solution, electrophoresis grade) was purchased from FisherScientific(Waltham,MA,USA),spermineandallotherchemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). A mixture of the four 2'-deoxynucleoside triphosphates (dNTPs) was purchased from Promega (Madison, WI, USA). Iron–EDTA–H2O2 footprinting (51–53), QTOF-MS (53–55), LC–MS/MS (53,55) and phi-29 (d29) DNA polymerase primer extension reactions (56) were conducted as described in published procedures, with minor modifications. Detailed experimental protocols for these experiments are provided in the Supplementary Data.

Representative procedures for generation of the nicked duplex C and its conversion to the nicked cross-linked duplex D

In these experiments, 2’-deoxyuridine-containing oligonucleotides were used as precursors for the enzymatic generation of Ap-containing oligonucleotides (57,58). The single-stranded, uracil-containing 2’-deoxy-oligonucleotides were α-32P 5’-end-labeled using standard procedures (59), and then annealed with the complimentary strand. The labeled duplex DNA was treated with the UDG (200 units/ml, final concentration) in Tris–HCl buffer (20 mM, pH 8) containing DTT (1 mM), EDTA (1 mM) and spermine (1 mM). After incubation at 37°C for 45–60 min, the UDG enzymewas removed by phenol–chloroform extraction (59) and the DNA ethanol precipitated, resuspended in formamide loading buffer, loaded onto a 2 mm thick 20% denaturing polyacrylamide gel, and the cross-link isolated as described above.

RESULTS AND DISCUSSION

Generation of nicked DNA duplexes containing a 3’ddr5p sugar remnant and gel electrophoretic evidence for the formation of nicked cross-linked DNA duplexes

Nicked DNA duplexes containing 32P-labeled, nicked duplex D was incubated in a buffer composed of HEPES (50 mM, pH 7.4) and NaCl (100 mM) at 37°C for 72 h. NaBH4 was added (100 mM, final concentration), and the pH of the resulting solution adjusted by addition of aqueous NaOH to a final concentration of 0.01 N. The mixture was incubated at 37°C for another 4 h, followed by neutralization with HCl (0.1 N). The DNA was ethanol precipitated, resuspended in formamide loading buffer, loaded onto a 20% denaturing polyacrylamide gel, and the cross-link isolated as described above.
Figure 2. Formation of the nicked cross-linked duplex D by incubation of nicked duplex C in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 37°C. Lane 1, size-marker consisting of the 5'-32P labeled uracil-containing oligonucleotide precursor to duplex A; Lane 2, freshly-prepared Ap-containing duplex A; Lane 3, Ap-containing duplex A subjected to piperidine work-up (1 M, 95°C, 25 min) to induce strand cleavage; Lane 4, formation of full-length, cross-linked duplex B by incubation of duplex A in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 37°C for 120 h; Lane 5, freshly-prepared, nicked 3'ddR5p-containing duplex C; Lane 6, formation of the nicked cross-linked duplex D by incubation of the nicked duplex C for 72 h. The 5'-32P-labeled oligodeoxynucleotides in these reactions were resolved by electrophoresis on a 20% denaturing polyacrylamide gel and the radioactivity in each band quantitatively measured by phosphorimager analysis.

piperidine (1 M, 95°C, 25 min) cleanly induced strand cleavage to give the expected (1,65) fast-migrating product with a 3'-phosphoryl terminus. In addition, incubation of duplex A, containing the intact Ap site, in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 37°C gave the expected slowly-migrating band (Figure 2, lane 4) corresponding to the previously characterized (55,63) full-length cross-linked duplex B (Figure 1, and structure 4 in Scheme 2).

Nicked duplex C, containing the 3'ddR5p end group was generated by addition of spermine (1 mM), to the UDG reaction (43,45,61). Spermine is an endogenous polyamine that is present at millimolar concentrations in the nuclei of cells (60). Spermine and other polyamines associate with chromatin (66) and also serve as regulators of cell growth and apoptotic cell death (67). The cleavage of abasic sites by spermine and other low molecular weight polyamines has been described previously (43-45,61). Ethanol precipitation of the freshly prepared nicked duplex C, followed by electrophoretic analysis, revealed two cleavage products (Figure 2, lane 5) whose gel mobilities were consistent with the presence of the 3'-end groups 1 and 3 (Scheme 1) (42,44,68). Further incubation of the spermine-nicked duplex C in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 37°C led to the formation of a major slowly-migrating band whose mobility on the denaturing polyacrylamide gel—intermediate between the full-length single strand from duplex A and the full-length cross-linked duplex B—was consistent with that expected for the nicked cross-link (duplex D, lane 6).

A separate experiment provided evidence for the intermediacy of the spermine adducts 2 and 3 (Scheme 1). Specifically, treatment of the Ap-containing duplex A with various concentrations of spermine in the presence of NaCNBH3 (25 mM) for 1 h gave rise to two new bands (Supplementary Figure S1) with retarded gel mobility consistent with that expected for products arising from reduction of the spermine adducts 2 and 3 (44). The band arising from reduction of the full length spermine adduct 2 predominate (Supplementary Figure S1). This result is consistent with a system in which reduction of the intermediate Ap-derived iminium ion 2 by NaCNBH3 competes effectively against strand cleavage to generate 3.

The yield of the nicked cross-link formed following spermine-mediated generation of the nicked duplex C was ~35%, with the half-maximal yield reached inside of 8 h at pH 7.4 and 37°C (Supplementary Figure S2). Easily detectable amounts of the cross-linked product were evident even at the earliest time points of our experiments, presumably formed during the generation of the nicked duplex C. It is noteworthy that a band consistent with the nicked cross-link was generated even in the absence of spermine (Figure 2, lane 3), albeit in lower yield, presumably arising via the relatively slow, spontaneous strand cleavage at the Ap site (31,38) to generate the 3'-ddR5p end product 1.

Gel shift analyses were used to provide evidence that the new, slowly-moving band generated from the nicked duplex C is a cross-linked species that contains three of the four ‘quadrants’ of the starting duplex A. We first showed that the new, slowly-migrating band has shed the oligonucleotide fragment on the 3’-side of the Ap site (light gray

Scheme 2. Generation of interstrand cross-links by reaction of an adenine residue with an intact Ap site in duplex DNA.
segments in Figures 1–3, and S3). We found that installation of a 5 nucleotide (nt) overhanging extension onto this end of the starting duplex caused no change in the mobility of the slowly-migrating band resulting from spermine-mediated strand cleavage (duplexes C and G, Figure 3). On the other hand, addition of 5 nt overhangs to the ends of the other four quadrants of the starting duplex caused retardation in the gel mobility of the slowly-migrating band resulting from spermine-nicked duplex (Figure 3). Overall, the gel electrophoretic results provided strong evidence that spermine-catalyzed cleavage of Ap sites in duplex DNA gave rise to a cross-linked species in which the 3'ddR5p sugar remnant is attached to the opposing strand of the duplex.

Iron–EDTA–H2O2 footprinting identifies the sites of cross-link attachment

To identify the location of the cross-link attachment in duplex D, we conducted an iron–EDTA–H2O2 footprinting experiment (51,52). The strand complementary to the 3’ddR5p-containing oligonucleotide was 5'-32P-labeled and the gel-purified, nicked cross-link resulting from this duplex subjected to cleavage by a mixture of iron–EDTA–H2O2 (51–53). In this experiment, the site of cross-linking appears as an interruption in the ‘ladder’ of strand cleavage products generated by the iron–EDTA–H2O2 system, because cleavages beyond the cross-link yield large, slowly-migrating DNA fragments that are connected to the opposing strand (51,52). Footprinting of the cross-linked duplex D provided evidence that interstrand attachments were located at the adenine residues in positions 52 and 53 of duplex D, with adenine 52 being the predominant site of attachment (Figure 4).

Mass spectrometric analysis of nicked, cross-linked duplexes

Analysis of the nicked cross-link generated in duplex E by nanospray QTOF-MS revealed a single major signal corresponding to an observed mass of 16115.65 amu (Supplementary Figure S4). This result combined with the data described above allowed us to suggest a reasonable mechanism for cross-link formation involving 1,4-addition of an adenine residue to the 3’ddR5p group (Scheme 3, calculated mass of 5 = 16115.44). Importantly, this proposed reaction is consistent with literature reports describing 1,4-conjugate addition of the N1-atom of the 2’-deoxyadenosine nucleoside to the low molecular weight H9251, H9252, unsaturated aldehyde, acrolein (69–71).

To further characterize the cross-linked DNA we undertook LC-MS analysis. In these experiments, we reasoned that reduction of the aldehyde group in the proposed adduct 5 would stabilize the cross-link against decomposition via a retro-Michael-type reaction (70) during sample handling and would also generate a diagnostic mass increase of 2 units. Toward this end, we added a solution of NaBH4 in NaOH to the cross-linking reaction after 72 h and incubated the mixture at 37°C for an additional 4 h, followed by neutralization with 0.1 N HCl, and isolation of the cross-linked DNA from a denaturing polyacrylamide gel. Digestion of the NaBH4-treated cross-linked DNA duplex E with a four-enzyme cocktail consisting of nuclease P1, alkaline phosphatase, and phosphodiesterases I and II, followed by LC-MS/MS analysis (63,72) revealed two peaks eluting at 23–26 min displaying the m/z 370 → 254 transition anticipated for the neutral loss of 2-deoxyribose (116 Da) from the reduced cross-link remnants 6 (Figures 5 and Supplementary.
Figure 4. Iron–EDTA footprinting of the nicked cross-linked duplex D. The cross-linked duplex D, unlabeled on the 3'ddR5p-containing strand and 32P-labeled on the longer strand, was isolated from a denaturing gel and subjected to cleavage by a mixture of iron–EDTA–H2O2. The 32P-labeled oligodeoxynucleotides were resolved on a denaturing 20% polyacrylamide gel (left side of Figure) and the radioactivity in each band quantitatively measured by phosphorimager analysis. The right side of the Figure shows densitometry traces of: Lane 1, Fe-EDTA footprinting reaction of the cross-linked duplex D (panel A); Lane 2, Fe-EDTA footprinting of the 5'-32P-labeled strand (panel B); Lane 3, Maxam–Gilbert G-specific sequencing reaction of the 32P-labeled strand (panel C); Lane 4 Maxam–Gilbert A+G sequencing reaction on the 32P-labeled strand (panel D).

Scheme 3. Formation of interstrand cross-links by reaction of an adenine residue with 3'ddR5p end groups arising from strand breakage at true Ap sites in duplex DNA.

Figure 5. LC–MS/MS and LC–MS/MS/MS analysis results of the nucleoside mixture arising from the enzymatic digestion of cross-linked duplex E. (A) The selected-ion chromatogram for the m/z 370→254 transition. (B) The selected-ion chromatogram for the m/z 370→254→136 transition. Shown in the insets are the MS/MS (A) and MS/MS/MS (B) averaged from the 23.8-min fraction. (C) Possible structures for the ions observed at m/z 570, 254 and 136.

Figure S5). The MS/MS/MS arising from the further cleavage of the ion of m/z 254 revealed fragment ions of m/z 236 and 218, which emanate from the neutral losses of one and two H2O molecules, respectively, along with the ion of m/z 136, which corresponds to the protonated adenine (Figures 5 and Supplementary Figure S5). The formation of these fragment ions was in line with the proposed structure for the cross-linked nucleobase remnant (Scheme 3). The presence of multiple peaks in the LC–MS/MS ion chromatogram was consistent with the anticipated diastereomeric nature of the nucleosidic cross-link remnant 6 (Scheme 3).

Overall, the mass spectrometric data are consistent with formation of a nicked, cross-linked duplex arising from 3'ddR5p derivatives generated by strand scission at the Ap site. The data further indicates that cross-linking involves attachment to adenine on the opposing strand. Finally,
the results are consistent with cross-link formation via 1,4-addition of adenine to the 3'ddR5p group.

The 3'ddR5p-derived cross-link blocks DNA replication by the strand-displacing φ29 DNA polymerase

The results described above indicate that the (unreduced) cross-link derived from the 3'ddR5p group is stable to sample handling and gel electrophoretic analysis (Figures 2 and 3). Nonetheless, we felt it was important to investigate whether the cross-linkage can withstand DNA processing enzymes that actively induce strand separation. To examine this issue, we used φ29 DNA polymerase, an enzyme that has been described as a hybrid helicase–polymerase (73). Bacteriophage φ29 DNA polymerase is a highly processive polymerase that couples synthesis and strand displacement to carry out DNA replication without assistance from helicases, clamp proteins, or other accessory factors (74).

We prepared a series of φ29 DNA polymerase substrates to examine whether the 3'ddR5p cross-link was able to block strand separation by the enzyme (Figure 6 and Supplementary Figure S6). We first carried out a series of control experiments showing that the enzyme, in combination with dNTPs fully extended a 15 nt 32P-labeled primer annealed to a 56 nt template strand (substrate M), a template strand with a 35 nt duplex region downstream of the primer (N), and a 56 nt template strand containing a 35 nt nicked duplex downstream of the primer (O, Figures 6 and Supplementary Figure S6). As expected based upon our previous work (56), extension of the primer in substrate R containing the dA-Ap cross-link 4 in the downstream duplex region was stalled immediately preceding the cross-link. Primer extension in substrate Q, containing the nicked 3’ddR5p cross-link in a downstream duplex region was almost completely stalled at last unmodified base preceding the cross-link. Extension of the primer in cross-linked substrate P, lacking the duplex region on the 3’-side of the cross-link was similarly stalled at the cross-link. Overall, the results provided evidence that the dA-3’ddR5p cross-link was stable against the powerful strand-separating properties (73) of the helicase-polymerase activity of the bacteriophage φ29 DNA polymerase.

CONCLUSIONS

Strand breaks at Ap sites in cellular DNA are generated by spontaneous and enzymatic processes (1,31–48). Interestingly, this reaction generates an electrophilic 3’dR5p derivative embedded within the nicked duplex (30,44,49,50). A few studies have shown that nucleophilic small molecules (34,37,75,76) and proteins (77–79) can react with the 3’dR5p sugar remnant. However, our work provides the first characterization of interstrand-cross-links resulting from the reaction of the 3’dR5p group with a nucleobase on the opposing strand. Catalano et al. previously proposed a product of this type to explain a band observed in the denaturing gel analysis of Ap-containing DNA duplexes (80) but information related to the structure, origin and properties of this cross-link have been lacking until now. In addition, cross-links of this type were proposed to explain high molecular weight impurities generated in the manufacture of therapeutic oligonucleotides, but again the structure, origin and properties of these cross-linked species were not characterized (81).

Our results provide evidence that spermine-catalyzed strand cleavage at a true Ap site in duplex DNA can lead to generation of a complex lesion consisting of an interstrand cross-link to adenine adjacent to a strand break. This reaction is distinct from previous work involving strand cleavage at oxidized Ap sites (52,82–85). The exact chemical structure of the interstrand attachment remains to be defined, but our data combined with literature precedents (69,71) allow us to propose a cross-link structure 5 derived from 1,4-addition of the N1-atom of adenine to the 3’dR5p residue (Scheme 3). Importantly, we provided evidence that this cross-link blocks DNA replication by φ29 DNA polymerase, a highly processive polymerase enzyme that couples synthesis and strand displacement (74). This forecasts the potential for the da-3’dR5p cross-link to block mammalian DNA replication and transcription. In addition,
cross-links of this type may present special challenges to repair systems (52,83). Cross-links could contribute to the toxicity and generation of slow-moving chromosomal DNA intermediates caused by increased levels of 3’ddR5p strand breaks in *Saccharomyces cerevisiae* mutants with dysregulated base excision repair pathways (86–88). It will be interesting to explore how spermine and other polyamines in the nucleus modulate the fate of Ap sites in genomic DNA. The polyamine-derived imines 2 and 3 (Scheme 1) are expected to be much more reactive than the parent aldehydes Ap and 1 (89–91). Indeed, the spermine-mediated formation of the nicked cross-links described here is substantially faster than the formation of the full-length dA-Ap cross-link described previously (4, Scheme 2) (55).

Chemical precursors involving the reaction of nucleosides with low molecular weight compounds such as HNE and acrolein show that all four of the canonical DNA bases have the potential to react with α,β-un saturated aldehydes (69,71,92–95). Thus, it seems likely that interstrand cross-links derived from the 3’ddR5p group in duplex DNA will prove to be a structurally diverse family of complex lesions that can form in a wide variety of sequence contexts. The LC–MS/MS methods developed here provide a powerful tool for studying the occurrence and properties of these cross-links in biochemical and biological systems (96,97).

**SUPPLEMENTARY DATA**

Supplementary data are available at NAR Online.

**ACKNOWLEDGEMENTS**

We thank Professor Marc Greenberg for helpful conversations on this topic.

**FUNDING**

National Institute of Environmental Health Sciences of the National Institutes of Health [ES021007 to K.S.G., Y.W.]; NRSA T32 institutional training grant [ES018827 to N.P.] (in part). Funding for open access charge: National Institutes of Health [ES021007 to K.S.G., Y.W.]; NRSA T32 institutional training grant [ES018827 to N.P.].

**REFERENCES**

1. Gates,K.S. (2009) An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylolation, and reactions with radicals. *Chem. Rev. Toxicol.*, 22, 1747–1760.

2. Lindahl,T. (1993) Instability and decay of the primary structure of DNA. *Nature*, 362, 709–715.

3. Potenski,C.J. and Klein,H.L. (2014) How the misincorporation of ribonucleotides into genomic DNA can be both harmful and helpful to cells. *Nucleic Acids Res.*, 42, 10226–10234.

4. De Bont,R. and van Larebeke,N. (2004) Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis*, 19, 169–185.

5. Barnes,D.E. and Lindahl,T. (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu. Rev. Genet.*, 38, 445–476.

6. Pham,P., Branstetter,R. and Goodman,M.F. (2005) Reward versus risk: DNA cytidine deaminases triggering immunity and disease. *Biochemistry*, 44, 2703–2715.

7. Marnett,L.J. and Burcham,P.C. (1993) Endogenous DNA adducts: potential and paradox. *Chem. Res. Toxicol.*, 6, 771–785.

8. Sweeney,J.A., Moeller,B.C., Gao,L., Upton,P.B., Nakamura,J. and Starr,T. (2011) Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology, and risk assessment. *Toxicol. Sci.*, 120, S130–S145.

9. Lindahl,T. and Wood,R.D. (1999) Quality control by DNA repair. *Science*, 286, 1897–1905.

10. Lindahl,T. and Barns,D.E. (2000) Repair of endogenous DNA damage. *Cold Spring Harb. Perspect. Biol.*, 65, 127–134.

11. Schärer,O.D. (2003) Chemistry and biology of DNA repair. *Annu. Chem. Int. Ed. Engl.*, 42, 2946–2974.

12. McKinnon,P.J. (2009) DNA repair deficiency and neurological disease. *Nat. Rev. Neurosci.*, 10, 100–112.

13. Hoeijmakers,J.H.J. (2009) DNA damage, aging, and cancer. *J. Engl. J. Med.*, 361, 1475–1485.

14. Borgesius,N.Z., de Waard,M., van der Pluijm,I., Omrani,Z., Zondag,G.C.M., ver der Horst,G.T.J., Melton,D.W., Hoeijmakers,J.H.J., Jaarsma,D. and Elgersma,Y. (2011) Accelerated age-related cognitive decline and neurodegeneration, caused by deficient DNA repair. *J. Neurosci.*, 31, 12543–12553.

15. Menck,C.F.M. and Munford,V. (2014) DNA repair diseases: what do they tell us about cancer and aging? *Genet. Mol. Biol.*, 37, 220–233.

16. Cline,S.D. (2012) Mitochondrial DNA damage and its consequences for mitochondrial gene expression. *Biochim. Biophys. Acta*, 1819, 979–991.

17. Friedberg,E.C., McDaniel,L.D. and Schultz,R.A. (2004) The role of endogenous and exogenous DNA damage and mutagenesis. *Curr. Opin. Genet. Dev.*, 14, 5–10.

18. Madabhushi,R., Pan,L. and Tsai,L.-H. (2014) DNA damage and its links to neurodegeneration. *Neuron*, 83, 266–282.

19. Schumacher,B., Garnis,G.A. and Hoeijmakers,J.H.J. (2007) Age to survive: DNA damage and aging. *Trends Genet.*, 24, 77–85.

20. Mehta,A. and Haber,J.E. (2014) Sources of DNA double-strand breaks and models of recombinational DNA repair. *Cold Spring Harb. Perspect. Biol.*, 6, a016428.

21. Clauson,C., Schärer,O.D. and Niedernhofer,L.J. (2013) Advances in understanding the complex mechanisms of DNA interstrand cross-link repair. *Cold Spring Harbor Perspect. Biol.*, 5, a012732.

22. Schärer,O.D. (2005) DNA interstrand crosslinks: natural and drug-induced DNA adducts that induce unique cellular responses. *ChemBioChem*, 6, 27–32.

23. Lawley,P.D. and Phillips,D.H. (1996) DNA adducts from chemotherapeutic agents. *Mutat. Res.*, 355, 13–40.

24. Grossman,K.F., Ward,A.M., Matkovic,M.E., Foliash,A.E. and Moses,R.E. (2001) S. cerevisiae has three pathways for DNA interstrand crosslink repair. *Mutat. Res.*, 487, 73–83.

25. Eccles,L.J., O’Neill,P. and Lomax,M.E. (2011) Delayed repair of radiation induced clustered DNA damage: friend or foe? *Mutat. Res.*, 711, 134–141.

26. Georgakilas,A.G., O’Neill,P. and Stewart,R.D. (2013) Induction and repair of clustered DNA lesions: what do we know so far? *Radiat. Res.*, 180, 100–109.

27. Nakamura,J. and Swenberg,J.A. (1999) Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues. *Cancer Res.*, 59, 2522–2526.

28. Guillet,M. and Bioteux,S. (2003) Origin of endogenous DNA abasic sites in Saccharomyces cerevisiae. *Mol. Cell Biol.*, 23, 8386–8394.

29. Tann,C., Shapiro,H.S., Lipshitz,R. and Chargaff,E. (1953) Distribution density of nucleotides within a deoxyribonucleic acid chain. *J. Biol. Chem.*, 199, 673–688.

30. Bayley,C.R., Brammer,K.W. and Jones,A.S. (1961) The nucleotide sequence in deoxyribonucleic acids. Part V. The alkaline degradation of apurinic sites. *Biochemistry*, 1903–1907.

31. Lindahl,T. and Anderson,A. (1972) Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid. *Biochemistry*, 11, 3618–3623.

32. Crine,P. and Verly,W.G. (1976) A study of DNA spontaneous degradation. *Biochim. Biophys. Acta*, 442, 50–57.

33. Luke,A.M., Chastain,P.D., Pachkowski,B.F., Afonin,V., Takeda,S., Kaufman,D.G., Swenberg,J.A. and Nakamura,J. (2010) Accumulation of true single strand breaks and AP sites in base excision repair deficient cells. *Mutat. Res.*, 694, 65–71.

34. Builloy,Y. and Verly,W.G. (1988) Importance of thiols in the repair mechanisms of DNA containing AP (apurinic/apyrimidinic) sites. *Nucleic Acids Res.*, 16, 9489–9497.
35. de Oliveira, M.P., Constant, J.-F., Peuchmaur, M., Pitta, I. and Decourt, J.-L. (2013) Antibiotic drugs aminoglycosides cleave DNA at abasic sites: shedding new light on their toxicity. *Chem. Res. Toxicol.*, 26, 1710–1719.

36. Mirzabekov, A.D., Shick, V.V., Belyavsky, A.V. and Baykov, S.G. (1978) Primary organization of nucleosome core particle of chromatin: Sequence of histone arrangement along DNA. *Proc. Natl. Acad. Sci. U.S.A.*, 75, 4184–4188.

37. Vasseur, J.-J., Rayner, B. and Imbach, J.-L. (1988) Apurinic DNA reactivity: modularisation of apurinic DNA breakage by phenylhydrazine and formation of pyrazole adduct. *J. Heterocyclic Chem.*, 25, 389–392.

38. Zhou, C., Szczepanski, J.T. and Greenberg, M.M. (2012) Mechanistic studies on histone catalyzed cleavage of apyrimidinic/apurinic sites in nucleosome core particles. *J. Am. Chem. Soc.*, 134, 16734–16741.

39. Brooks, S.C., Adhikary, S., Rubinson, E.H. and Eichman, B.F. (2013) Deoxyguanosine adducts of t-4-hydroxy-2-nonenal are endogenous DNA lesions in rodent liver and brain: detection and potential sources. *Cancer Res.*, 73, 1507–1511.

40. Sczepanski, J.T., Wong, R.S., McKnight, J.N., Bowman, G.D. and Greenberg, M.M. (2010) Rapid DNA-protein cross-linking and gamma-crosslink reactions in the repair of DNA containing AP (apurinic/apyrimidinic) sites in mammalian cells. *Biochem. J.*, 433, 553–559.

41. Price, N.E., McHugh, P.J. and Knowland, J. (1995) Novel reagents for chemical modification of abasic sites in DNA. *Methods Enzymol.*, 26, 495–500.

42. Raspaud, E., Chaperon, I., Leforestier, A. and Livolant, F. (1999) Sperminduced aggregation of DNA, nucleosome, and chromatin. *Biophys. J.*, 77, 1547–1555.

43. Wallace, H.M., Fraser, A.V. and Hughes, A. (2003) A perspective of polyamine metabolism. *Biochem. J.*, 376, 1–14.

44. Bally, V., Derydt, M. and Verly, W.G. (1989) E-Elmination in the repair of AP (apurinic/apyrimidinic) sites in DNA. *Biochem. J.*, 261, 304–307.

45. Chung, F.-L., Rath, R.G., Ocando, J., Nishikawa, A. and Zhang, L. (2006) Deoxyguanosine adducts of t-4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources. *Cancer Res.*, 66, 1507–1511.

46. Smith, R.A., Williamson, D.S., Cerny, R.L. and Cohen, S.M. (1990) Characterization of interstrand DNA-DNA cross-links formed between abasic sites and adenine residue in duplex DNA. *Biochemistry*, 29, 3434–3441.

47. Gamboa Varela, J. and Gates, K.S. (2016) Simple high-yield syntheses of DNA duplexes containing interstrand DNA-DNA cross-links between an N4-aminocytidine residue and an abasic site. *Carr. Protoc. Nucleic Acid Chem.*, 65, doi:10.1002/cpc3.

48. Maxam, A.M. and Gilbert, W. (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.*, 65, 495–500.

49. Sczepanski, J.T., Zhou, C. and Greenberg, M.M. (2011) Nucleosome core particle-catalyzed strand scission at abasic sites. *Biochemistry*, 50, 2157–2164.

50. Sugiyama, H., Fujisawa, T., Ura, A., Tashiro, T., Yamamoto, K., Kawashima, S. and Saito, I. (1994) Chemistry of thermodegradation of abasic sites in DNA: mechanistic investigation on thermal DNA strand cleavage of alkylated DNA. *Chem. Rev.*, 7, 673–683.

51. Johnson, K.M., Price, N.E., Wang, J., Fekry, M.I., Dutta, S., Seiner, D.R., Wang, Y. and Gates, K.S. (2013) On the formation and properties of interstrand DNA-DNA cross-links forged by reaction of an abasic site with the opposing guanine residue of 5'-CAp sequences in duplex DNA. *J. Am. Chem. Soc.*, 135, 1015–1025.

52. Gamboa Varela, J. and Gates, K.S. (2015) A simple, high-yield synthesis of DNA duplexes containing a covalent, thermally-reversible interstrand cross-link at a defined location. *Angew. Chem. Int. Ed. Engl.*, 54, 7666–7669.

53. Price, N.E., Johnson, K.M., Wang, J., Fekry, M.I., Le, M., Chang, D.-K. and Lown, I.W. (1987) Structure of the adduct formed between 3-amino carbazole and the apurinic site oligonucleotide model d[Tp(Ap)pT]. *J. Org. Chem.*, 52, 4994–4998.

54. Sczepanski, J.T., Wong, R.S., McKnight, J.N., Bowman, G.D. and Greenberg, M.M. (2010) Rapid DNA-protein cross-linking and...
strand scission by an abasic site in a nucleosome core particle. *Proc. Natl. Acad. Sci. U.S.A.*, **107**, 22475–22480.

78. Pruss, D., Gravin, I. M., Melnik, S. and Bavykin, S. G. (1999) DNA-protein cross-linking applications for chromatin studies in vitro and in vivo. *Methods Enzymol.*, **304**, 516–533.

79. Prasad, R., Horton, J. K., Chastain, P. D. II, Gassman, N. R., Freudenthal, B. D., Hou, E. W. and Wilson, S. H. (2014) Suicidal cross-linking of PARP-1 to AP site intermediates in cells undergoing base excision repair. *Nucleic Acids Res.*, **42**, 6337–6351.

80. Catalano, M. J., Price, N. E. and Gates, K. S. (2016) Effective molarity in a nucleic acid controlled reaction. *Bioorg. Med. Chem. Lett.*, **26**, 2627–2630.

81. Kurata, C., Bradley, K., Gaus, H., Luu, N., Cedillo, I., Ravikumar, V. T., Sooy, K. V., Mc Ardle, J. V. and Capaldi, D. C. (2006) Characterization of high molecular weight impurities in synthetic phosphorothioate oligonucleotides. *Bioorg. Med. Chem. Lett.*, **16**, 607–614.

82. Sczepanski, J. T., Jacobs, A. C. and Greenberg, M. M. (2008) Self-promoted DNA interstrand cross-link formation by an abasic site. *J. Am. Chem. Soc.*, **130**, 9646–9647.

83. Ghosh, S. and Greenberg, M. M. (2014) Synthesis of cross-linked DNA containing oxidized abasic site analogues. *J. Org. Chem.*, **79**, 5948–5957.

84. Sun, J. and Tang, X. (2015) Photouncaged sequence-specific interstrand DNA cross-linking with photolabile 4-oxo-enal-modified oligonucleotides. *Sci. Rep.*, **5**, doi:10.1038/srep10473.

85. Regulus, P., Duroux, B., Bayle, P.-A., Favier, A., Cadet, J. and Ravanat, J.-L. (2007) Oxidation of the sugar moiety of DNA by ionizing radiation or bleomycin could induce the formation of a cluster DNA lesion. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 14032–14037.

86. Guillet, M. and Boiteux, S. (2002) Endogenous DNA abasic sites cause cell death in the absence of Apn1, Apn2, and Rad1/Rad10 in *Saccharomyces cerevisiae*. *EMBO J.*, **21**, 2833–2841.

87. Ma, W., Westmoreland, J. W., Gordenin, D. A. and Resnick, M. A. (2011) Alkylation base damage is converted into repairable double-strand breaks and complex intermediates in G2 cells lacking AP endonuclease. *PLoS Genet.*, **7**, e1002059.

88. Boiteux, S. and Guillet, M. (2004) Abasic sites in DNA: repair and biological consequences in *Saccharomyces cerevisiae*. *DNA Repair*, **3**, 1–12.

89. Bender, M. L. and Williams, A. (1966) Ketimine intermediates in amine-catalyzed enolization of acetone. *J. Am. Chem. Soc.*, **88**, 2502–2508.

90. Erkkilä, A., Majander, I. and Pihko, P. M. (2007) Iminium ion catalysis. *Chem. Rev.*, **107**, 5416–5470.

91. Appel, R., Chelli, S., Tokuyasu, T., Troshin, K. and Mayr, H. (2013) Electrophilicities of benzaldehyde-derived iminium ions: quantification of the electrophilic activation of aldehydes by iminium formation. *J. Am. Chem. Soc.*, **135**, 6579–6587.

92. Pawlowicz, A. J., Klika, K. D. and Kronberg, L. (2007) The structural identification and conformational analysis of the products from the reaction of acrolein with 2'-deoxyctydine, 1-methylcytosine and calf thymus DNA. *Eur. J. Org. Chem.*, **2007**, 1429–1437.

93. Pawlowicz, A. J. and Kronberg, L. (2008) Characterization of adducts formed in the reactions of acrolein with thymidine and calf thymus DNA. *Chem. Biodivers.*, **5**, 177–188.

94. Chung, F.-L., Young, R. and Hecht, S. M. (1984) Formation of cyclic 1,N2-propanodeoxyguanosine adducts in DNA upon reaction with acrolein or crotonaldehyde. *Cancer Res.*, **44**, 990–995.

95. Kowaleczk, P., Ciesla, J. M., Komisarski, M., Kusmiercz, J. and Tudek, B. (2004) Long-chain adducts of trans-4-hydroxy-2-nonenal to DNA bases cause recombination, base substitutions and frameshift mutations in M13 phage. *Mutat. Res.*, **550**, 33–48.

96. Liu, S. and Wang, Y. (2015) Mass spectrometry for the assessment of the occurrence and biological consequences of DNA adducts. *Chem. Soc. Rev.*, **44**, 7829–7854.

97. You, C. and Wang, Y. (2016) Mass spectrometry-based quantitative strategies for assessing the biological consequences and repair of DNA adducts. *Acc. Chem. Res.*, **49**, 205–213.