


 Cite this: *RSC Adv.*, 2023, **13**, 15490

Mass spectrometry-based assays for assessing replicative bypass and repair of DNA alkylation in cells

 Jiaxian Li, Zhihai Hu, Dandan Liu* and Pengcheng Wang *

Endogenous metabolism and environmental exposure can give rise to DNA alkylation, which can elicit deleterious biological consequences. In the search for reliable and quantitative analytical methods to elucidate the impact of DNA alkylation on the flow of genetic information, mass spectrometry (MS) has attracted increasing attention, owing to its unambiguous determination of molecular mass. The MS-based assays obviate conventional colony-picking methods and Sanger sequencing procedures, and retained the high sensitivity of postlabeling methods. With the help of the CRISPR/Cas9 gene editing method, MS-based assays showed high potential in studying individual functions of repair proteins and translesion synthesis (TLS) polymerases in DNA replication. In this mini-review, we have summarized the development of MS-based competitive and replicative adduct bypass (CRAB) assays and their recent applications in assessing the impact of alkylation on DNA replication. With further development of MS instruments for high resolving power and high throughput, these assays should be generally applicable and efficient in quantitative measurement of the biological consequences and repair of other DNA lesions.

Received 31st December 2022

Accepted 15th May 2023

DOI: 10.1039/d2ra08340j

rsc.li/rsc-advances

1. Introduction

DNA is intrinsically unstable and its integrity in cells is constantly challenged by various endogenous and exogenous chemicals, resulting in a plethora of DNA lesions.^{1–3} For instance, it can undergo spontaneous deamination and depurination under physiological conditions. In addition, DNA, as a poly-nucleophile, can react with many electrophile species, leading to covalently bound adducts.^{3,4} Among them, DNA

alkylation constitutes a major type of DNA damage due to the ubiquitous presence of alkylating agents in the environment and within cells.⁵ If not repaired, the alkylated DNA may perturb the efficiency and fidelity of DNA replication and transcription, thereby compromising the flow of genetic information and conferring adverse human health consequences.^{5,6} Indeed, it has been demonstrated that DNA alkylation can be cytotoxic, teratogenic, and carcinogenic.⁷

To counteract the DNA alkylation and reduce its subsequent deleterious effects, cells have equipped diverse cellular repair pathways to collectively modulate alkylation, such as direct removal, base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination

Institute of Surface Analysis and Chemical Biology, University of Jinan, Jinan, Shandong, 250022, P. R. China. E-mail: 202111100006@stu.ujn.edu.cn; ila_wangpc@ujn.edu.cn



Jiaxian Li received his M.S. in Chemistry from the University of Jinan under the supervision of Prof. Pengcheng Wang. His research mainly involves the development of MS methods and the study of molecular interactions.



Zhihai Hu received his B.S. in Biotechnology from the Zhengzhou University of Light Industry and is pursuing his M.S. degree at the University of Jinan. His research focuses on molecular interactions between biomolecules such as DNA and proteins.



(HR), and non-homologous end joining (NHEJ).^{1,2,4,5} In addition, the translesion DNA synthesis (TLS) pathway utilizes low-fidelity DNA polymerases to bypass lesions in case the replication machinery is stalled by alkylation blockage.^{8,9} However, thus far, the roles of specific TLS polymerase in mutagenesis still largely remain elusive. Understanding the replicative bypass of DNA alkylation and involvement of repair proteins as well as TLS polymerases necessitates the investigation of how this type of lesions perturbs the flow of genetic information in cells under various gene manipulation.

The synthesis and construction of site-specifically modified genomes facilitate pinpointing mutation spectra of specific lesions with accuracy and certainty.^{10,11} Conventionally, extensive colony picking and Sanger sequencing procedures are required to assess the biological consequences of lesions and elucidate the roles of DNA polymerases and repair proteins.^{10,12–14} However, the relatively small sample size may result in some mutations of low frequencies being overlooked.¹⁵ Recent years witnessed the advancement of mass spectrometry (MS) in investigating the occurrence and biological consequences of DNA lesions.^{16–18} Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been successfully employed as an efficient approach in assessing how DNA lesions compromise DNA replication *in vitro* and *in vivo*, which has been reviewed by others.^{17–19} Here, we place emphasis on recent discoveries and compare the replicative bypass of different types of alkyl lesions in cells.

2. MS-based assay for replication study in cells

With the availability of site-specifically modified oligodeoxyribonucleotides (ODN), Essigmann and co-workers introduced a lesion bypass and mutagenesis assay to assess how DNA replication is compromised by certain lesions.^{20,21} In that assay, the bypass efficiency and mutation frequency of a defined lesion can be quantitatively determined by restriction digestion and postlabeling. This assay is then further developed by Wang's lab and the resultant competitive and replicative adduct bypass (CRAB) assay has been employed to evaluate the genetic

perturbations caused by DNA lesions during replication in *E. coli* as well as in mammalian cells.^{22–24}

As shown in Fig. 1a, in *E. coli* cells, the experimental procedure begins with the synthesis and construction of lesion-containing and lesion-free M13 phage genomes in addition to a competitor M13 genome which possesses three additional nucleotides. As transfection efficiency for each independent experiment is not consistent, the competitor genome is pre-mixed with a lesion-containing or lesion-free genome and serves as an internal standard in the calculation of bypass efficiencies with the assumption that the bypass efficiency of the corresponding control (lesion-free) M13 genome is 100%. After co-transfection and replication in *E. coli*, the progeny M13 genomes are harvested and the region of interest containing either the initial lesion site or the lesion-free control, or the competitor is amplified by PCR. The resultant PCR products are digested by appropriate restriction enzymes and subjected to LC-MS/MS analysis (Fig. 1b). In addition, with postlabeling, the digested products can also be analyzed by polyacrylamide gel electrophoresis (PAGE). Furthermore, by switching the order of restriction enzyme addition, the lesion-situated strand or its complementary strand can be selectively labeled and four potential types of replication products can be resolved from each other with two electrophoreses (Fig. 1c).^{15,25–27} Note that TLS polymerases in *E. coli* cells can be induced due to SOS response, which is named after the distress signal “SOS” in the Morse alphabet.²⁸ Thus, to investigate the roles of specific TLS polymerases, the *E. coli* cells are generally treated with UV irradiation to initiate the SOS response.²⁸

The CRAB assay also extended to mammalian cells by changing the single-stranded phage plasmid to a double-stranded shuttle vector that is capable of propagating in mammalian cells (*cf.* Fig. 2). Similarly, lesion-containing/lesion-free and competitor plasmids are constructed first *via* a gapped vector-based strategy. As the lesion may exert an impediment to replication, the non-lesion strand in the double-stranded plasmid usually is preferentially replicated, rendering it difficult to accurately determine the mutation frequencies and bypass efficiencies. As a result, a mismatch is introduced into the double-stranded plasmid containing the lesion site, enabling the independent assessment of the products arising



Dandan Liu earned her M.S. in Chemistry from the University of Jinan in 2021 under the supervision of Prof. Pengcheng Wang. She is now pursuing her Ph.D. degree at the University of Jinan. Her research focuses on MS analysis and biosensor development.



Pengcheng Wang received his B.S. and M.S. from Wuhan University, China. He earned his Ph.D. in Environmental Toxicology from the University of California Riverside in 2016. After post-doctoral training in the USA, he started his independent career at the University of Jinan, China. His research interests are focused on MS-based DNA analysis and surface plasmon resonance (SPR)-based biomarker detection.



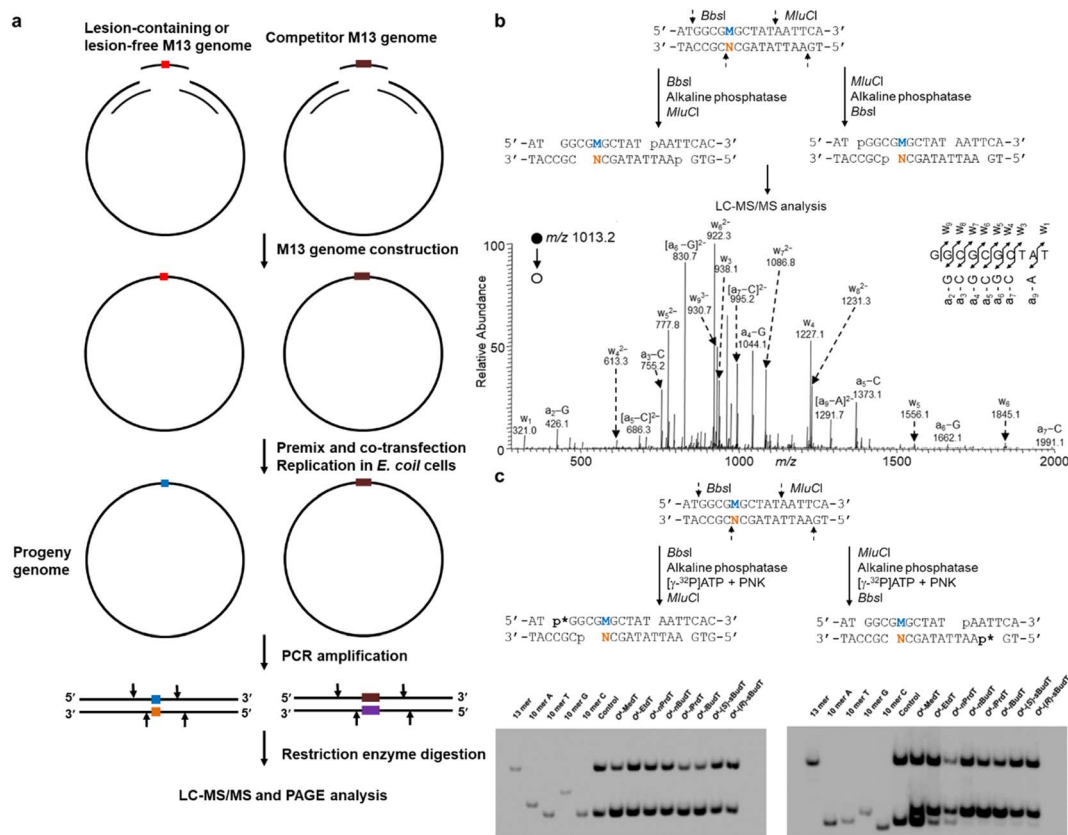


Fig. 1 (a) Schematic illustration of experimental procedures of CRAB in *E. coli* cells. (b) Sequential restriction enzyme digestion for MS-based replication assay in *E. coli* cells (top) and a representative MS spectrum (bottom) showing the T → C mutation elicited by O⁴-methylthymidine (O⁴-MedT). (c) Sequential restriction enzyme digestion for the selective labeling of the strand initially bearing the lesion or the complementary strand, and the representative images of PAGE gel. The 'M' labeled in blue and 'N' labeled in orange indicate the nucleotide at the lesion-situated strand and lesion-complementary strand after replication, respectively. Reproduced from ref. 25 with permission from Oxford, copyright 2015.

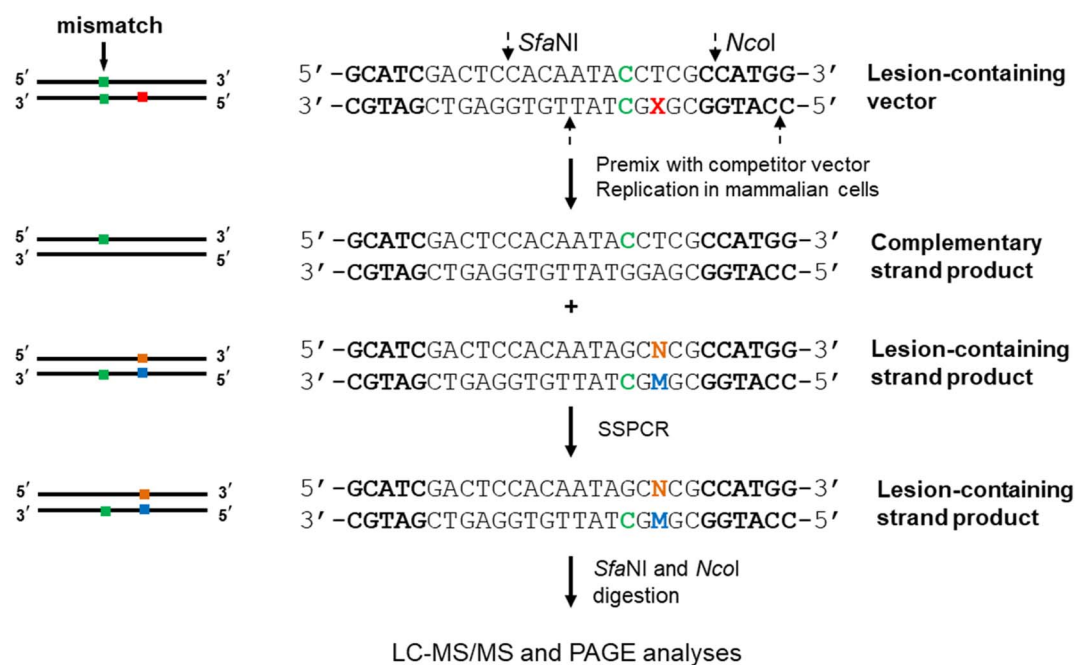


Fig. 2 Experimental procedures for MS-based replication assay in mammalian cells. The C/C mismatch site, the lesion site, and the incorporated nucleotides at the lesion site and opposite lesion site are marked in green, red, blue, and orange. The *Sfa*NI and *Nco*II sites are highlighted in bold.



Table 1 Summary of experimental procedures

<i>E. coli</i> cells	<ol style="list-style-type: none"> (1) The lesion-containing, lesion-free, and competitor M13 genomes are constructed by incorporation of specific ODNs into the <i>EcoRI</i>-linearized M13 genome with two scaffolds (2) The scaffolds and unligated M13 genomes are degraded with T4 DNA polymerase (3) The lesion-containing or lesion-free M13 genome is premixed with the competitor genome (4) The competent <i>E. coli</i> cells are transfected with the premixed M13 genomes (5) The transfected <i>E. coli</i> cells are cultured in Lysogeny Broth (LB) media (6) The culture media are centrifuged, and the cell pellets are harvested (7) The progeny M13 genomes are extracted (8) The region of interest in the purified single-stranded M13 genome is PCR amplified using two primers (9) The PCR products are digested with sequential restriction enzymes (10) The resultant digestion mixture is subjected to LC-MS analysis and postlabeling for PAGE
Mammalian cells	<ol style="list-style-type: none"> (1) The double-stranded shuttle vector is nicked with <i>Nt.Bst</i>NBI to produce a gapped vector (2) The gap is filled with lesion-containing, lesion-free, or competitor ODN and ligated with T4 DNA ligase (3) The supercoiled plasmid is purified by agarose gel electrophoresis (4) The lesion-containing or lesion-free plasmid is premixed with the competitor plasmid (5) The overnight-cultured cells are transfected with the premixed plasmids (6) The transfected cells are harvested after 24 h, and the progenies of the plasmid are isolated (7) The residual unreplicated plasmid is removed by <i>DpnI</i> digestion (8) The region of interest in the progeny genomes is amplified with SPCR (9) The PCR products are digested with sequential restriction enzymes (10) The resultant digestion mixture is subjected to LC-MS analysis and postlabeling for PAGE

from the replication of the lesion-bearing and lesion-free complementary strands. The progeny genomes of the plasmid are isolated from host cells, and the residual unreplicated plasmid is removed by *DpnI* digestion. The progeny plasmids are then amplified by PCR. The region of interest in the progeny plasmids is amplified with strand-specific PCR (SSPCR) so that the strand complementary to the lesion-containing can be selectively amplified. The resulting PCR products are restriction digested and subjected to LC-MS/MS and PAGE analyses.^{29–33} A summary of experimental procedures of CRAB in *E. coli* and mammalian cells are shown in Table 1.

3. Applications of MS-based replication assays

In the past few years, a number of discoveries have been made using the abovementioned strategy to assess the effect of DNA alkylation on replication in cells.^{25–27,29–36} Here, we discuss the effects of alkyl lesions on replication in *E. coli* and mammalian cells and compare the difference between the major groove and minor groove lesions. In addition, alkylation on the phosphate backbone will also be described.

3.1 Alkyl lesions in the major groove

The *O*⁴-alkylthymidine (*O*⁴-alkyldT) and *O*⁶-alkyl-2'-deoxyguanosine (*O*⁶-alkyl dG) lesions represent two types of alkyl lesions on pyrimidine and purine bases. Systematic experiments have been performed to assess how the size of the alkyl group, from methyl to butyl, at the *O*⁴ position of thymine and *O*⁶ position of guanine (*cf.* Fig. 3) affects the efficiency and fidelity of DNA replication in *E. coli* cells.^{25,26} It is found that *O*⁴-alkyl dT lesions direct exclusively misincorporation of 2'-deoxyguanosine opposite the lesion site,²⁵ while *O*⁶-alkyl dG lesions elicit only G → A mutation,²⁶ which can be attributed to their

distinct base-pairing properties. In addition, both types of lesions are not strong impediments to DNA replication in *E. coli* cells and the SOS-induced polymerases play redundant roles in bypassing these lesions, except for *O*⁴-sBudT, which exhibits moderate blockage to DNA replication and requires polymerase (Pol) V to facilitate efficient bypass.^{25,26}

Direct removal by *O*⁶-alkylguanine DNA alkyltransferase (MGMT) is an important repair pathway for *O*⁴-alkyl dT and *O*⁶-alkyl dG lesions.⁵ In *E. coli* cells, two types of MGMT are encoded, *i.e.*, Ogt and Ada.³⁷ Thus, their impact on the repair of these two types of lesions was also evaluated, and found that Ogt but not Ada is involved in repairing *O*⁴-alkyl dT lesions²⁵ while both of them can repair *O*⁶-alkyl dG lesions with Ogt more efficiently than Ada.²⁶

*O*⁴-alkyl dT and *O*⁶-alkyl dG lesions also elicit exclusively T → C and G → A mutation in mammalian cells, respectively. However, different from *E. coli* cells, these two types of lesions exert some blockage to DNA replication (*cf.* Fig. 4).^{29,30} It is shown that *O*⁴-alkyl dT lesions are moderately blocking DNA replication, with bypass efficiencies ranging from 20% to 33%. For *O*⁶-alkyl dG lesions, they also show moderate impediment to DNA replication with the exception of *O*⁶-MedG. The DNA adduct, 4-(3-pyridyl)-4-oxobut-1-yl (POB), was also studied, which is generated by tobacco-specific nitrosamine.³¹ It was found that while *O*⁴-POB dT still directs only T → C mutation, *O*⁶-POB dG elicited primarily the G → A transition (~75%) together with a low frequency of the G → T transversion (~3%). In addition, *O*⁶-POB dG exerts a higher blockage effect on DNA replication compared to *O*⁴-POB dT.³¹

The roles of repair proteins and TLS polymerases in replicative bypass and mutagenesis were also investigated in mammalian cells. For *O*⁴-alkyl dT lesions, deficiency in Pol η or Pol ζ, but not Pol κ or Pol ι, leads to pronounced drops in bypass efficiencies for all the *O*⁴-alkyl dT lesions. In addition, depletion



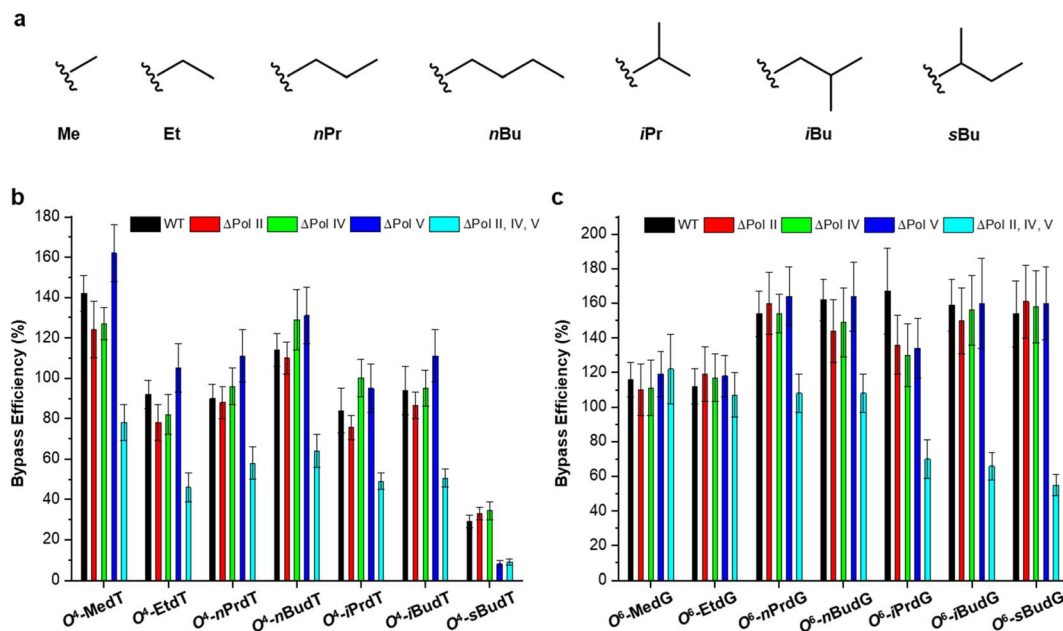


Fig. 3 Chemical structures of the examined alkyl groups (a) and replicative bypass efficiencies of O²-alkyldT and O⁴-alkyldG lesions (b and c) in *E. coli* cells. Reproduced from ref. 25 with permission from Oxford, copyright 2015 and ref. 26 with permission from Elsevier, copyright 2018.

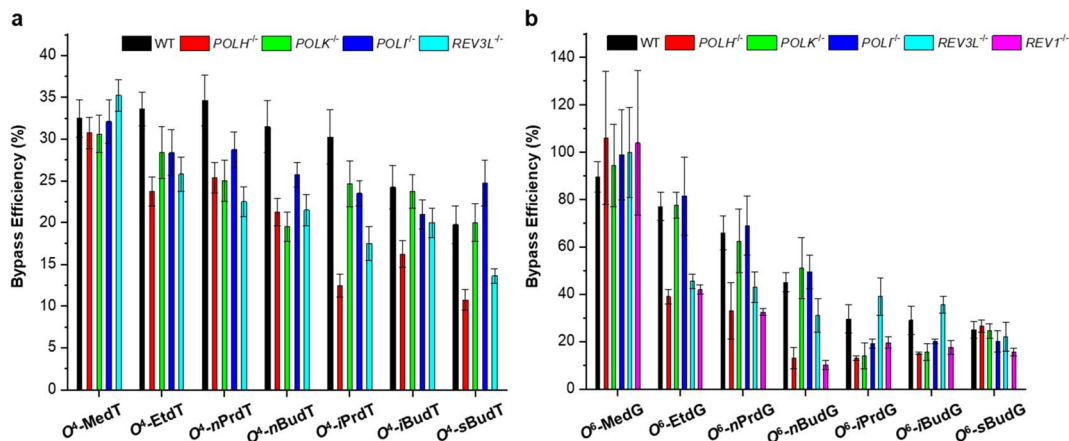


Fig. 4 Replicative bypass efficiencies of O⁴-alkyldT (a) and O⁶-alkyl-dG lesions (b) in mammalian cells. Reproduced from ref. 29 with permission from Oxford, copyright 2016 and ref. 30 with permission from Elsevier, copyright 2019.

of Pol η, Pol κ, or Pol ι does not significantly alter the T → C mutation for any of the O⁴-alkyldT lesions, with the exception that loss of Pol η results in significant decreases in T → C mutation for O⁴-EtdT and O⁴-nPrdT. Moreover, the depletion of Pol ζ results in significant drops in T → C mutation frequencies for all O⁴-alkyldT lesions except O⁴-MedT and O⁴-nBudT. For O⁶-alkyldG lesions, depletion of REV1 significantly decreases the bypass efficiency of all lesions except for O⁶-MedG. In addition, individual ablation of Pol η or Pol ζ confers a pronounced reduction in bypass efficiency for all straight-chain lesions. In terms of replication fidelity, Pol η, and Pol ζ are involved in the error-prone bypass of the straight-chain lesions, whereas Pol κ favors the accurate bypass of the branched-chain lesions. Moreover, it was found that MGMT is effective in removing the

smaller alkyl groups from the O⁶ position of guanine, whereas repair of the branched-chain lesions relies on NER. Interestingly, the roles of TLS polymerases involved in replicative bypassing of these two types of lesions are similar to other major groove lesions, such as N⁷-guanine and N⁶-adenine cross-links.^{38,39} Several reports indicated that Pol η is required for efficient bypass of DNA adducts in the major groove.^{29,30,38,39}

3.2 Alkyl lesions in the minor groove

Two representative minor-groove alkyl lesions were systematically investigated using the CRAB assay, *i.e.*, O²-alkylthymidine (O²-alkyldT) and N²-alkyl-2'-deoxyguanosine (N²-alkyldG).^{27,32,33} These two types of lesions direct promiscuous mutations, but the results are not consistent in *E. coli* and in mammalian cells.



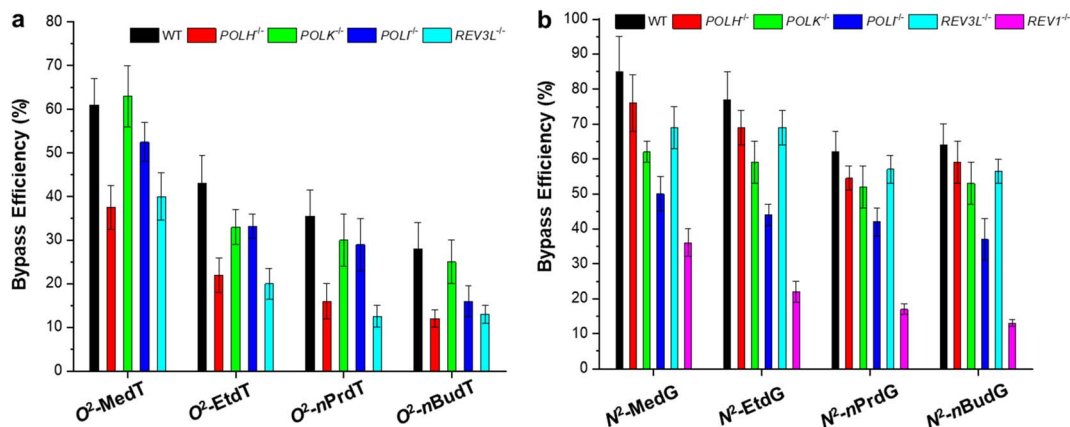


Fig. 5 Replicative bypass efficiencies of O^2 -alkyl dT (a) and N^2 -alkyl dG lesions (b) in mammalian cells. Reproduced from ref. 32 with permission from Elsevier, copyright 2018 and ref. 33 with permission from American Chemical Society, copyright 2019.

In *E. coli* cells, all four nucleotides can be inserted opposite the O^2 -alkyl dT lesion site,⁴⁰ while T \rightarrow C mutation is absent in mammalian cells.³² For N^2 -alkyl dG, it is nonmutagenic in *E. coli* cells,²⁷ while it directs G \rightarrow A and G \rightarrow C mutations in mammalian cells.³³

As shown in Fig. 5, both lesions are strong impediments to DNA replication in mammalian cells,^{32,33} which is consistent with the results obtained in *E. coli* cells.^{27,40} In addition, the bypass efficiencies decrease with the increase in the size of the alkyl group. The involvement of TLS polymerases was also evaluated by conducting experiments in isogenic cells engineered with CRISPR/Cas9 genome editing method. It was found that the replicative bypass of O^2 -alkyl dT lesions requires Pol η and Pol ζ , which resulted in higher mutation frequencies. In contrast, Rev1, Pol ι , and Pol κ are involved in bypassing N^2 -alkyl dG lesions, and ablation of these three polymerases elicits substantial frequencies of G \rightarrow A transition and G \rightarrow T transversion. Interestingly, further depletion of Pol ζ in Pol κ - or Pol ι -deficient cells elevated G \rightarrow A and G \rightarrow T mutations but decreased bypass efficiencies. Different from major-groove lesions, the roles of TLS polymerases are lesion-specific in bypassing adducts in minor groove.

3.3 Alkyl lesions on the phosphate backbone

Apart from alkylation on nucleobases, the oxygen atoms of the internucleotide phosphate group can also be alkylated by alkylating agents and give rise to phosphotriester adducts (PTEs),⁴¹ which are known to be persistent in mammalian cells.⁴² As the phosphotriester contains a chiral phosphorus center, two configurations (S_p or R_p) can be formed depending on the non-carbon-bound oxygen atom that is alkylated (*cf.* Fig. 6).

Using the CRAB assay, Wu *et al.* found that the S_p diastereomer of the alkyl-PTE lesions could be efficiently bypassed in *E. coli* cells, while the R_p counterparts moderately impede DNA replication.³⁴ In addition, the bypass of the alkyl-PTE lesions does not require any of the three TLS polymerases (Pol II, Pol IV, and Pol V) and is not modulated by SOS induction. It was found that at the flanking TT dinucleotide site, S_p -Me-PTE induces TT

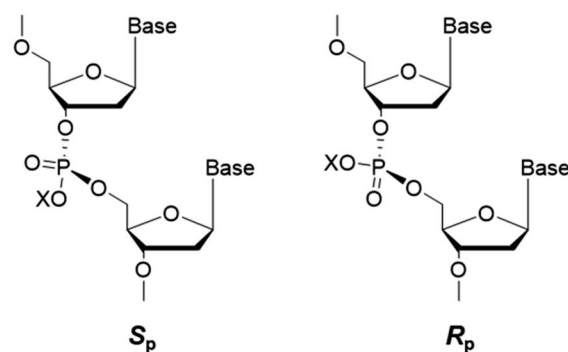


Fig. 6 S_p and R_p diastereomers of alkyl phosphotriester residues in DNA. The 'X' indicates the substituted alkyl group.

\rightarrow GT and TT \rightarrow GC mutations, and the induction of these mutations requires Ada protein. Additionally, the mutation frequencies are not affected by individual depletion of TLS polymerases, though simultaneous ablation of all three polymerases results in a slight decrease in TT \rightarrow GT mutation.³⁴

The replication bypass and fidelity of alkyl-PTE lesions were also investigated at different flanking dinucleotide sites, *i.e.*, XT and TX (X = A, C, or G).³⁵ It was shown that DNA replication was highly efficient, and the replication products contain 85–90% AT and 5–10% TG for S_p -Me-PTE in the sequence contexts of 5'-XT-3', largely independent of the flanking base. In addition, the R_p diastereomer of Me-PTEs at XT sites and both diastereomers of Me-PTEs at TX sites exhibited error-free replicative bypass. Moreover, both diastereomers of *n*Bu-PTEs at TX and XT sites are non-mutagenic.³⁵ In another report, both diastereomers of POB-PTEs exhibit low blocking effects on DNA replication with bypass efficiencies higher than 80%, and neither diastereomer is mutagenic.³⁶

4. Conclusions and perspectives

The advances in MS instrumentation have rendered it a viable and efficient tool for elucidating the biological consequences of



DNA adducts.^{16,18,19,43} In this review, we summarized the development of MS-based strategies for assessing replicative bypass and mutagenesis of alkylated DNA lesions, and recent findings in *E. coli* and mammalian cells. These MS-based assays demonstrate comparable sensitivity to restriction endonuclease and postlabeling (REAP) assay and are highly reproducible that a mutation frequency of around 0.5% can be reliably determined with a standard deviation being $\sim 0.1\%$.^{20,21,24,44} As all these assays use the entire progeny genomes and obviate the tedious colony counting, their quantitative results would be more reliable. The better reliability has been manifested by the low G \rightarrow T transversion conferred by O⁶-POBdG which was detected using MS-based assays but absent in the conventional method.⁴⁵ It should be noted that PAGE (*cf.* Fig. 1c), though can distinguish the four potential types of replication products as aforementioned, requires a specific sequence of lesion-containing strands. Hence, the PAGE analysis lacks the ability to determine the involvement of flanking base. However, these MS-based assays still cannot fully assess the impact of sequence context on DNA replication, as the pool of ODN sequences is confined by the restriction sites recognized by enzymes. Another limitation of MS-based assays is that these assays generally cannot provide kinetic information about nucleotide insertion and the interaction between DNA and proteins. Therefore, methods, such as surface plasmon resonance⁴⁵ and biolayer interferometry,⁴⁶ that can reveal the binding affinity and kinetics between lesions and repair proteins/polymerases would complement MS-based assays.

Although DNA alkylation is generally considered deleterious, alkylating agents are a class of widely used drugs in chemotherapy.^{5,47} Understanding how alkylating agents exert their therapeutic effects and how cells respond to chemotherapeutic alkylating agents would be important for drug discovery and development.⁵ In addition, it will provide insight into the proper use of chemotherapeutic drugs. All these necessitate elucidation of the roles of repair proteins and TLS polymerases in coping with alkyl DNA lesions. MS displays high potential in these applications, owing to its unambiguous quantification. Moreover, by utilizing the CRISPR/Cas9 genome editing method,^{48–50} manipulating single and even multiple genes involved in DNA replicative bypass becomes more facile and proficient, enabling the investigation of individual or synergistic roles of repair proteins and TLS polymerases. Furthermore, MS-based assays have also been adopted in the evaluation of the impact of DNA lesions on transcription, rendering them more versatile in analytical method development.^{51–54}

Owing to the importance of understanding the biological consequence of DNA lesions, the development, and applications of lesion bypass and mutagenesis assays have attracted numerous attention.^{10,55} Unfortunately, the CRAB assays described here are of relatively low throughput and the analysis costs hours even days to complete. In addition, it cannot provide genome-wide information on DNA adducts. Thus, many other biological assays for the determination of DNA lesion bypass and mutation have been developed, such as chromatin immunoprecipitation (ChIP)-based assay⁵⁶ and next-generation sequencing (NGS)-based assay.⁴⁴ For instance, the involvement

of polymerases ν and θ in the replicative bypass of 8-oxo-7,8-dihydroguanine was assessed with high-throughput, using the construction of a shuttle vector-based NGS assay.⁵⁷ However, MS-based assays can still be a complementary tool to pinpoint the exact type of mutations including frameshift mutations.^{43,58} As the mutagenesis caused by environmental exposure will be more complicated in the human body,^{55,59} MS with high resolving power is indispensable in quantitative and/or qualitative measurement. Therefore, combining MS and other analytical methods would be an effective way to circumvent the above-mentioned limitations. In addition, improvement of sample preparation techniques, development of programmable software, and application of high-resolution MS (*e.g.*, Orbitrap and time-of-flight) would be of top priority. With the development of online separation and automatic injection, we envision that high throughput and unattended analysis can be achieved in the future.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the financial support from the Natural Science Foundation of China (No. 21906065), and the Shandong Provincial Grant for the Talent-Leading Teams.

References

- 1 N. Chatterjee and G. C. Walker, *Environ. Mol. Mutagen.*, 2017, **58**, 235–263.
- 2 E. C. Friedberg, G. C. Walker, W. Siede, R. D. Wood, R. A. Schultz and T. Ellenberger, *DNA Repair and Mutagenesis*, ASM Press, Washington, D.C., 2005.
- 3 K. S. Gates, *Chem. Res. Toxicol.*, 2009, **22**, 1747–1760.
- 4 N. C. Bauer, A. H. Corbett and P. W. Doetsch, *Nucleic Acids Res.*, 2015, **43**, 10083–10101.
- 5 D. Fu, J. A. Calvo and L. D. Samson, *Nat. Rev. Cancer*, 2012, **12**, 104–120.
- 6 Y. Yu, P. Wang, Y. Cui and Y. Wang, *Anal. Chem.*, 2018, **90**, 556–576.
- 7 L. P. Bignold, *Anticancer Res.*, 2006, **26**, 1327–1336.
- 8 X. Ma, T.-S. Tang and C. Guo, *Environ. Mol. Mutagen.*, 2020, **61**, 680–692.
- 9 S. Fujii and R. P. Fuchs, *Microbiol. Mol. Biol. Rev.*, 2020, **84**, e00002–e00020.
- 10 A. K. Basu and J. M. Essigmann, *Chem. Res. Toxicol.*, 2022, **35**, 1655–1675.
- 11 H. O. Smith, C. A. Hutchison III, C. Pfannkoch and J. C. Venter, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 15440–15445.
- 12 J. C. Delaney and J. M. Essigmann, *Chem. Res. Toxicol.*, 2008, **21**, 232–252.
- 13 O. Ziv, N. Diamant, S. Shachar, A. Hendel and Z. Livneh, *Methods Mol. Biol.*, 2012, **920**, 529–542.



Review

- 14 A. L. Livingston, V. L. O'Shea, T. Kim, E. T. Kool and S. S. David, *Nat. Chem. Biol.*, 2008, **4**, 51–58.
- 15 P. Wang, J. Leng and Y. Wang, *J. Biol. Chem.*, 2019, **294**, 3899–3908.
- 16 S. Liu and Y. Wang, *Chem. Soc. Rev.*, 2015, **44**, 7829–7854.
- 17 C. You and Y. Wang, *Acc. Chem. Res.*, 2016, **49**, 205–213.
- 18 M. Dizdaroglu, E. Coskun and P. Jaruga, *Free Radical Res.*, 2015, **49**, 525–548.
- 19 J. Guo and R. J. Turesky, *High-Throughput*, 2019, **8**, 13.
- 20 J. C. Delaney, L. Smeester, C. Wong, L. E. Frick, K. Taghizadeh, J. S. Wishnok, C. L. Drennan, L. D. Samson and J. M. Essigmann, *Nat. Struct. Mol. Biol.*, 2005, **12**, 855–860.
- 21 J. C. Delaney and J. M. Essigmann, *Methods Enzymol.*, 2006, **408**, 1–15.
- 22 H. Hong, H. Cao and Y. Wang, *Nucleic Acids Res.*, 2007, **35**, 7118–7127.
- 23 B. Yuan, T. R. O'Connor and Y. Wang, *ACS Chem. Biol.*, 2010, **5**, 1021–1027.
- 24 B. Yuan, H. Cao, Y. Jiang, H. Hong and Y. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 8679–8684.
- 25 P. Wang, N. J. Amato, Q. Zhai and Y. Wang, *Nucleic Acids Res.*, 2015, **43**, 10795–10803.
- 26 P. Wang and Y. Wang, *J. Biol. Chem.*, 2018, **293**, 15033–15042.
- 27 Y. Wang, J. Wu, J. Wu and Y. Wang, *Chem. Res. Toxicol.*, 2021, **34**, 695–698.
- 28 C. Janion, *Int. J. Biol. Sci.*, 2008, **4**, 338–344.
- 29 J. Wu, L. Li, P. Wang, C. You, N. L. Williams and Y. Wang, *Nucleic Acids Res.*, 2016, **44**, 9256–9265.
- 30 H. Du, P. Wang, L. Li and Y. Wang, *J. Biol. Chem.*, 2019, **294**, 11144–11153.
- 31 H. Du, J. Leng, P. Wang, L. Li and Y. Wang, *J. Biol. Chem.*, 2018, **293**, 11100–11108.
- 32 J. Wu, P. Wang, L. Li, C. You and Y. Wang, *J. Biol. Chem.*, 2018, **293**, 8638–8644.
- 33 J. Wu, H. Du, L. Li, N. E. Price, X. Liu and Y. Wang, *ACS Chem. Biol.*, 2019, **14**, 1708–1716.
- 34 J. Wu, P. Wang and Y. Wang, *Nucleic Acids Res.*, 2018, **46**, 4013–4021.
- 35 J. Wu, J. Yuan, N. E. Price and Y. Wang, *J. Biol. Chem.*, 2020, **295**, 8775–8783.
- 36 J. Wu and Y. Wang, *Chem. Res. Toxicol.*, 2020, **33**, 308–311.
- 37 M. C. Wilkinson, P. M. Potter, L. Cawkwell, P. Georgiadis, D. Patel, P. F. Swann and G. P. Margison, *Nucleic Acids Res.*, 1989, **17**, 8475–8484.
- 38 S. Wickramaratne, S. Ji, S. Mukherjee, Y. Su, M. G. Pence, L. Lior-Hoffmann, I. Fu, S. Broyde, F. P. Guengerich, M. Distefano, O. D. Scharer, Y. Y. Sham and N. Tretyakova, *J. Biol. Chem.*, 2016, **291**, 23589–23603.
- 39 P. P. Ghodke, G. Gonzalez-Vasquez, H. Wang, K. M. Johnson, C. A. Sedgeman and F. P. Guengerich, *J. Biol. Chem.*, 2021, **296**, 100444.
- 40 Q. Zhai, P. Wang, Q. Cai and Y. Wang, *Nucleic Acids Res.*, 2014, **42**, 10529–10537.
- 41 G. D. D. Jones, R. C. Le Pla and P. B. Farmer, *Mutagenesis*, 2010, **25**, 3–16.
- 42 L. Den Engelse, G. J. Menkveld, R. J. De Brij and A. D. Tates, *Carcinogenesis*, 1986, **7**, 393–403.
- 43 J. R. Edwards, H. Ruparel and J. Ju, *Mutat. Res.*, 2005, **573**, 3–12.
- 44 S.-c. Chang, B. I. Fedeles, J. Wu, J. C. Delaney, D. Li, L. Zhao, P. P. Christov, E. Yau, V. Singh, M. Jost, C. L. Drennan, L. J. Marnett, C. J. Rizzo, S. S. Levine, F. P. Guengerich and J. M. Essigmann, *Nucleic Acids Res.*, 2015, **43**, 5489–5500.
- 45 P. G. Stockley and B. Persson, *Methods Mol. Biol.*, 2009, **543**, 653–669.
- 46 J. K. Barrows and M. W. Van Dyke, *PLoS One*, 2022, **17**, e0263322.
- 47 Y. Peng and H. Pei, *J. Zhejiang Univ., Sci., B*, 2021, **22**, 47–62.
- 48 Y. Ma, L. Zhang and X. Huang, *FEBS J.*, 2014, **281**, 5186–5193.
- 49 D. Gupta, O. Bhattacharjee, D. Mandal, M. K. Sen, D. Dey, A. Dasgupta, T. A. Kazi, R. Gupta, S. Sinharoy, K. Acharya, D. Chattopadhyay, V. Ravichandiran, S. Roy and D. Ghosh, *Life Sci.*, 2019, **232**, 116636.
- 50 P. D. Hsu, E. S. Lander and F. Zhang, *Cell*, 2014, **157**, 1262–1278.
- 51 C. You, X. Dai, B. Yuan, J. Wang, J. Wang, P. J. Brooks, L. J. Niedernhofer and Y. Wang, *Nat. Chem. Biol.*, 2012, **8**, 817–822.
- 52 X. He, P. Wang and Y. Wang, *Anal. Chem.*, 2021, **93**, 1161–1169.
- 53 Y. Tan, S. Guo, J. Wu, H. Du, L. Li, C. You and Y. Wang, *J. Am. Chem. Soc.*, 2021, **143**, 16197–16205.
- 54 Y. Tan, J. Wu, G. Clabaugh, L. Li, H. Du and Y. Wang, *DNA*, 2022, **2**, 221–230.
- 55 D. H. Phillips, *DNA Repair*, 2018, **71**, 6–11.
- 56 D. Wu, A. Banerjee, S. Cai, N. Li, C. Han, X. Bai, J. Zhang and Q.-E. Wang, *DNA Repair*, 2021, **108**, 103230.
- 57 X. Zheng, D. Chen, Y. Zhao, X. Dai and C. You, *Anal. Chem.*, 2022, **94**, 11627–11632.
- 58 P. Kowalczyk, J. M. Ciesla, M. Komisarowski, J. T. Kusmieriek and B. Tudek, *Mutat. Res.*, 2004, **550**, 33–48.
- 59 N. Saini and D. A. Gordenin, *Environ. Mol. Mutagen.*, 2018, **59**, 672–686.

