Distinct pathways for repairing mutagenic lesions induced by methylating and ethylating agents

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DNA alkylation damage can be repaired by nucleotide excision repair (NER), base excision repair (BER) or by direct removal of alkyl groups from modified bases by O6-alkylguanine DNA alkyltransferase (AGT; E.C. 2.1.1.63). DNA mismatch repair (MMR) is also likely involved in this repair. We have investigated alkylation-induced mutagenesis in a series of NER- or AGT-deficient Escherichia coli strains, alone or in combination with defects in the MutS, MutL or MutH components of MMR. All strains used contained the F’prolac from strain CC102 (F’CC102) epism capable of detecting specifically lac GC to AT reverse mutations resulting from O6-alkylguanine. The results showed the repair of O6-methylguanine to be performed by AGT > MMR > NER in order of importance, whereas the repair of O6-ethylguanine followed the order NER > AGT > MMR. Studies with double mutants showed that in the absence of AGT or NER repair pathways, the lack of MutS protein generally increased mutant frequencies for both methylating and ethylating agents, suggesting a repair or mutation avoidance role for this protein. However, lack of MutL or MutH protein did not increase alkylation-induced mutagenesis under these conditions and, in fact, reduced mutagenesis by the N-alkyl-N-nitrosoureas MNU and ENU. The combined results suggest that little or no alkylation damage is actually corrected by the mutHLS MMR system; instead, an as yet unspecified interaction of MutS protein with alkylated DNA may promote the involvement of a repair system other than MMR to avoid a mutagenic outcome. Furthermore, both mutagenic and antimutagenic effects of MMR were detected, revealing a dual function of the MMR system in alkylation-exposed cells.

Introduction

Alkylation is a common class of DNA damage, which can be induced by various environmental chemicals and possibly endogenous substances. From bacteria to humans, many types of DNA repair systems operate to repair the damage, such as nucleotide excision repair (NER), base excision repair (BER) and a system to remove alkyl groups directly from modified bases as in the case with O6-alkylguanine DNA alkyltransferase (AGT; E.C. 2.1.1.63). Mismatch repair systems (MMRS) may also be involved in this repair, although their precise role is still unclear. MMRS play an important role in removing mismatched base pairs and small insertion/deletion loops during DNA replication and in maintaining high fidelity of genome DNA (1–4). MMRS also target alkylated base lesions, O6-alkylguanine-containing base pairs caused by treatments with alkylating agents are recognised by MutS, a protein recognising mismatch lesions, and become subjects of MMRS (5). We previously demonstrated that deficiency of MutS increased the mutation frequency induced by methylating agents more than that of ethylating agents, a finding that correlated well with the observed higher MutS binding to methylated guanine base pairs than to corresponding ethylated base pairs (6). Recently, Lupari et al. (7) reported that translesion synthesis (TLS) polymerase-related rescue of cytotoxicity induced by methylating agents depended on MMRS. Among the DNA repair systems, AGT is thought to play a major role to protect organisms from alkylation damage (8,9). Escherichia coli AGT consists of an inducible enzyme, Ada, and a constitutive enzyme, Ogt. Both enzymes efficiently remove alkylated bases by transferring the alkyl group from DNA to themselves in a suicidal process resulting in enzyme inactivation (10). NER is also well known to repair a broad range of DNA lesions including alkylated bases (11). However, it is unclear which repair system is primarily involved in the repair of each alkylated damage created by various methylating and ethylating agents. In this study, we examine the mutant frequencies induced by alkylating agents in various repair backgrounds to estimate their relevant contributions. Rye et al. (12) reported that MMR proteins collaborate with AGT in the repair of O6-methylguanine. On the other hand, lack of AGT is known to enhance methylation-induced cell damage such as apoptosis and chromosomal instability through futile cycles of MMR or through direct signalling by MMR proteins (8,13–16). As repair pathways likely compete, one informative way to examine the role of MMR is to study its effects on alkylation mutagenesis in the absence of AGT or NER. For this reason, we constructed MMR-deficient strains additionally deficient in AGT or NER activity and measured the mutant frequencies for a series of alkylating agents. The results suggested that little or no alkylation-containing base pairs are corrected by the MMRs itself. Nevertheless, alkylated base pairs are subject to interaction with MutS protein, as we observed that mutS deficiency increased the mutagenic activity of methyl methanesulphonate (MMS), ethyl methanesulphonate (EMS), N-methyl-N’-nitro-N-nitrosoguanidine (MNGG) and N-ethyl-N’-nitro-N-nitrosoguanidine (ENNG). However, these increases were not seen in the case of mutL or mutH deficiencies. We, therefore, suggest that MutS interaction with alkylated base pairs leads indirectly to repair or mutation avoidance mediated by a repair system other than MMR.

Materials and methods

Chemicals

N-methyl-N-nitrosourea (MNU) (CAS 684-93-5) and ENNG (CAS 63885-23-4) were purchased from Nakarai Tesque (Kyoto, Japan) and EMS (CAS 62-50-0) from Wako Pure Chemicals (Osaka, Japan). N-ethyl-N-nitrosourea (ENU)
Bacterial strains and plasmids

The bacterial strains used in this study are shown in Table I. All strains used in the mutagenesis experiments are derived from strain KA796 (ara, thi and apro-lac) (17) and also containing the F’prolac from strain CC102 (F’CC102) (25) that permits crossing of lac GC to AT transitions, as described (17,25). The wild-type, NR10832; the MMR-deficient derivatives, NR12896 (mutS201::Tn5), NR11102 (mutL211::Tn5), NR12897 (mutH471::Tn5); and the NER-deficient derivative, NR12999 (uvrA277::Tn10) have been described by Negishi et al. (17). AGT-deficient strains GW5352 (ada-10::Tn10) (18) and KT233 (ada::kan and ogt::cat) (19) were gifts from Drs S. Cohen (Massachusetts Institute of Technology) and M. Sekiguchi (Kyushu University), respectively. Mutants doubly deficient in MMR and either AGT or NER were constructed in this study. PI transductions, performed as described previously (20), were used to introduce the ada and ogt disruptions from GW5352 and KT233, respectively, and the uvrA277::Tn10 marker from NR12999, using selection for antibiotic resistance (tetracycline or chloramphenicol resistance for Tn10 or cat gene insertions, respectively) (see also Figure 1). Disruption of the genes in the final strains was confirmed by observed loss of PCR products amplified from the ada, ogt and nvrE genes, using as specific primers: 5'-gagatgccattaagcgtc-3' (forward) and 5'-ccctcagagcagcaacaa-3' (reverse) for ada; 5'-taatgatgcaagccgct-3' (forward) and 5'-aaatggttgcaccaagc-3' (reverse) for ada; and 5'-gctcgctttcagacctatat-3' (forward) and 5'-ccttcatggcgcaataacca-3' (reverse) for uvrA (see Figure 2). To confirm that the transduced genes were inserted in the correct locations in the E. coli genome, we performed Southern blot analysis as previously described (20). Results are shown in Figure 3. Probes used were a 533-bp PCR-amplified region within the cat gene (forward primer 5'-ttatttcgctccttcgct-3' and reverse primer 5'-atcccaatggcatcgtaaag-3'), a 1000-bp PCR-amplified region within the cat gene (forward primer 5'-tatttctgcctgctgct-3' and reverse primer 5'-catctgcaagcagcgagag-3'), and a 200-bp probe within the mutS201 gene (forward primer 5'-agaagcagcgatcagcgg-3' and reverse primer 5'-catctgcaagcagcgagag-3'). PCR products were purified using the GenElute™ Clean-Up Kit (Sigma–Aldrich Chemicals, St Louis, MO, USA) and labelled with digoxigenin using a PCR DIG Probe Synthesis Kit (Roche Diagnostics, Indianapolis, IN, USA) in accordance with the manufacturer’s protocols. Genomic DNA extracted from each E. coli strain was digested with two restriction enzymes, EcoRI and PstI (Takara Bio Inc., Otsu, Japan), at 37°C overnight. Digested samples were electrophoresed on a 0.8% agarose gel and transferred to a nitrocellulose membrane (Hybond-N'; GE Healthcare Life Sciences, Tokyo, Japan). The membrane was baked at 80°C for 2h and then subjected to pre-hybridisation and hybridisation in DIG Easy Hyb (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer’s protocol. Two bands hybridising with cat gene-specific probes were observed only in strains in which the cat gene should be inserted (KT11141, KT21181 and KT31131), whereas the bands were not observed in the parental strains and uvrA-disrupted strains. For the Tn10-carrying strains, 7-kb and 1-kb bands were detected in ada-deficient strains and 7-kb and 4.5-kb bands in the uvrA-deficient strains. (As the sequence of the region annealed by the probes contained an EcoRI site, two bands hybridised to the probe.) Taken together, the results show that the newly constructed E. coli strains are, as expected, deficient in AGT or NER function, in addition to their MMR deficiency. The double-mutant strains were as resistant to the toxicity of MNNG as the single MMR-deficient strains (data not shown). The spontaneous mutant frequencies of E. coli strains used in this study are shown in Table I. MMR-deficient strains show mutant frequencies 20–100 times higher than the proficient strains.

The plasmids containing each wild-type MMR gene were introduced into corresponding double mutants by electroporation (Electroporator EC-100, Thermo Quest, Milan, Italy). Plasmid pMQ315 contains the mutS gene (21), plasmid pMQ350 contains the mutL* gene and pRH71-17 (22) contains the mutH* gene (23). All three plasmids are derivatives of plasmid pBR322 (24). Cells transformed with plasmid were selected on Luria–Bertani agar containing ampicillin. The transformation of plasmid pMQ315 (mutS* and pRH71-17) into uvrA277-deficient E. coli (KT11141 or KT10021U) decreased the spontaneous mutant frequency, whereas the frequency was not influenced by the transfection of pBR322, although the extent of spontaneous mutation did not decrease to the level of parental AGT-deficient strain (KT01121). When plasmids pMQ350 (mutL*) and pRH71-17 (mutH*) were introduced in their corresponding deficient strains, spontaneous mutant frequencies also decreased (data not shown).

**Results**

**Mutagenic activities of alkylating agents in MMR-deficient strains**

To estimate how much the various repair systems contribute to the repair of alkylated bases, we measured mutant frequencies induced by methylating agents (MMS, MNU and MNNG) and ethylating agents (EMS, ENU and ENNG) in E. coli strains deficient in AGT, NER or MMR. As shown in Figure 4 and Table II, mutation frequencies induced by each methylating agent increased >100 times in an AGT-deficient strain compared with the wild-type exposed to the same agent. The frequencies were also elevated modestly (3–5-fold) in the single MMR-deficient strains (mutS, mutL and mutH). The NER deficiency enhanced the mutagenesis induced by MNU (7.5-fold) but not by MMS or MNNG (Table II and Figure 4).

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**Table I. E. coli strains used in this study and their spontaneous mutant frequencies**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Spontaneous mutant frequency (×10^-7)</th>
<th>References</th>
</tr>
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<tr>
<td>NR10832</td>
<td>KA796, wild-type</td>
<td>0.3±0.1</td>
<td>[17]</td>
</tr>
<tr>
<td>NR12896</td>
<td>KA796, mutS201::Tn5</td>
<td>34.3±3.3</td>
<td>[17]</td>
</tr>
<tr>
<td>NR11102</td>
<td>KA796, mutL211::Tn5</td>
<td>27.5±2.4</td>
<td>[17]</td>
</tr>
<tr>
<td>NR12897</td>
<td>KA796, mutH471::Tn5</td>
<td>28.9±8.1</td>
<td>[17]</td>
</tr>
<tr>
<td>NR12999</td>
<td>KA796, uvrA277::Tn10</td>
<td>0.4±0.1</td>
<td>[17]</td>
</tr>
<tr>
<td>GW5352</td>
<td>AB1157, ada-10::Tn10</td>
<td>—</td>
<td>[18]</td>
</tr>
<tr>
<td>KT233</td>
<td>AB1157, ada::kan, ogt::cat</td>
<td>—</td>
<td>[19]</td>
</tr>
<tr>
<td>KT01121</td>
<td>NR10832, ada-10::Tn10, ogt::cat</td>
<td>1.1±0.6</td>
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<td>KT11141</td>
<td>NR12896, mutS201::Tn5, ada-10::Tn10, ogt::cat</td>
<td>41.7±7.4</td>
<td>This study</td>
</tr>
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<td>KT21181</td>
<td>NR11102, mutL211::Tn5, ada-10::Tn10, ogt::cat</td>
<td>21.1±0.5</td>
<td>This study</td>
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<td>KT31131</td>
<td>NR12897, mutH471::Tn5, ada-10::Tn10, ogt::cat</td>
<td>32.6±5.8</td>
<td>This study</td>
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<td>KT01021U</td>
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<td>43.0±9.4</td>
<td>This study</td>
</tr>
<tr>
<td>KT20021U</td>
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<td>13.1±2.1</td>
<td>This study</td>
</tr>
<tr>
<td>KT30013U</td>
<td>NR12897, mutH471::Tn5, uvrA277::Tn10</td>
<td>34.7±4.3</td>
<td>This study</td>
</tr>
</tbody>
</table>

*All strains, except GW5352 and KT233, also contain the F’CC102 episome (17.25).*

*Spontaneous mutant frequencies as measured in this study.*
A

Wild-type

Ogt deficient

Primers

Ogt-F: 5'-gatgageaatcgcaggtcg-3'  Ogt-R: 5'-cctgtgagcaaaaatca-3'
Cat-F: 5'-caacgctacaaaccgaaga-3'  Cat-R: 5'-tctgaatggttctccgc-3'

B

Wild-type

Ada deficient

Primers

Ada-F: 5'-taactgatcataagctg-3'  Ada-R: 5'-aaagtgtgccacaccgg-3'
TetA-F: 5'-ttattcggaagaatt-3'  TetA-R: 5'-ctctcctatgttgatg-3'

C

Wild-type

UvrA deficient

Primers

uvrA-F: 5'-gcctgttctcattatat-3'  uvrA-R: 5'-acacgctacttaaggaag-3'
TetA-F: 5'-ttattcggaaatgat-3'  TetA-R: 5'-ctcttcctattgatgaa-3'

Fig. 1. Ogt, Ada and UvrA knockouts of E. coli used in this study. Also shown are the primer sequences and their locations as used for their confirmation after P1 transduction into the relevant tester strains. See Materials and methods for details. (A) Disruption of the ogt gene by replacement of the chloramphenicol resistance gene (cat), (B) disruption of ada gene introduced by tetracycline resistant gene (tetR and tetA) and (C) disruption of the uvrA gene as in (B).
For the ethylating agents, AGT is much less effective, whereas NER becomes more important (Figure 5 and Table II): EMS- and ENU-induced mutant frequencies were elevated in the *uvrA* strain by 9.6- and 17.5-fold, respectively. The frequency of ENNG-induced mutagenesis was increased to a similar extent (about 3-fold) in AGT- and NER-deficient *E. coli* (Table II). Mutagenesis by the ethylating agents was little affected by MMR deficiency. Taken together, methylated guanine residues appear repaired mainly by the AGT system, whereas ethylated guanines are subject to effective repair by the NER system. The contribution of MMR to the repair of alkylated lesions is modest, but appears to be significant at least in the repair of methylated lesions, as summarised in Table II.

**Mutagenic activities of alkylating agents in mutants doubly deficient in MMR and AGT/NER**

The precise involvement of MMR in mutagenesis induced by alkylating agents is difficult to estimate because it appears significantly smaller than AGT in case of methylation damage or than NER for ethylation damage (Table II). To gain more insight into this question, we thought it helpful to measure the effects of MMR deficiency in the absence of either main repair pathway. To do so, we constructed AGT- or NER-deficient mutants that were additionally defective in either MutH, MutL or MutS activity. The results presented in Figure 6 show that the mutant frequency induced by MMS and MNNG increased in the AGT-deficient *mutS* strain, but not in the corresponding *mutL* or *mutH* strains (Figure 6A and B and Table III). In fact, for the case of MNU, the addition of the *mutL* or *mutH* defect decreased the mutant frequency (Figure 6C and Table III).

Similarly, EMS and ENNG induced higher mutant frequencies in the NER-deficient *mutS* strain compared with either single defect (Figure 6D and E and Table III), whereas no such effect was seen for the corresponding *mutL* or *mutH* defects. The case of ENU appeared somewhat different, as no enhancing effect of *mutS* was observed in the NER background. However,
Repair of alkylated lesions

in this case the mutL or mutH defect decreased mutability (Figure 6F and Table III). Thus, an interesting parallel is uncovered for the case of alkylation by MNU and ENU (Figure 6C and F); no increase was observed for the mutS deficiency, but a decrease for the mutL and mutH deficiencies was observed (see also Table III).

Recovery of MMR function by introduction of plasmids containing MMR genes

To confirm that the deficiency of MMR function is responsible for the effects observed above, the relevant MMR genes were reintroduced into the various strains on pBR322-based plasmids. When mutS+ gene was reintroduced in mutS- deficient KT11141 (AGT-deficient) or KT10021U (NER deficient), MMS- and EMS-induced mutation decreased to the level of parent AGT- or NER-deficient strains KT01121 or NR12999, as shown in Figure 7A and B. Introduction of the mutL+ or mutH+ genes to the corresponding mutL or mutH-deficient strains KT21181 (AGT deficient) or KT31131 (NER deficient) increased the MNNG-induced mutant frequencies to the level of the MMR-proficient strain (Figure 7C). In all cases, empty vector pBR322 showed no effects (Figure 7A–C).

Discussion

Many alkylating agents induce mutation via formation of O6-alkylguanine, resulting in GC to AT transitions, and the repair pathway(s) of O6-alkylguanine are critical for mutagenesis. Previously, we demonstrated that MutS protein, whose function involves the recognition of mismatched lesions in DNA, recognises O6-MeG:T base pairs more efficiently than O6-EtG:T pairs (6), and further that, consistent with this notion, MutS deficiency increased mutagenesis by methylating agents, while affecting mutagenesis by ethylating agents to a much smaller extent (6).
In this study, we used a set of *E. coli* strains, containing the F’prolac (F’CC102) episome that can detect specifically the GC to AT transition induced by O⁶-alkylguanine (25). We examined which repair pathways work mainly to repair O⁶-alkylguanine in DNA, using AGT-, NER- and MMR-deficient strains. Mutant frequencies for methylating agents were highest (increased by several 100-fold) in the AGT-deficient strain, followed by the MMR- and NER-deficient strains, whereas for the ethylating agents frequencies were the highest in the NER-defective strain, followed by the AGT and MMR deficiencies. Thus, the repair of O⁶-methylguanine appears to be performed by AGT » MMR > NER in order of contribution, whereas the repair of O⁶-ethylguanine may be performed in the order of NER > AGT > MMR.

It is well documented that AGT protects cells and organisms from genotoxicity and carcinogenicity due to the presence of O⁶-methylguanine (9). However, the role of AGT in repair of O⁶-ethylguanine is less clear. Our results indicate that the role of AGT in O⁶-ethylguanine repair is small compared with that of O⁶-methylguanine. In contrast, the NER system works inefficiently for O⁶-methylguanine, whereas it is the most efficient pathway for O⁶-ethylguanine (Table II). With regard to the role of MMR, a deficiency in this process affects mutagenesis by methylating agents more strongly than for ethylating...
agents (Table II), in agreement with our previous observations on MutS protein binding to $O^6$-MeG:T versus $O^6$-EtG:T base pairs (6). Feitsma et al. (26) reported that ENU-induced mutation in the zebrafish germ line was not affected by deficiency of Msh6, a MutS homologue. In our experiments with ENU, we likewise found that the lack of MMR proteins did not affect mutagenesis. In fact, a modest reduction (up to 2-fold) may be seen (Table II). On the other hand, lack of MMR proteins promoted mutagenesis by the methylating agents significantly (up to 5-fold) (Table II).

To further dissect the role of MMR, we investigated its effect in the absence of the major AGT or NER repair pathway. Interestingly, in newly constructed strains lacking these major repair pathways we observed that mutant frequencies with MMS, EMS, MNNG and ENNG were increased by a MutS deficiency, suggesting a clearly protective or antimutagenic role of MutS protein. On the other hand, for these cases no increases in mutagenesis were observed in mutL- or mutH-deficient strains (Figure 6 and Table III). In case of normally functioning MMR, MutS protein would recognise an alkylated base or mismatch at the replication fork and complete repair through recruitment of MutL and MutH proteins, all three being indispensable for the process. Therefore, our results suggest that MutS might play an antimutagenic role that is different from triggering MutHLS-dependent MMR. For example, it is possible that the recognition of the alkylated site by MutS
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could lead to initiation or promotion of another (error-free) repair pathway, such as excision repair or recombinational repair.

On the other hand, the case of MNU and ENU is clearly different. The MNU- and ENU-induced mutant frequencies in \textit{mutS}-deficient and -proficient \textit{E. coli} are similar, but frequencies decreased in the \textit{mutL}- and \textit{mutH}-deficient strains. This suggests that the full action of MMR might actually be mutagenic for the case of the two nitrosoureas at least when the major AGT or NER repair pathways are absent. Lehner and Jinks-Robertson (27) in a study of spontaneous mutagenesis in \textit{Saccharomyces cerevisiae} reported that the MMR system was responsible for a subgroup of the observed mutations. Specifically, MMR was proposed to increase mutagenesis by diverting replication forks stalled at an (as yet hypothetical) spontaneous DNA lesion away from error-free resolution by DNA recombination into an error-prone, Pol zeta-dependent TLS pathway. The antirecombinational properties of the MMR system, through its breakdown of recombination intermediates, have been described (28). A similar mechanism may underlie the current observations in \textit{E. coli}. In this study, replication forks are likely to be stalled at least some of the DNA alkylation sites induced by MNU and ENU. Strains lacking the major AGT or NER repair pathways will suffer from high levels of unrepaired alkylation damage, and the recombinational repair of stalled replication forks may be a major mode of damage and mutation avoidance. If so, the antirecombinogenic action of the MutHLS system will likewise promote TLS of the alkylated bases. Such TLS might involve the replicative polymerase Pol III holoenzyme or any one of the \textit{E. coli} accessory DNA polymerases Pol II, Pol IV or Pol V. TLS by the latter two would be expected to be particularly mutagenic as these enzymes lack exonucleolytic proofreading. These two polymerases are also induced as part of the SOS response, which can be activated by high levels of alkylating damage. The validity of this model may be investigated by further genetic studies with recombination-defective strains, like \textit{recA}, and/or strains lacking one or more of the TLS polymerases.

It is worth re-emphasising that, at least for the case of MNU and ENU exposure, the apparent effects of the MMR system are different in the repair-deficient backgrounds (AGT and NER) and the wild-type background. Although in the wild-type background the MMR effect is clearly antimutagenic (Figures 4 and 5), it is mutagenic in the repair-deficient backgrounds (Figures 6 and 7). In the simplest model, this reflects the dual aspects of MMR that are likely operating concurrently: (i) the correction of replication errors (a mutation-prevention function) and (ii) the breakdown of recombination intermediates (a mutagenic function). With increasing DNA damage, the balance between the
Fig. 7. Complementation of MMR deficiencies by plasmid-carried wild-type mutS, mutL, and mutH genes. (A) MMS mutagenesis. Strains used are 1 (grey bar) KT01121 (ada ogt); 2 (white bar) KT11141 (mutS ada ogt); 3 (black bar) KT11141 (mutS ada ogt) with pMQ315 (mutS); and 4 (dark grey bar) KT11141 (mutS ada ogt) with pBR322. (B) EMS mutagenesis. Strains are 5 (grey bar) NR12999 (uvrA); 6 (white bar) KT10021U (mutS uvrA); 7 (black bar) KT10021U (mutS uvrA) with pMQ315 (mutS); 8 (dark grey bar) KT10021U (mutS uvrA) with pBR322. (C) MNNG mutagenesis. Strains are 1 (grey bar) KT01121 (ada ogt); 2 (white bar) KT21181 (mutL ada ogt); 3 (black bar) KT21181 (mutL ada ogt) with pMQ350 (mutL); 4 (dark grey bar) KT21181 (mutL ada ogt) with pBR322; 5 (white bar) KT31131 (mutH ada ogt); 6 (black bar) KT31131 (mutH ada ogt) with pRH71-17 (mutH); 7 (dark grey bar) KT31131 (mutH ada ogt) with pBR322. Statistical analysis was performed using the Student’s t test. **P > 0.01 and *P > 0.05 compared with the mutant frequency for each parental strain with MMR deficiency.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MMS (5 mM)</th>
<th>MNNG (0.005 mM)</th>
<th>MNU (5 mM)</th>
<th>EMS (50 mM)</th>
<th>ENNG (0.5 mM)</th>
<th>ENU (25 mM)</th>
</tr>
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<tbody>
<tr>
<td>Wild-type</td>
<td>1 (7.4 ± 1.0)</td>
<td>1 (3.4 ± 0.9)</td>
<td>1 (11.9 ± 3.8)</td>
<td>1 (117.5 ± 4.7)</td>
<td>1 (226.2 ± 55.3)</td>
<td>1 (81.6 ± 6.8)</td>
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<td>ada, ogt</td>
<td>173.3</td>
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<td>2.3</td>
<td>0.5</td>
<td>10.3</td>
<td>3.1</td>
<td>5.9</td>
</tr>
</tbody>
</table>

*Shown are mutant frequencies obtained at the highest dose of each mutagen tested relative to the frequency for the wild-type strain at the same dose (i.e. wild-type = 1).
two components might shift from an overall antimutagenic role to a mutagenic role. It will be worthwhile to further investigate this balance, including the possible role of the SOS system, as well as the separate, protective role of MutS protein outside of its role within the MutHLS system.

Finally, it is an important question to ask why the effect of MMR deficiencies varied so significantly among the different alkylating agents used. DNA can be alkylated at a variety of base oxygen and nitrogen atoms, and each alkylating agent will produce its own profile of DNA modifications. In addition, the agents may modify proteins as well as the NTP or dNTP nucleotide pools that serve as RNA and DNA precursors. It is likely that each of these factors may affect, differentially, the manner in which cells can recover from the exposure and resume DNA replication with either more or less mutations.

In summary, our data have revealed distinct pathways for repairing mutagenic lesions induced by methylating and ethylating agents, including differential mutagenic and antimutagenic effects of the MutHLS system, and have suggested the existence of a novel, additional role of MutS protein in maintaining genome stability.

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References