

# Mismatch correction at $O^6$ -methylguanine residues in *E. coli* DNA

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*Escherichia coli* has a correction system which removes mismatched bases from DNA<sup>1</sup>. Mutants (*dam*) which lack the major DNA adenine methylase<sup>2</sup> are hypersensitive to the effects of base analogue mutagens such as 2-aminopurine<sup>3</sup> and appear to be defective in mismatch correction. Phenotypic revertants of *dam* mutants to base analogue resistance include second site mutations in *mutL* or *mutS* genes<sup>4</sup>, which are also part of the correction system. We report here that *E. coli dam* mutants are also sensitive to the DNA methylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), which introduces  $O^6$ -methylguanine ( $m^6G$ ) into the DNA. This sensitivity, however, was not observed using methylating agents which generate only low amounts of this alkylated base. Furthermore, the introduction of either a *mutL* or a *mutS* mutation into *dam* strains abolished the sensitivity to MNNG. These results suggest that mismatch correction occurs at  $m^6G$  residues in DNA. These lesions miscode in DNA polymerase I-mediated DNA synthesis *in vitro*<sup>5</sup> and are known to be mutagenic *in vivo*<sup>6</sup>. Nevertheless, it appears that mismatch correction at  $m^6G$  residues in DNA does not lead to reduced induction of mutation by MNNG.

The major repair pathway for the promutagenic base  $m^6G$  in *E. coli* is via a methyltransferase induced as part of the adaptive response to alkylating agents<sup>7-9</sup>. The adaptive response is induced by exposure to sublethal concentrations of alkylating agents and renders cells resistant to the lethal and mutagenic effects of subsequent treatment with such agents<sup>9</sup>. To allow the maximum possible interaction between  $m^6G$  and the mismatch correction system, except when specifically stated, all the experiments reported were performed in conditions in which the adaptive response is not induced. Figure 1a shows that the *dam-3* strain GM113 is more sensitive than its parental strain GM112 (*dam*<sup>+</sup>) to treatment with MNNG for 5 min. For MNNG, a fairly high proportion (7%) of the total alkylation products are  $m^6G$  (ref. 10). To determine whether the sensitivity of *dam* strains to MNNG is related to the presence of this base, we tested the sensitivity of GM113 (*dam-3*) to dimethyl sulphate (DMS) for which  $m^6G$  constitutes only a very minor proportion (0.5%) of the total products<sup>11</sup>. Figure 1b shows that *dam* strains are not significantly more sensitive to DMS than *dam*<sup>+</sup> strains; this agrees with a previous report that *dam* strains are only slightly hypersensitive to methylmethanesulphonate (MMS)<sup>12</sup> which also induces only a low proportion of  $m^6G$  residues in DNA.

**Table 1** MNNG-induced mutation to rifampicin resistance

| [MNNG] ( $\mu\text{g ml}^{-1}$ )  | Mutation frequency<br>(Rif <sup>r</sup> mutants per $10^8$ bacteria) |       |       |        |
|-----------------------------------|--|-------|-------|--------|
|                                   | 0  | 5     | 10    | 20     |
| Strain                            |  |       |       |        |
| AB1157 (wild type)                | 2.6  | 2,900 | 4,900 | 33,000 |
| GM112 ( <i>dam</i> <sup>+</sup> ) | 1.3  | 1,100 | 6,700 | 25,000 |
| GM113 ( <i>dam-3</i> )            | 94   | 1,100 | 3,900 | 9,600  |
| GM150 ( <i>mutL dam-3</i> )       | 270  | 2,000 | 6,200 | 23,000 |

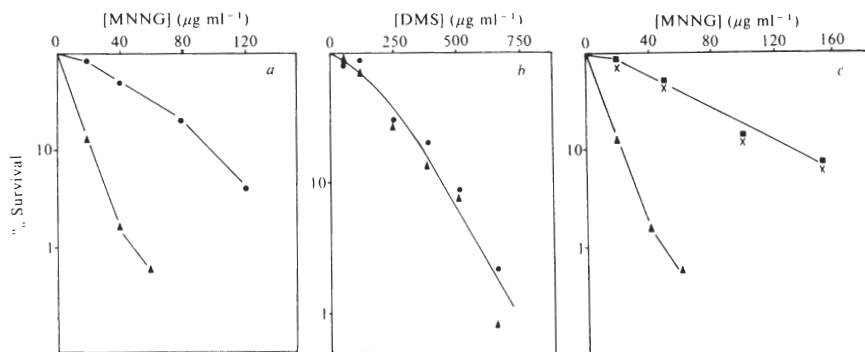
Cultures (1 ml) containing  $\sim 10^8$  cells  $\text{ml}^{-1}$  in supplemented minimal medium were treated with MNNG at the concentrations shown at 37 °C for 5 min. After treatment, cells were collected, washed and resuspended at  $10^7$  cells  $\text{ml}^{-1}$  in supplemented minimal medium and grown at 37 °C for 16–20 h. Appropriate dilutions were plated onto nutrient agar containing rifampicin ( $100 \mu\text{g ml}^{-1}$ ). Total viable bacteria were determined by plating onto nutrient agar plates. The mutation frequency is expressed as Rif<sup>r</sup> colonies per  $10^8$  viable cells.

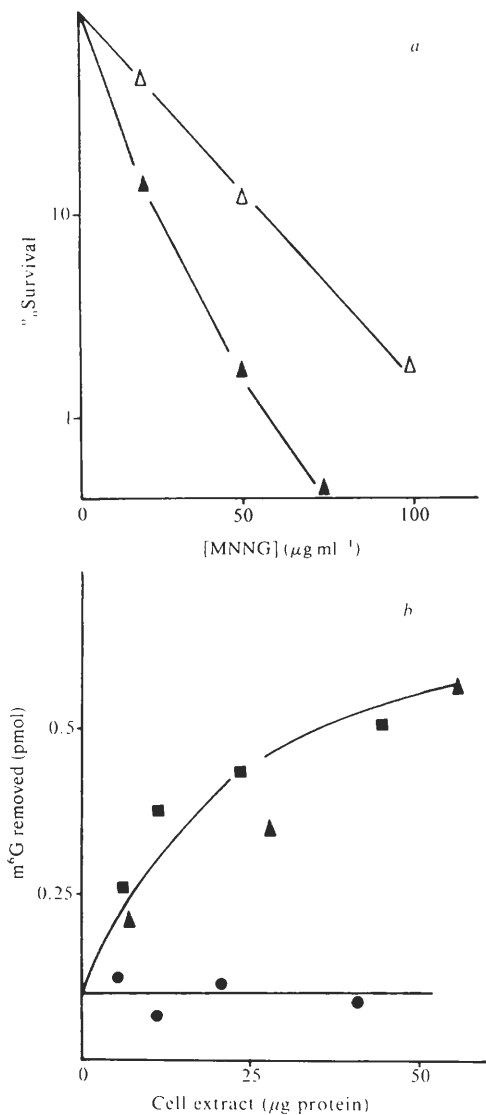
As the introduction of a mutation into any one of several mutator loci abolishes the sensitivity of *dam* strains to killing by the base analogue 2-aminopurine<sup>3,4</sup>, we examined the effect on MNNG sensitivity of these second-site mutations. GM150 (*mutL-451 dam-3*) and GM169 (*mutS-453 dam-3*) were more resistant to MNNG treatment than GM113 (*dam-3*) and were similar in sensitivity to wild-type strains (Fig. 1c). By analogy to the effect of 2-aminopurine on these strains, we suggest that *dam* strains are sensitive to MNNG because of an attempted mismatch correction at base pairs containing  $m^6G$ . In the absence of the requisite control normally present in wild-type (*dam*<sup>+</sup>) strains, this leads to cell death in a high proportion of cases. The introduction of the *mut* loci prevents the occurrence of this abortive correction mechanism, thus restoring wild-type survival levels. A reduction in the amount of  $m^6G$  in *dam* strains should therefore result in a decreased frequency of these 'death-prone' repair events and thus enhanced survival after MNNG treatment. This reduction in the level of  $m^6G$  may be brought about by pre-adaptation of the cells. Adapted *dam* strains should show enhanced survival after challenge with MNNG, but Jeggo *et al.*<sup>13</sup> were unable to demonstrate such adaptation of *dam* strains to MNNG challenge. However, using a slightly different adaptation protocol, we have demonstrated enhanced survival in these strains after MNNG challenge (Fig. 2a). As normal levels of the  $m^6G$  methyltransferase are induced in GM113 (*dam-3*) after adaptation (Fig. 2b), we conclude that at least part of the observed adaptation for survival in this strain is due to an enhanced removal of  $m^6G$ .

The abolition of sensitivity to 2-aminopurine by the introduction of a second-site *mut* mutation into *dam* strains is accompanied by an increased frequency of both spontaneous and base analogue-induced mutagenesis<sup>14</sup> presumably due to the absence of a mismatch correction system. It was expected, therefore, that MNNG-induced mutagenesis should be higher in the *mutL-451 dam-3* strain than in wild-type strains. Table 1 shows that this is not the case. The frequency of spontaneous mutation to

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**Fig. 1** Sensitivity of *dam-3* strains to alkylating agents. Cultures in the exponential growth stage in minimal salts medium supplemented with 0.5% glucose, 0.1% casamino acids and 0.00003% thiamine were exposed to alkylating agent at the concentrations shown at 37 °C for 5 min. After dilution in 10 mM K phosphate pH 7.0, cells were plated onto nutrient agar. Survival was determined after growth at 37 °C for 24 h. a, MNNG sensitivity of ●, GM112 (*dam*<sup>+</sup>) and ▲, GM113 (*dam-3*). b, DMS sensitivity of ●, GM112 (*dam*<sup>+</sup>) and ▲, GM113 (*dam-3*). c, MNNG sensitivity of ■, GM150 (*mutL-451 dam-3*); ×, GM169 (*mutS-453 dam-3*); and ▲, GM113 (*dam-3*).





**Fig. 2** Effect of adaptation on *dam* strains. *a*, MNNG sensitivity. Unadapted cultures ( $\blacktriangle$ ) were grown and treated as described in Fig. 1 legend. Adapted cultures ( $\Delta$ ) were grown in medium containing  $0.1 \mu\text{g ml}^{-1}$  MNNG for 3 h followed by  $1 \mu\text{g ml}^{-1}$  MNNG for 1 h immediately before treatment. *b*, Induction of m<sup>6</sup>G methyltransferase activity in adapted *dam-3* strains. Adapted and unadapted cultures were grown as described above and then cells were collected, washed and disrupted by sonication. The demethylation of <sup>3</sup>H-labelled m<sup>6</sup>G in DNA treated with <sup>3</sup>H-methylnitrosourea was assayed using these crude cell extracts as described elsewhere<sup>7</sup>.  $\bullet$ , GM113 (*dam-3*) unadapted;  $\blacktriangle$ , GM113 (*dam-3*) adapted;  $\blacksquare$ , GM1150 (*mutL dam-3*) adapted.

rifampicin resistance of GM150 (*mutL-451 dam-3*) is ~100–200-fold greater than that of wild-type strains as expected. However, the MNNG-induced frequency of mutation to rifampicin resistance is not significantly different between the wild-type strains, AB1157 and GM112, and GM150 (*mutL-451 dam-3*). GM113 (*dam-3*) exhibits the smaller but consistent spontaneous mutator phenotype characteristic of *dam* mutants<sup>15</sup> but again is not hypermutable by MNNG. It appears,

therefore, that while the relative sensitivity to MNNG and DMS of GM113 (*dam-3*), GM150 (*mutL-451 dam-3*) and the parental GM112 (*dam*<sup>+</sup>) suggests that mismatch correction does occur in wild-type *E. coli* at m<sup>6</sup>G-containing base pairs, this correction system does not reduce miscoding mutagenesis due to this lesion. This interpretation is supported by the data of Sklar and Strauss<sup>16</sup> which indicate that *E. coli uvrE* mutants, which are also deficient in mismatch correction, are normally mutable by MNNG.

The ineffectiveness of the mismatch correction system in reducing MNNG-induced mutagenesis at m<sup>6</sup>G may be explained by the presence of the miscoding lesion in the parental DNA strand and by the ambiguous nature of m<sup>6</sup>G coding. After incorporation of 2-aminopurine or bromouracil, the 'incorrect' base remains in the daughter strand. In wild-type cells, the mismatch correction system will recognize the parental strand by its methylation pattern, and will thus always correct the mispair in the correct orientation. However, replication at m<sup>6</sup>G will direct the incorporation of T or C into daughter DNA. We propose that neither m<sup>6</sup>G:C nor m<sup>6</sup>G:T is a sufficiently good base pair to avoid subsequent recognition by the mismatch correction system. In normal conditions, the mismatch correction system will operate by removing the incorporated T or C, thus allowing a second attempt at the incorporation of the correct base. This second attempt will again result in an imperfect match as no 'correct' base exists. This removal/insertion cycle will presumably continue without check until the *dam*<sup>+</sup> gene-encoded DNA methylase methylates the GATC sequences in daughter DNA thus removing the strand discrimination necessary for mismatch correction. If the incorporation of C or T opposite m<sup>6</sup>G occurs in approximately the same ratio for the polymerase system involved in replication and in mismatch correction, no net decrease in misincorporation and thus no decrease in mutagenesis will result from the operation of mismatch correction at m<sup>6</sup>G-containing base pairs.

There are, however, circumstances in which operation of mismatch correction at base pairs containing m<sup>6</sup>G should be effective in avoiding mutation. If the m<sup>6</sup>G methyltransferase operates during base removal and replacement and before the action of the *dam* encoded DNA methylase, the resulting G-C or G-T base pair will be either recognizably correct or a true mismatch and dealt with accordingly. In this case, the adaptive response, in addition to its role in removing m<sup>6</sup>G residues before DNA replication, may interact with the mismatch correction system to further reduce mutation frequency.

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