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*Communication*

# Mutator specificity of *Escherichia coli alkB117* allele

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**The *Escherichia coli* AlkB protein encoded by *alkB* gene was recently found to repair cytotoxic DNA lesions 1-methyladenine (1-meA) and 3-methylcytosine (3-meC) by using a novel iron-catalysed oxidative demethylation mechanism that protects the cell from the toxic effects of methylating agents. Mutation in *alkB* results in increased sensitivity to MMS and elevated level of MMS-induced mutations. The aim of this study was to analyse the mutational specificity of *alkB117* in a system developed by J.H. Miller involving two sets of *E. coli lacZ* mutants, CC101–106 allowing the identification of base pair substitutions, and CC107–CC111 indicating frameshift mutations. Of the six possible base substitutions, the presence of *alkB117* allele led to an increased level of GC→AT transitions and GC→TA and AT→TA transversions. After MMS treatment the level of GC→AT transitions increased the most, 22-fold. Among frameshift mutations, the most numerous were –2CG, –1G, and –1A deletions and +1G insertion. MMS treatment appreciably increased all of the above types of frameshifts, with additional appearance of the +1A insertion.**

**Keywords**: *E. coli*, *alkB117*, MMS, mutational specificity, *lacZ* → Lac$^+$ reversion

## INTRODUCTION

Alkylating agents introducing a variety of lesions into DNA are wide spread in the environment (Taverna & Sedgwick, 1996; Sedgwick, 2004). To protect their DNA, organisms possess mechanisms that abolish the harmful effects of alkylanes. In *Escherichia coli* alkylating agents induce so called adaptive response involving the *ada*, *alkB*, *alkA* and *aidB* genes (Samson & Cairns, 1977). The *alkB*-encoded AlkB protein is a dioxygenase that oxidatively demethylates 1meA and 3meC in DNA in a reaction that involves α-ketoglutarate, $O_2$, and Fe(II) (Trewick *et al*., 2002; Begley & Samson, 2003; Falnes & Rognes, 2003).

MMS (methyl methanesulfonate) is an $S_N2$ type alkylating agent which predominantly methylates nitrogen in DNA purines generating $N^7$-methylguanine (7-meG), $N^3$-methyladenine (3-meA), $N^7$-methyladenine (7-meA), $N^1$-methyladenine (1-meA), and $O^6$-methylguanine ($O^6$-meG) (Grzesiuk, 1998). Enzymatic or spontaneous loss of 3-meA, 3-meG and 7-meG leads to the formation of apurinic sites which may induce AT→TA or GC→TA transversions involving polymerase V-directed translesion synthesis (Grzesiuk & Janion, 1994; Fuchs *et al*., 2004).

Damage to *alkB* greatly increases the sensitivity of bacteria to MMS, diminishes the ability to reactivate MMS-treated single-stranded phage DNA (Dinglay *et al*., 2000) and markedly increases MMS-induced mutagenesis in *E. coli* AB1157 cells (Kataoka *et al*., 1983; Nieminuszczy *et al*., 2006).

The aim of this study was to analyse the mutational specificity of the *alkB117* mutation in a system developed by Cupples and Miller (1989) and Cupples *et al*. (1990). This system includes a set of eleven *lacZ* mutants of *E. coli* CC101–CC111, and allows for the identification of all six types of base substitutions and five types of frameshift mutations.

## MATERIALS AND METHODS

**Bacterial strains.** The bacteria used in this study were *E. coli* CC101–CC111 strains constructed in Miller's laboratory (Cupples & Miller, 1989; Cup-

**Abbreviations**: MMS, methyl methanesulfonate; 7-meG, $N^7$-methylguanine; 3-meA, $N^3$-methyladenine; 7-meA, $N^7$-methyladenine; 1-meA, $N^1$-methyladenine; $O^6$-meG, $O^6$-methylguanine.

ples *et al*., 1990). Strains CC101–CC106 contain base substitution mutations in the *lacZ* gene (phenotype Lac$^-$). Reversion of these mutations leads to the ability to metabolise lactose (phenotype Lac$^+$) (Borden *et al*., 2002). In strains CC107–CC111 the altered sequences are in runs of six or seven repeated base pairs creating frameshift mutations. Reversion to Lac$^+$ can occur by addition or deletion of the altered sequences and recovery of the proper reading frame for β-galactosidase synthesis (Cupples *et al*., 1990). A detailed description of the CC101–CC111 strains and the pathways of Lac$^+$ reversions is shown in Fig. 1.

The *alkB117* gene was introduced into the CC101–CC111 strains by P1-mediated transduction (Miller, 1972). The donor strain for *alkB117*::Tn*3* was BS87 (Sedgwick, 1992). The transductants were selected on LB plates containing carbenicillin (50 μg/ml), and then their desired phenotype was ascertained.

**Mutational specificity assay.** The appropriate bacteria (CC101–CC111 and their *alkB117* derivatives) were grown overnight at 37°C with shaking in minimal C-salts medium consisting of C-salts (Vogel & Bonner, 1956) supplemented with 0.5% glucose, 0.2% casamino acids and 2 μg/ml thiamine. When cultures reached (2–4) × $10^8$ cells/ml they were treated with 0.17% (20 mM) MMS for 15 min, centrifuged, washed twice, and resuspended in the same volume of C-salts medium without glucose. Aliquots of 0.1 ml diluted to $10^{-6}$ were plated on LB-plates and of 0.1 ml on C-salts plates enriched with 0.5% lactose and 2 μg/ml thiamine. Plates were incubated at 37°C and screened after one day for viable cells, or after two days for Lac$^+$ revertants. Only the Lac$^+$ revertants give readily visible colonies on selective plates with lactose as the only source of carbon.

Reversion frequencies for each Lac$^-$ strain were calculated by dividing the number of Lac$^+$ revertants by the total number of viable cells. For each strain experiments were repeated 5 times and standard deviations (±S.D.) were calculated.

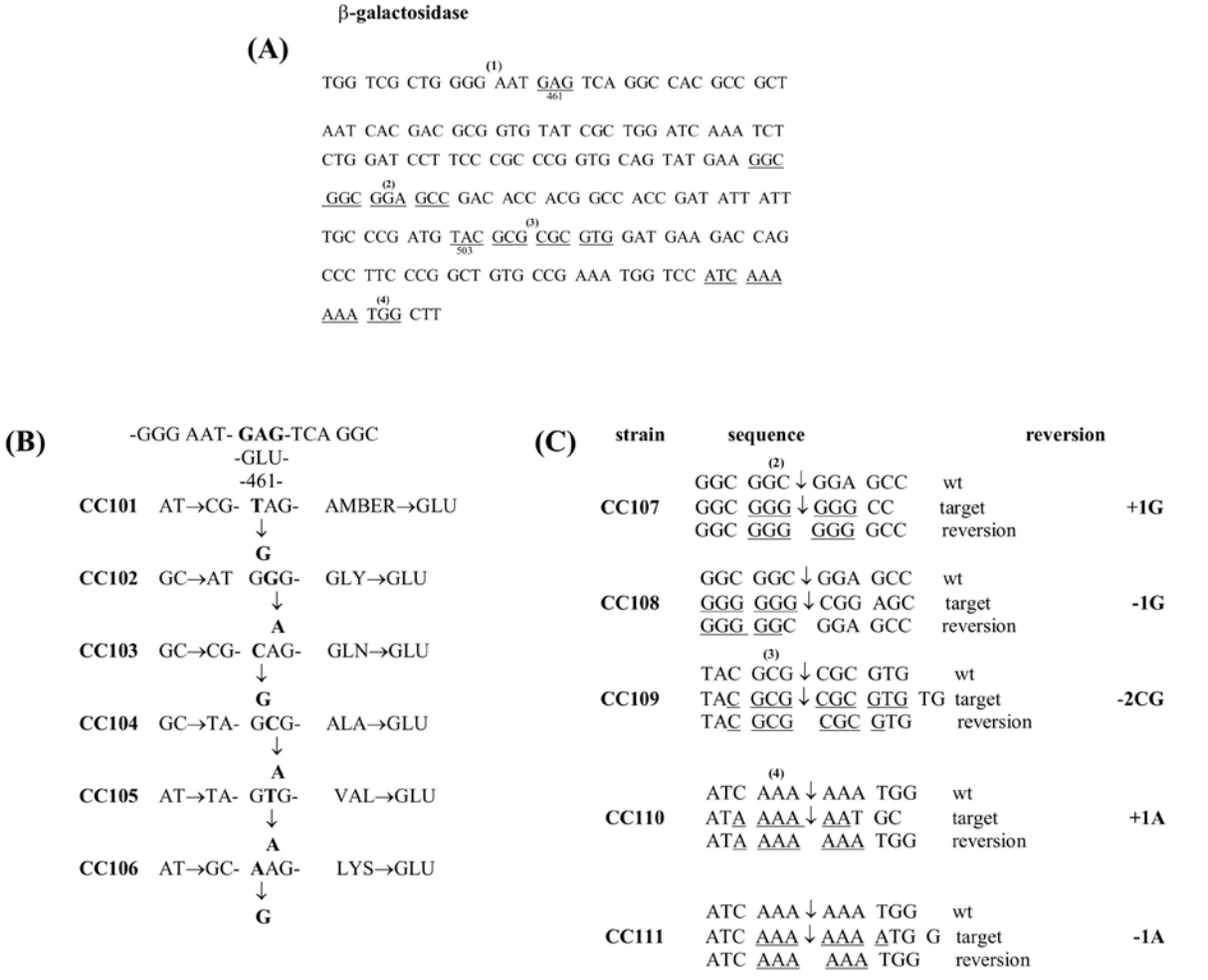


**Figure 1. Location of target sites in *lacZ* gene.**
(A) The portion of the *lacZ* gene containing the following sites: (1) Glu–461 codon that was altered to yield strains CC101–CC106 for detecting base substitutions (Cupples & Miller, 1989); (2), (3) and (4) sequences that were altered to yield strains for detecting frameshift mutations (Cupples *et al*., 1990): CC107 and CC108 at site (2), CC109 at site (3) and CC110 and CC111 at site (4). (B) Base substitutions required to restore codon 461 to GAG in CC101–CC106 strains. (C) Frameshift mutations required to restore the correct amino-acid sequence in CC107–CC111 strains.

**Plate test for quick screening of *alkB* mutants.** An aliquot of 0.1 ml of overnight culture in LB was added to 5 ml of 0.5% top agar and poured onto LB plate. Blotting paper disc (5 mm diameter) with 2 µl of MMS were then placed on the plate surface and incubated for one day at 37°C. The zones of bacterial growth inhibition were measured.

## RESULTS AND DISCUSSION

We have shown that in the *argE3*→Arg$^+$ reversion system MMS induces an extremely high level of Arg$^+$ revertants in AB1157 strains mutated in the *alkB* gene and that these revertants are mainly GC→AT transitions and AT→TA transversions (Nieminuszczy *et al*., 2006).

Here, we used *lacZ*→Lac$^+$ reversion to estimate the specificity of *alkB117* mutation. The *alkB117* allele was introduced into a set of *E. coli* CC101–CC111 strains. The presence of *alkB117* was proved by plate test showing an increased sensitivity of CC101–CC111 *alkB117* bacteria to MMS.

The CC101–CC111 strains by themselves showed a very low level of spontaneous Lac$^+$ revertants. These revertants arose by base substitutions: GC→TA in CC104 strain (0.21 Lac$^+$ revertants per $10^8$ cells) and AT→TA in CC105 strain (0.19 Lac$^+$ revertants per $10^8$ cells) or by frameshifts: +1G (CC 107), –1G (CC108), –2CG (CC109), and –1A (CC111); respective mutation frequencies were 24.3, 16.1, 38.6, and 12.1 Lac$^+$ revertants per $10^8$ cells.

The presence of the *alkB117* mutation increased the frequency and variety of spontaneous Lac$^+$ revertants (Figs. 2 and 3). The most numerous among base substitutions were GC→AT transitions (1.25 Lac$^+$ revertants per $10^8$ cells). Also a new class of base substitutions, namely GC→TA transversions appeared in the CC104 *alkB117* strain. The frequency of this class of mutations was 0.61 Lac$^+$ revertants per $10^8$ cells.

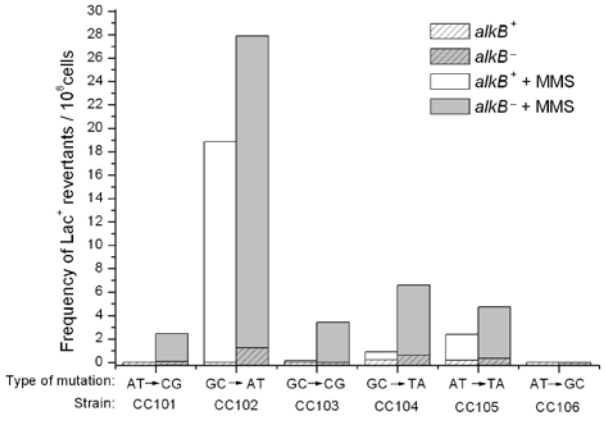


**Figure 2. The specificity of spontaneous and MMS-induced base substitution mutations in CC101–CC106 wt (*alkB*$^+$) strains and their *alkB117* (*alkB*$^-$) derivatives.**

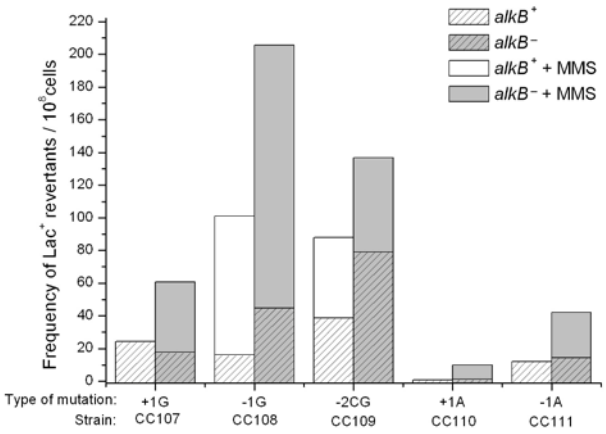


**Figure 3. The specificity of spontaneous and MMS-induced frameshift mutations in CC107–CC111 wt (*alkB*$^+$) strains and their *alkB117* (*alkB*$^-$) derivatives.**

Introduction of *alkB117* to the CC107–CC111 strains measuring frameshift mutations led to increased level of two types of frameshifts, –1G (44.6 Lac$^+$ revertants per $10^8$ cells, which is 3-fold higher in comparison to CC108 wt) and –2CG (79.0 Lac$^+$ revertants per $10^8$ cells, which is 2-fold higher in comparison to CC109 wt) (Fig. 3).

In the CC101–CC106 wt strains MMS induces mainly GC→AT transitions (27.9 Lac$^+$ revertants per $10^8$ cells) and, to a lesser degree, AT→TA transversions (4.7 Lac$^+$ revertants per $10^8$ cells) (Fig. 2); among the CC107–CC111 wt strains, MMS induces first of all –2CG frameshifts (in CC109, 79.0 Lac$^+$ revertants per $10^8$ cells) and –1G (in CC108, 44.6 Lac$^+$ revertants per $10^8$ cells). The respective values for +1G (CC107), –1A (CC111), and +1A (CC110) were 17.8, 14.3, and 2.1 Lac$^+$ revertants per $10^8$ cells (Fig. 3).

In the *alkB117* derivatives of the CC101–CC106 strains MMS treatment led to an induction of 5 out of 6 base substitutions with noticeable prevailing of mutations arising by GC→AT transitions measured in the CC102 *alkB117* strain (27.9 Lac$^+$ revertants per $10^8$ cells) (Fig. 2). The highest mutator effect of *alkB117* after MMS treatment was noted for frameshift mutations. Compared to the CC107–CC111 wt strains the level of +1G, –1G, –2CG, +1A, and –1A increased 3.4-, 4.6-, 1.7-, 5.0-, and 3.0-fold, respectively (Fig. 3).

The specificity of the *alkB117* mutation has been already measured by Dinglay and co-workers (2000) and Nieminuszczy and co-workers (2006). The first group used the Miller's strains but only those identifying base substitutions (CC101–CC106), the second one used T4 mutants. The phage system identifies only two base substitutions and in this respect the present results are in agreement with the previous ones. However, base substitutions are a minor group of MMS-induced *alkB*-specific mutations. We found that most of these mutations arise

by all types of frameshift measured, but especially the –1G and –2CG deletions.

It is worth noticing that of the two *alkB* mutants available, HK82 (*alkB22*) and BS87 (*alkB117*), HK82 has shown a 10-fold higher level of MMS-induced *argE3*→Arg$^+$ revertants compared to BS87 (Nieminuszczy *et al*., 2006). Our unpublished results suggest the presence of an additional mutation in HK82 strain. This could explain the differences in the specificity of the *alkB* mutation in HK82 (Delaney & Essigmann, 2004) and assayed here, in the BS87 strain.

## CONCLUSIONS

Using the *argE3*→Arg$^+$ reversion system we have found that mutation in *alkB* gene followed by an inability to repair 1meA/3meC in DNA greatly increases the mutagenic potency of MMS. Here we found that mutations specific for *alkB117* are due to GC→AT, GC→TA, and AT→TA base substitutions, and –1G and –2CG frameshifts. These results indicate that A and C residues could be the target of MMS attack.

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