A Novel Mutator of *Escherichia coli* Carrying a Defect in the *dgt* Gene, Encoding a dGTP Triphosphohydrolase

Damian Gawel, Michael D. Hamilton, and Roel M. Schaaper*

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Received 8 July 2008/Accepted 26 August 2008

A novel mutator locus in *Escherichia coli* was identified from a collection of random transposon insertion mutants. Several mutators in this collection were found to have an insertion in the *dgt* gene, encoding a previously characterized dGTP triphosphohydrolase. The mutator activity of the *dgt* mutants displays an unusual specificity. Among the six possible base pair substitutions in a *lacZ* reversion system, the G • C → C • G transversion and A • T → G • C transition are strongly enhanced (10- to 50-fold), while a modest effect (two- to threefold) is also observed for the G • C → A • T transition. Interestingly, a two- to threefold reduction in mutant frequency (antimutator effect) is observed for the G • C → T • A transversion. In the absence of DNA mismatch repair (*mutL*) some of these effects are reduced or abolished, while other effects remain unchanged. Analysis of these effects, combined with the DNA sequence contexts in which the reversions take place, suggests that alterations of the dGTP pools as well as alterations in the level of some modified dNTP derivatives could affect the fidelity of in vivo DNA replication and, hence, account for the overall mutator effects.

The mechanisms used by cells to maintain genomic stability are of considerable interest. There are many potential sources of mutation, and cells use an array of different mechanisms to prevent their frequent production. One major source for mutations is the process of DNA replication, which proceeds with high but not infinite accuracy. The accuracy of DNA replication is controlled at several levels, including the fidelity of base selection by the DNA polymerases and their associated proofreading abilities (29). Following DNA synthesis, the newly synthesized DNA is surveyed by proteins of the DNA mismatch repair (MMR) system, which find DNA mismatches and restore the correct sequence using the parental strand as a matrix (30).

Another aspect of replication fidelity that has received renewed attention is the mechanism(s) that ensures the quantity and quality of the deoxynucleoside triphosphate DNA precursors (dNTPs). dNTP levels are precisely controlled, and deviations in both absolute and relative dNTP amounts can lead to an increase in mutations (32, 37, 39). Damage to the dNTPs, by endogenous or exogenous factors, may lead to alternative dNTP forms that have ambiguous base pairing properties and will be mutagenic if incorporated by the DNA polymerase. The best known example of such a mutagenic derivative is 8-oxodGTP, an oxidative damage product of dGTP. Many mutators in this collection were found to have an insertion in the *dgt* gene, encoding a previously characterized dGTP triphosphohydrolase. The mutator activity of the *dgt* mutants displays an unusual specificity. Among the six possible base pair substitutions in a *lacZ* reversion system, the G • C → C • G transversion and A • T → G • C transition are strongly enhanced (10- to 50-fold), while a modest effect (two- to threefold) is also observed for the G • C → A • T transition. Interestingly, a two- to threefold reduction in mutant frequency (antimutator effect) is observed for the G • C → T • A transversion. In the absence of DNA mismatch repair (*mutL*) some of these effects are reduced or abolished, while other effects remain unchanged. Analysis of these effects, combined with the DNA sequence contexts in which the reversions take place, suggests that alterations of the dGTP pools as well as alterations in the level of some modified dNTP derivatives could affect the fidelity of in vivo DNA replication and, hence, account for the overall mutator effects.

The present study is concerned with a member of another class of dNTP hydrolyzing enzymes, the dNTP triphosphohydrolases, which hydrolyze dNTPs to the corresponding deoxyribonucleotides (dNTPs). Three members of this group have been investigated in some detail, the *E. coli* dgt gene product (1, 48) and the *Thermus thermophilus* T71383 protein (25). The *E. coli* protein has a preference for hydrolyzing dGTP (dGTP → dG + PPP) and is generally referred to as a dGTPase (1, 48). Genetic and biochemical examinations revealed interesting properties of this enzyme (1, 21, 38, 48, 52, 53). The enzyme strongly prefers dGTP among the dNTPs and has little activity toward rNTPs, including GTP (1, 38, 48). Among tested dNTP analogs, it degrades 8-BrGTP at a comparable rate to dGTP, but other analogs, including 8-oxoGTP, are poor substrates (48, 53). Loss of Dgt function (*dgt* mutant) leads to an approximately twofold increase in dGTP levels (40), while its overexpression (*optA1* mutant) leads to an about fivefold lowering of the dGTP level (36, 40).

*Corresponding author. Mailing address: Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709. Phone: (919) 541-4250. Fax: (919) 541-7613. E-mail: schaaper@niehs.nih.gov. Published ahead of print on 5 September 2008.*
Another interesting, and likely relevant, feature of Dgt protein is that it possesses a rather strong DNA binding activity (1, 53).

Despite the described known features of the Dgt enzyme, no biological function for the protein has been established so far. Seto et al. (48) have considered the possibility that dGTP might not be the physiological substrate; instead, the possibility was raised that Dgt performs a dNTP pool-sanitizing function. However, supporting evidence for such a function has not been found. No alternative Dgt substrate has been identified and, importantly, no mutator phenotype could be identified for a dgt-defective strain when measuring mutation frequencies for rifampin or nalidixic acid resistance (40, 53).

In the present study, we describe the discovery of a mutator phenotype for a dgt mutant. This discovery was made, serendipitously, during the screening of a library of random transposon insertions for a mutator phenotype using a lac A·T→G·C reversion system. We then found that a large fraction of the obtained mutants carried an insertion in the dgt gene. We report on the nature and mutational specificity of the dgt mutator phenotype and on its interaction with certain other E. coli genes as a first step in identifying the precise molecular and metabolic basis for the phenotype.

**MATERIALS AND METHODS**

**Strains and constructions.** The strains used in this study are listed in Table 1.

With the exception of YG7207, all are derivatives of KA796 (ara thi Δprolac) (45) or MG1655 (49). The series of F′prolac episomes from strains CC101 through CC116 (7) were introduced into KA796 and its derivatives by conjugation. The lacZ marker on these F′ episomes contains one of six defined mutations that can revert to lac+ by only one defined base substitution event (7). All other markers were introduced by P1 transduction using PlacΦ4. The mut::Tn10 and mut::Tn5 markers were transferred from strains NR1963 (44) and NR9559 (5), respectively, selecting for tetracycline or kanamycin resistance. NR13138 is an tpx9777 derivative of KA796 (18). The Δdgt::cat allele of NR16093 was generated by the gene replacement method of Datsenko and Wanner (6). The following 70-mer primers were used to generate a PCR product on plasmid pKD3 (8), which was the source of the cat gene: 5′-ATGACGACAGATTTAGTCCGAAA AAAATTAAACTGGCAGCTGGTATTGATGCAACGACGGCTT-3′ and 5′-TTGGTGGCTCCTGGCAAGGTGCTGCTTATCAGAAGCGCT-3′ and in the (italicized) 3′ residues are complementary to the cat gene of pKD3, while the flanking 5′ sequences correspond to the beginning and end of the dgt gene, respectively. The PCR product was transformed into strain KA796 bearing plasmid pKD46 (8), and chloramphenicol-resistant colonies were selected. Transformants were checked for the correct chromosomal deletion/insertion by PCR. P1 transduction was then used to generate NR16093 (Table 1), which served as the P1 donor for all other Δdgt::cat constructions. The ΔdlnB::kan allele was obtained from strain YG7207 (23). The F′CCI103/dnb, F′CCI104/dnb, and F′CCI106/dnb episomes, which contain the ΔdlnB::kan allele on the F′ episome (strains NR13243, NR13244, and NR13246) (Table 1), were constructed by introduction of the ΔdlnB::kan marker into strains CC103, CC104, and CC106 and by testing the Kan' transductants in an F′ transfer test for their abilities to simultaneously transfer the pro and kan markers. Chromosomal ΔdlnB::kan markers were created in F′ strains and strains carrying deletions of both the chromosomal and episomal dnb gene were created by transferring the F′prolac/dnb episomes into ΔdlnB strains using proline selection. Where needed, appropriate collector strains were used to serve as intermediates in the various F′ transfers. The recA56 allele of strain NR11264 was derived from strain UTH2 (51) using linkage with strains CC103, CC104, and CC106 (Table 1). We first transduced MG1655 to become zai-403::Tn10 proC (proline requiring) using strain SG1039 (obtained from S. Gottesman) as a donor, yielding NR11584. NR11584 was then transduced with P1 lysates prepared on strains CC101 through CC106 to become proC+, and the transductants were tested for the

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC101</td>
<td>F′CC101 7</td>
<td></td>
</tr>
<tr>
<td>CC102</td>
<td>F′CC102 7</td>
<td></td>
</tr>
<tr>
<td>CC103</td>
<td>F′CC103 7</td>
<td></td>
</tr>
<tr>
<td>CC104</td>
<td>F′CC104 7</td>
<td></td>
</tr>
<tr>
<td>CC105</td>
<td>F′CC105 7</td>
<td></td>
</tr>
<tr>
<td>CC106</td>
<td>F′CC106 7</td>
<td></td>
</tr>
<tr>
<td>KA796</td>
<td>ara thi Δprolac 45</td>
<td></td>
</tr>
<tr>
<td>NR1958</td>
<td>mut::Tn5 proC 44</td>
<td></td>
</tr>
<tr>
<td>NR559</td>
<td>mut::Tn5 proC 44</td>
<td></td>
</tr>
<tr>
<td>NR1036</td>
<td>F′CC106 26</td>
<td></td>
</tr>
<tr>
<td>NR1264</td>
<td>recA56 rec-560::Tn10 This work</td>
<td></td>
</tr>
<tr>
<td>NR1593</td>
<td>zai-403::Tn10 proC+ This work</td>
<td></td>
</tr>
<tr>
<td>NR12403</td>
<td>lac Z103 9</td>
<td></td>
</tr>
<tr>
<td>NR12404</td>
<td>lac Z104 9</td>
<td></td>
</tr>
<tr>
<td>NR12406</td>
<td>lac Z106 9</td>
<td></td>
</tr>
<tr>
<td>NR1318</td>
<td>ara thi Δprolac tprE9777 18</td>
<td></td>
</tr>
<tr>
<td>NR13144</td>
<td>ΔdlnB::kan 7</td>
<td></td>
</tr>
<tr>
<td>NR13145</td>
<td>mut::Tn10 7</td>
<td></td>
</tr>
<tr>
<td>NR13244</td>
<td>F′CCI104/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR13246</td>
<td>F′CCI104/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR13436</td>
<td>F′CCI106/dnb mini-Tn10/kam 7</td>
<td></td>
</tr>
<tr>
<td>NR16897</td>
<td>F′CCI101 mut::Tn5 9</td>
<td></td>
</tr>
<tr>
<td>NR16898</td>
<td>F′CCI101 mut::Tn5 9</td>
<td></td>
</tr>
<tr>
<td>NR16899</td>
<td>F′CCI101 mut::Tn5 9</td>
<td></td>
</tr>
<tr>
<td>NR16900</td>
<td>F′CCI104 mut::Tn5 9</td>
<td></td>
</tr>
<tr>
<td>NR16901</td>
<td>F′CCI104 mut::Tn5 9</td>
<td></td>
</tr>
<tr>
<td>NR16902</td>
<td>F′CCI106 mut::Tn5 9</td>
<td></td>
</tr>
<tr>
<td>NR16939</td>
<td>Δdgt::cat 7</td>
<td></td>
</tr>
<tr>
<td>NR16942</td>
<td>F′CCI101 7</td>
<td></td>
</tr>
<tr>
<td>NR16943</td>
<td>F′CCI102 7</td>
<td></td>
</tr>
<tr>
<td>NR16946</td>
<td>F′CCI104 7</td>
<td></td>
</tr>
<tr>
<td>NR16947</td>
<td>F′CCI105 7</td>
<td></td>
</tr>
<tr>
<td>NR16948</td>
<td>F′CCI106 7</td>
<td></td>
</tr>
<tr>
<td>NR16949</td>
<td>F′CCI107 7</td>
<td></td>
</tr>
<tr>
<td>NR16950</td>
<td>F′CCI108 7</td>
<td></td>
</tr>
<tr>
<td>NR16952</td>
<td>F′CCI109 7</td>
<td></td>
</tr>
<tr>
<td>NR16953</td>
<td>F′CCI110 7</td>
<td></td>
</tr>
<tr>
<td>NR17040</td>
<td>dnbB F′CCI103/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR17041</td>
<td>dnbB F′CCI104/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR17042</td>
<td>dnbB F′CCI106/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR17044</td>
<td>dnbB F′CCI107/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR17045</td>
<td>dnbB F′CCI108/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR17046</td>
<td>dnbB F′CCI109/dnb 7</td>
<td></td>
</tr>
<tr>
<td>NR17227</td>
<td>F′CCI101 mut::Tn10 d gt 9</td>
<td></td>
</tr>
<tr>
<td>NR17228</td>
<td>F′CCI102 mut::Tn10 d gt 9</td>
<td></td>
</tr>
<tr>
<td>NR17229</td>
<td>F′CCI103 mut::Tn10 d gt 9</td>
<td></td>
</tr>
<tr>
<td>NR17230</td>
<td>F′CCI104 mut::Tn10 d gt 9</td>
<td></td>
</tr>
<tr>
<td>NR17231</td>
<td>F′CCI105 mut::Tn10 d gt 9</td>
<td></td>
</tr>
<tr>
<td>NR17232</td>
<td>F′CCI106 mut::Tn10 d gt 9</td>
<td></td>
</tr>
<tr>
<td>NR17535</td>
<td>lac Z103 mut::Tn5 7</td>
<td></td>
</tr>
<tr>
<td>NR17536</td>
<td>lac Z104 mut::Tn5 7</td>
<td></td>
</tr>
<tr>
<td>NR17537</td>
<td>lac Z105 mut::Tn5 7</td>
<td></td>
</tr>
<tr>
<td>NR17538</td>
<td>lac Z103 dgt 7</td>
<td></td>
</tr>
<tr>
<td>NR17539</td>
<td>lac Z104 dgt 7</td>
<td></td>
</tr>
<tr>
<td>NR17540</td>
<td>lac Z106 dgt 7</td>
<td></td>
</tr>
<tr>
<td>NR17541</td>
<td>lac Z103 mut::Tn5 d gt 7</td>
<td></td>
</tr>
<tr>
<td>NR17542</td>
<td>lac Z104 mut::Tn5 d gt 7</td>
<td></td>
</tr>
<tr>
<td>NR17543</td>
<td>lac Z106 mut::Tn5 d gt 7</td>
<td></td>
</tr>
<tr>
<td>NR17558</td>
<td>F′CCI103 recA56 9</td>
<td></td>
</tr>
<tr>
<td>NR17582</td>
<td>F′CCI103 recA56 9</td>
<td></td>
</tr>
<tr>
<td>NR17583</td>
<td>F′CCI106 recA56 9</td>
<td></td>
</tr>
<tr>
<td>NR17584</td>
<td>F′CCI107 recA56 9</td>
<td></td>
</tr>
<tr>
<td>NR17587</td>
<td>F′CCI104 recA56 9</td>
<td></td>
</tr>
<tr>
<td>NR17588</td>
<td>F′CCI104 recA56 9</td>
<td></td>
</tr>
</tbody>
</table>

*With the exception of YG7207, all strains are also ara thi Δprolac derivatives of MG1655 (indicated with an asterisk). See Materials and Methods for details.

The designations F′CCI101, F′CCI102, F′CCI103, F′CCI104, F′CCI105, and F′CCI106 refer to the F′prolac originally present in strains CC101, CC102, CC103, CC104, CC105, and CC106, which permit measurement of A·T→C·G, G·C→A·T, G·C→C·G, G·C→C·T, A·T→G·C, and A·T→C·G reversion, respectively (7). The lacZ designations lacZ101, lacZ104, and lacZ106 refer to the lacZ alleles originally present on F′prolac in strains CC103, CC104 and CC106 but now located on the chromosome. The designations F′CCI103/dnbB, F′CCI104/dnbB, and F′CCI106/dnbB indicate deletion of the dnbB gene on the F′ episome.

**TABLE 1. E. coli strains used in this study**
determined by plating 0.1 ml of a 10-fold dilution of a culture (1 ml) were initiated from single colonies (one colony per tube). The recombinants display normal linkage between lac and proC (~20%). DNA sequencing of the lacZ genes revealed the expected missense mutation as described previously for the various CC strains (7).

**Media.** Strains were maintained on Luria-Bertani (LB) rich medium. Rifampin (100 µg/ml) and nalidixic acid (40 µg/ml) were added to LB plates to score Rifr and Nalr mutation frequencies, respectively. Antibiotic selections were made on minimal medium containing chloramphenicol (25 µg/ml), or tetracycline (20 µg/ml). Reversion to Lac+ was scored on minimal medium (MM) plates containing Vogel-Bonner salts (50), lactose (0.2%), and thiamine (2.5 µg/ml). Viable cell counts were scored on MM plates containing 0.2% glucose. XPG plates used for papillation studies were MM plates containing 0.2% glucose, 50 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 0.05% phenyl-β-D-galactopyranoside (P-Gal), and 2.5 µg/ml thiamine.

**Mutator screen.** A random transposon insertion library was created in strain KA796 by using the mini-Tn10cam transposon from phage λKNI1324 as described by Kleckner et al. (24). A P1 lysate prepared from this library was then used to transduce strain NR10836 to yield chloramphenicol-resistant colonies. The frequency of CmR transductants was determined by plating onto rich medium containing chloramphenicol, and appropriate dilutions were then plated on XPG medium containing chloramphenicol to yield approximately 250 transductants per plate (14,000 colonies total). These plates were incubated for a total of 8 days and examined at daily intervals for colonies with increased reversion to Lac+, as visualized by the number of blue (lac-) papillae appearing per colony. Putative mutator clones were stained and rescreened. Bona fide mutators were then characterized by DNA sequencing of the transposon insertion point.

**DNA sequencing.** Total DNA from mutator colonies was isolated using EasyDNA kits (Invitrogen), and the location of the mini-Tn10cam insertion was determined for each clone using arbitrary primed PCR analysis (6). Briefly, a first round of PCR analysis was performed on genomic DNA of each mutant using the following three primers: two primers with random 3′ ends, ARB1 (5′-GGG CACGCGTGTGACTGATCGACNN#####NNNAGAT-3′) and ARB2 (5′-GGG CACGCGTGTGACTGATCGACNN#####NNNAGAT-3′), and a third primer complementary to the mini-Tn10cam sequence, CmExt (5′-CAGGTCTCCCCGTTGAGG-3′). A second round of PCR was then performed with primers ARB2 (5′-GGG CACGCGTGTGACTGATCGACNN#####NNNAGAT-3′) and CmInt (5′-CAGGTCTCCCCGTTGAGG-3′). The final PCR product was purified with a QiAquick spin PCR purification kit (Qiagen) and sequenced using ARB2 primer and the dRhodamine dye terminator cycle DNA sequencing kit (PE Biosystems, Warrington, UK). The sequence surrounding the transposon insertion site was analyzed using BLAST searches identifying the gene in which the transposon was inserted.

**Mutant frequency determinations.** For each strain, 12 to 18 independent LB cultures (1 ml) were initiated from single colonies (one colony per tube). The colonies were taken from several independent isolates for each strain. Cultures were grown to saturation at 37°C on a rotary wheel. The total cell count was determined by plating 0.1 ml of a 10^-4 dilution on MM-glucose plates. Appropriate dilutions of each culture were plated separately onto selective plates (MM-lactose, LBrif, or LBNal). Plates were incubated at 37°C (24 h for LB plates; 36 to 40 h for MM plates). To calculate mutant frequencies, the number of mutants per plate was divided by the number of total cells. Occasional jackpot cultures were removed from the analysis. Average frequencies with standard errors were determined using the statistical software program Prism (GraphPad).

**RESULTS**

**Isolation of dgt as a mutator.** The discovery of the dgt gene as a mutator locus was a side product of an experiment that was aimed at obtaining chloramphenicol-resistant alleles of established mutator genes, particularly the mutHLS MMR genes, by transposon insertion. In this experiment (see Materials and Methods for details), we created a library of random mini-Tn10cam transposon insertions (24) and screened this library for mutators using a lacZ papillation assay. In this assay, colonies of certain β-galactosidase (lacZ)-deficient strains are grown on minimal glucose plates additionally containing both P-Gal and X-Gal. After exhaustion of the glucose, the alternative carbon source P-Gal will permit continued growth for any lacZ+ revertants created within the colonies, while the resulting mini-colonies within the larger colony will be colored blue due to the presence of X-Gal. In this manner, mutator colonies may be distinguished from normal colonies by the presence of an increased number of blue mini-colonies (papillae) (Fig. 1).

The papillation screen was performed for strain NR10836 (Table 1), which carries the lacZ missense allele originally present in strain CC106 (7) and which reverts to lac+ uniquely by an A→T→G→C transition mutation. We screened ~14,000 colonies and retrieved 50 putative mutators, of which 33 were sequenced. Fourteen separate insertions were found in at least four different genes. These included dam (one time), mutL (five times), and uvrD (five times), which are all involved in MMR. To our surprise, 17 out of 33 isolates, representing six different insertions, were located in the dgt gene, a gene not previously implicated in mutagenesis. An example of the increased level of papillation provided by the dgt deficiency is shown in Fig. 1. The map of the recovered transposon insertions in the dgt gene is shown in Fig. 2. The insertions are distributed throughout the gene and are oriented in both directions. It was considered likely that the mutator effect results directly from the loss of dgt function, and we subsequently created a complete dgt gene deletion (see Materials and Methods). The resulting Δdgt::cat allele behaved similarly to the mini-Tn10 insertions and was used for all further studies.

**Specificity of the dgt mutator.** We first tested the specificity of the dgt mutator using the complete set of lacZ alleles that permit detection of each of the six base substitution mutations (7). The data in Table 2, comparing dgt to the wild-type strain, show that dgt caused a modest increase in the frequency of
G·C→A·T transitions (threefold) and strong increases in the G·C→C·G transitions (20-fold) and A·T→G·C transitions (40-fold). The last increase is consistent with the initial discovery of the dgt mutator mutants using the lac A·T→G·C allele. Interestingly, a threefold decrease (anti-mutator effect) was observed for the G·C→T·A transition. Little or no change was noted for the remaining base substitutions (A·T→C·G and A·T→T·A).

The possible mutator activity of a dgt mutant was tested previously by Quirk et al. (40) and Wurgler and Richardson (53), and it was concluded that the dgt mutant strain did not show an increase in mutation rate. These studies used the frequency of rifampin- or nalidixic acid-resistant mutants as their indicator for mutator activity. We therefore tested the frequency of Rifr or Nalr mutants in our dgt strains. Likewise, we did not find any significant difference between the wild-type and dgt strains for either antibiotic resistance phenotype, as follows: (11 ± 1)×10⁻⁸ for Rifr for both strains and (0.3 ± 0.1)×10⁻⁸ and (0.4 ± 0.1)×10⁻⁸ for Nalr for the wild-type and dgt strains, respectively. In view of the defined specificity of the dgt mutator as observed using the lacZ reversion system, including its antimutator activity for at least one specific base pair substitution, the forward nature of the Rifr or Nalr targets may preclude detection of the mutator in these systems. Alternatively, the distinction may lie in the chromosomal (Rifr and Nalr) versus F’ episomal (lacZ) location of the mutational markers (see below).

The dgt mutator effect in a MMR-deficient background. A generally useful tool for studying mutational mechanisms is strains defective in the postreplicative (mutHLS) MMR system. A comparison of mutabilities in wild-type and MMR-defective strains provides information about the correctness of the mutational intermediates and may yield insight into their possible nature and origin as replication error. The data in the lower half of Table 2 show that in the MMR-defective mutL background, the dgt mutator activity (comparing mutL dgt to mutL) is no longer apparent for the lac G·C→A·T transition but is still observed for G·C→C·G and A·T→G·C substitutions. The dgt mutator activity for G·C→C·G transversions is actually enhanced (about fivefold) in the mutL background, suggesting that the mispairs responsible for the dgt-induced G·C→C·G mutations are susceptible to MMR (in contrast to the mispairings responsible for G·C→C·G in the dgt⁺ background). The dgt mutator effect for the A·T→G·C transition, while clearly observed (~2.5-fold), is significantly lower than the corresponding effect in the mutL⁺ background (~40-fold). Finally, and importantly, the dgt antimutator effect observed for the lac G·C→T·A transversions is reproduced in the MMR-defective strain (two- to threefold), despite the increases in overall frequencies. The combined data suggest that dgt-induced mutations are likely to result at least in part from mispairings made during DNA replication, as they are subject to mismatch correction. Furthermore, the fact that there are quantitative differences between the dgt mutator effects in mutL and mutL⁺ strains suggests that the actual nature of the mispairings may differ between wild-type and dgt strains. These observations need to be taken into account when trying to explain the nature of the dgt mutator effect (see Discussion).

Effect of dgt on chromosomal lac markers. As the dgt mutator effect is observed for several lacZ markers residing on F’prolac while no such effect was apparent for the chromosomal Rifr and Nalr markers, we also investigated the dgt effect on lacZ markers when residing on the chromosome. We used a set of strains containing the set of lacZ markers originally present on F’prolac in strains CC101 through CC106 (7) now placed at the normal lac position on the chromosome (8.4 min) (see Materials and Methods). The results in Table 3 show that for three lac markers that respond clearly to dgt in their episomal configuration, little or no effect could be demonstrated in this system. Thus, the dgt mutator (and antimutator) effect appears to work preferentially on the F’ episome.

Effect of dinB and recA on the dgt mutator. The preferential production of mutations on the F’ episome presents a possible parallel to the process of “adaptive” mutation, which also occurs preferentially on the F’ (12, 13). Adaptive mutations are defined by their appearance upon prolonged (4- to 10-day) incubation on minimum lactose plates (stationary phase mutants) and are further characterized by their dependence on the dinB (encoding DNA polymerase IV [Pol IV]) and recA genes (11, 14, 19, 20, 34). Our lac⁺ mutants were counted within 48 h of incubation and should represent, in large majority, mutants produced during growth in the liquid cultures prior to plating. Nevertheless, a mechanistic connection between the two modes of mutagenesis might exist. We therefore

---

<table>
<thead>
<tr>
<th>Genotype</th>
<th>lac A·T→C·G</th>
<th>lac G·C→A·T</th>
<th>lac G·C→C·G</th>
<th>lac G·C→T·A</th>
<th>lac A·T→G·C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.2 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>0.02 ± 0.02</td>
<td>3.4 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>dgt</td>
<td>0.2 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>mutL</td>
<td>1.1 ± 0.2</td>
<td>147 ± 12</td>
<td>0.03 ± 0.03</td>
<td>14.3 ± 1.4</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>dgt mutL</td>
<td>1.1 ± 0.2</td>
<td>133 ± 36</td>
<td>2.0 ± 0.5</td>
<td>7.2 ± 0.8</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

a Mutant frequencies were determined as described in Materials and Methods. The six different lac alleles used to measure the reversion frequency are indicated by the specific base pair substitution by which they revert (see text for details). The strains used were NR16942 to NR16947 (wild type), NR16948 to NR16953 (dgt), NR16897 to NR16902 (mutL), and NR17227 to NR17232 (dgt mutL) (Table 1).
tested the effect of $dinB$ and $recA$ deficiencies on the $dgt$ mutator activity.

The results shown in Table 4 indicate that there are, at best, modest reductions in the $dgt$ mutant effect in the $dinB$-deficient background. For the $lac$ A $\cdot$ T $\rightarrow$ G $\cdot$ C transition, the reduction is about twofold (observed in several experiments), whereas the effect on the G $\cdot$ C $\rightarrow$ G $\cdot$ C transversion is less than that. Despite these reductions, the $dgt$ mutant effect for the G $\cdot$ C $\rightarrow$ G $\cdot$ C and A $\cdot$ T $\rightarrow$ G $\cdot$ C substitutions in the $dinB$-deficient background is still around 10-fold.

The case of the lac G $\cdot$ C $\rightarrow$ T $\cdot$ A transversion is interesting, not only because of the $dgt$ antimutator effect for this lac allele, but also because it has been previously demonstrated to be $dinB$ dependent (18, 28), a result reproduced here (Table 4). The present data also suggest that the $dgt$ and $dinB$ antimutator effects both operate independently to reduce the overall frequency by fourfold.

The data in Table 5 for the $recA$-deficient background (using the $recA56$ allele) present an interesting picture. Notably, for the G $\cdot$ C $\rightarrow$ G $\cdot$ C allele, the reversion frequency in the $dgt$ $recA$ strain is actually increased (by about fourfold) over the single $dgt$ strain. A similar increase (~2.5-fold) was observed in another experiment using a $\Delta recA$ allele (data not shown). Thus, the lack of RecA function enhances the $dgt$ mutant effect for this lac transversion. For the lac A $\cdot$ T $\rightarrow$ G $\cdot$ C transition, the $recA$ deficiency causes a reduction in the $dgt$ mutant effect (about fourfold). Nevertheless, in the $recA$ background, an about fourfold $dgt$ mutant effect still remains.

A most interesting result is obtained for the case of the Rif$^R$ mutations. As noted before, no $dgt$ mutant was detected for this chromosomal marker, but a clear mutant effect can be observed in the $recA$-deficient background (52 versus 15 $\times$ 10$^{-8}$) (Table 5). This result was obtained in several repeated experiments, using both the $recA56$ and $\Delta recA$ allele. Both $recA$ alleles were transferred into the $dgt$ background by cotransduction with the $sr1::Tn10$ marker, and therefore, also several $recA^{-}$ $sr1::Tn10$ isolates were included in the analysis. No effect on the $dgt$ mutant effect was observed using those isolates, further indicating that the effects are due to the $recA$ deficiency per se. Overall, these combined results seem to indicate that the $dgt$ mutant effect has more than one component and, possibly, more than one mechanism.

**DISCUSSION**

Dgt, a mutator with unusual characteristics. The discovery of new mutant alleles is important, as mutators can provide unique insight into cellular mutation avoidance processes and their underlying mechanisms. Here we describe a novel mutator phenotype resulting from a deficiency of the *E. coli* $dgt$ gene. This gene has been known to encode a dNTP triphosphohydrolase (or dGTPase, based on its preference for dGTP) among the four dNTPs (1, 48), but the physiological function of this activity has not been clear. Our present results indicate that the dNTPase activity has a fidelity function inside the cell.

The $dgt$ mutator has several features that distinguish it from other known *E. coli* mutators, and these should be taken into account when considering the possible mechanisms. First, among the six studied lac reversion pathways, the $dgt$ mutant effect is specific for the G $\cdot$ C $\rightarrow$ G $\cdot$ C and A $\cdot$ T $\rightarrow$ G $\cdot$ C transition (although a modest effect is also seen for the G $\cdot$ C $\rightarrow$ A $\cdot$ T transition); this is a combination not reported before. Second, there is an antimutator effect for at least one base substitution, the lac G $\cdot$ C $\rightarrow$ T $\cdot$ A transversion. Third, the mutator effect seems to have a strong preference for events occurring on the F$^+$ episome. Fourth, the introduction of a $recA$ deficiency enhances the mutator activity for the G $\cdot$ C $\rightarrow$ G $\cdot$ C transversion as well as for the chromosomal Rif$^R$ target, which is an unusual finding. The $recA$ effect on the Rif$^R$ mutations also makes it clear that the mutator activity of $dgt$ is not necessarily restricted to the F$^+$ episome. Fifth, more than one mechanism may operate as suggested by the differential effects of the $mutL$ (Table 2) and $recA$ deficiencies (Table 5).

**A model for $dgt$ based on dGTP pool changes.** While at this time we do not know the precise mechanism underlying the $dgt$ mutator effect, it seems appropriate in a first approach to analyze the mutational data in terms of $dgt$-mediated effects on the cellular dGTP pool. Measurements of the dNTP pools in $dgt$ mutants have revealed an approximately twofold increase in intracellular dGTP relative to each of the other dNTPs (40). Conversely, Dgt overproduction (*E. coli* optA1 strain) yielded a five- to eightfold lowering of the dGTP concentration (36, 40). Thus, the dGTPase is capable of affecting the cellular dGTP level, and any dGTP changes will have certain mutational consequences. A second consideration, as already suggested in the earlier studies (40, 48), is that while dGTP is preferred among the canonical dNTPs, it may not be the physiological Dgt substrate. Thus, it cannot be excluded that the loss of the $dgt$ activity may lead to even stronger increases in the levels of certain modified dNTPs, which cause mispairings and mutations when incorporated into DNA (sanitation function).

As shown in Fig. 3, we have analyzed the potential conse-

---

**TABLE 4. Mutability (mutants per 10$^8$ cells) of $dgt$ strains in $dinB$ genetic background**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>lac G · C→C · G</th>
<th>lac G · C→T · A</th>
<th>lac A · T→G · C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.02 ± 0.02</td>
<td>2.4 ± 0.2</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>$dinB$</td>
<td>0.02 ± 0.02</td>
<td>1.1 ± 0.1</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>$dgt$</td>
<td>0.35 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.55 ± 0.1</td>
</tr>
<tr>
<td>$dinB$ $dgt$</td>
<td>0.20 ± 0.1</td>
<td>0.55 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

* The wild-type strains used were NR16944, NR16945, and NR16947 for the three lac reversions, respectively; the $dinB$ strains were NR17040, NR17041, and NR17042; the $dgt$ strains were NR16950, NR16951, and NR16953; and the $dinB$ $dgt$ strains were NR17044, NR17045, and NR17046 (Table 1). The lac gene in these strains resides on F$^+$prolac.

**TABLE 5. Mutability (mutants per 10$^8$ cells) of $dgt$ strains in a $recA$-deficient background**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>lac G · C→C · G</th>
<th>lac G · C→T · A</th>
<th>lac A · T→G · C</th>
<th>Rif$^R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.02 ± 0.02</td>
<td>1.6 ± 0.02</td>
<td>0.04 ± 0.03</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>$recA56$</td>
<td>0.02 ± 0.02</td>
<td>0.38 ± 0.07</td>
<td>0.04 ± 0.03</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>$dgt$</td>
<td>0.35 ± 0.03</td>
<td>0.51 ± 0.06</td>
<td>0.6 ± 0.1</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>$recA56$ $dgt$</td>
<td>1.2 ± 0.2</td>
<td>0.44 ± 0.08</td>
<td>0.15 ± 0.04</td>
<td>52 ± 2</td>
</tr>
</tbody>
</table>

* Mutant frequencies were determined as described in Materials and Methods. The wild-type strains used were NR16944, NR16945, and NR16947; the $recA56$ strains were NR17581, NR17587, and NR17583; the $dgt$ strains were NR16950, NR16951, and NR16953; and the $recA56$ $dgt$ strains were NR17582, NR17588, and NR17584 for the three lac alleles, respectively (Table 1). The lac gene in these strains resides on F$^+$prolac.
For the G·C→A·T transition, no effect is predicted for the case of the G·T mismatch, while a mixed prediction results for the C·A mismatch. At the C·A misinsertion step, dGTP is the correct nucleotide and increased levels will be antimutagenic, while at the extension step, dGTP is the next correct dNTP and increased levels will be mutagenic. Our observation of a modest (about threefold) mutator effect of dgt is not inconsistent with this set of predictions.

For the G·C→C·G transversion, dGTP would be mutagenic for the G·A mispairings (dGTP is the next nucleotide) but antimutagenic for the C·T mispairings (dGTP is the correct nucleotide). Which effect will dominate depends on whether G·A or C·T mispairings are the predominant contributors. Previous work from our laboratory (10, 33) has provided arguments and data consistent with C·T mispairings being the major event at this site. Therefore, the antimutator effect observed for the lac G·C→C·T·A allele (Table 2) is consistent with the predictions made here.

For the A·T→C·G transversions, an increase may be expected if the A·G mismatch were to be a significant contributor to the overall mispairings (dGTP is the incorrect nucleotide). However, no dgt mutator effect is observed. This may be because the predominant mismatch at this site is not A·G but T·C or, alternatively, A·8-oxoG, as in fact was previously suggested (16). In either case, any enhancing effects of dgt on A·G mispairings may be obscured.

For the A·T→C·G transversion, a mutator effect is predicted for the case of the A·A mismatching (dGTP is the next nucleotide), while no effect is predicted for the case of the T·T mispairings. As T·T mispairings have been argued to be the predominant factor (10, 33), the lack of any effect on this lac allele is consistent with the elevated dGTP* level hypothesis.

In summary, an interpretation of the mutational specificity of the dgt mutator in terms of increased dGTP (dGTP*) levels provides a set of predictions that is largely consistent with the observations, and this model for dgt action must be considered. As noted earlier, the large mutator effects for the lac G·C→C·G and A·T→G·C markers are more consistent with large increases in dGTP* concentrations rather than modest increases in dGTP itself. In this model, Dgt has a sanitizing function. However, it is likely that effects of both dGTP and dGTP* could be occurring in parallel. The experiments with the MMR-defective mutL strain give further clues to this (see below).

The dgt mutator and DNA MMR. The mutHLS MMR system of E. coli corrects replication errors with variable efficiency depending on the type of error. In general, transition errors are well corrected (200- to 400-fold), while transversion errors are corrected much less efficiently (20- to 30-fold) (43, 46), although this factor depends on the precise transition or transversion and on the DNA sequence context (22, 27). Errors containing damaged DNA bases are also generally poorly recognized. For example, A·8-oxoG mispairings do not appear to be subject to detectable correction (44). Thus, data on the correctness of certain errors may provide information on the nature of the mismatches involved. Our data in Table 2 on the dgt effect may be used for this purpose.

For the lac G·C→A·T transition, the dgt mutator effect observed in the mutL+ strain is no longer apparent in the mutL strain. A simple explanation for this would be that while the dgt
mutator effect in the mutL+ background results from enhanced extension of C·A mispairings (Fig. 3) this mispair is, in fact, as a primary polymerase error, a minor component compared to the more frequent G·T errors (10). However, the greater MMR efficiency for the G·T errors makes it possible for the C·A errors to make a sufficient contribution to the observed G · C→A · T transitions in the repair-proficient background. In the MMR-defective mutL strain, we see effectively only the more frequent G · T component, for which no dgt effect is predicted (Fig. 3).

The case of the G · C→C · G transversions is most interesting. The frequency of these events is normally very low (at the detection limit) regardless of the cell’s MMR status. Hence, no statement can be made about the possible origin or nature of these errors. In the dgt background, G · C→C · G mutations are strongly enhanced, about 60-fold in the MMR-defective mutL background. In this case, it appears that the underlying errors are reduced by about fourfold by MMR, a factor not inconsistent with an origin from G · G or G · G* errors (22, 27). The observed 60-fold increase in these errors would be most consistent with the latter type of error.

The case of the G · C→T · A transversions is unique because of the dgt antimutator effect. In the previous section, this antimutator effect was proposed to result from a decrease in C · T errors (dGTP is the correct nucleotide). As C · T errors have been argued to be the predominant mismatch (over the G · A alternative), the antimutator effect should also be observable in the MMR defective background, as is the case (Table 2).

No effect is observed for the lac A · T→T · A allele, consistent with all expectations.

The A · T→G · C transitions represent another interesting case. The 40-fold dgt mutator effect observed in the MMR-proficient strain is reduced to a twofold effect (two- to fourfold in different experiments) in the mutL-defective background. A reasonable explanation for this finding lies in the predominance of T · G over A · C as primary replication errors, in conjunction with the highly efficient correction of the T · G errors by the MMR system (42, 47). In the MMR defective background, the dgt mutator effect may simply result from enhanced T · G errors (dGTP is the incorrect nucleotide) (Fig. 3). On the other hand, in the MMR-proficient background the T · G mispairs are effectively removed and, instead, the dgt-induced T · G* errors may contribute most strongly to the observed mutations.

In summary, it appears that the combined data on the dgt mutator/antimutator effect can be fitted within a framework describing the effects as resulting from enhanced dGTP and dGTP* levels.

The effects of RecA and Pol IV. The effects of the recA and dinB gene products were investigated in view of the preferential activity of the dgt mutator on the episomal lac genes, a phenomenology characteristic of the process of adaptive mutation, which is to a large extent dependent on these two gene functions. In addition, such experiments may provide insight into the role of error-prone polymerases Pol IV and Pol V, independent of any mechanistic connection to adaptive mutation. In particular, Pol IV, at its basal level, has been implicated in several mutational processes, including a role in producing the F’ episomal G · C→T · A transversions, even in growing cells (18, 28). The combined results (Tables 4 and 5) provide a mixed picture that may be indicative of more than one type of error processing.

The case of the G · C→C · G transversions is most intriguing. In the dgt+ background, no effect of dinB or recA can be discerned at the detection limit. The dgt mutator effect for this lac allele is strong (~20-fold) and is modestly reduced by the loss of Pol IV. It is, surprisingly, increased by the recA deficiency. The dinB result may indicate that Pol IV is involved in producing or fixing at least part of the underlying mispairs. This may not be surprising within the suggested view that the responsible mispairings include a modified base (G · G*). The approximately fourfold increase of the mutator effect in the recA-deficient background might suggest that replication stalling can occur upon G* incorporation and that recombinational repair constitutes an error-free pathway to avoid the mutagenic consequences of G*. As far as we know, no precedent for this kind of mutation avoidance exists. The mutator effect for the recA deficiency is also clearly seen for the chromosomal RifR mutations (Table 5), indicating that this effect of recA is a more general phenomenon.

The lac G · C→T · A transversions on F’prolac are known to be dinB dependent (18, 28), and this effect is reproduced here (Table 4). However, we now demonstrate that these events are also recA dependent (Table 5). Thus, the combined dinB/recA dependency of the G · C→T · A transversions parallels that of adaptive mutagenesis. Possibly, the recombinational-associated DNA replication events on the F’ episome, which are proposed to dominate the production of adaptive mutations in the stationary phase (12), may also occur to some extent in the growing stage (or in any initial replications on the plate) and may numerically account for all observed G · C→T · A transversions. Under such conditions, lowered levels of dGTP will be antimutagenic (C · T mispair). In the absence of recA (or dinB) function, the majority of observed mutations may no longer be of the C · T error type and the dgt-mediated antimutator effect may no longer be observable.

Finally, the G · C→A · T transitions also show a dependency on recA and dinB, although only in the dgt background. This result emphasizes the different genesis of these transitions in the dgt and dgt+ backgrounds. As indicated earlier, the responsible mispairings in the dgt background may be T · G* errors, and these may be created during recombinational-depenent events on F’prolac.

Role of dgt in the cell. While our analysis of the dgt mutational specificity and its genetic requirements has yielded a more or less consistent picture that permits us to link the mutational outcomes to increased concentrations of dGTP or dGTP*, it is likely that this provides only a partial glimpse of the functioning of dgt in the cell. One important issue that requires further thought is the role of the DNA binding activity of Dgt (1, 53). In the simplest case of a sanitation enzyme, no need for a DNA binding activity would exist. For the Dgt enzyme, one might speculate that DNA binding serves to activate the protein’s dGTPase activity (21). In such a model, the dGTPase activity would normally be restricted but lead to strongly diminished dGTP levels in the presence of sufficient amounts of single-stranded DNA, such as what might arise during certain DNA repair activities. This diminishement in dGTP levels could in turn lead to restrictions on DNA repli-
cation or, alternatively, make DNA synthesis (including repair synthesis) increasingly accurate. In this kind of model, Dgt could serve as a checkpoint function. The strongly enhanced dgt mutator activity for episomal G:C→C·G transversions and chromosomal Rif* mutations in the recA-deficient background (Table 5) provides a further connection to a role of Dgt during DNA repair. This will be an interesting area for further investigation.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

We thank S. Covo and Z. Pursell of the NIEHS for their helpful review of the manuscript for this paper and Sean Moore for his help in constructing the strains containing the chromosomal lacZ alleles.

REFERENCES


