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

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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 proof-reading 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 DNA polymerases will accept 8-oxodGTP as a substrate for insertion opposite template adenines, leading to characteristic A · T → C · G transversions. In the bacterium *Escherichia coli*, the MutT enzyme, a nucleoside triphosphate pyrophosphohydrolase, converts 8-oxodGTP to 8-oxodGMP and pyrophosphate (PP_i). The importance of this system is evidenced by the 10,000-fold or more increase in A · T → C · G transversion mu-

tations in the absence in the *mutT* function (15, 16). The MutT enzyme is the founding member of a large family of enzymes that hydrolyze nucleotide diphosphates (Nudix hydrolases), splitting off a PP_i moiety (3, 54). These enzymes are generally assigned a “sanitizing” or “housecleaning” function, removing exogenous compounds or toxic compounds resulting from normal metabolism (by-products) (17). Certain pool-sanitizing enzymes outside the Nudix family have also been identified (17). Examples are dUTPase, which removes dUTP, and the RdgB enzyme, which removes dITP and dXTP (and the corresponding ribonucleotides) (4, 5). These enzymes are likewise pyrophosphohydrolases, converting the triphosphate to the corresponding monophosphate.

The present study is concerned with a member of another class of dNTP hydrolyzing enzymes, the dNTP triphosphohydrolases, which hydrolyze dNTPs to the corresponding deoxynucleoside and tripolyphosphate (PPP_i). Two members of this group have been investigated in some detail, the *E. coli* *dgt* gene product (1, 48) and the *Thermus thermophilus* TT1383 protein (25). The *E. coli* protein has a preference for hydrolyzing dGTP (dGTP → dG + PPP_i) 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-BrdGTP at a comparable rate to dGTP, but other analogs, including 8-oxodGTP, 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). Certain mutants of bacteriophages T4 (*dexA* mutant) or T7 (*I.2* mutant) are incapable of growing in such *optA1* strains because of dGTP limitation (36, 41). Notably, the T7 *I.2* gene product is a specific Dgt inhibitor, and a T7 *I.2* mutant, lacking the inhibitor, is not able to grow in the overproducer (21, 38).

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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 mutators 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* Δ prolac) (45) or MG1655 (49). The series of F'prolac episomes from strains CC101 through CC106 (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 P1virA. The *mutL*::Tn10 and *mutL*::Tn5 markers were transferred from strains NR9163 (44) and NR9559 (9), respectively, selecting for tetracycline or kanamycin resistance. NR13138 is a *trpE*9777 derivative of KA796 (18). The Δ dgt::cat allele of NR16939 was generated by the gene replacement method of Datsenko and Wanner (8). 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'-ATGGCAGATTGATTTCGGAA AAAAATAAACTGGCATCGTTCACCGTGTAGGCTGGAGCTGC TT-3' and 5'-TTATTGTTCTACGGCCATCAGACGTCGGTATTCATCCCA CGCATAGAGGTCATATGAATATCTCTCTAG-3', in which 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 NR16939 (Table 1), which served as the P1 donor for all other Δ dgt::cat constructions. The Δ dinB::kan allele was obtained from strain YG7207 (23). The F'CC103/*dinB*, F'CC104/*dinB*, and F'CC106/*dinB* episomes, which contain the Δ dinB::kan allele on the F' episome (strains NR13243, NR13244, and NR13246) (Table 1), were constructed by introduction of the Δ dinB::kan marker into strains CC103, CC104, and CC106 and by testing the Kan^r transductants in an F' transfer test for their abilities to simultaneously transfer the *pro* and *kan* markers. Chromosomal Δ dinB::kan markers were created in F⁻ strains, and strains carrying deletions of both the chromosomal and episomal *dinB* gene were created by transferring the F'prolac/*dinB* episomes into Δ dinB strains using proline selection. Where needed, appropriate collector strains were used to serve as intermediates in the various F' transfers. The *recA*56 allele of strain NR11264 was derived from strain UTH2 (51) using linkage with nearby *srl*::Tn10. Selection was for tetracycline resistance, followed by testing for UV sensitivity. Strains NR12403, NR12404, and NR12406 are derivatives of MG1655 that carry the *lac* allele of strains CC103, CC104, and CC106 chromosomally instead of on the F' episome. These strains were constructed by P1 transduction, making use of the linkage of the chromosomal *lac* genes with *proC* (2). We first transduced MG1655 to become *zaj*-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 *pro*⁺, and the transductants were tested for the

TABLE 1. *E. coli* strains used in this study

Strain ^a	Relevant genotype ^b	Reference or source
CC101	F'CC101	7
CC102	F'CC102	7
CC103	F'CC103	7
CC104	F'CC104	7
CC105	F'CC105	7
CC106	F'CC106	7
KA796	<i>ara</i> <i>thi</i> Δ (<i>prolac</i>)	45
NR9163	<i>mutL</i> 218::Tn10	44
NR9559	<i>mutL</i> 211::Tn5	9
NR10836	F'CC106	26
NR11264	<i>recA</i> 56 <i>srl</i> -360::Tn10	This work
NR11584*	<i>zaj</i> -403::Tn10 <i>proC</i>	This work
NR12403*	<i>lacZ</i> 103	This work
NR12404*	<i>lacZ</i> 104	This work
NR12406*	<i>lacZ</i> 106	This work
NR13138	<i>ara</i> <i>thi</i> Δ (<i>prolac</i>) <i>trpE</i> 9777	18
NR13144	Δ dinB::kan	NR13138 × P1/YG7207
NR13145	<i>mutL</i> ::Tn10	NR13138 × P1/NR9163
NR13243	F'CC103/ <i>dinB</i>	This work
NR13244	F'CC104/ <i>dinB</i>	This work
NR13246	F'CC106/ <i>dinB</i>	This work
NR13436	F'CC106 <i>dgt</i> ::mini-Tn10cam	This work
NR16897	F'CC101 <i>mutL</i> ::Tn10	NR13145 × F'CC101
NR16898	F'CC102 <i>mutL</i> ::Tn10	NR13145 × F'CC102
NR16899	F'CC103 <i>mutL</i> ::Tn10	NR13145 × F'CC103
NR16900	F'CC104 <i>mutL</i> ::Tn10	NR13145 × F'CC104
NR16901	F'CC105 <i>mutL</i> ::Tn10	NR13145 × F'CC105
NR16902	F'CC106 <i>mutL</i> ::Tn10	NR13145 × F'CC106
NR16939	Δ dgt::cat	NR13138 × P1/ Δ dgt::cat
NR16942	F'CC101	NR13138 × F'CC101
NR16943	F'CC102	NR13138 × F'CC102
NR16944	F'CC103	NR13138 × F'CC103
NR16945	F'CC104	NR13138 × F'CC104
NR16946	F'CC105	NR13138 × F'CC105
NR16947	F'CC106	NR13138 × F'CC106
NR16948	F'CC101 <i>dgt</i>	NR16939 × F'CC101
NR16949	F'CC102 <i>dgt</i>	NR16939 × F'CC102
NR16950	F'CC103 <i>dgt</i>	NR16939 × F'CC103
NR16951	F'CC104 <i>dgt</i>	NR16939 × F'CC104
NR16952	F'CC105 <i>dgt</i>	NR16939 × F'CC105
NR16953	F'CC106 <i>dgt</i>	NR16939 × F'CC106
NR17040	<i>dinB</i> F'CC103/ <i>dinB</i>	NR13144 × F'NR13243
NR17041	<i>dinB</i> F'CC104/ <i>dinB</i>	NR13144 × F'NR13244
NR17042	<i>dinB</i> F'CC106/ <i>dinB</i>	NR13144 × F'NR13246
NR17044	<i>dinB</i> <i>dgt</i> F'CC103/ <i>dinB</i>	NR17040 × P1/NR16939
NR17045	<i>dinB</i> <i>dgt</i> F'CC104/ <i>dinB</i>	NR17041 × P1/NR16939
NR17046	<i>dinB</i> <i>dgt</i> F'CC106/ <i>dinB</i>	NR17042 × P1/NR16939
NR17227	F'CC101 <i>mutL</i> ::Tn10 <i>dgt</i>	NR16948 × P1/NR9163
NR17228	F'CC102 <i>mutL</i> ::Tn10 <i>dgt</i>	NR16949 × P1/NR9163
NR17229	F'CC103 <i>mutL</i> ::Tn10 <i>dgt</i>	NR16950 × P1/NR9163
NR17230	F'CC104 <i>mutL</i> ::Tn10 <i>dgt</i>	NR16951 × P1/NR9163
NR17231	F'CC105 <i>mutL</i> ::Tn10 <i>dgt</i>	NR16952 × P1/NR9163
NR17232	F'CC106 <i>mutL</i> ::Tn10 <i>dgt</i>	NR16953 × P1/NR9163
NR17535*	<i>lacZ</i> 103 <i>mutL</i> ::Tn5	NR12403 × P1/NR9559
NR17536*	<i>lacZ</i> 104 <i>mutL</i> ::Tn5	NR12404 × P1/NR9559
NR17537*	<i>lacZ</i> 106 <i>mutL</i> ::Tn5	NR12406 × P1/NR9559
NR17538*	<i>lacZ</i> 103 <i>dgt</i>	NR12403 × P1/NR16939
NR17539*	<i>lacZ</i> 104 <i>dgt</i>	NR12404 × P1/NR16939
NR17540*	<i>lacZ</i> 106 <i>dgt</i>	NR12406 × P1/NR16939
NR17541*	<i>lacZ</i> 103 <i>mutL</i> ::Tn5 <i>dgt</i>	NR17535 × P1/NR9559
NR17542*	<i>lacZ</i> 104 <i>mutL</i> ::Tn5 <i>dgt</i>	NR17536 × P1/NR9559
NR17543*	<i>lacZ</i> 106 <i>mutL</i> ::Tn5 <i>dgt</i>	NR17537 × P1/NR9559
NR17581	F'CC103 <i>recA</i> 56	NR16944 × P1/NR11264
NR17582	F'CC103 <i>dgt</i> <i>recA</i> 56	NR16950 × P1/NR11264
NR17583	F'CC106 <i>recA</i> 56	NR16947 × P1/NR11264
NR17584	F'CC106 <i>dgt</i> <i>recA</i> 56	NR16953 × P1/NR11264
NR17587	F'CC104 <i>recA</i> 56	NR16945 × P1/NR11264
NR17588	F'CC104 <i>dgt</i> <i>recA</i> 56	NR16951 × P1/NR11264
YG7207	Δ (<i>dinB</i> -yafN)::kan	23

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

^b The designations F'CC101, F'CC102, F'CC103, F'CC104, F'CC105, and F'CC106 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 → T · A, A · T → T · A, and A · T → G · C reversions, respectively (7). The *lacZ* designations *lacZ*103, *lacZ*104, and *lacZ*106 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'CC103/*dinB*, F'CC104/*dinB*, and F'CC106/*dinB* indicate deletion of the *dinB* gene on the F' episome.

FIG. 1. Increased papillation of *E. coli* colonies lacking the *dgt* gene, indicating a *dgt* mutator activity for *lac* A · T→G · C transitions. Representative wild-type (wt) and *dgt* colonies are shown. See text for details.

desired *lac* genotype. Because in the CC donor strains, *proC* is located on the chromosome while *lac* resides on the F'*prolac* episome, production of *proC*⁺ *lac* recombinants may require a double recombination event or some other less-frequent type of occurrence. Nevertheless, the desired recombinants were obtained at an average frequency of ~0.2%. (All recombinants were also tetracycline sensitive.) When tested in backcrosses with NR11584, the new recombinants displayed 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 Rif^r and Nal^r mutation frequencies, respectively. Antibiotic selections were performed on LB medium with kanamycin (50 µg/ml), 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 λNK1324 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 Cam^r 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 purified and retested. Bona fide mutators were then characterized by DNA sequencing of the transposon insertion point.

DNA sequencing. Total DNA from mutator colonies was isolated using Easy-DNA 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'-GGC CACGCGTCGACTAGTACNNNNNNNNNGATAT-3') and ARB6 (5'-GGC CACGCGTCGACTAGTACNNNNNNNNNACGCC-3'), and a third primer complementary to the mini-Tn10cam sequence, CmExt (5'-CAGGCTCTCCCC GTGGAGG-3'). A second round of PCR was then performed with primers ARB2 (5'-GGCCACGCGTCGACTAGTAC-3') and CmInt (5'-CTGCCTCCC AGAGCTG-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, United Kingdom). 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 rotator wheel. The total cell count was determined by plating 0.1 ml of a 10⁻⁶ 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

FIG. 2. Mini-Tn10cam transposon insertion sites in the *dgt* locus yielding an A · T→G · C mutator phenotype. The six different insertion sites found are indicated by nucleotide position in the gene. The orientation of the transposon is indicated by direction of the arrow, indicating the direction of transcription of the *cat* gene.

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 Δ *dgt*::*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

TABLE 2. Mutability (*lac* revertants per 10^8 cells) of wild-type and *dgt* strains in MMR-proficient or MMR-deficient (*mutL*) backgrounds^a

Genotype	<i>lac</i> A · T→C · G	<i>lac</i> G · C→A · T	<i>lac</i> G · C→C · G	<i>lac</i> G · C→T · A	<i>lac</i> A · T→T · A	<i>lac</i> A · T→G · C
Wild type	0.2 ± 0.1	1.6 ± 0.1	0.02 ± 0.02	3.4 ± 0.2	0.6 ± 0.1	0.03 ± 0.02
<i>dgt</i>	0.2 ± 0.1	3.6 ± 0.2	0.4 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	1.2 ± 0.2
<i>mutL</i>	1.1 ± 0.2	147 ± 12	0.03 ± 0.03	14.3 ± 1.4	1.6 ± 0.3	14.1 ± 1.2
<i>dgt mutL</i>	1.1 ± 0.2	133 ± 36	2.0 ± 0.5	7.2 ± 0.8	1.6 ± 0.3	31.0 ± 1.5

^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).

G · C→A · T transitions (threefold) and strong increases in the G · C→C · G transversions (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 (antimutator effect) was observed for the G · C→T · A transversion. 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 Rif^r or Nal^r 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: $(11 \pm 1) \times 10^{-8}$ for Rif^r for both strains and $(0.3 \pm 0.1) \times 10^{-8}$ and $(0.4 \pm 0.1) \times 10^{-8}$ for Nal^r 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 Rif^r or Nal^r targets may preclude detection of the mutator in these systems. Alternatively, the distinction may lie in the chromosomal (Rif^r and Nal^r) 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 correctability 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 in-

creases 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 Rif^r and Nal^r 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 3. Mutant frequencies (mutants per 10^8 cells) for *dgt* strains with *lac* alleles residing on the *E. coli* chromosome^a

Genotype	<i>lac</i> G · C→C · G	<i>lac</i> G · C→T · A	<i>lac</i> A · T→G · C
Wild type	0.02 ± 0.02	0.3 ± 0.1	0.02 ± 0.02
<i>dgt</i>	0.02 ± 0.02	0.3 ± 0.1	0.02 ± 0.02
<i>mutL</i>	0.08 ± 0.03	0.9 ± 0.2	13.5 ± 0.9
<i>dgt mutL</i>	0.09 ± 0.04	0.9 ± 0.2	16.9 ± 1.3

^a The wild-type strains used were NR12403, NR12404, and NR12406 for the three *lac* alleles, respectively; the *dgt* strains were NR17538, NR17539, and NR17540; the *mutL* strains were NR17535, NR17536, and NR17537; and the *dgt mutL* strains were NR17541, NR17542, and NR17543 (Table 1). Mutant frequencies were determined as described in Materials and Methods.

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

Genotype	<i>lac</i> G · C→C · G	<i>lac</i> G · C→T · A	<i>lac</i> A · T→G · C
Wild type	0.02 ± 0.02	2.4 ± 0.2	0.02 ± 0.02
<i>dinB</i>	0.02 ± 0.02	1.1 ± 0.1	0.02 ± 0.02
<i>dgt</i>	0.35 ± 0.1	1.1 ± 0.1	0.55 ± 0.1
<i>dinB dgt</i>	0.20 ± 0.1	0.55 ± 0.1	0.3 ± 0.1

^a 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*.

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* mutator effect in the *dinB*-deficient background. For the *lac* A · T→G · C transition, the reduction is about twofold (observed in several experiments), whereas the effect on the G · C→C · G transversion is less than that. Despite these reductions, the *dgt* mutator effect for the G · C→C · G and A · T→G · C substitutions in the *dinB*-deficient background is still around 10-fold.

The case of the *lac* G · C→T · 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 · C→C · G 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* mutator effect for this *lac* transversion. For the *lac* A · T→G · C transition, the *recA* deficiency causes a reduction in the *dgt* mutator effect (about fourfold). Nevertheless, in the *recA* background, an about fourfold *dgt* mutator effect still remains.

A most interesting result is obtained for the case of the Rif^r mutations. As noted before, no *dgt* mutator was detected for this chromosomal marker, but a clear mutator effect can be observed in the *recA*-deficient background (52 versus 15×10^{-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 *srl::Tn10* marker, and therefore, also several *recA*⁺ *srl::Tn10* isolates were included in the analysis. No effect on the *dgt* mutator 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* mutator effect has more than one component and, possibly, more than one mechanism.

DISCUSSION

Dgt, a mutator with unusual characteristics. The discovery of new mutator alleles is important, as mutators can provide

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

Genotype	<i>lac</i> G · C→C · G	<i>lac</i> G · C→T · A	<i>lac</i> A · T→G · C	Rif ^r
Wild type	0.02 ± 0.02	1.6 ± 0.02	0.04 ± 0.03	15 ± 2
<i>recA56</i>	0.02 ± 0.02	0.38 ± 0.07	0.04 ± 0.03	15 ± 2
<i>dgt</i>	0.35 ± 0.03	0.51 ± 0.06	0.6 ± 0.1	14 ± 2
<i>recA56 dgt</i>	1.2 ± 0.2	0.44 ± 0.08	0.15 ± 0.04	52 ± 2

^a 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*.

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* mutator effect is specific for the G · C→C · G and A · T→G · C transition (although a modest effect is also seen for the G · C→A · T transition); this is a combination not reported before. Second, there is an antimutator effect for at least one base substitution, the *lac* G · C→T · 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 · C→C · G 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 *dgt* 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-

<i>lac</i> allele	Substitution	Mispair 1	Next dNTP	Mispair 2	Next dNTP
CC101	A·T→C·G	A·(G/T)	dATP	T·(C/A)	dATP
CC102	G·C→A·T	G·(T/C)	dCTP	C·(A/G)	dGTP
CC103	G·C→C·G	G·(G/C)	dATP	C·(C/G)	dATP
CC104	G·C→T·A	G·(A/C)	dGTP	C·(T/G)	dCTP
CC105	A·T→T·A	A·(A/T)	dGTP	T·(T/A)	dCTP
CC106	A·T→G·C	A·(C/T)	dATP	T·(G/A)	dATP

FIG. 3. Schematic to illustrate predicted effects of increased dGTP level on the production of each of the 12 mispairs, leading to reversion of the six investigated *lac* alleles. The format for the mispairs is as follows: template base · (wrong base/correct base). In red, increases of dGTP predicted to promote increased misinsertion (dGTP is incorrect nucleotide) or increased mispair extension (dGTP is next dNTP). In blue, increases of dGTP predicted to disfavor misinsertion (dGTP is correct nucleotide). The wild-type DNA sequence at the site of *lac* reversion is 5'-AATGAGAGT-3', while in strains CC101 through CC106, the GAG codon (encoding the essential glutamic acid residue) is replaced by TAG, GGG, CAG, GCG, GTG, or AAG, respectively. Only the single-base change to GAG will restore the Lac⁺ phenotype. See text for further details.

quences of increased dGTP levels on the production of the various DNA polymerase errors that may be responsible for each of the six studied *lac* base substitution events. This analysis takes into account the following: (i) the competition between correct and incorrect nucleotides at the site of reversion and (ii) the effect of increased dGTP levels when dGTP is the next correct nucleotide to be incorporated following the mismatch. This latter, so-called “next nucleotide” effect is an established determinant of polymerase error rates, as high levels of the next correct incoming dNTP promote mismatch extension and counteract exonucleolytic removal by the polymerase proofreading activity (31, 35).

Describing one example in detail, the A · T→G · C transition (Fig. 3, bottom row) must be created by either an A · C mismatch or a T · G mismatch, depending on the DNA strand considered (here and below we follow the convention of noting the template base first). In the case of the A · C mismatch, the competition is between dTTP (correct) and dCTP (incorrect), and the next nucleotide to be incorporated is dATP. In the simplest model, increased levels of dGTP are not expected to influence the frequency of these A · C events, as dGTP is not a participant in either the misinsertion or the mispair extension step. For the T · G mismatch, which is the presumed preferred mismatch (10, 33), the competition is between dATP (correct) and dGTP (incorrect), and the next nucleotide is dATP. Here, an increased level of dGTP is predicted to promote the frequency of the T · G mispairings and the resulting A · T→G · C transition, as indeed observed (Table 2). This logic would also hold if the error-producing dNTP is not dGTP itself but a modified derivative (dGTP^{*}). Indeed, one may argue that the large mutator effect seen for the A · T→G · C (and also G · C→C · G) substitutions (20- to 70-fold) (Table 2) is more consistent with a strong elevation of some unidentified dGTP^{*} rather than a modest elevation of dGTP itself.

For the G · C→C · G transversion, a mutator effect can be predicted for the case of the G · G mispairings, while an antimutator effect is predicted for the C · C mismatches. The strong mutator effect observed is certainly consistent with an increase in G · G or G · G^{*} mispairings.

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→T · A 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→T · A allele (Table 2) is consistent with the predictions made here.

For 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→T · A 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 mispairs do not appear to be subject to detectable correction (44). Thus, data on the correctability 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 Rif^r 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 recombination-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 recombination-dependent 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 diminishment 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^r 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.

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