Examination of the Role of DNA Polymerase Proofreading in the Mutator Effect of Miscoding tRNAs

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We previously described *Escherichia coli* mutator tRNAs that insert glycine in place of aspartic acid and postulated that the elevated mutation rate results from generating a mutator polymerase. We suggested that the proofreading subunit of polymerase III, ε, is a likely target for the aspartic acid-to-glycine change that leads to a lowered fidelity of replication, since the altered ε subunits resulting from this substitution (approximately 1% of the time) are sufficient to create a mutator effect, based on several observations of *mutD* alleles. In the present work, we extended the study of specific *mutD* alleles and constructed 16 altered *mutD* genes by replacing each aspartic acid codon, in series, with a glycine codon in the *dnaQ* gene that encodes ε. We show that three of these genes confer a strong mutator effect. We have also looked for new mutator tRNAs and have found one: a glycine tRNA that inserts glycine at histidine codons. We then replaced each of the seven histidine codons in the *mutD* gene with glycine codons and found that in two cases, a strong mutator phenotype results. These findings are consistent with the ε subunit playing a major role in the mutator effect of misreading tRNAs.

Mutator genes confer elevated rates of spontaneous mutations. Mutators have been used to help define DNA repair systems and to identify pathways of mutagenesis in prokaryotic as well as eukaryotic cells (24). The findings that certain human inherited cancer susceptibilities result from mutator phenotypes caused by defects in mismatch repair systems (5, 12, 18, 30) have sparked renewed interest in mutators. Defects in the mismatch repair genes lead to a strong mutator phenotype (25), as do combined defects in the GO system that repairs or prevents the incorporation of 7,8-dihydro-8-oxoguanidine in DNA (19, 20, 22). One of the strongest bacterial mutators in *Escherichia coli* results from a defect in the ε subunit of DNA polymerase III (Pol III), which is responsible for the editing function of Pol III (10, 31). The *mutD/dnaQ* gene encodes the ε subunit, and mutations in this gene result in the observed mutator effect. Some of these mutations give very strong effects; an example is the *mutD5* allele, which increases the mutation frequency as much as 10^5-fold in rich medium (6, 9). There are surprisingly few published sequence changes resulting from *mutD* mutations, and some of the published assignments have been disputed (15, 33).

Although mutators have been actively investigated for several decades, new pathways of mutagenesis are still being discovered. We recently described a novel pathway of mutagenesis mediated by mutator tRNAs that are encoded by the *E. coli* genes *mutA* and *mutC* (21, 32). We postulated that the mutator tRNA effect is exerted by mistranslation that generates a mutator polymerase, through an alteration in the ε subunit of DNA Pol III (32). The mutator tRNAs are glycine tRNAs with anticodons altered to read the aspartic acid codon and which insert glycine at approximately 1% efficiency. There are 16 aspartic acid codons in the ε subunit. In this study, we investigated their importance by replacing each of the 16 co-

dons, in series, with a glycine codon. Three cases resulted in a strong mutator. We also report the construction of an additional mutator tRNA that inserts glycine at histidine codons. We describe the replacement of seven histidine codons in the *mutD* gene with glycine codons and show that two of the constructed mutants are strong mutators. We discuss these results with regard to the mechanism of the mutator tRNAs and the primary structure of the ε subunit.

MATERIALS AND METHODS

Bacterial strains and methods. *E. coli* CC105 has been described by Michaels et al. (21) and Cupples and Miller (7). Strain XL2-Blue MRF′ (Stratagene, La Jolla, Calif.) was used for cloning. All genetic methods were as described in reference 23.

DNA synthesis and sequencing. Oligonucleotides were synthesized on a Beckman oligo1000 DNA synthesizer using solid-phase cyanoethyl phosphoramidite chemistry. All oligonucleotides were deprotected in ammonium hydroxide and used without further purification. DNA sequencing was carried out by using [α-³²P]dATP and a SequiTherm cycle sequencing kit (Epicentre Technologies, Madison, Wis.) with reagents supplied by the manufacturer. For sequencing cDNA clones we used PCR conditions as described by the manufacturer.

Cloning of the *mutD* gene. The DNA encoding *dnaQ/mutD* was PCR amplified from chromosomal DNA from CC105, isolated by standard procedures. Primers for amplification were based on sequences from GenBank (accession no. X04027, K00985, and M30201), and the gene, along with an extra 202 bp from the 5′ end and 28 bp from the 3′ end, was amplified. The 202-bp-long fragment from the 5′ end of the PCR-amplified DNA segment contained the promoter of the *mutD* gene and 137 bp of the 5′ end of the *rnh* gene. The conditions for PCR were as described previously (32), and we used PCR SuperMix (Gibco/BRL, Life Technologies, Gaithersburg, Md.). The PCR-amplified DNA segment was end polished by using chemicals and procedures supplied by Stratagene and blunt-end cloned into the *Pvu*II site of pBR329 (GenBank accession no. L08859 and J01753). After cloning, the entire insert was sequenced to ensure that there were no PCR-introduced point mutations.

Cloning of tRNA genes. The tRNA genes *glyV*, *glyW*, *glyU*, *glyT*, *tyrT*, *serU*, and *pheV* were cloned into the *Pvu*II site of pBR329 by the same strategy as used for cloning the *mutD* gene.

Site-directed mutagenesis of the *mutD* gene. We performed site-directed mutagenesis by overlap extension as described by Ho et al. (14). For each mutant, two primers complementary to both strands of the *mutD* gene were synthesized. Primers were complementary to the *mutD* sequence except for the desired nucleotide change: GA(T/C)→GG(T/C) for the Asp→Gly replacement and CA(T/C)→GG(T/C) for the His→Gly replacement. Primers covered about 10 to 15 bases from the targeted nucleotide codon on each side and always ended before

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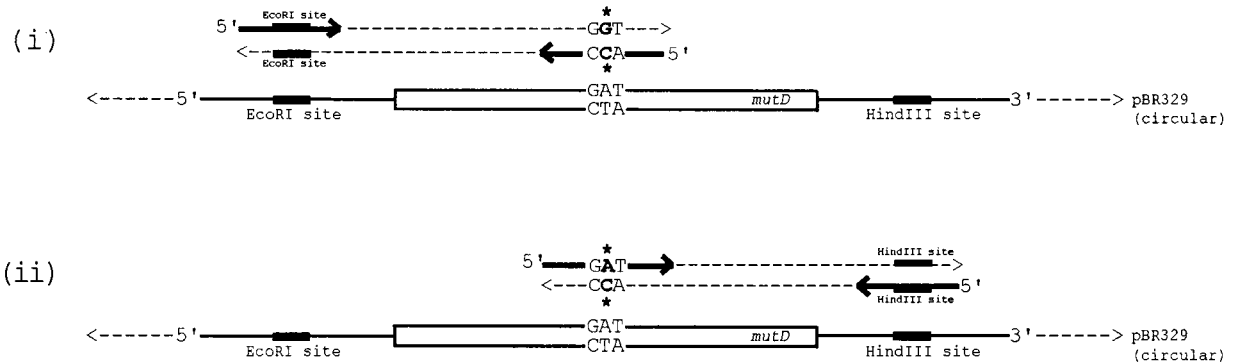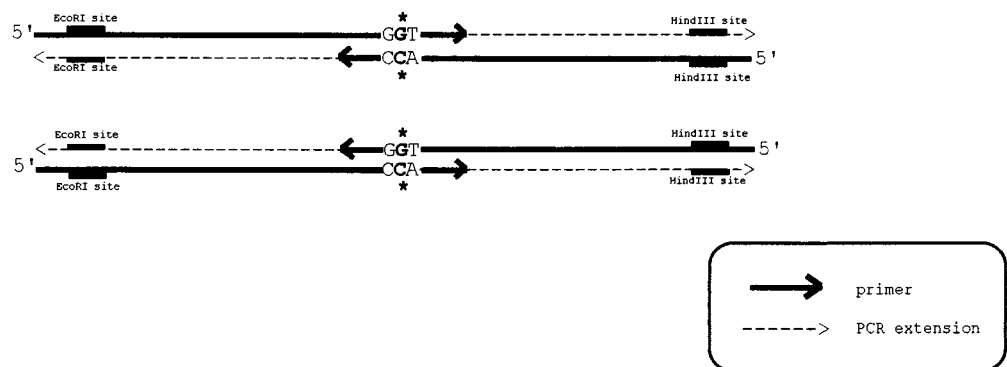
(a) First PCR

(b) Second PCR

FIG. 1. Strategy for site-directed mutagenesis. For each mutant, two primers complementary (except for a desired mutation) to the sequence of *mutD* were synthesized. We also made primers complementary to the sequence of pBR329 in the region of *Eco*RI and *Hin*dIII sites. In the first PCR, we obtained two fragments of the *mutD* gene, each containing the desired mutation. These fragments were agarose purified and used in the second PCR, in which the entire *mutD* gene (together with flanking pBR fragments) was amplified. The DNA obtained in the second PCR was digested with *Eco*RI and *Hin*dIII and cloned into pBR329.

an A in the sequence. In addition, we made two primers complementary to the opposite strands of pBR329 and covering the restriction sites *Eco*RI and *Hin*dIII that flank the *mutD* insert in the recombinant plasmid. These four primers were used in specific combinations of a two-step PCR amplification which yielded a DNA segment containing the mutated *mutD* gene flanked by the fragments of pBR329 with the *Eco*RI and *Hin*dIII sites (Fig. 1). The fragment was digested with *Eco*RI and *Hin*dIII and cloned into pBR329; each mutated clone was sequenced to ensure that the desired mutation was introduced and that there were no extra point mutations. Conditions for PCR amplification were as in reference 32. Plasmids containing mutated *mutD* genes were next transformed into CC105 to measure mutation frequencies.

Site-directed mutagenesis of tRNA genes. For site-directed mutagenesis of tRNA genes, we used the same strategy as for site-directed mutagenesis of the *mutD* gene. Since we had problems with recovery of all the site-directed tRNA genes we attempted to synthesize, we did three independent ligations for each site-directed mutagenized tRNA, for each ligation performed two transformations, and sequenced on average five clones for the tRNA.

Mutation frequency measurements. Mutation frequency measurements were determined as described in reference 23.

Nucleotide sequence accession numbers. The GenBank accessions numbers for the sequences of the tRNA genes cloned in this study are as follows: *glyV*, X53236; *glyW*, J01624; *glyU*, M54893 and M38664; *glyT*, J01717; *serU*, M10746 and M10748; *tyrT*, X90989, K01197, J01720, K01198, K01217, K01300, and M10704; and *pheV*, X52799.

RESULTS

***mutD* mutants constructed by replacement of aspartic acid with glycine.** In a previous report (32), we described a mutator effect of *E. coli* tRNAs that insert glycine in place of aspartic acid. We suggested that the effect is exerted by generating a mutator ε subunit of *E. coli* DNA Pol III. In this study, we investigated the number of crucial aspartic acids in the ε protein that are sensitive to substitution by glycine. Using site-directed mutagenesis (see Materials and Methods), we replaced each aspartic acid codon in the *mutD* gene with a glycine codon, constructing 16 *mutD* genes, each with a single amino acid substitution. The newly constructed *mutD* genes were cloned onto pBR329 plasmids and transformed into strain CC105, which carries a *lacZ* mutation that reverts via the AT→TA transversion (7). We compared the mutator phenotype of the constructed *mutD* genes by measuring the frequency of Rif[r] and Lac[+] mutants in strains harboring the plasmid with the *mutD* genes (Table 1). Of 16 *mutD* genes encoding different glycine-for-aspartic acid substitutions, three (D12G, D103G, and

TABLE 1. Mutation frequencies in strain CC105 harboring
pBR329-derived plasmids with cloned mutated *mutD* genes[a]

Strain	Mutation frequency (10^8)	
	Rif[r]	Lac[+]
Wild type	3.8	0.9
D12G	17,664.0	713.0
D55G	8.1	0.7
D59G	6.1	0.6
D70G	10.6	4.4
D75G	5.8	3.5
D84G	3.6	1.1
D88G	4.7	0.8
D103G	4,133.3	326.0
D108G	2.7	0.7
D117G	6.1	0.6
D129G	254.4	82.0
D146G	17.3	1.6
D155G	5.3	1.0
D167G	5,173.3	290.4
D219G	5.1	1.0
D230G	4.1	0.9

[a] Each mutant carries an altered *mutD* gene, which results in an aspartic acid-to-glycine change at the indicated amino acid position in the ε subunit. Between 4 and 10 single colonies of each strain were inoculated into LB medium with tetracycline or ampicillin and grown overnight. Samples were plated on LB medium with rifampin and on lactose minimal plates to measure mutants and on LB medium without rifampin to measure total viable cells. The Lac[+] revertants of CC105 strain result from AT→TA transversions (7).

D167G) resulted in strong mutator effects, with increases in Rif[r] frequency over the wild-type value on the order of 1,000- to 5,000-fold. D129G displayed a weaker effect. The same constructs also showed an increase in Lac[+] reversion, where the frequency of the AT→TA transversion at one specific site was measured (Table 1). Note that these effects are seen in the presence of the chromosomal wild-type *mutD/dnaQ* gene. Many *mutD* mutations are dominant in partial heterodiploids (6).

Construction of additional synthetic tRNA genes. We attempted to synthesize 20 different misreading tRNAs by altering the tRNA-encoding genes *glyW, glyV, gluU, glyT, tyrT, serU,* and *pheV*. However, we recovered only 13 of the desired constructs. In nine of these cases, the plasmids recovered after ligation contained different extra point mutations in the tRNA-encoding genes. The same extra point mutations were not recovered repeatedly. In seven other cases, we simply failed to recover any clones. It is possible that synthesizing fully expressed misreading tRNAs on a multicopy plasmid is lethal in many cases. Table 2 summarizes these results. We tested the mutator phenotype of each of the misreading tRNAs that we successfully recovered. In one case we found a phenotype similar to that previously described for the *mutA* and *mutC* genes. Here, the *glyU* gene was altered so that a glycine tRNA now recognizes histidine codons, inserting glycine in place of histidine. Although we found an extra point mutation in the promoter region (Fig. 2), it still displays a mutator effect in both the papillation assay (Fig. 3) and the Lac[+] reversion test in strain CC105 (49.9 ± 24.7 Lac[+] revertants/10^8 cells, compared with 0.81 ± 0.4 for CC105 cells with pBR). The mutator effect of the altered *glyU* tRNA was smaller (3.0 ± 0.5 Lac[+] revertants/10^8 cells) than in the case of the *mutC* mutator but still significant.

***mutD* mutants constructed by replacement of histidine with glycine.** We changed each histidine codon in the *mutD* gene to a glycine codon, generating seven additional *mutD* genes. After plasmids containing these genes were transformed into CC105,

we compared Rif[r] and Lac[+] mutation frequencies and found that two mutants (H98G and H162G) showed a strong mutator phenotype and one (H66G) displayed a very weak effect (Table 3).

DISCUSSION

In this report, we have described 23 mutants with an engineered change in the ε subunit of *E. coli* DNA Pol III. In each of 16 cases, one of the aspartic acid residues in the ε subunit was ultimately changed to glycine. This allowed us to determine whether any sites in this protein are sensitive to the exact substitution caused by miscoding tRNAs present in *mutA* and *mutC* strains. The altered tRNAs in *mutA* and *mutC* strains insert glycine at aspartic acid codons approximately 1% of the time and result in mutation rates about 1% of that found in *mutD* strains defective in the ε subunit. We postulated that the mutator effect seen in *mutA* and *mutC* strains was in fact due to generating 1% altered ε subunits. Murphy and Humayun (27) have shown that *mutA* and *mutC* strains also display a UVM (UV modulator of mutagenesis) phenotype, which results in an increase of mutagenesis at the site of a 3,N^4-ethenocytosine lesion in M13 single-stranded DNA transfected into *E. coli* and that the *mutA* and *mutC* mutator effect is *recA* dependent. They argued that the UVM phenotype could not result from a minor fraction (1%) of the ε subunits being altered to create a *mutD* effect. However, it is possible that the mutator effect of *mutA* and *mutC* does not occur by the same mechanism as the UVM effect. Moreover, Grollman and co-workers (26) have shown that a *mutD* strain indeed displays the UVM phenotype.

The dominance of many strong *mutD* mutator alleles (6) indicates that altered ε subunits can cause mutations even in the presence of normal subunits. Are there targets in the ε protein for the Asp-to-Gly change produced by *mutA* and *mutC* that would yield a mutator subunit? As Table 1 shows, there are three aspartic acid residues which yield a mutator ε subunit when exchanged for glycine.

We searched for other tRNAs which would give mutator effects, but despite the construction and examination of numerous tRNA genes with altered anticodons (Table 2), we detected a mutator effect in only one case, that of a *glyU* gene

TABLE 2. Miscoding tRNAs

Type of tRNA	Description
Constructed	Phe (*pheV*) reading Leu (UUG)
	Tyr (*tyrT*) reading Phe (UUC)
	Ser (*serU*) reading Leu (UUG)
	Phe (*pheV*) reading Thr (ACU)
Failed to construct	Ser (*serU*) reading Phe (UUC)
	Gly (*glyW*) reading Leu (CUC)
	Gly (*glyW*) reading His (CAC)
	Gly (*glyV*) reading Phe (UUC)
	Gly (*glyV*) reading Glu (GAA)
	Gly (*glyW*) reading Glu (GAA)
	Gly (*glyT*) reading Lys (AAG)
Constructed but carrying extra point mutations	Gly (*glyU*) reading His (CAC)
	Gly (*glyU*) reading Phe (UUC)
	Tyr (*tyrT*) reading His (CAC)
	Gly (*glyV*) reading His (CAC)
	Gly (*glyW*) reading Phe (UUC)
	Gly (*glyT*) reading Arg (CGA)
	Gly (*glyV*) reading Ser (AGC)
	Phe (*pheV*) reading Ala (GCC)
	Gly (*glyT*) reading Cys (UGU)

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glyU                                                              -35              -10
AAAATGCTGATGGCGAATTAATCAGCAGTCAGCAGCGTGGCACTTGCTAAGGAGAGCGTAAGGTTTATAATGCCTTACGCATCTCGAAGCGGGCGTAGTT


glyU reading His
AAAATGCTGATGGCGAATTAATCAGCAGTCAGCAGCGTGGCACTTGCTAAGGAGAGCGTAAGGTTTACAATGCCTTACGCATCTCGAAGCGGGCGTAGTT
```
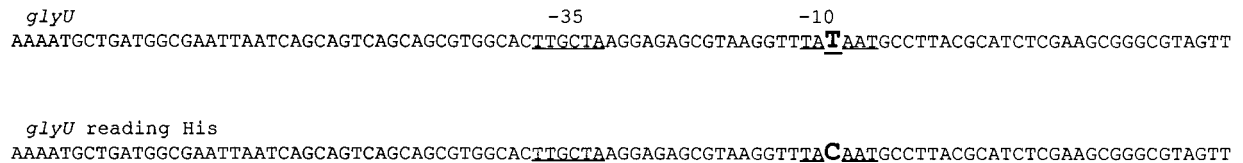
FIG. 2. The promoter region of the *glyU* gene and the change found in the *glyU* gene that reads histidine codons. The diagram of the sequence is adopted from reference 17.

altered so that the resulting tRNA now inserts glycine in place of histidine. The resulting mutator effect is somewhat weaker than that seen for *mutA* and *mutC* but is nonetheless significant (Results and Fig. 3). We therefore altered each of the codons specifying histidine in the *mutD* gene so that in each of seven cases the ε subunit would carry a glycine in place of one of the histidines. Table 3 shows that replacement of either of two histidines with glycine results in a strain with a strong mutator effect.

There are two explanations why the effect of a tRNA that inserts glycine in place of histidine is smaller than the effect of a tRNA which replaces aspartic acids with glycine. First, there are three strong *mutD* mutants made by replacement of aspartic acids with glycine and only two *mutD* mutants made by replacement of histidines with glycine, which means there are more targets resulting in the mutator phenotype for the tRNA inserting glycine in place of aspartic acid than for the tRNA inserting glycine in place of histidine. In addition, the aspartic acid replacement gives a stronger mutator, especially in the case of the D12G replacement (Table 1). Another explanation for the smaller mutator effect of the *glyU* reading histidine tRNA would be the point mutation in the promoter region of *glyU* gene that reads histidine, resulting in lower levels of this tRNA. The continued failure to clone this gene without secondary point mutations raises the possibility that the cell does not tolerate the elevated expression of this missense tRNA and that the only way to maintain the plasmid in the cell is through lowering of expression by a change in the promoter.

The results presented above shed some light on the conflicting interpretation of the primary structure of the ε subunit. The existence of three conserved motifs in a large number of polymerase-associated exonucleases (Exo motifs) was proposed by Bernad et al. (3) on the basis of the protein alignment of different proofreading polymerases with the Klenow fragment of *E. coli* Pol I, whose three-dimensional structure was elucidated by Steitz and coworkers (2, 13, 29) and Derbyshire et al. (8). Each Exo motif contains highly conserved amino acids

involved in the two-metal-ion catalysis reaction defined by Steitz and coworkers for the Klenow fragment of Pol I. For many proofreading polymerases, the role of critical amino acids in the three Exo motifs was confirmed by site-directed mutagenesis (for a review, see reference 16). For the ε subunit, the two main Exo I residues were predicted to be D12 and E14 (reference 3 and Fig. 4). Replacement of D12 and E14 with alanine results in an ε protein with a strong, dominant mutator phenotype, and cells containing the sole copy of the mutated gene (*dnaQ926*) are inviable unless they also carry a *dnaE* antimutator or a plasmid with the mismatch repair gene *mutL* (11). The *mutD5* allele, which produces a strong mutator effect, generates the T15I substitution and *mutD14* generates the T16I substitution (11, 15). In the work reported here, the D12G substitution resulted in a very strong mutator phenotype. For the Exo II motif, the crucial amino acids were predicted to be N99 and D103 (reference 3 and Fig. 4). As expected, both H98G and D103G turned out to be strong mutators (Table 1).

There are at least three different protein alignments of the Exo III region within the ε subunit. Two different regions of the ε protein have been proposed (3, 4), pointing to Y152 and D155 or V215 and D219, respectively (Fig. 4), as the residues that parallel Y497 and D501 of Exo III from the Klenow fragment. On the other hand, Barnes et al. (1) examined the structure of the Exo site of Pol III from *Bacillus subtilis* by site-directed mutagenesis and proposed the existence of an alternative Exo IIIε motif. They showed that three residues of this domain, His565, Asp533, and Asp574, are crucial for proofreading activity. These authors also point out that these residues are highly conserved among the Pol III Exo domains of three gram-positive bacteria, *B. subtilis*, *Staphylococcus aureus*, and *Mycoplasma pulmonis*, and that the same motif can be found in the ε subunit of *E. coli* Pol III (1).

For the *E. coli* ε protein, the critical amino acids of the Exo IIIε domain according to Bernad et al. (3) would be H162, D129, and D167 (Fig. 4). Our results support this hypothesis since all three changes (H162G, D129G, and D167G) diminish
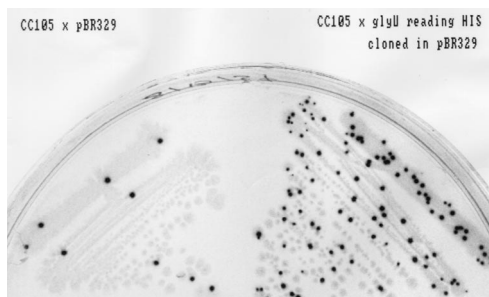
FIG. 3. Mutator phenotype of *glyU* that reads histidine codons. CC105 with plasmid pBR329 (control) and with plasmid pBR329 with the cloned altered *glyU* was plated on minimal glucose, P-Gal (phenyl β-D-galactoside), X-Gal (5-bromo-4-chloro-3-indolyl β-D-galactoside), and tetracycline plates. Single colonies of the strain with the altered *glyU* gene contain more blue papillae (28), since they form more Lac$^+$ revertants than the strain with pBR329 alone.

TABLE 3. Mutation frequencies in strain CC105 harboring pBR329 plasmids with cloned mutated *mutD* genes[a]

Strain	Mutation frequency (10^8)	
	Rif^r	Lac$^+$
Wild type	3.8	0.9
H24G	2.5	0.4
H28G	7.2	5.2
H49G	4.4	2.7
H66G	49.0	27.7
H98G	1,282.2	142.4
H162G	3,680.4	66.6
H225G	4.0	0.5

[a] Each mutant carries an altered *mutD* gene, which results in a histidine-to-glycine change at the indicated amino acid position in the ε subunit. For details, see the footnote to Table 1.

```
ATG AGC ACT GCA ATT ACA CGC CAG ATC GTT CTC GAT ACC GAA    42
 M   S   T   A   I   T   R   Q   I   V   L   D   T   E      14

ACC ACC GGT ATG AAC CAG ATT GGT GCG CAC TAT GAA GGC CAC    84
 T   T   G   M   N   Q   I   G   A   H   Y   E   G   H      28

AAG ATC ATT GAG ATT GGT GCC GTT GAA GTG GTG AAC CGT CGC   126
 K   I   I   E   I   G   A   V   E   V   V   N   R   R      42

CTG ACG GGC AAT AAC TTC CAT GTT TAT CTC AAA CCC GAT CGG   168
 L   T   G   N   N   F   H   V   Y   L   K   P   D   R      56

CTG GTG GAT CCG GAA GCC TTT GGC GTA CAT GGT ATT GCC GAT   210
 L   V   D   P   E   A   F   G   V   H   G   I   A   D      70

GAA TTT TTG CTC GAT AAG CCC ACG TTT GCC GAA GTA GCC GAT   252
 E   F   L   L   D   K   P   T   F   A   E   V   A   D      84

GAG TTC ATG GAC TAT ATT CGC GGC GCG GAG TTG GTG ATC CAT   294
 E   F   M   D   Y   I   R   G   A   E   L   V   I   H      98

AAC GCA GCG TTC GAT ATC GGC TTT ATG GAC TAC GAG TTT TCG   336
 N   A   A   F   D   I   G   F   M   D   Y   E   F   S     112

TTG CTT AAG CGT GAT ATT CCG AAG ACC AAT ACT TTC TGT AAG   378
 L   L   K   R   D   I   P   K   T   N   T   F   C   K     126

GTC ACC GAT AGC CTT GCG GTG GCG AGG AAA ATG TTT CCC GGT   420
 V   T   D   S   L   A   V   A   R   K   M   F   P   G     140

AAG CGC AAC AGC CTC GAT GCG TTA TGT GCT CGC TAC GAA ATA   462
 K   R   N   S   L   D   A   L   C   A   R   Y   E   I     154

GAT AAC AGT AAA CGA ACG CTG CAC GGG GCA TTA CTC GAT GCC   504
 D   N   S   K   R   T   L   H   G   A   L   L   D   A     168

CAG ATC CTT GCG GAA GTT TAT CTG GCG ATG ACC GGT GGT CAA   546
 Q   I   L   A   E   V   Y   L   A   M   T   G   G   Q     182

ACG TCG ATG GCT TTT GCG ATG GAA GGA GAG ACA CAA CAG CAA   588
 T   S   M   A   F   A   M   E   G   E   T   Q   Q   Q     196

CAA GGT GAA GCA ATT CAG CGC ATT GTA CGT CAG GCA AGT       630
 Q   G   E   A   I   Q   R   I   V   R   Q   A   S         210

AAG TTA CGC GTT GTT TTT GCG ACA GAT GAA GAG ATT GCA GCT   672
 K   L   R   V   V   F   A   T   D   E   E   I   A   A     224

CAT GAA GCC CGT CTC GAT CTG GTG CAG AAG AAA GGC GGA AGT   714
 H   E   A   R   L   D   L   V   Q   K   K   G   G   S     238

TGC CTC TGG CGA GCA TAA                                    732
 C   L   W   R   A   .                                     243
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FIG. 4. Nucleotide and amino acid sequences of the *mutD*/*dnaQ* gene and the ε protein. For the *mutD* nucleotide sequence, numbering is given at the right relative to the ATG translation start site (+1); the amino acid sequence of ε, given below in single-letter code, is also numbered at the right. Putative Exo I, Exo II (3), and two Exo III motifs (3, 4) are underlined; Exo IIIε (1) is underlined with a dotted line (the C-terminal homology of the ε subunit with gram-positive bacteria ends somewhere between E192 and V206 in the alignment of Barnes et al. [1]), and crucial amino acids in all putative Exo motifs are doubly underlined.

the proofreading activity of the ε subunit, whereas replacements of the amino acids (D155 and D219) as predicted by Bernad et al. (3) and Blanco et al. (4) do not affect the proofreading activity of ε. The results presented above are also in agreement with the work of Horner and Cox (15), who found that D167N and H162T replacements had a mutator effect. On the other hand, Horner and Cox have found a mutator *mutD* gene with the H225T replacement, whereas we found that H225G had no mutagenic effect (Table 3). All of the strong mutator effects described above are explained by changes in the Exo motifs of proofreading polymerases.

ACKNOWLEDGMENT

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