The Mutational Specificity of Two *Escherichia coli dnaE* Antimutator Alleles as Determined From *lacI* Mutation Spectra

Roel M. Schaaper

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

Manuscript received December 22, 1992
Accepted for publication April 5, 1993

ABSTRACT

In a companion study we have described the isolation of a series of mutants of *Escherichia coli* that replicate their DNA with increased fidelity. These mutants carry a mutation in the *dnaE* gene, encoding the α (polymerase) subunit of DNA polymerase III holoenzyme, which is responsible for the faithful replication of the bacterial chromosome. The mutants were detected as suppressors of the high mutability of a *mutL* strain (defective in postreplicative mismatch correction), in which mutations may be considered to arise predominantly from errors of DNA replication. To investigate the specificity of these antimutator effects, we have analyzed spectra of forward mutations in the N-terminal part of the *lacI* gene (i^{-d} mutations) for two of the *mutL dnaE* derivatives (*dnaE911* and *dnaE915*), as well as the control *mutL* strain. DNA sequencing of over 600 mutants revealed that in the *mutL* background both antimutator alleles reduce specifically transition mutations (A·T → G·C and G·C → A·T). However, the two alleles behave differently in this respect. *dnaE911* reduces A·T → G·C more strongly than it does G·C → A·T, whereas the reverse is true for *dnaE915*. Second, *dnaE911* does not appear to affect either transversion or frameshift mutations, whereas *dnaE915* displays a distinct *mutator* effect for both. This mutator effect of *dnaE915* for frameshift mutations was confirmed by the frequency of reversion of the *trpE9777* frameshift mutation. The discovery that *dnaE* antimutator alleles possess distinct specificities supports the notion that DNA polymerases discriminate against errors along multiple pathways and that these pathways can be influenced independently.

THE mechanisms by which cells faithfully replicate their DNA and, hence, maintain low mutation rates are of considerable interest. Extensive knowledge about this process is available in the bacterium *Escherichia coli*, where numerous genes and gene products involved in DNA replication fidelity have been characterized (COX 1976; MCHENRY 1988; ECHOLS and GOODMAN 1991). The replication of the chromosome is performed by DNA polymerase III holoenzyme, a dimeric complex composed of some 20 subunits (MCHENRY 1991). Two subunits in this complex are of particular importance to fidelity (MCHENRY 1988). The α subunit, encoded by the *dnaE* gene, is the polymerase subunit, which discriminates against errors by selecting against incorrect nucleotides during the insertion step. The ϵ subunit, encoded by the *dnaQ* gene, represents the 3′ → 5′ exonuclease activity, which performs a proofreading function, preferentially removing incorrect bases inserted by the polymerase subunit. Following DNA replication, the *mutHLS* mismatch-repair system scrutinizes the newly synthesized DNA, searching for and correcting base-pair mismatches, using the transient undermethylation (at GATC sequences) of the newly replicated strand to distinguish the correct from the incorrect half of the mismatch (for a review of mismatch repair, see MODRICH 1991).

In a companion study (FIJALKOWSKA, DUNN and SCHAAPER 1993) we have described the isolation and some of the properties of a series of mutants of *E. coli* carrying mutations in the *dnaE* gene. These mutants were isolated as suppressors of the high mutability of the mismatch repair-deficient *mutL* strain (FIJALKOWSKA, DUNN and SCHAAPER 1993). In a *mutL* strain, most mutations may be assumed to result from DNA replication errors, in contrast to a wild-type strain where mutations may originate from a variety of sources (SCHAAPER and DUNN 1991). On this basis we have argued that these new derivatives represent strains that replicate their DNA with increased fidelity and may be considered antimutators for DNA replication errors. It is our intention to use these strains for two purposes. One is to help further understand the precise mechanisms by which DNA polymerases select against errors, the other is to use replication-specific antimutators to dissect the role of DNA replication errors in a variety of mutagenic processes, including spontaneous mutation.

To understand how DNA polymerases discriminate against a variety of possible errors, it is important to ask what the specificity of such antimutators is. Because mutations in mismatch repair-deficient strains are largely transitions (SCHAAPER and DUNN 1987a),

the antimutators may be assumed to reduce transition errors. Whether other mutations, such as transversions, are also affected was initially addressed by studying the effect in a *mutT*-defective strain (FIJALKOWSKA, DUNN and SCHAAPER 1993). *mutT* mutators produce exclusively A·T → C·G transversions resulting from A·G mispairings during DNA replication (SCHAAPER and DUNN 1987b; AKIYAMA *et al.* 1989; MAKI and SEKIGUCHI 1992). Our results showed that the antimutators were capable of reducing the *mutT* mutator activity, indicating that the antimutators were also capable of reducing this type of error. On the other hand, experiments in a *recA730* background, where a mutator effect results from the constitutive induction of the SOS system (WITKIN *et al.* 1982; WALKER 1984) and mutations are likely to be transversions as well (MILLER and LOW 1984; YATAGAI, HALLIDAY and GLICKMAN 1991), showed little or no susceptibility of this type of mutagenesis to the antimutators (FIJALKOWSKA, DUNN and SCHAAPER 1993). Thus, antimutators appear to have a defined specificity which could depend on both the type of mutation and the genetic background in which it is generated.

To more systematically address the question of the specificity of the antimutator effects, we have analyzed by DNA sequencing the spectra of forward mutations in the *E. coli lacI* gene for two of the antimutator alleles, *dnaE911* and *dnaE915*. This was again done in the *mutL* background so that the results may be interpreted in terms of DNA replication errors. The analysis of these spectra, in comparison to that of the control *mutL* strain, should allow an assessment of the specificity of the antimutator effects. The *lacI* target used (the N-terminal region) allows detection of mutations at some 120 base-substitution sites, including all six base substitutions, in addition to frameshift, deletion and duplication mutations. The results show that in the *mutL* background the antimutator effects are largely limited to transition mutations. Furthermore, the two antimutators are quite distinct from each other. In one of them, both mutator and antimutator effects occur. The results may have significance for the mechanisms by which DNA polymerases discriminate against the various possible DNA replication errors.

MATERIALS AND METHODS

Strains and media: The strains used for recording the *lacI* spectra were NR9562 (*ara, thi, mutL*::Tn*5*, Δ*prolac*, F′*prolac*), NR9563 (as NR9562, but *dnaE911 zae502*::Tn*10*) and NR9566 (as NR9562, but *dnaE915 zae502*::Tn*10*). All strains are derived from KA796 (*ara, thi*, Δ*prolac*) (SCHAAPER, DANFORTH and GLICKMAN 1985). Into this strain *mutL*::Tn*5* was introduced by P1 transduction from donor strain ES1293 (SIEGEL *et al.* 1982). The *dnaE911* and *dnaE915* antimutator alleles were then introduced by P1 transduction using their linkage with transposon *zae502*::Tn*10* as described (FIJALKOWSKA, DUNN and SCHAAPER 1993). Transductants carrying the antimutator allele were detected by backcrossing *zae502*::Tn*10* into strain NR9360 and testing for antimutator phenotype using the papillation assay as described (FIJALKOWSKA, DUNN and SCHAAPER 1993). Finally, F′*prolac* (F′128-27), which provides the target for scoring *lacI* forward mutations and whose features have been described (SCHAAPER and DUNN 1991), was introduced by conjugation. NR3835 (*ara, thi, trpE9777*, Δ*prolac*, F′*prolac*) has been described (GLICKMAN 1979; TODD and GLICKMAN 1982). Its derivatives NR9464 (*mutL*::Tn*5*), NR9465 (*mutL*::Tn*5 dnaE911 zae502*::Tn*10*) and NR9466 (*mutL*::Tn*5 dnaE915 zae502*::Tn*10*) were constructed as described above for the KA796 derivatives. Strains CSH52 and S90C, used for the genetic analysis and sequencing of *lacI* mutants, have been described (SCHAAPER, DANFORTH and GLICKMAN 1985, 1986; SCHAAPER and DUNN 1987a).

LB broth and minimal media (MM) were as described (SCHAAPER, DANFORTH and GLICKMAN 1985). When needed, antibiotics were added as follows: kanamycin 25 μg/ml; tetracycline 15 μg/ml; streptomycin 100 μg/ml; rifampicin 100 μg/ml; and nalidixic acid 40 μg/ml. Phenyl-β-D-galactopyranoside (Pgal) (Bachem Fine Chemicals) plates used to select *lacI* mutants contained 750 μg/ml Pgal as sole carbon source.

Mutant frequency determinations: To determine the frequency of rifampicin-resistant, nalidixic-acid-resistant, and *lacI* mutants, strains NR9562, NR9563 and NR9566 were each grown as 18 independent 1-ml cultures started from circa 500 cells in LB plus kanamycin. After growth overnight at 37°, aliquots of appropriate dilutions were plated on LB or MM plates to determine the numbers of viable cells, and on LBRif, LBNal and Pgal plates to determine the numbers of mutants. To determine the frequency of *trpE9777* revertants, strains NR3835, NR9464, NR9465 and NR9466 were each grown as 15 independent cultures started by toothpicking individual colonies in 1 ml LB plus appropriate antibiotic and grown overnight at 37° while shaking. The cultures were centrifuged, resuspended in 1 ml 0.9% NaCl and plated in appropriate dilutions on MM without tryptophan to determine the number of Trp^+ revertants and on MM plus tryptophan to determine the total number of cells.

Collection of *lacI* mutants and DNA sequencing: For each of the three strains NR9662, NR9563 and NR9566, three independent isolates (transductants) were grown to saturation in LB plus kanamycin. Each culture was diluted in LB plus kanamycin to ~500/ml and distributed in 200-μl aliquots over the wells of four 96-well microtiter dishes. After overnight growth at 37° on a shaking platform, 5 μl from each well was spread on a quarter section of a Pgal plate. On these plates only mutants that constitutively express the *lac* operon form colonies. Plates were incubated for 60 hr at 37°. One mutant was taken from each quarter section and toothpicked in a gridded pattern on a Pgal plate (384 mutants per culture, 1152 per strain). After overnight growth these plates were replica-mated into strain CSH52 to identify the dominant (negatively complementing) mutants, as described (SCHAAPER and DUNN 1987a, 1991). For each strain, 300 dominant mutants were regridded and replica-mated into strain S90C. The resulting conjugants were infected with phage mRS81 as described elsewhere (SCHAAPER, DANFORTH and GLICKMAN 1985, 1986). Recombinant phage, carrying the mutant *lacI* (or *lacO*) genes, were purified and their DNA sequenced as described (SCHAAPER, DANFORTH and GLICKMAN 1985, 1986).

RESULTS

The *lacI* system: The specificity of the antimutator effects was investigated by DNA sequence analysis of

TABLE 1

Mutant frequencies for *mutL*, *mutL dnaE911* and *mutL dnaE915* strains (per 10^8 cells)

Strain	rif^R	nal^R	*lacI*$^-$
mutL	12 ± 5	2.8 ± 1.6	101 ± 31
mutL dnaE911	3.8 ± 0.8 [3.2]	0.45 ± 0.24 [6.2]	32 ± 13 [3.2]
mutL dnaE915	7.2 ± 1.5 [1.7]	0.28 ± 0.15 [10]	56 ± 14 [1.8]

Values within brackets indicate the fold reduction in frequency compared to the *mutL* strain. Frequencies (±SD) are the averages for 18 independent cultures.

a large number of mutations arising in the bacterial *lacI* gene as a mutational target. The *lacI* gene encodes the repressor of the *lac* operon and provides a forward mutational target in which many types of mutations can be scored (FARABAUGH *et al.* 1978; SCHAAPER, DANFORTH and GLICKMAN 1986). Because the gene is relatively large (1100 bp), the DNA sequence analysis is often restricted to mutations in the N-terminal (DNA-binding) domain of the protein. This provides a DNA target of convenient size (~240 bp) that contains a high density of detectably mutable sites (SCHAAPER and DUNN 1991). Mutants residing in the N-terminal domain can be scored by means of a genetic complementation test in which they are distinguished by their dominance over *lacI*$^+$ (MILLER 1978). The dominant mutants (termed i^{-d}) are then crossed to the single-stranded phage vector mRS81 (SCHAAPER, DANFORTH and GLICKMAN 1985, 1986) and sequenced.

From the series of seven antimutators (FIJALKOWSKA, DUNN and SCHAAPER 1993), two (*dnaE911* and *dnaE915*) were selected for spectral analysis because they presented the most consistent antimutator effects when inspecting different mutational markers (FIJALKOWSKA, DUNN and SCHAAPER 1993). The spontaneous mutant frequencies obtained with these alleles in the *mutL* background are presented in Table 1. The two *dnaE* alleles reduced the mutant frequency for Rif^R (2–3-fold) and Nal^R (6–10-fold). The *lacI* forward frequencies were reduced 3.2–1.8-fold for *dnaE911* and *dnaE915*, respectively. Whereas Rif^R and Nal^R represent small targets where only a limited number of DNA sequence changes can be detected, the *lacI* gene presents a large mutational target. Thus, the antimutator effects, while moderate in overall magnitude, appear to be broadly based and to apply to large forward targets as well. The latter may be contrasted to antimutators isolated in bacteriophage T4, which, although strong antimutators for specific reversion pathways, do not display significant effects in a forward system (DRAKE 1993).

DNA sequence analysis: The test for dominance among the *lacI* mutants revealed an essentially identical proportion of dominant mutants for the three strains (35%, 36% and 36%). Two hundred and fifty-two independent i^{-d} mutants from the control *mutL* strain and 200 i^{-d} mutants from each of the antimutator derivatives were taken for DNA sequence analyses. The results of the sequencing effort are summarized in Table 2. For all three strains the mutations are virtually exclusively base substitutions and frameshifts. The three strains differ, however, in their ratio of base substitutions to frameshifts, which is reduced in the antimutator strains. The ratios are 13.4 for the *mutL* strain, 2.7 for the *mutL dnaE911* strain, and 1.7 for the *mutL dnaE915* strain.

TABLE 2

Summary of i^{-d} mutations in *mutL*, *mutL dnaE911* and *mutL dnaE915* strains

Mutation	*mutL*	*mutL dnaE911*	*mutL dnaE915*
Base substitution	228	143	122
Transition			
A·T → G·C	126	70	72
G·C → A·T	98	65	34
Transversion			
A·T → C·G	2	5	9
A·T → T·A	1	1	1
G·C → T·A	0	0	2
G·C → C·G	1	2	4
Frameshift	17	53	72
Deletion	0	1	0
Duplication	0	0	1
Total	245	197	195

Included are three cases where a double mutation was observed. For the mutL strain, there were two double mutants (A → G at 145 and C → T at 174; G → A at 185 and G → A at 197). The mutations at positions 145 and 197 have not been observed as single mutations (among >4000 sequenced mutants) and are considered silent. They were not included in further analyses. For the *mutL dnaE911* strain there was one case of a double-frameshift mutation (see Table 3). No mutation was found in case of nine, four, and five mutants for the three strains, respectively.

The complete base-substitution spectra are presented in Figure 1. The spectrum in the *mutL* strain is strongly dominated by transitions (A·T → G·C and G·C → A·T), with only 1.8% being transversions, consistent with earlier results (SCHAAPER and DUNN 1987a). However, larger percentages of transversions are observed for the two antimutator strains (5.6% for the *dnaE911* strain, 13.1% for the *dnaE915* strain). A further distinction between the three strains is the ratio of A·T → G·C and G·C → A·T transitions. Consistent with a previous study (SCHAAPER and DUNN 1987a), A·T → G·C transitions are more frequent than G·C → A·T (125/97 = 1.29) in the *mutL* strain. In the two antimutator strains this ratio is either reduced (*dnaE911*: 70/65 = 1.08) or enhanced (*dnaE915*: 72/34 = 2.11).

Table 3 tabulates the frameshift mutations. All frameshifts represented the loss of a single base. [Note that (+1) frameshifts are generally not detected as dominant mutations (SCHAAPER and DUNN 1991)]. As before (SCHAAPER and DUNN 1987a), a frameshift hotspot exists at the run of five A·T base pairs (nucleotides 135–139), which is present in each of the three strains (50–60% of all frameshifts). For each of the

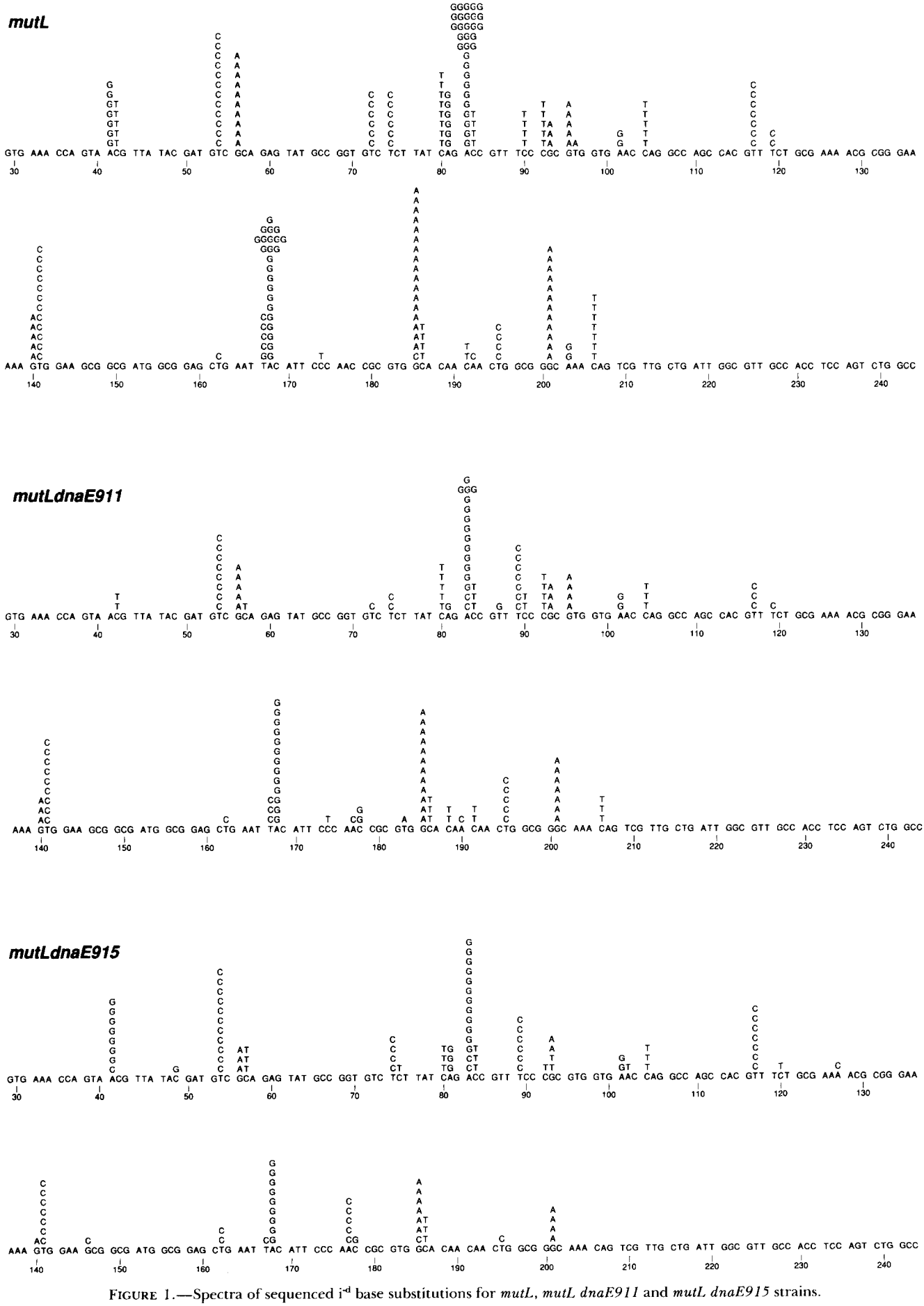

FIGURE 1.—Spectra of sequenced i[d] base substitutions for *mutL*, *mutL dnaE911* and *mutL dnaE915* strains.

TABLE 3

i^{-d} frameshift mutations in *mutL*, *mutL dnaE911* and *mutL dnaE915* strains

Nucleotide	Mutation	Strain		
		mutL	*mutL dnaE911*	*mutL dnaE915*
40–41	–A	0	1	1
44–45	–T	0	1	0
65	–G	0	0	1
66–67	–C	1	0	0
68–69	–G	0	0	1
76–77	–T	0	1	0
78	–A	0	0	1
87–89	–T	1	3	4
90–92	–C	0	0	5
93	–G	0	1	0
101–102	–A	0	0	2
105	–A	0	1	0
108–109	–C	2	0	4
110	–A	0	0	1
117–119	–T	0	1	0
120	–C	0	2[a]	0
125–128	–A	1	1	5
132–134	–G	1	2	2
135–139	–A	9	32[a]	36
144–145	–A	0	2	1
148–149	–G	0	0	2
173–175	–C	0	2	0
183	–T	0	0	1
189–190	–A	0	0	1
196–197	–G	0	1	0
199–201	–G	1	2	2
203–205	–A	0	0	1
207	–A	0	0	1
241–242	–G	1	0	0
Total		17	53	72

[a] One mutant contained two frameshift mutations: –C at 120 and –A at 135–139.

TABLE 4

Frequencies for each of the classes of mutations in *mutL*, *mutL dnaE911* and *mutL dnaE915* strains (per 10^6 cells)

Class	*mutL*	*mutL dnaE911*	*mutL dnaE915*
i^{-d}	37	12 (3.1)	20 (1.9)
Base substitutions	35	8.7 (4.0)	13 (2.7)
Transitions	34	8.2 (4.1)	11 (3.1)
A·T → G·C	19	4.2 (4.5)	7.4 (2.6)
G·C → A·T	15	3.9 (3.8)	3.5 (4.3)
Transversions	0.61	0.48 (1.3)	1.6 (0.38)
Frameshifts	2.6	3.2 (0.81)	7.4 (0.35)

Frequencies were calculated by multiplying the overall *lacI* frequencies (Table 1) by the fraction of dominant mutations (0.35, 0.36 and 0.36, respectively) and the fraction of the respective mutations derived from Table 2. The numbers in parentheses indicate the decrease compared to the *mutL* strain (*i.e.*, the strength of the antimutator effect). A factor lower than indicates a mutator effect.

TABLE 5

Effect of *dnaE* alleles on *trpE9777* frameshift reversion

Experiment	Strain	Trp^+ (per 10^8 cells)
1	Wild type	0.0046
	mutL	6.0
	mutL dnaE911	5.4
	mutL dnaE915	19.4 (3.2)
2	*mutL*	5.0
	mutL dnaE911	5.9
	mutL dnaE915	20.9 (4.1)

Strains were NR3835 (=wild type) with the indicated *mut* or *dnaE* alleles. In parentheses, the fold increase (mutator effect) compared to the *mutL* level.

three frameshift spectra, the majority (>90%) is associated with a run of at least two identical base pairs. As a whole, no major differences among the three frameshift spectra are apparent.

Specificity of the antimutator effects: The data presented above indicate significant differences in the composition of the three *lacI* spectra, most notably in the ratio of base substitutions to frameshifts, the ratio of transitions to transversions, and the ratio of A·T → G·C to G·C → A·T transitions. Thus, the antimutators do not lower mutation spectra across the board, but possess a defined specificity. To further evaluate this specificity, absolute frequencies were calculated for each class of mutation. From these, the ratio of frequencies (*mutL/mutL dnaE*) was calculated to reveal the specific antimutator effect for each class (Table 4).

For both *dnaE* alleles, both transitions are reduced, although not equally (2.6–4.5-fold). In the case of *dnaE911*, A·T → G·C transitions are reduced more strongly than G·C → A·T transitions, while in case of *dnaE915* the reverse is true. In contrast, no antimutator effects are apparent for the transversions (the four types of transversions are combined), although the limited number of transversions observed in the *mutL* background makes this conclusion tentative. Interestingly, in the case of *dnaE915* transversions are enhanced 2.6-fold. A similar situation exists for the frameshift mutations. *dnaE911* shows no significant effect on the frameshift frequency, whereas *dnaE915* displays a 2.9-fold mutator effect. Thus, the antimutator effects for the two *dnaE* alleles appear specific for transition errors. In addition, *dnaE915* shows a mutator activity for both transversions and frameshifts.

Reversion of the *trpE9777* frameshift allele: To independently confirm the above observations on frameshift mutations, we measured the effect of the two *dnaE* alleles on the reversion of the *trpE9777* mutation. In this mutant, an extra A·T base pair has been added to a run of five A·T base pairs (BRONSON and YANOFSKY 1974), and reversion to tryptophan independence occurs by loss of the extra pair (SIEGEL and VACCARO 1978; GLICKMAN 1979). Table 5 shows that also in this case *dnaE911* does not affect the *mutL*-induced reversion frequency, while *dnaE915* clearly enhances it, confirming the results from the *lacI* spectra.

DISCUSSION

The data presented in this paper define the specificity of the antimutator effect for two *E. coli* strains that are characterized by a reduced level of DNA replication errors. Their spectra of forward mutations in the *lacI* gene were recorded in the mismatch repair-deficient *mutL* background. The data may therefore be interpreted in terms of the specific effects on DNA replication errors. The information on the specificity of the antimutators should provide insights into the mechanisms by which DNA polymerases discriminate against replication errors. The data show that the two tested antimutator alleles have discrete specificities, as discussed below.

The specificity of the antimutator effects: The reduction in replication errors by the two investigated antimutators is specific for the class of transition errors, no reductions being apparent for transversions and frameshifts. The reduction in transitions is not unexpected in view of the method used to isolate the *dnaE* alleles, namely as suppressors of the high mutability of a strain defective in mismatch repair (FIJALKOWSKA, DUNN and SCHAAPER 1993). In such strains, the majority of mutations are transitions (CHOY and FOWLER 1985; SCHAAPER and DUNN 1987a). More precisely, the antimutators were isolated by scoring the reversion rate of the *galK2* marker which reverts in the *mutL* background specifically via an A·T → G·C transition (OLLER, FIJALKOWSKA and SCHAAPER 1993). Therefore, by necessity, all isolates are likely antimutators for this transition. Whether other base substitutions (including the reciprocal G·C → A·T) or non-base-substitution mutations are reduced as well, remained to be answered. The observation that, in the *mutL* background, the two antimutators reduce both G·C → A·T and A·T → G·C transitions, but not the transversions or frameshifts, suggests that the two transitions have aspects in common that are not shared by the transversions or frameshifts.

Interestingly, antimutator effects were observed in case of the *mutT* mutator (FIJALKOWSKA, DUNN and SCHAAPER 1993), indicating that the specific A·T → C·G transversions in this strain are subject to the antimutator effect. Recent data (MAKI and SEKIGUCHI 1992) have indicated that the A·G mispairing that creates this transversion involves a modified substrate (8-oxo-dGTP), and this result may therefore not be characteristic for transversions in general. Our data suggest that (i) the mechanism by which the DNA polymerase discriminates against the *mutT*-induced A·T → C·G transversion has aspects in common with that for the transitions, and (ii) that this mechanism is distinct from those discriminating against other transversion errors.

A second point of interest is that the two antimutators are in fact quite distinct from each other. First, the two transitions are differentially affected: *dnaE911* reduces A·T → G·C slightly more efficiently than G·C → A·T (4.5- *vs.* 3.8-fold), whereas *dnaE915* does the opposite (2.6- *vs.* 4.3-fold). Second, a 2.8-fold transversion mutator effect was found for *dnaE915*. Third, a (−1) frameshift mutator effect was observed for *dnaE915* (2.9-fold for frameshifts in the *lacI* gene and 3.2- to 4.1-fold for reversion of the *trpE* frameshift marker). Clearly, the mechanisms by which the DNA polymerase discriminates against the various types of errors can be affected differentially. Further, a single amino acid substitution is capable of reducing one set of pathways, while enhancing another.

Antimutators in T4 DNA polymerase: Antimutators are known in bacteriophage T4, and the responsible mutations have been shown in nearly all cases to reside in the gene encoding the DNA polymerase (DRAKE *et al.* 1969; REHA-KRANTZ 1988). The strongest antimutators reside in the C-terminal region of the protein (REHA-KRANTZ 1988) and some display increased apparent exonuclease-to-polymerase activity ratios (MUZYCZKA, POLAND and BESSMAN 1972; GILLIN and NOSSAL 1976a; CLAYTON *et al.* 1979). Specificity studies (DRAKE *et al.* 1969; RIPLEY 1975, 1982; RIPLEY, GLICKMAN and SHOEMAKER 1983; DRAKE 1993) have revealed exceptionally strong (several orders of magnitude) antimutator effects for A·T → G·C transitions, but little effect, or even weak mutator effects, for G·C → A·T transitions or any of the transversions. Frameshift mutations occurring in runs of identical bases were generally reduced, whereas those in non-runs were either not affected or increased. This highly specific character of the T4 antimutator alleles was confirmed using a forward mutational assay, in which all tested T4 antimutators proved noneffective in reducing overall mutation rates or were, in fact, weak mutators (DRAKE 1993). Thus, both similarities and differences between the T4 and the *E. coli* antimutators are apparent. The similarity is that in both cases antimutator effects are observed for one group of mutations but not for others, while the dissimilarities pertain to the precise specificities. The T4 antimutators are highly specific for A·T → G·C transitions, whereas the *dnaE* antimutators reduce both A·T → G·C and G·C → A·T. It is possible that these dissimilarities reflect differences in the mechanisms by which the different DNA polymerases discriminate against errors. DNA polymerases from different sources, as determined from *in vitro* DNA polymerase fidelity assays, display quite different error specificities (KUNKEL and ALEXANDER 1986; KUNKEL 1992).

Base selection *vs.* proofreading: The observation that antimutators possess defined specificities has two additional consequences. First, it serves as a strong additional argument against the possibility that these strains would be affected in their ability to express mutant phenotypes rather than possessing a true an-

timutator phenotype (FIJALKOWSKA, DUNN and SCHAAPER 1993). Second, it might be used to gain insights into the possible mechanism(s) by which the antimutator effect is exerted. Three general possibilities have been suggested (FIJALKOWSKA, DUNN and SCHAAPER 1993): increased DNA polymerase accuracy at the level of base selection (insertion step) or increased exonucleolytic proofreading. The latter could take place either directly (because the polymerase might actively stimulate proofreading) or indirectly (because the mutant polymerase might be somehow catalytically slowed which, by default, would permit increased time for proofreading). In deciding among these models, the observed specificity of the antimutator alleles should be taken into account. Analysis of *lacI* mutational spectra in proofreading-deficient *E. coli* (SCHAAPER 1988) in addition to studies with purified polymerases (*e.g.*, SINHA 1987; BEBENEK *et al.* 1990) have indicated that many classes of mutations (including transitions, transversions and frameshifts) are subject to proofreading. Furthermore, recent data from our laboratory (I. FIJALKOWSKA, unpublished data) demonstrate that the present *dnaE* alleles are also capable of reducing the mutability of proofreading-deficient *dnaQ49* mutator strains (TAKANO *et al.* 1986). Thus, these observations might be used to argue that the present antimutator effects do not simply result from enhanced proofreading and that, instead (or in addition), mechanisms including improved base selection should be considered (see for example, HALL *et al.* 1985). A similar situation may exist for the T4 antimutator DNA polymerases, whose specificity is unique in that they exclusively reduce A·T → G·C transitions (DRAKE 1993). Impaired strand displacement leading to enhanced exonuclease-to-polymerase ratios has been suggested as on explanation to account for the antimutator effects (GILLIN and NOSSAL 1976a,b; REHA-KRANTZ 1988). However, since proofreading by T4 DNA polymerase presumably affects many types of errors (*e.g.*, SINHA 1987), more complex explanations may be required.

Antimutator effects and the kinetics of DNA polymerization: A possible explanation for antimutator specificity may result from a consideration of the kinetics of DNA replication, with which the fidelity of DNA polymerization is tightly intertwined (ECHOLS and GOODMAN 1991; EGER and BENKOVIC 1992). In the best studied case, *E. coli* DNA polymerase I (Klenow fragment), discrimination against incorrect nucleotides proceeds at several different levels, including (i) the binding of dNTPs to the enzyme-DNA complex, (ii) the rate of the chemical bond formation, (iii) a conformational change that follows product formation and (iv) extension of the new primer terminus. The first two steps comprise the insertion fidelity, while the last two contribute to fidelity through the exonuclease pathway (because of their slower rates for incorrectly inserted nucleotides). However, it is unlikely that all mutational intermediates (such as the 12 different mispairs responsible for the six possible base substitutions) traverse the cycle of discriminating steps in the same quantitative manner. For example, experiments with a derivative of Klenow fragment in which the invariant residue Tyr766 is replaced by a serine residue (POLESKY *et al.* 1990; CARROLL, COWART and BENKOVIC 1991) have revealed that this derivative displays increased incorporation of incorrect nucleotides, *i.e.*, a *mutator* effect at the insertion level [steps (i) and (ii) above]. However, the conformational change following the chemical step and the extension from the mismatched primer [steps (iii) and (iv) above] are slowed, suggesting that there is likely to be an *antimutator* effect during the proofreading steps. Thus, both mutator and antimutator effects could occur, the net effect depending on the balance of the two.

Perspectives: We intend to continue the analysis of DNA polymerase III by attempting to link defined amino acid substitutions with defined changes in the specificity of mutagenesis. We have recently isolated an additional set of antimutators in the *dnaE* gene (J.-Y. MO and R. M. SCHAAPER, unpublished data) and are currently analyzing their specificity. The amino acid changes responsible for these antimutator alleles are known (FIJALKOWSKA and SCHAAPER 1993, and unpublished data). We expect that the combined information on amino acid changes and specificity, in conjunction with increased insight into the structure of DNA polymerases, will contribute to a more detailed understanding of the mechanisms by which DNA polymerases achieve high fidelity of DNA synthesis.

I thank RONNIE DUNN for his assistance in this project. K. BEBENEK, J. DRAKE, I. FIJALKOWSKA, T. KUNKEL, A. OLLER and Y. PAVLOV are thanked for helpful comments on the manuscript.

LITERATURE CITED

AKIYAMA, M., H. MAKI, M. SEKIGUCHI and T. HORIUCHI, 1989 A specific role of MutT protein: to prevent dG·dA mispairing in DNA replication. Proc. Natl. Acad. Sci. USA **86:** 3949–3952.

BEBENEK, K., C. M. JOYCE, M. P. FITZGERALD and T. A. KUNKEL, 1990 The fidelity of DNA synthesis catalyzed by derivatives of *Escherichia coli* DNA polymerase I. J. Biol. Chem. **265:** 13878–13887.

BRONSON, M. J., and C. YANOFSKY, 1974 Characterization of mutations in the tryptophan operon of *Escherichia coli* by RNA nucleotide sequencing. J. Mol. Biol. **88:** 913–916.

CARROLL, S. S., M. COWART and S. J. BENKOVIC, 1991 A mutant of DNA Polymerase I (Klenow fragment) with reduced fidelity. Biochemistry **30:** 804–813.

CHOY, H. E., and R. G. FOWLER, 1985 The specificity of base-pair substitution induced by the *mutL* and *mutS* mutators in *E. coli*. Mutat. Res. **142:** 93–97.

CLAYTON, L. K., M. F. GOODMAN, E. W. BRANSCOMB and D. J. GALAS, 1979 Error induction and correction by mutant and wild type T4 DNA Polymerases. Kinetic error discrimination mechanisms. J. Biol. Chem. **254:** 1902–1912.

COX, E. C., 1976 Bacterial mutator genes and the control of

spontaneous mutation. Annu. Rev. Genet. **10:** 135–156.

DRAKE, J. W., 1993 General antimutators are improbable. J. Mol. Biol. **229:** 8–13.

DRAKE, J. W., E. F. ALLEN, S. A. FORSBERG, R.-M. PREPARATA and E. O. GREENING, 1969 Genetic control of mutation rates in bacteriophage T4. Nature **221:** 1128–1132.

ECHOLS, H., and M. F. GOODMAN, 1991 Fidelity mechanisms in DNA replication. Annu. Rev. Biochem. **60:** 477–511.

EGER, B. T., and S. J. BENKOVIC, 1992 Minimal kinetic mechanism for misincorporation by DNA polymerase I (Klenow fragment). Biochemistry **31:** 9227–9236.

FARABAUGH, P. J., U. SCHMEISSNER, M. HOFER and J. H. MILLER, 1978 Genetic studies of the *lac* repressor. VII. On the molecular nature of spontaneous hotspots in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **126:** 847–863.

FIJALKOWSKA, I. J., R. L. DUNN and R. M. SCHAAPER, 1993 Mutants of *Escherichia coli* with increased fidelity of DNA replication. Genetics **134:** 1023–1030.

FIJALKOWSKA, I. J., and R. M. SCHAAPER, 1993 Antimutator mutations in the α subunit of *Escherichia coli* DNA polymerase III: Identification of the responsible mutations and alignment with other DNA polymerases. Genetics **134:** 1039–1044.

GILLIN, F. D., and N. G. NOSSAL, 1976a Control of mutation frequency by bacteriophage T4 DNA polymerase. I. The CB120 antimutator DNA polymerase is defective in strand displacement. J. Biol. Chem. **251:** 5219–5224.

GILLIN, F. D., and N. G. NOSSAL, 1976b Control of mutation frequency by bacteriophage T4 DNA polymerase. II. Accuracy of nucleotide selection by the L88 mutator, CB120 antimutator and wild type phage T4 DNA Polymerases. J. Biol. Chem. **251:** 5225–5232.

GLICKMAN, B. W., 1979 Spontaneous mutagenesis in *Escherichia coli* strains lacking 6-methyladenine residues in their DNA. An altered mutational spectrum in *dam*$^-$ mutants. Mutat. Res. **61:** 153–162.

HALL, J. D., FURMAN, P. A., M. H. ST. CLAIR and C. W. KNOPF, 1985 Reduced *in vivo* mutagenesis by mutant herpes simplex DNA Polymerase involves improved nucleotide selection. Proc. Natl. Acad. Sci. USA **82:** 3889–3893.

KUNKEL, T. A., 1992 DNA replication fidelity. J. Biol. Chem. **267:** 18251–18254.

KUNKEL, T. A., and P. S. ALEXANDER, 1986 The base substitution fidelity of eucaryotic DNA polymerases. Mispairing frequencies, site preferences, insertion preferences and base substitution by dislocation. J. Biol. Chem. **261:** 160–166.

MAKI, H., and M. SEKIGUCHI, 1992 MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature **355:** 273–275.

MCHENRY, C. S., 1988 DNA polymerase III holoenzyme of *Escherichia coli*. Annu. Rev. Biochem. **57:** 519–550.

MCHENRY, C. S., 1991 DNA polymerase III holoenzyme. Components, structure and mechanism of a true replicative complex. J. Biol. Chem. **266:** 19127–19130.

MILLER, J. H., 1978 The *lacI* gene: its role in *lac* operon control and its use as a genetic system, pp. 31–88 in *The Operon*, edited by J. H. MILLER and W. S. REZNIKOFF. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.

MILLER, J. H., and K. B. LOW, 1984 Specificity of mutagenesis resulting from the induction of the SOS system in the absence of mutagenic treatment. Cell **37:** 675–682.

MODRICH, P., 1991 Mechanisms and biological effects of mismatch repair. Annu. Rev. Genet. **25:** 229–253.

MUZYCZKA, N., R. L. POLAND and M. H. BESSMAN, 1972 Studies on the biochemical basis of spontaneous mutation. I. A comparison of the deoxyribonucleic acid polymerases of mutator, antimutator and wild type strains of bacteriophage T4. J. Biol. Chem. **247:** 7116–7122.

OLLER, A. R., I. J. FIJALKOWSKA and R. M. SCHAAPER, 1993 The *Escherichia coli galK2* papillation assay: its specificity and application to seven newly isolated mutator strains. Mutat. Res. (in press).

POLESKY, A. H., T. A. STEITZ, N. D. F. GRINDLEY and C. M. JOYCE, 1990 Identification of residues critical for the polymerase activity of the Klenow fragment of DNA polymerase I from *Escherichia coli*. J. Biol. Chem. **265:** 14579–14591.

REHA-KRANTZ, L. J., 1988 Amino acid changes coded by bacteriophage T4 DNA Polymerase mutator mutants. Relating structure to function. J. Mol. Biol. **202:** 711–724.

RIPLEY, L. S., 1975 Transversion mutagenesis in bacteriophage T4. Mol. Gen. Genet. **141:** 23–40.

RIPLEY, L. S., 1982 The specificity of infidelity of DNA polymerase, pp. 85–116 in *Induced Mutagenesis: Molecular Mechanisms and their Implications for Environmental Protection*, edited by C. W. LAWRENCE, L. PRAKASH and F. SHERMAN. Plenum Press, New York.

RIPLEY, L. S., B. W. GLICKMAN and N. B. SHOEMAKER, 1983 Mutator *versus* antimutator activity of a T4 DNA Polymerase mutant distinguishes two different frameshifting mechanisms. Mol. Gen. Genet. **189:** 113–117.

SCHAAPER, R. M., 1988 Mechanisms of mutagenesis in the *Escherichia coli* mutator *mutD5*: role of DNA mismatch repair. Proc. Natl. Acad. Sci. USA **85:** 8126–8130.

SCHAAPER, R. M., B. N. DANFORTH and B. W. GLICKMAN, 1985 Rapid repeated cloning of mutant *lac* repressor genes. Gene **39:** 181–189.

SCHAAPER, R. M., B. N. DANFORTH and B. W. GLICKMAN, 1986 Mechanisms of spontaneous mutagenesis: an analysis of the spectrum of spontaneous mutation in the *Escherichia coli lacI* gene. J. Mol. Biol. **189:** 273–284.

SCHAAPER, R. M., and R. L. DUNN, 1987a Spectra of spontaneous mutations in *Escherichia coli* strains defective in mismatch correction: The nature of *in vivo* DNA replication errors. Proc. Natl. Acad. Sci. USA **84:** 6220–6224.

SCHAAPER, R. M., and R. L. DUNN, 1987b *Escherichia coli mutT* mutator effect during *in vitro* DNA synthesis. Enhanced A·G replicational errors. J. Biol. Chem. **262:** 16267–16270.

SCHAAPER, R. M., and R. L. DUNN, 1991 Spontaneous mutation in the *Escherichia coli lacI* gene. Genetics **129:** 317–326.

SIEGEL, E. C., and K. K. VACCARO, 1978 The reversion of *trp* frameshift mutations in *mut*, *polA*, *lig* and *dnaE* mutant strains of *Escherichia coli*. Mutat. Res. **50:** 9–17.

SIEGEL, E. C., S. L. WAIN, S. F. MELTZER, M. L. BINION and J. L. STEINBERG, 1982 Mutator mutations in *Escherichia coli* induced by the insertion of phage Mu and the transposable resistance elements Tn*5* and Tn*10*. Mutat. Res. **93:** 25–33.

SINHA, N. K., 1987 Specificity and efficiency of editing of mismatches involved in the formation of base-substitution mutations by the 3′ → 5′ exonuclease activity of phage T4 DNA Polymerase. Proc. Natl. Acad. Sci. USA **84:** 915–919.

TAKANO, K., Y. NAKABEPPU, H. MAKI, T. HORIUCHI and M. SEKIGUCHI, 1986 Structure and function of *dnaQ* and *mutD* mutators of *Escherichia coli*. Mol. Gen. Genet. **205:** 9–13.

TODD, P. A., and B. W. GLICKMAN, 1982 Mutational specificity of UV light in *Escherichia coli*: indications for a role of DNA secondary structure. Proc. Natl. Acad. Sci. USA **79:** 4123–4127.

WALKER, G. C., 1984 Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:** 60–93.

WITKIN, E. M., J. O. MCCALL, M. R. VOLKERT and I. I. WERMUNDSEN, 1982 Constitutive expression of SOS functions and modulation of mutagenesis resulting from resolution of genetic instability at or near the *recA* locus of *Escherichia coli*. Mol. Gen. Genet. **185:** 43–50.

YATAGAI, F., J. A. HALLIDAY and B. W. GLICKMAN, 1991 Specificity of *recA441*-mediated (*tif-1*) mutational events. Mol. Gen. Genet. **230:** 75–80.

Communicating editor: N. R. DRINKWATER