Mutants of *Escherichia coli* With Increased Fidelity of DNA Replication

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ABSTRACT

To improve our understanding of the role of DNA replication fidelity in mutagenesis, we undertook a search for *Escherichia coli* antimutator strains with increased fidelity of DNA replication. The region between 4 and 5 min of the *E. coli* chromosome was mutagenized using localized mutagenesis mediated by bacteriophage P1. This region contains the *dnaE* and *dnaQ* genes, which encode, respectively, the DNA polymerase (α subunit) and 3′ exonucleolytic proofreading activity (ϵ subunit) of DNA polymerase III holoenzyme, the enzyme primarily responsible for replicating the bacterial chromosome. The mutated bacteria were screened for antimutator phenotype in a strain defective in DNA mismatch repair (*mutL*), using a papillation assay based on the reversion of the *galK2* mutation. In a *mutL* strain, mutations result primarily from DNA replication errors. Among 10,000 colonies, seven mutants were obtained whose level of papillation was reduced 5–30-fold. These mutants also displayed decreased mutation frequencies for rifampicin or nalidixic acid resistance as well as for other markers. Mapping by P1 transduction and complementation showed each to reside in *dnaE*. These observations support the idea that the mutants represent antimutators which replicate their DNA with increased fidelity. Mutation rates were reduced in both *mutL* and *mutT* backgrounds, but mutagenesis by ultraviolet light was not significantly affected, suggesting that the antimutator effect may be largely restricted to normal DNA replication.

IN the study of mutagenesis, mutator strains–derivatives that display higher mutation frequencies than the wild-type strain–have been useful in defining genes and activities that control mutation (COX 1976; RYDBERG 1978; GLICKMAN and RADMAN 1980; NGHIEM *et al.* 1988; SCHAAPER 1988). Their counterparts, antimutators–displaying lower mutation frequencies than the wild-type strain–have been used less frequently, mostly because few such strains have been available. In principle, antimutators have a unique usefulness that is not shared by mutators. While mutator effects can be caused by mechanisms that may or may not be related to the major pathways of mutagenesis, antimutator effects can arise only through a reduction of a major existing pathway. Thus, antimutators may be particularly useful for addressing questions regarding the prevalent pathway(s) of mutagenesis under the condition of interest.

Only a few studies have described the isolation of antimutators (DRAKE 1993). Antimutator alleles of T4 DNA polymerase (DRAKE and ALLEN 1968; DRAKE *et al.* 1969) reduced transitions mutations (especially A·T → G·C), from both spontaneous and base analog-induced origin (DRAKE and GREENING 1970). Lower spontaneous mutation rates have also been reported for mutants in yeast (ESPOSITO, BOLOTIN-FUKUHARA and ESPOSITO 1975; QUAH, VON BORSTEL and HASTINGS 1980) and Neurospora (DE SERRES 1971). GEIGER and SPEYER (1977) reported the first antimutator mutant in *Escherichia coli*, which mapped to the *purB* locus. Addition of adenine or adenosine to the growth medium eliminated the antimutator phenotype, as did growth at 24°. Results from our laboratory, however, suggest that its antimutator phenotype is only apparent and results from the delayed growth of mutants under selective conditions (R. M. SCHAAPER and R. L. DUNN, unpublished data). QUIÑONES and PIECHOCKI (1985) isolated of some 20 *E. coli* mutants based on their reduced mutability by the base analog 2-aminopurine and which were subsequently observed to also reduce spontaneous mutation. The responsible genes were mapped at a variety of loci, but were not further characterized.

One area where antimutators might be particularly helpful is analysis of the mechanisms of spontaneous mutation. The origins of spontaneous mutation, while of considerable interest, are essentially unknown. Possible sources (SCHAAPER and DUNN 1991; SMITH 1992) include errors of DNA replication, unrepaired DNA damage (of which many types are known), transposable elements, and others. Antimutators could play an important role in distinguishing among these possible sources. Two different experimental approaches may be attempted. First, generalized antimutators may be sought that lower the overall spontaneous mutation rate. Identification of the mechanism or

pathway that is responsible for the effect then identifies the pathway as a main source for spontaneous mutations. Second, mutants may be sought which lower the production of mutations arising through one selected pathway. These pathway-specific antimutators can than be analyzed for their effect on overall mutation. In the present study, we have followed the latter approach, focusing on antimutators specific for errors of DNA replication.

DNA polymerase III holoenzyme is primarily responsible for replicating the *E. coli* chromosome (McHENRY 1991; KORNBERG and BAKER 1992). The enzyme contains a core consisting of the α, ϵ and θ subunits, of which α and ϵ are involved in maintaining the high replicational fidelity (McHENRY 1988; ECHOLS and GOODMAN 1991). The α subunit, encoded by *dnaE*, contains the polymerase activity. The ϵ subunit, encoded by *dnaQ*, provides 3′ exonucleolytic proofreading. We therefore decided to search for antimutators residing in either the *dnaE* or *dnaQ* gene. Because these genes are located closely together in the 4–5 min region of the *E. coli* chromosome (BACHMANN 1990), we performed localized mutagenesis of this region of the chromosome. This was done in a *mutL* mutator strain, which is deficient in *mutHLS*-dependent postreplicative mismatch repair (for review, see MODRICH 1991). In such a strain mutations arise primarily from replication errors and, hence, any antimutator effects are likely directed toward this type of error. We report on the isolation of seven such mutants, each in *dnaE*, that possess reduced rates of DNA replication errors.

MATERIALS AND METHODS

Strains and media: The *E. coli* strains used in this study and their derivation are listed in Table 1. All strains were grown at 37°. P1 transductions were performed using P1*virA*. Transformations with plasmids pBR322, pMM5 (*dnaQ*$^+$) and pMK9 (*dnaE*$^+$) were performed by electroporation with a BioRad Gene Pulser and protocol provided by the manufacturer. VB minimal medium (1xE) and L broth (LB) were standard recipes (MILLER 1972). Minimal medium (MM) was supplemented with 0.4% glucose (MM), galactose (MMGal) or lactose (MMLac) as a carbon source, 5 μg/ml of thiamine and 50 μg/ml amino acids as required. Antibiotics were added as follows: ampicillin (Amp), 50 μg/ml; tetracycline (Tet), 15 μg/ml; kanamycin (Kan), 25 μg/ml; chloramphenicol (Cam), 10 μg/ml; rifampicin (Rif), 100 μg/ml; nalidixic acid (Nal), 40 μg/ml. Solid media contained 1.5% Bacto agar. MacConkeyGal plates used for papillation assays were prepared using MacConkey agar base (Difco) supplemented with 0.4% galactose and antibiotics as needed.

Localized mutagenesis and papillation assay: Mutagenesis was performed by the method of HONG and AMES (1971). Bacteriophage P1*virA* was grown on strain JW353 (*zae-502*::Tn*10*) to 10^{10}–10^{11} ml^{-1} and treated with hydroxylamine to yield ~0.1% phage survival. This phage preparation was then used to transduce strain NR9360 (*mutL*::Tn*5*, *galK2*) to tetracycline resistance on LBTetKan plates. Ten thousand independent transductants were toothpicked in a gridded pattern (100 per plate) on LB TetKan plates. The next day, the plates were replicated to MacConkeyGal plates containing tetracycline and kanamycin. On MacConkeyGal plates, Gal$^-$ colonies are colorless while Gal$^+$ colonies are red. Gal$^+$ revertants arising during growth of a Gal$^-$ colony appear as red subcolonies (papillae) within the main colony. After 3 days, the plates were inspected for an increased (mutator) or decreased (antimutator) number of papillae. The *mutL* control strain produced ~35–50 papillae per colony. A 3-fold or larger increase or decrease in the number of papillae was used as a criterion for selecting putative mutators and antimutators, respectively. A rough correlation exists between the size of a colony on MacConkeyGal and the number of papillae (RYAN and WAINWRIGHT 1954; and our observations). As a general rule, colonies that were significantly smaller than the control were ignored.

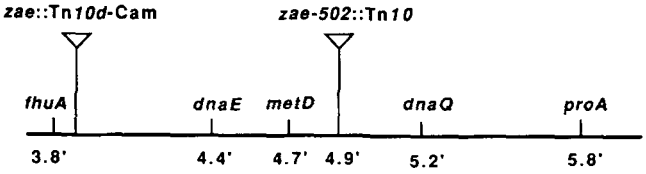

FIGURE 1.—The *dnaE/dnaQ* region of the *E. coli* chromosome. The order of the genes and map positions in minutes are from BACHMANN (1990). The position of transposon insertion *zae*::Tn*10d*-Cam, which confers chloramphenicol resistance, is from DICKER and SEETHARAM (1991). The position of *zae-502*::Tn*10* relative to *metD* and *dnaQ* was determined in our laboratory (R. M. SCHAAPER, unpublished data).

Mutation frequency determinations: Twelve colonies from each strain were toothpicked into 1 ml of LB medium and grown overnight with agitation. Colonies were taken from two or three independent isolates of each strain. Aliquots of appropriate dilutions were then plated on selective and nonselective plates to determine mutant and total cell counts. Mutant frequencies were determined in most cases by dividing the median mutant count by the average total cell count.

Ultraviolet light mutagenesis: Overnight cultures of wild-type and antimutator strains were diluted 20-fold in 50 ml fresh LB, grown to (2–3) × 10^8 cells/ml and placed on ice. Cells were then centrifuged and resuspended in half the volume of ice-cold 0.9% (w/v) NaCl. Portions (12 ml) were irradiated in a thin layer with gentle agitation in a Petri dish with ultraviolet light provided by a 782L10 bulb from the American Ultraviolet Company at an approximate dose rate of 1.2 Jm^{-2}s^{-1}. After irradiation for 0 or 60 sec, the cells were returned to ice and appropriate dilutions plated on LB plates to determine survival. To determine mutant frequencies, 10 ml of the cells were added to 25 ml of LB and grown overnight. All procedures were performed in subdued light to prevent photoreactivation. The next day, appropriate dilutions were plated on LB, MM, LBRif, and MMGal plates to determine mutant frequencies.

RESULTS

Localized mutagenesis and selection of antimutators: To search for antimutators acting at the level of DNA replication, we performed localized mutagenesis of the *dnaE-dnaQ* region of the *E. coli* chromosome (Figure 1) using the method of HONG and AMES (1971). Phage P1 was grown on strain JW353 carrying transposon *zae-502*::Tn*10* which is linked (30–50% cotransduction) to both *dnaE* and *dnaQ*. This phage

TABLE 1

Bacterial strains and plasmids

Strain	Genotype [derivation]	Source or reference
Bacteria		
AB1976	*ara-9, fhuA1,*Δ*(gpt-proA)62, lacY1* or *lacZ4, tsx-3, supE44, GalK2,* λ⁻, *hisG4(Oc), rfbD1?, trp3(Oc), rpsL8* or *rpsL9, malA1* (λ^R), *metE46, mtl-1, thi-1*	B. BACHMANN
CD4	Hfr, *metD88, proA3,* Δ*(lacI-Y)6, tsx-76,* λ⁻, *relA1, malA36*(λ^R), *metB1*	B. BACHMANN
ES1293	*mutL*::Tn*5*	SIEGEL *et al.* (1982)
GC4540	*pyrD, sulA*::Tn*5, thr, lac, his, tsl, rpsL*	S. GOTTESMAN
Hfr3000U118	*thi-1, relA1, lacZ118*(Oc), λ⁻, *spoT1*	B. BACHMANN
ID19	*galK2, lac-74, rpsL200, zae*::Tn*10d*-Cam	DICKER and SEETHARAM (1991)
JM30	*srlC*::Tn*10, recA730, thr-1, leuB6,* Δ*(gpt-proA)62, his-4, argE3, galK2, ilv*(TS), *sulA211, ara-14, xyl-5, tsx-33?, supE44*	E. WITKIN
JW353	*thr1, leuB6, zae-502*::Tn*10, thyA6, met-89, thi-1, deoC1, lacY1, rpsL67, tonA21,* λ⁻, *supE44*	B. BACHMANN
KA796	*ara, thi,* Δ*prolac*	SCHAAPER and DUNN (1987b)
NK6033	Hfr, Δ*(gpt-lac)5, nadA50*::Tn*10,* λ⁻, *relA1, spoT1, thi-1*	B. BACHMANN
NR9082	*ara, thi, mutT1, azi*	SCHAAPER and DUNN (1987b)
NR9355	AB1976, but *pro*⁺ *gpt*⁺ [P1(SG13082) → *pro*⁺]	This work
NR9360	NR9355, but *mutL*::Tn*5*[P1(ES1293) → kan^r]	This work
NR9418	NR9355, but *leu*::Tn*10* [P1(SG13082) → tet^r]	This work
NR9419	NR9418, but *leu*⁺, *mutT1* [P1(NR9082) → *leu*⁺]	This work
NR9485	NR9360, but *lac*⁺ [P1(W3110) → *lac*⁺]	This work
NR9489	NR9485, but *proC, zaj-403*::Tn*10* [P1(SG1039) → tet^r]	This work
NR9606	NR9489, but *pro*⁺, Tet^S, *lacZ118*(Oc) [P1(Hfr3000 U118) → *pro*⁺]	This work
NR9694	CD4, but *mutL*::Tn*5* [P1(ES1293) → kan^r]	This work
NR9760	NR9355, but *nadA50*::Tn*10* [P1(NK6033) → tet^r]	This work
NR9777	NR9694, but *nadA50*::Tn*10*, galK2 [P1(NR9760) → tet^r]	This work
NR9789	NR9777, but *nad*⁺ Tet^S [spontaneous *nad*⁺]	This work
NR9800	NR9789, but *zae*::Tn*10d*-Cam [P1(ID19) → cam^r]	This work
SG1039	Δ*lac, proC, zaj-403*::Tn*10*	S. GOTTESMAN
SG13082	*sulA366, his, leu*::Tn*10, lon-100*	S. GOTTESMAN
W3110	λ⁻, IN(*rrnD-rrnE*)1	B. BACHMANN
Plasmids		
pMM5	*E. coli dnaQ*⁺(*rnh*⁺) inserted into *Eco*RI site of pBR322	H. MAKI
pMK9	*E. coli dnaE*⁺ inserted into *Eco*RI site of pBR325	H. MAKI

Derivation [in brackets] denotes the P1 donor used to construct the strain, the arrow pointing toward the selected marker.

was then treated with hydroxylamine (see MATERIALS AND METHODS) and used to transduce strain NR9360 to tetracycline resistance. By this procedure, the region comprising *dnaE* and *dnaQ* is specifically targeted for a high level of mutations, some of which may result in an antimutator phenotype. Strain NR9360 (Table 1) carries the *galK2* and *mutL* mutations, both of which are important features of the screen for antimutators, as described below. The *mutL* mutation abolishes the postreplicative *MutHLS* mismatch repair system which recognizes and corrects DNA mismatches that result from replication errors (for review, see MODRICH 1991). As a consequence, a *mutL* strain has a much higher spontaneous mutation rate than the wild-type strain, and most mutations observed in this strain may be assumed to result from replication errors (SCHAAPER and DUNN 1987a).

Second, the presence of the *galK2* marker permits the visual scoring of mutation rates via papillation on MacConkeyGal medium. On MacConkeyGal plates, reversion of *galK2* during growth of the colony produces red papillae that outgrow the colorless parental strain due to their regained ability to ferment galactose (Figure 2). The *galK2* system has been used previously (RYDBERG 1978) to isolate mutators for base analog-induced mutagenesis. These derivatives proved subsequently to be also spontaneous mutators and to include mismatch repair-deficient strains. It should be noted that the *GalK2* reversion system scores exclusively base-pair substitution mutations (RYDBERG 1978; OLLER, FIJALKOWSKA and SCHAAPER 1993). Furthermore, mismatch repair-deficient strains produce largely transitions (SCHAAPER and DUNN 1987a) and the search for antimutators using the *galK2* system is therefore selective for antimutators capable of reducing this type of error (see DISCUSSION).

In the *mutL* strain, the average number of papillae per colony was about 30–40, compared to about 0.1 in a wild-type strain (Figure 2). Putative antimutators were scored as colonies having at least 3-fold fewer papillae. Among 10,000 transductants, 76 tentative candidates were obtained. These were further tested by direct mutation frequency measurements testing

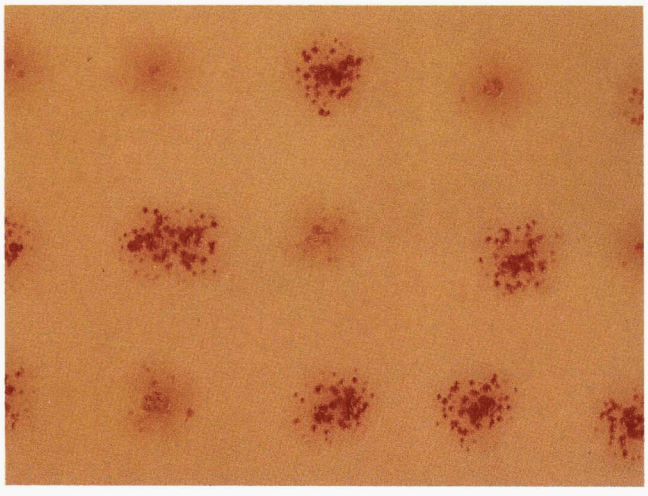

FIGURE 2.—Visual scoring of antimutators on MacConkeyGal plates. Colonies of strain NR9360 (*mutL*) produce a large number of red-colored papillae (Gal$^+$ revertants). Mutants with reduced mutation rates may be detected as colonies that produce few or no papillae. On this plate a mixture was gridded of NR9360 and one of its antimutator derivatives discovered in this study (*dnaE911*, see RESULTS).

for a reduction in Rif^R and Nal^R mutations and by backcrosses to strain NR9360 to test for linkage of the presumed antimutator phenotype with transposon *zae502*::Tn*10*. This yielded a total of seven linked antimutator candidates, which were able to consistently decrease the number of papillae between 5- and 30-fold (Table 2). During the initial scoring, mutator colonies (>3-fold increase in papillae) were also observed. These were collected as well and, following the same procedures, seven Tn*10*-linked mutators were obtained displaying a 3–10-fold increase over the *mutL* level (Table 2).

Mapping and complementation: The antimutator (and mutator) isolates were found to be linked 30–50% to the transposon *zae-502*::Tn*10* (data not shown). This linkage is consistent with a location in either the *dnaE* or *dnaQ* gene (Figure 1). More precise mapping was accomplished by *zae-502*-linked transduction into recipient strain NR9800 (*mutL*::Tn*5*, *galK2*, *proA6*, *zae*::Tn*10d*-Cam). Transposon *zae*::Tn*10d*-Cam (Figure 1) provides resistance to chloramphenicol (DICKER and SEETHARAM 1991). Tetracycline-resistant transductants of this strain were selected and tested for antimutator phenotype, chloramphenicol resistance, and proline prototrophy. Residence in *dnaE* would predict the antimutator mutation to be carried by a high percentage of all chloramphenicol-sensitive transductants, but by few of the Pro$^+$ transductants. In contrast, residence in the *dnaQ* gene would predict none of the chloramphenicol-sensitive transductants, but virtually all of the Pro$^+$ transductants, to carry the mutation. Using this logic, all seven antimutators were mapped at or near *dnaE*. A different result was obtained for the seven mutators:

TABLE 2

Newly isolated antimutator (amu) and mutator (mut) strains

Isolate	Papillae/colony	Gene	Allele
Control	35		
amu-1	3	*dnaE*	*dnaE911*
amu-12	3.5	*dnaE*	*dnaE912*
amu-36	5	*dnaE*	*dnaE913*
amu-40	10	*dnaE*	*dnaE914*
amu-59	0	*dnaE*	*dnaE915*
amu-66	5	*dnaE*	*dnaE916*
amu-69	3	*dnaE*	*dnaE917*
mut-3	>>100	*dnaQ*	*dnaQ918*
mut-13	>100	*dnaE*	*dnaE919*
mut-62	>100	*dnaQ*	*dnaQ920*
mut-98	>100	*dnaQ*	*dnaQ921*
mut-99	>100	*dnaQ*	*dnaQ922*
mut-102	>100	*dnaQ*	*dnaQ923*
mut-103	>100	*dnaQ*	*dnaQ924*

All strains are also *mutL*::Tn*5* (NR9360). Papillation data are averages of 10 colonies. For gene designations, see RESULTS. ">" indicates that the indicated number is exceeded, ">>" indicates that it is exceeded by severalfold.

only one resided at *dnaE*, the remaining six at *dnaQ*.

To confirm the mapping, complementation experiments were performed using plasmids carrying *dnaE*$^+$ or *dnaQ*$^+$ (MARUYAMA *et al.* 1983). Introducing pMK9(*dnaE*$^+$) into the antimutators in the *mutL* background restored the number of papillae to that of the *mutL* strain, confirming the location in the *dnaE* gene. For the seven mutators, six were complemented by pMM5(*dnaQ*$^+$) and one by pMK9(*dnaE*$^+$), again fully confirming the transductional mapping. (Plasmid pBR322, used as a control in this experiment, did not affect the extent of papillation in any of the strains, nor did the presence of pMK9 or pMM5 in the *mutL* control strain.) The data also demonstrate that the new alleles are recessive to the wild-type gene, at least when the latter is present on a multicopy plasmid.

Mutation frequencies of the antimutators in a *mutL* background: The seven antimutators were tested further for their ability to affect the level of mutagenesis in the *mutL* background using several mutational markers. Mutation frequencies were reduced 2–5-fold for Rif^R (Figure 3A), 2–7-fold for Nal^R (Figure 3B), 4–35-fold for Gal reversion (Figure 3C), and 2–10-fold for LacZ118(Oc) reversion (Figure 3D). However, no significant effects were observed for reversion of the Trp-3(Oc) marker (data not shown).

Mutagenesis in the *mutT* background: In a *mutL* background, transitions predominate (SCHAAPER and DUNN 1987a). To test if the antimutators can also act against other types of mutations, we transduced the antimutator alleles into the *mutT* strain NR9419. *E. coli mutT* is a strong mutator, which specifically enhances A·T → C·G transversions (YANOFSKY, COX

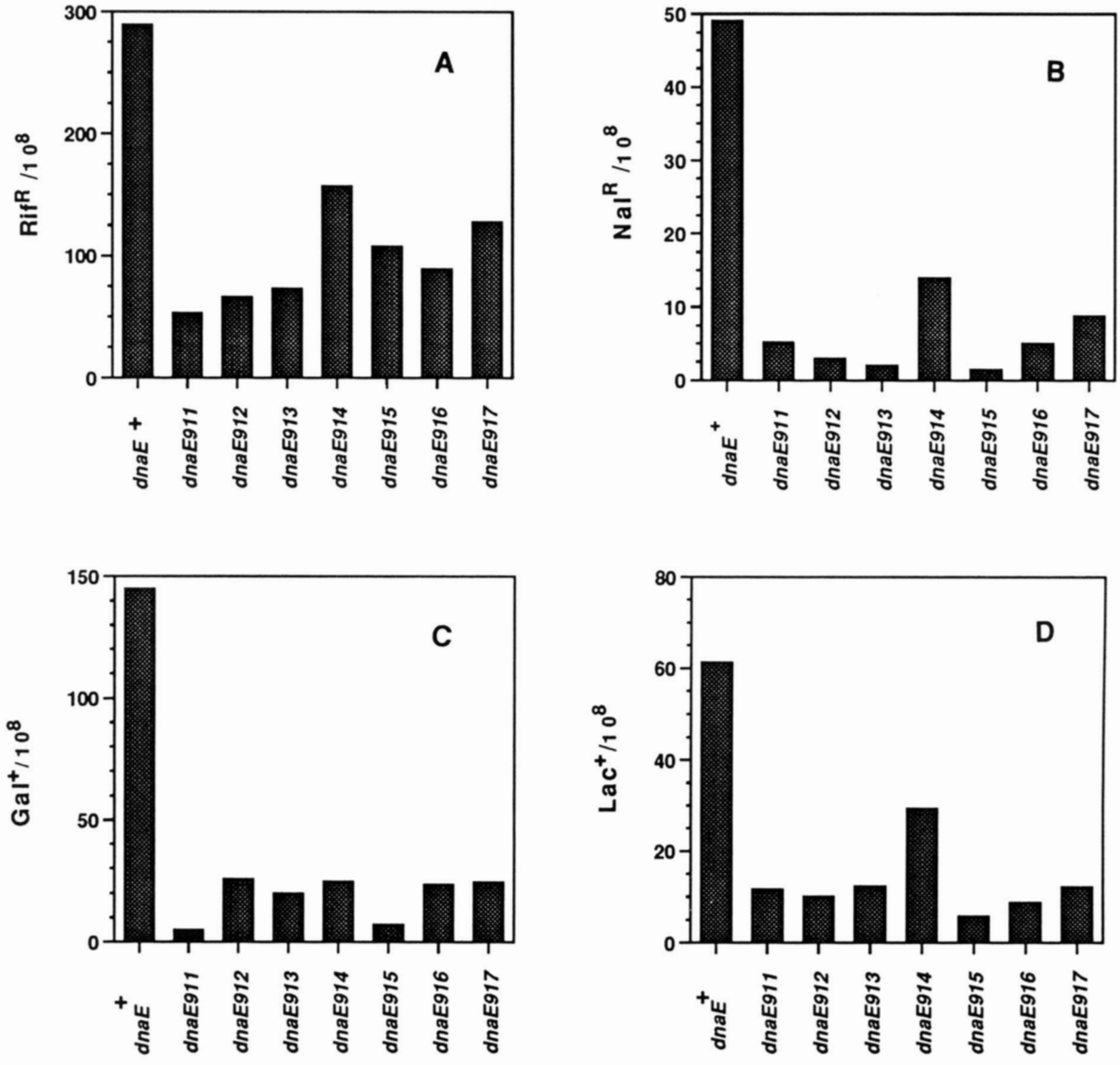

FIGURE 3.—Mutation frequencies to rifampicin resistance (A), nalidixic-acid resistance (B), *galK2* reversion (C) or *lacZ118* reversion (D) in strain NR9606 (*mutL*) and its various *dnaE* antimutator derivatives. The values represent the medians for 12 independent cultures for each strain.

and HORN 1966; COX 1976). Recent evidence has suggested that the high frequency of A·T → C·G transversions in a *mutT* strain results from its reduced ability to remove 8-oxo-dGTP from the cellular dNTP pool (MAKI and SEKIGUCHI 1992). This modified nucleotide has a strong base-pairing potential with template adenines, the resulting A·G mispairs being responsible for the high frequency of A·T → C·G transversions. Figure 4 shows that each of the antimutator alleles decreased the Nal^R mutant frequency in this background by 2–20-fold. For two antimutators, *dnaE911* and *dnaE915* the *mutT*-specific antimutator effect was further investigated using additional markers. Rifampicin-resistance was reduced 3.5-fold in both strains, while streptomycin resistance was reduced 7- and 17-fold, and Gal$^+$ papillation 3- and 28-fold for *dnaE911* and *dnaE915*, respectively (data not shown).

Antimutator effects under conditions of SOS mutagenesis: To further test the generality of the antimutator effects, we tested their mutability by ultraviolet light. Table 3 shows that there were no significant effects of the antimutator alleles on this type of mutagenesis using Rif^R and Gal$^+$ selection. Antimutator effects were observed using these same markers in a *mutL* background (Figure 3). Experiments were also performed measuring spontaneous mutation in strains containing the *recA730* allele. This allele causes constitutive expression of the SOS system, which results in a spontaneous mutator phenotype (WITKIN *et al.* 1982; WALKER 1984). No significant effects of the *dnaE* alleles were apparent for either Rif^R or Gal$^+$ (data not shown). These results suggest that SOS-

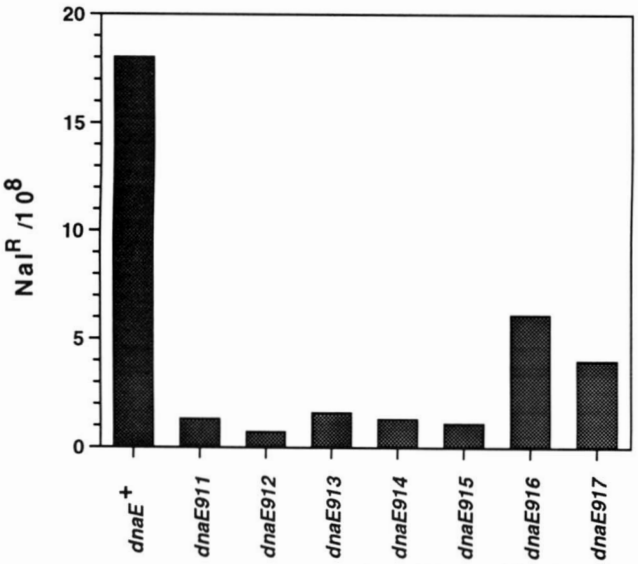

FIGURE 4.—Mutation frequencies for nalidixic-acid resistance in strain NR9419 (*mutT*) and its *dnaE* antimutator derivatives. Values are the medians for 12 independent cultures for each strain.

related mutagenesis is not subject to the antimutagenesis effects observed for the same markers in *mutL* (or *mutT*) strains, and that the effect of the antimutators may be restricted to normal DNA replication.

DISCUSSION

In this paper we describe the isolation and characterization of a set of specific *E. coli* antimutators. These mutants were obtained by localized mutagenesis of the *dnaE-dnaQ* region of the chromosome. Each was subsequently shown to reside in the *dnaE* gene encoding the α subunit (polymerase subunit) of DNA polymerase III holoenzyme. The mutants were isolated in a *mutL* strain in which most of the observed mutations may be assumed to result from DNA replication errors. On this basis, we suggest that we have isolated mutants of *E. coli* that possess increased accuracy during *in vivo* DNA replication.

Antimutators *vs.* expression artifacts: In contrast to the case of mutator strains, where the appearance of increased numbers of mutants may be readily interpreted as increased mutant production, some caution is needed in case of presumed antimutators. An alternative explanation would be that mutants are produced at their normal rate, but their expression is abnormal or significantly delayed. This could occur if the antimutator mutation interfered with the expression of the tested phenotypes or if it conferred a generalized "slow growth" phenotype (particularly under selective conditions) causing delayed appearance of the mutants. It is difficult to rigorously rule out such possibilities. However, the present strains appear generally healthy, displaying normal colony sizes and growth rates (the exception being *dnaE915*, which produces slightly smaller colonies). In addition, the

TABLE 3

UV-induced mutagenesis

Genotype	Survival (%)	Mutants per 10^6 cells	
		Rif^R	Gal$^+$
dnaE$^+$	17	5.2 ± 1.0	0.70 ± 0.34
dnaE911	18	8.1 ± 2.3	0.81 ± 0.07
dnaE912	21	5.1 ± 1.4	0.44 ± 0.08
dnaE913	31	10.8 ± 1.6	1.30 ± 0.54
dnaE914	30	5.7 ± 1.6	0.39 ± 0.03
dnaE915	22	8.8 ± 1.5	0.46 ± 0.08
dnaE916	20	9.4 ± 2.6	0.59 ± 0.13
dnaE917	25	13.8 ± 0.1	0.35 ± 0.04

Frequencies are means (±SD) for four independent cultures per strain. The strains were NR9355 (Table 1) carrying the indicated *dnaE* allele. Gal$^+$ revertants were assayed on MMGal plates. The UV dose was 72 Jm^{-2}. Frequencies for unirradiated cells were approximately 0.025×10^{-6} and 0.0016×10^{-6} for Rif^R and Gal$^+$, respectively.

antimutator effect is observed for several mutational markers (Rif^R, Nal^R, Gal$^+$, Lac$^+$), suggesting that the effect is not simply a marker-related expression artifact. On the other hand, no effect was observed for at least one marker (Trp reversion), arguing against any generalized expression difficulty. Furthermore, the antimutator effects for given markers, for example Rif^R or Gal$^+$, are observed in some cases (*mutL* or *mutT* strains), but not in others (UV irradiation or *recA730*). Together, these findings argue that the responsible mechanism operates at the level of mutant production rather than mutant expression.

Mutators *vs.* antimutators: Among 10,000 transductants isolated after mutagenesis of the *dnaE-dnaQ* region, we obtained equal numbers (seven) of antimutators and mutators. Intriguingly, all antimutators were located within *dnaE*, while most (6/7) of the mutators resided in *dnaQ*. This dichotomy is unlikely to be the result of a chance distribution because additional experiments (R. M. SCHAAPER, unpublished data) have yielded similar results. The bias of mutators for the *dnaQ* gene may be rationalized on the basis of target size. The proofreading activity provided by the *dnaQ* gene is not essential for viability (TAKANO *et al.* 1986; LANCY *et al.* 1989) and mutators, impairing or disabling the *dnaQ* function, could arise throughout this gene. In contrast, the DNA polymerase function provided by the *dnaE* gene is essential (WECHSLER and GROSS 1971; MAKI, HORIUCHI and SEKIGUCHI 1985; MCHENRY 1991) and mutators in this gene may arise at only a few sites. The bias among antimutators toward the *dnaE* gene is less readily explained. One possible interpretation is that the DNA polymerase, but not the proofreading activity, is rate-limiting in replication fidelity, *i.e.*, increased exonuclease proficiency does not necessarily lead to increased fidelity. Alternatively, the target size within the *dnaQ* gene for such changes is small.

The most interesting comparison results when focusing on *dnaE*. Six times more antimutators than mutators were obtained in this gene. This difference does not result from a detectional bias, because the two phenotypes are equally detectable in the papillation assay, and *dnaE* mutators have been reported in numerous other studies (HALL and BRAMMAR 1973; KONRAD 1978; SEVASTOPOULOS and GLASER 1977; MAKI, MO and SEKIGUCHI 1991). In the simplest view, assuming that fidelity is an evolved and specialized function, one might argue that one would be more likely to obtain DNA polymerases with lower fidelity (mutators) than with higher fidelity (antimutators). That the reverse is observed may have special significance with regard to the mechanisms by which DNA polymerases achieve high fidelity. Less likely, a significant fraction of *dnaE* mutators might be lethal in combination with the *mutL* mutation.

Specificity of the antimutator effect: Mutations occurring in a *mutL* strain have a defined specificity. Studies in the *lacI* gene of *E. coli* (SCHAAPER and DUNN 1987a) showed the main class of mutation in a *mutL* strain to be base-pair substitutions, among which transitions (A·T → G·C and G·C → A·T) were strongly favored over transversions (96% *vs.* 4%). Thus, the present set of antimutators, isolated in a *mutL* background, must be capable of reducing transition errors. More precisely, we report elsewhere (OLLER, FIJALKOWSKA and SCHAAPER 1993) that the *galK2* mutation represents a TAA (ochre) mutation which, in the *mutL* background, reverts by A·T → G·C transition. It is an interesting question whether other types of mutations, such as G·C → A·T transitions or the various transversions or frameshifts are also subject to the antimutator effect. Results described in detail in a companion paper (SCHAAPER 1993) suggest that for at least two of the antimutators, in the *mutL* background, both A·T → G·C and G·C → A·T transitions are reduced, but not (overall) transversions and frameshifts. Thus, the *dnaE* antimutators, like the previously reported T4 antimutators (DRAKE 1993), possess a defined specificity. Of interest is the ability of the present antimutator to also reduce the frequency mutation in a *mutT* mutator strain, where A·T → C·G transversions are uniquely induced. These transversions have been suggested to result from A·G mispairs involving 8-oxo-dGTP (MAKI and SEKIGUCHI 1992). It is likely that a detailed study of these specificities, including both normal and modified nucleotide substrates, will provide further insights into the mechanisms by which DNA polymerases discriminate against various errors.

Possible antimutator mechanisms: We can envision three different (but not necessarily exclusive) mechanisms by which a mutation in the DNA polymerase can increase replication accuracy. First, the mutant DNA polymerase may possess greater selectivity during the insertion step of DNA polymerization. Second, the mutation may lead to enhanced proofreading due to increased communication (or interaction) between the polymerizing and proofreading subunits. Third, the mutant polymerase may simply represent a catalytically less proficient enzyme which, by default, provides increased time for exonucleolytic proofreading. Both the first and the last possibility have been advanced to explain the antimutator phenotype of the T4 DNA polymerase mutant CB120 (GILLIN and NOSSAL 1976a,b). We will further address the possible mechanism(s) of the present antimutators in two companion papers.

Perspectives: The discovery of mutants of *E. coli* with increased accuracy of DNA replication should be helpful in analyzing the mechanisms by which DNA polymerases avoid errors, and in analyzing the role of DNA replication in various types of mutagenesis, such as spontaneous mutation in a wild-type strain. Preliminary results have indicated that at least one of the antimutators (*dnaE911*), reduces the spontaneous frequency for certain markers in a wild-type strain by 2–3-fold. Values of this magnitude are of significance. For example, a 2-fold reduction in the level of spontaneous mutations could mean that at least 50% of all mutations result from this particular source. Two companion studies describe the detailed specificity of some of the antimutators (SCHAAPER 1993) as well as the cloning and sequencing of the responsible mutant *dnaE* genes (FIJALKOWSKA and SCHAAPER 1993).

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