JOURNAL OF BACTERIOLOGY, Dec. 1997, p. 7507–7514 Vol. 179, No. 23
0021-9193/97/$04.00+0

Escherichia coli Cells Expressing a Mutant *glyV* (Glycine tRNA) Gene Have a UVM-Constitutive Phenotype: Implications for Mechanisms Underlying the *mutA* or *mutC* Mutator Effect

HOLLY S. MURPHY AND M. ZAFRI HUMAYUN*

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey 07103-2714

Received 1 August 1997/Accepted 30 September 1997

Transfection of M13 single-stranded viral DNA bearing a 3,N^4-ethenocytosine lesion into *Escherichia coli* cells pretreated with UV results in a significant elevation of mutagenesis at the lesion site compared to that observed in untreated cells. This response, termed UVM, for UV modulation of mutagenesis, is induced by a variety of DNA-damaging agents and is distinct from known cellular responses to DNA damage, including the SOS response. This report describes our observation, as a part of our investigation of the UVM phenomenon, that *E. coli* cells bearing a *mutA* or *mutC* allele display a UVM-constitutive phenotype. These mutator alleles were recently mapped (M. M. Slupska, C. Baikalov, R. Lloyd, and J. H. Miller, Proc. Natl. Acad. Sci. USA 93:4380–4385, 1996) to the *glyV* (*mutA*) and *glyW* (*mutC*) tRNA genes. Each mutant allele was shown to arise by an identical mutation in the anticodon sequence such that the mutant tRNAs could, in principle, mistranslate aspartate codons in mRNA as glycine at a low level. Because a UVM-constitutive phenotype resulting from a mutation in a tRNA gene was unexpected, we undertook a series of experiments designed to test whether the phenotype was indeed mediated by the expression of mutant glycine tRNAs. We placed either a wild-type or a mutant *glyV* gene under the control of a heterologous inducible promoter on a plasmid vector. *E. coli* cells expressing the mutant *glyV* gene displayed all three of the following phenotypes: (i) missense suppression of a test allele, (ii) a mutator phenotype measured by mutation to rifampin resistance, and (iii) a UVM-constitutive phenotype. These phenotypes were not associated with cells expressing the wild-type *glyV* gene or with cells in which the mutant allele was present but was not transcriptionally induced. These observations provide strong support for the idea that expression of mutant tRNA can confer a mutator phenotype, including the UVM-constitutive phenotype observed in *mutA* and *mutC* cells. However, our data imply that low-level mistranslation of the epsilon subunit of polymerase III probably does not account for the observed UVM-constitutive phenotype. Our results also indicate that *mutA* Δ*recA* double mutants display a normal UVM phenotype, suggesting that the *mutA* effect is *recA* dependent. The observations reported here raise a number of intriguing questions and raise the possibility that the UVM response is mediated through transient alteration of the replication environment.

Chemical and physical mutagens can induce mutations through two distinct pathways. In the direct pathway, the DNA lesions inflicted by the mutagen are misreplicated. In the second pathway, DNA lesions inflicted by the mutagen transiently alter the cellular physiology such as to affect mutation-fixation at the cognate lesions, at noncognate (i.e., heterologous) lesions, and possibly at unmodified bases. The *Escherichia coli* SOS response is believed to be an example of the second, inducible mutagenesis pathway. In the SOS response, DNA damage blocks DNA replication and generates a signal that ultimately derepresses the approximately 20 genes that constitute the SOS regulon (11). SOS genes encode mostly DNA repair, replication, and recombination functions, with the *lexA* and *recA* gene products providing, respectively, the repression and derepression functions. The RecA protein is believed to have multiple additional functions during the SOS response.

3,N^4-Ethenocystine (εC) is an exocyclic DNA lesion induced by carcinogens such as vinyl chloride (2) and ethylcarbamate (15). It appears that εC lesions may also be induced by end products of lipid peroxidation and may thus be part of the spontaneous DNA damage burden (6). We have previously shown that εC is a highly mutagenic DNA lesion that has in vitro and in vivo template characteristics expected for a noninstructive lesion (26, 28). However, mutagenesis at this lesion was *recA* independent, an anomaly that led us to investigate the mechanisms by which εC induces mutations. Further investigation showed that when M13 single-stranded DNA (ssDNA) bearing a site-specific εC lesion was transfected into *E. coli* preirradiated with UV, mutation fixation at the εC site was elevated significantly (23, 24). This enhancement in mutagenesis proved not to require the *recA*, *umuD*, or *umuC* gene and was therefore independent of the SOS response (27). This response, termed UVM (UV modulation of mutagenesis), was subsequently shown to be induced by all major classes of DNA-damaging agents (32, 33) and to be distinct from most known cellular responses to DNA damage (21, 27, 33).

As part of our initial characterization of UVM, we screened *E. coli* mutator strains defective for the *mutH*, *mutL*, *mutS*, *mutM*, and *mutY* genes and concluded that the UVM response

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, UMD-New Jersey Medical School, 185 South Orange Avenue MSB F607, Newark, NJ 07103-2714. Phone: (973) 972-5217. Fax: (973) 972-3644. E-mail: humayun@umdnj.edu.

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

Strain	Relevant genotype	Source (reference)
E. coli strains		
KH2	Sup^0 Δ(*lac-pro*) *trpE9777* F′ *lacI*^{q}*ZΔM15 pro*$^+$	This laboratory (26)
KH2R	Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) in KH2	This laboratory (26)
SR100	Δ(*umuDC*) Cm^r in KH2	This laboratory (unpublished data)
CC105	[*ara* Δ(*lac proB*)*xiii*] F′ *lacIZ proB*$^+$	J. Miller (16)
CC105*mutA*	*mutA590C* in CC105	J. Miller (16)
CC105*mutC*	*mutC* (Tet^r) in CC105	J. Miller (16)
HM105	Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) in CC105*mutA*	This work
HM106	Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) in CC105*mutC*	This work
FTP5378[a]	*trpA*(GAU234) *glyV55* IN (*rrnD-rrnE*) *rph-1*	E. Murgola (19)
Plasmids		
pSE380	Amp^r (vector)	R. Maurer (4)
pHM22	pSE380 with wild-type glycine tRNA	This work
pHM11	pSE380 with mutant glycine tRNA	This work

[a] The *glyV55* mutation in FTP5378 codes for a tRNA species that can read GGA/G codons and permits the recovery of *glyT* mutants (5, 20, 31). Note that wild-type *E. coli* has a total of four identical genes specifying the same tRNA species (three at the *glyV* locus and one at the *glyW* locus).

did not require any of those genes (21). However, *mutA* and *mutC*, two relatively uncharacterized mutator strains originally isolated by Miller and coworkers (16), showed a UVM-constitutive phenotype in that mutagenesis at an εC lesion borne on transfected M13 ssDNA was high in uninduced, as well as in UVM-induced, cells (this work). Slupska et al. (30) recently showed that although *mutA* and *mutC* mapped, respectively, to loci at 95 and 42 min on the *E. coli* chromosome, both alleles appeared to encode identical copies of a glycine tRNA molecule each bearing the same base substitution in the anticodon loop. In *mutA* cells, one of the three identical *glyV* genes located in a tandem cluster at 95 min was mutated, whereas in *mutC* cells, the single *glyW* gene at 42 min was mutated. In each case, the mutation changed the 3′-CCG anticodon (which recognizes the glycine 5′-GGU and 5′-GGC codons) to a 3′-CUG anticodon that can, in principle, mistranslate the aspartate codons 5′-GAU and 5′-GAC as glycine. Thus, the mutant tRNA could act as a missense suppressor that mistranslates a normal wild-type codon. Slupska et al. (30) have hypothesized that the increased mutation frequency of the strain may be attributed to a transient mutator effect imposed by mistranslation of catalytically important aspartic acid residues of the DNA polymerase III (pol-III) holoenzyme ε subunit that normally functions to edit base insertion errors.

Because a UVM-constitutive phenotype resulting from mutations in tRNA genes was unexpected, we have sought to obtain definitive evidence that expression of a mutant glycine tRNA can cause the observed UVM phenotype. Our results provide strong support for the notion that a tRNA mutation can indeed have a mutator effect and indicate that the *recA* gene is required for mediation of the *mutA-mutC*-encoded phenotypes. These data also raise the possibility that the transient mutator phenotypes displayed in UVM-induced cells and in *mutA* or *mutC* cells result from distinct pathways that may converge at a common effector mechanism.

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 lists the *E. coli* strains and plasmids used in this study.

Strain construction. Plasmid pHM11 was constructed by cloning a 122-bp-long chemically synthesized tRNA gene under the control of an inducible (P_{trc}) promoter in the pSE380 vector (4) as described below. The pSE380 vector DNA was linearized with *Nco*I, and the ends were filled in with T4 DNA polymerase in the presence of all four deoxynucleoside triphosphates. The DNA was cut with *Hin*dIII to obtain a large (4,118-bp) vector fragment bearing a promoter-proximal blunt end and a staggered *Hin*dIII-compatible end. Eight oligonucleotides constituting the synthetic tRNA gene (which also includes its own transcription termination sequence derived from the *E. coli rrnC* gene) were 5′ phosphorylated with T4 polynucleotide kinase in the presence of ATP. The phosphorylated oligonucleotides (150 pmol of each oligonucleotide) were annealed at 80°C for 5 min in 100 mM NaCl in a volume of 75 μl (22). The assembled sequence had a promoter-proximal blunt end, the other end being a *Hin*dIII-compatible staggered end (Fig. 1). A fivefold molar excess of the assembled DNA fragment bearing the mutant tRNA gene was ligated into the vector in a volume of 100 μl for 16 h at 16°C with 1 U of T4 DNA ligase (Gibco-BRL) in an ATP-containing buffer provided by the vendor. One microgram of the ligated DNA was transformed into *E. coli* FTP5378 cells (19), and plasmid DNAs from 17 transformants selected on Luria-Bertani (LB) plates supplemented with ampicillin (Sigma) at 50 μg/ml were analyzed for restriction fragment patterns predicted for the tRNA gene clone. DNA sequencing was performed on one plasmid (pHM11) as a final confirmation of the cloned sequence (data not shown). All of the enzymes used in the plasmid construction procedures described above were from New England Biolabs. Plasmid pHM22, a control plasmid containing a wild-type copy of the tRNA gene, was constructed exactly as described above, except that appropriate oligonucleotides specifying the wild-type version of the tRNA were substituted for the corresponding mutant oligonucleotides (see legend for Fig. 1).

The *E. coli* Δ*recA mutA* (HM105) and Δ*recA mutC* (HM106) strains were constructed by P1 transduction (17) of the Δ(*srlR-recA*)*306*::Tn*10*(Tet^r) locus from *E. coli* KH2R to the CC105*mutA* or CC105*mutC* strain. Transductants were found to display the expected UV sensitivity due to loss of *recA* function.

Background mutation frequency and missense suppression. Background mutation frequency was determined from the number of spontaneously arising rifampin-resistant mutants as previously described (17). The ability of pHM11 to function as a missense suppressor was tested as follows. *E. coli* FTP5378 *trpA* cells harboring pHM11 (or appropriate control cells) were streaked on the following minimal plates: (i) minimal A medium (17), (ii) minimal A medium plus tryptophan at 20 μg/ml, (iii) minimal A medium plus 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma), and (iv) minimal A medium plus trypto-

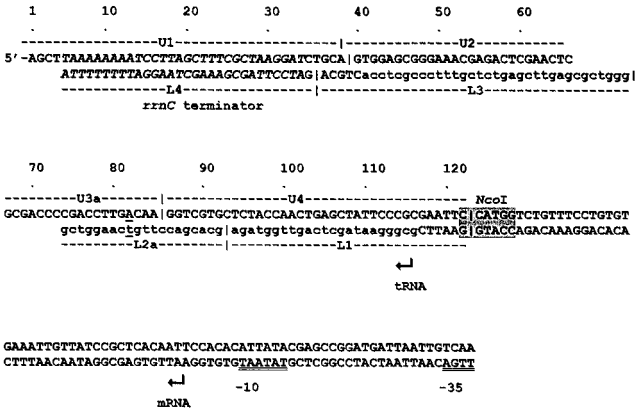

FIG. 1. DNA sequences of synthetic mutant and wild-type *glyV* tRNA genes in plasmids pHM11 and pHM22 showing major features and strategies used in their construction. The mutant tRNA gene in pHM11 was assembled by annealing eight 5′-phosphorylated deoxyribooligonucleotides (U1, U2, U3a, and U4 for the upper strand and L4, L3, L2a, and L1 for the lower strand). The sequence of the tRNA molecule extending from position 116 (5′ end) through position 41 (3′ end) is indicated in lowercase letters on the bottom strand. The sequence of the *rrnC* transcription terminator (base numbers 5 through 34) is indicated by uppercase italicization. The predicted −35, −10, and transcription start sites are indicated. The location of the base substitution mutation is indicated by the underlined base pair at position 82. The wild-type *glyV* tRNA gene was similarly assembled, except that the 19-mer 5′-GCGACCCCGACCTTGGCAA (U3b) and the 20-mer 3′-GCTGGAACCGTTCCAGCACG (L2b) were used to replace, respectively, the U3a and L2a oligonucleotides shown. As a result, the AT base pair at sequence position 82 in the mutant tRNA gene (pHM11) changes to GC in the wild-type version (pHM22).

phan at 20 μg/ml and 1 mM IPTG. Plates were examined for growth after incubation at 37°C for 24 and 48 h.

Construction of M13 ssDNA bearing a site-specific εC lesion and UVM assays. The construction of εC-containing vector ssDNA (εC-DNA) and that of control ssDNA have been described previously in detail (23, 27, 33). LB medium (usually 100 ml) was inoculated with 0.01 volume of a fresh overnight saturated culture of the appropriate *E. coli* strain, and the cells were grown to exponential phase (about 10^8 cells/ml) at 37°C with vigorous aeration. The cultures were exposed to *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) at 0, 1, 5, or 10 μg/ml for 10 min at 37°C (33). The treated cells were pelleted at 3,600 × *g* at 4°C and washed with an equal volume of ice-cold LB medium. The cells were resuspended in 1/10 of the original culture volume of ice-cold transformation and storage solution (10% polyethylene glycol 3350, 5% dimethyl sulfoxide, and 20 mM $MgCl_2$ in LB medium) and then incubated at 4°C for 40 min (23). To induce the expression of the tRNA gene, all of the above steps (starting from the preparation of fresh overnight cultures) were carried out in the presence of 1 mM IPTG. One-milliliter aliquots of competent cells prepared as described above were transfected with 50 ng of εC-DNA or control ssDNA as previously described (23). Effect of the lesion on survival was determined by plating for infectious centers. Pooled progeny phage DNA from each transfection was prepared either as previously described (23, 25) or by use of Qiaprep Spin M13 kits from Qiagen in accordance with the instructions provided by the vendor. Mutation fixation at the lesion site was analyzed by a quantitative multiplex DNA sequence analysis described previously (23, 25) and summarized in Fig. 2.

RESULTS

Experimental system. The UVM response is detected as increased mutagenesis at the site of an εC lesion borne on an M13 ssDNA vector. As summarized in Fig. 2, the system consists of (i) pretreatment of cells in the exponential growth phase with a UVM-inducing agent, (ii) transfection of ssDNA bearing the lesion, (iii) plating of transfected cells for infectious centers to monitor survival effects of the lesion, (iv) preparation of pooled progeny phage DNA from each transfection, and (v) multiplex sequence analysis of the pooled progeny DNA.

***mutA* and *mutC* cells have a UVM-constitutive phenotype.** Figure 3A shows an example of a normal UVM response as observed in *E. coli* CC105 cells. When untreated cells are transfected with εC-DNA (Fig. 3A, MNNG at 0 μg/ml) mutagenesis at the εC lesion is relatively low, as indicated by light 21- and 22-mer bands representing C→T and C→A mutations, respectively. Pretreatment of cells with MNNG dramatically enhances the intensity of the 21- and 22-mer bands such that approximately 50% of the progeny are mutant at an MNNG concentration of 5 μg/ml (see Table 2, experiment A, for a quantitative summary). Figure 3B and Table 2, experiment B, show that mutation fixation is very high in untreated CC105*mutA* cells (Fig. 3B, lane 0 μg/ml). Pretreatment of cells brings about relatively small changes in mutation frequency and specificity. A similar result is obtained with CC105*mutC* cells (Fig. 3C and Table 2, experiment C), except that there is a further enhancement of mutagenesis in response to pretreatment with MNNG at 5 or 10 μg/ml.

Construction and functional characterization of a plasmid vector expressing a mutant *glyV* tRNA gene. To test whether the observed UVM-constitutive phenotype of *mutA* cells is attributable to the expression of a mutant *glyV* tRNA, we constructed pHM11, a pSE380-derived plasmid in which a synthetic mutant *glyV* tRNA gene (Fig. 1 shows the DNA sequence) is placed under the control of the IPTG-inducible P_{trc} promoter (4). Plasmid pHM22 is an identical construct, except that a wild-type *glyV* sequence has been inserted in place of the mutant tRNA gene. To test whether expression of the mutant tRNA is capable of acting as a missense suppressor tRNA, we transformed *E. coli* FTP5378 *trpA*(GAU234) with plasmid pHM11 or pHM22. FTP5378 is a missense suppressor test strain that is auxotrophic for tryptophan because of a glycine-to-aspartate change (resulting from a G · C→A · T base substitution) at position 234 of the *trpA* gene that specifies the

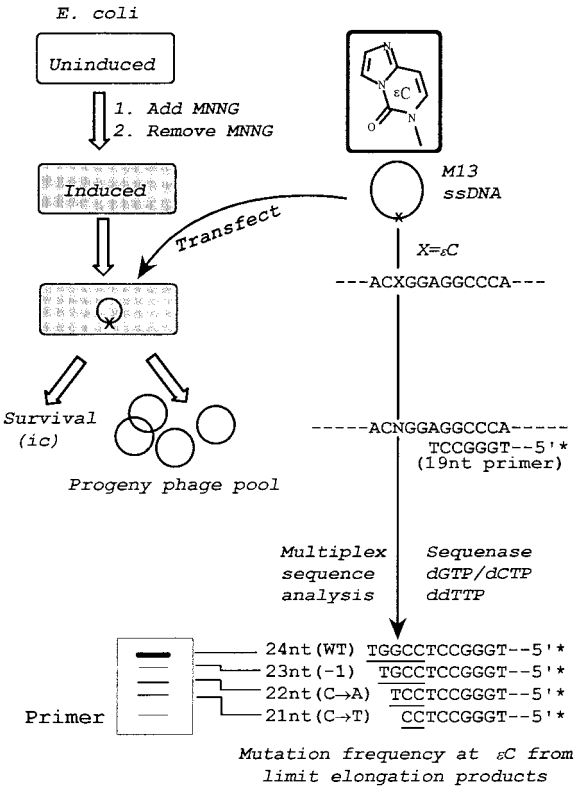

FIG. 2. Summary of procedures used to detect the UVM response. Cells grown in the presence or absence of 1 mM IPTG to the exponential phase were exposed to MNNG at 0, 1, 5, or 10 μg/ml for 10 min at 37°C and processed to render them transfection competent as described in Materials and Methods. Competent cells (1 ml) were transfected with 50 ng of M13 ssDNA bearing a site-specific εC residue (see inset at top right for structure). M13 ssDNA survival was determined by plating a 0.1- or 0.2-ml aliquot of transfected cells for infectious centers (*ic*), and the remainder was used to grow progeny phage as previously described (23, 25). Pooled progeny phage DNA was isolated and subjected to quantitative multiplex sequence analysis to determine the mutation frequency and specificity as described in detail elsewhere (23, 25) and summarized at the right, where the immediate DNA sequence context of the lesion is shown (*X*=ε*C*). A 5′ ^{32}P-end-labeled 19-mer primer (0.1 pmol) is annealed to pooled progeny phage DNA (0.2 pmol) and elongated in the presence of dGTP and dCTP (each at 1 μM) and ddTTP (10 μM) by T7 DNA polymerase devoid of 3′→5′ exonuclease activity (Sequenase 2.0; U.S. Biochemicals). The absence of dATP and the replacement of dTTP with ddTTP result in the production of limit elongation products whose length reflects the sequence of the template, as shown at the bottom. Thus, the 24-mer band derives from the wild-type (WT) fraction while the 21-mer, 22-mer, and 23-mer bands derive, respectively, from C→T, C→A, and −1-nucleotide (nt) mutant fractions. The elongation products are separated by high-resolution denaturing gel electrophoresis, and the autoradiographic signal in the bands is quantitated by computing densitometry as previously described (23, 25). Mutation frequency is deduced as the ratio of combined signal in the mutant bands to that in all four bands as previously described (21). Assays include elongation reactions in which standard mixtures of mutant and wild-type DNAs are analyzed to monitor the accuracy of each assay.

α subunit of tryptophan synthetase (19). This strain can be reverted to tryptophan prototrophy by missense suppression such as to insert glycine or alanine at the mutated (Gly→Asp) codon. Figure 4 shows that FTP5378/pHM11 cells can grow in the absence of tryptophan, provided IPTG is included in the plate (cf. sectors b and e of plate C with those in plate A, the no-IPTG control). In contrast, FTP5378/pHM22 cells expressing the wild-type *glyV* tRNA do not grow in minimal medium with IPTG (Fig. 4, plate C, sector d) or without IPTG (plate A, sector d). These observations suggest that expression of the mutant tRNA (but not that of wild-type tRNA) confers the

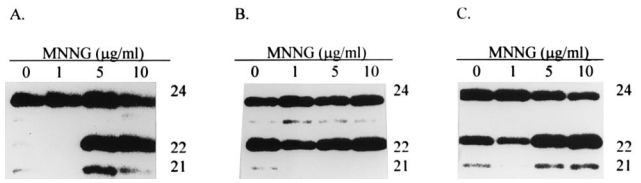

FIG. 3. Examples of multiplex analyses of mutation fixation at an εC residue on M13 ssDNA transfected into *mutA* or *mutC* cells. Panels: A, wild-type (CC105) cells; B, *mutA* (CC105*mutA*) cells; C, *mutC* (CC105*mutC*) cells. Untreated and treated cells were transfected with ssDNA bearing εC, and phage progeny was subjected to multiplex sequence analysis as described in Fig. 2. Mutagenesis is low in uninduced wild-type cells (panel A, MNNG at 0 μg/ml), as indicated by light mutant bands (21-mer, 22-mer, and 23-mer) in comparison with the wild-type (24-mer) band. There is a striking increase in mutagenesis in response to pretreatment with MNNG at 5 or 10 μg/ml. Mutagenesis in uninduced *mutA* cells (panel B, MNNG at 0 μg/ml) is very high and does not appear to significantly increase further in response to MNNG pretreatment. Mutagenesis in uninduced *mutC* cells (panel C, MNNG at 0 μg/ml) is also much higher than that observed in wild-type cells; however, a further increase in mutagenesis in response to MNNG pretreatment can be seen in *mutC* cells. The values to the right of each panel are the lengths in nucleotides of the limit elongation products.

missense suppressor activity predicted by the anticodon mutation.

Table 3, experiments B and C, shows that background mutagenesis, measured as the frequency of rifampin-resistant colonies, increases (as expected for mutator cells) for *mutA* and *mutC* cells compared with that of the wild-type parent (Table 3, experiment A). Table 3, experiment D, shows that expression of mutant glycine tRNA, but not that of wild-type glycine tRNA, elevates background mutagenesis significantly. Elevated mutagenesis in KH2/pHM11 cells (no IPTG) is probably due to basal expression of the tRNA gene on a multicopy vector plasmid (the estimated copy number per cell is 30 [1]) under the experimental conditions used.

Expression of a mutant glycine tRNA gene confers a UVM-constitutive phenotype. Figure 5B and Table 2, experiment I, show very high mutagenesis in IPTG-induced, untreated *E. coli* KH2/pHM11 cells (Fig. 5B, lane marked 0 μg/ml), in contrast to the normal UVM response observed in IPTG-induced *E. coli* KH2/pHM22 cells (Fig. 5A and Table 2, experiment H). The UVM-constitutive phenotype of KH2/pHM11 cells required IPTG (cf. data in Table 2, experiments F and I), showing that expression of the mutant tRNA was necessary for the phenotype. Pretreatment of cells with MNNG also elevates the survival (number of infectious centers per microgram) of transfected DNA (e.g., Table 2, experiments B and J), as noted previously (33). However, this stimulation (termed UVM reactivation) may represent a poorly understood effect on phage replication (see reference 32 for a discussion).

The UVM-constitutive phenotype conferred by mutant glycine tRNA expression, or by the *mutA* or *mutC* mutation, requires a functional *recA* gene but not a functional *umuD* or *umuC* gene. Figure 5D and Table 2, experiment P, show that UVM induction is normal in IPTG-induced *E. coli* KH2R/pHM11 cells, as evidenced by low mutagenesis at εC in untreated cells and a strong induction of mutations in cells pretreated with MNNG at 5 or 10 μg/ml. Because *E. coli* KH2R is a Δ*recA* derivative of KH2, these results imply that the UVM-constitutive phenotype of cells expressing the mutant glycine tRNA is *recA* dependent. Figure 5E and Table 2, experiment R, show that the UVM-constitutive phenotype of *mutA* cells is not observed in Δ*recA mutA* double-mutant cells, as evidenced by low mutagenesis in untreated cells (cf. these data and those in Fig. 5B and Table 2, experiment I) and strong induction in pretreated cells. Figure 5F and Table 2, experiment S, show essentially similar results for the Δ*recA mutC* double mutant.

Because SOS induction requires the *recA* gene, it was interesting to investigate if *umuD* and *umuC*, two genes that are believed to play an essential role in SOS mutagenesis, are also required for the UVM-constitutive phenotype displayed by *mutA* or *mutC* cells. An analysis of *E. coli* SR100 Δ*umuD* or -*C* cells harboring pHM11 showed a UVM-constitutive phenotype when mutant tRNA gene expression was induced by IPTG (Table 2, experiment Q), showing that the mutant tRNA-mediated phenotype did not require the *umuD* and *umuC* genes.

The data in Table 3, experiment E, show the *recA* dependence of the mutator phenotype conferred by the expression of mutant glycine tRNA measured as spontaneous mutation to rifampin resistance. Thus, the pHM11 plasmid in the presence of IPTG fails to elevate mutagenesis in Δ*recA* cells compared to the strong effect observed in *recA*$^+$ cells (cf. Table 3, experiments D and E). The data in Table 3, experiment F, show that pHM11 (plus IPTG) does elevate mutation to rifampin resistance in Δ*umuDC* cells, showing that the *umuD* and *umuC* genes are not required for the mutator phenotype. Finally, the data in Table 3, experiments G and H, show that in a Δ*recA* background, neither the *mutA* nor the *mutC* allele confers a mutator phenotype.

DISCUSSION

In this investigation, we sought definitive evidence that the UVM-constitutive phenotype we observed in *mutA* and *mutC* cells was linked to the expression of a mutant glycine tRNA gene. To that end, we constructed plasmid clones of the wild-type (*glyV*) and mutant (*mutA*) versions of the glycine tRNA gene such as to place the tRNA gene expression under the control of an IPTG-inducible promoter. The results described here satisfactorily link three phenotypes to the expression of the mutant tRNA gene: (i) missense suppression of a bona fide *E. coli* tester strain bearing a Gly→Asp mutation, (ii) a mutator phenotype measured as spontaneously arising rifampin-resistant mutants, and (iii) a UVM-constitutive phenotype. Therefore, the data presented here provide strong support for the unexpected finding by Slupska et al. (30) that expression of a mutant tRNA can confer a mutator phenotype. However, as discussed below, these data do not support the hypothesis that low-level mistranslation of a subunit of pol-III can account for the mutator phenotypes.

The *E. coli* pol-III holoenzyme is an asymmetric dimeric complex consisting of 18 polypeptides built around two pol-III core assemblies. Each pol-III core is a stable complex of three polypeptides: the α subunit is the actual DNA polymerase, and the ε subunit is the editing 3′→5′ exonuclease, whereas the function of the third subunit, θ, remains unknown (13). The dimeric arrangement appears to reflect the functional requirements for coordinated replication of the leading and lagging strands. Point mutations in the ε subunit that abolish the editing 3′→5′ exonuclease activity confer very strong mutator phenotypes (7, 9). Because deletion of the gene encoding the ε subunit (*dnaQ*) makes cells very sick (29) and because the pol-III core is a very stable αεθ heterotrimer, the ε subunit may have additional structural and functional roles in the pol-III holoenzyme complex. The assembly of the pol-III holoenzyme appears to begin with the binding of the γ complex to a primed template, followed by the recruitment of a β clamp and the pol-III core (13). The β ring confers such remarkable processivity on the holoenzyme that the enzyme is believed to remain stably associated with the replication fork until replication is complete. Analysis of the active sites of the ε subunit has identified a number of amino acid residues (including three

TABLE 2. Effects of MNNG pretreatment of *E. coli* strains on mutagenesis at a site-specific εC residue borne on transfected M13 ssDNA

Expt, strain[a] (presence of IPTG [1 mM])	MNNG concn (μg/ml)[b]	No. of ic/μg[c]	Mean mutation frequency[d] ± SD		
			Total	C→A	C→T
A, CC105 (−)	0	148	4 ± 1	2 ± 1	2 ± 0
	1	2,270	7 ± 1	4 ± 1	2 ± 1
	5	7,120	51 ± 1	43 ± 0	8 ± 1
	10	8,240	68 ± 2	62 ± 3	5 ± 1
B, CC105*mutA* (−)	0	873	60 ± 5	53 ± 4	8 ± 1
	1	2,978	40 ± 2	35 ± 3	5 ± 2
	5	5,995	54 ± 6	51 ± 5	3 ± 1
	10	7,550	62 ± 2	58 ± 1	4 ± 1
C, CC105*mutC* (−)	0	5,095	45 ± 2	33 ± 1	12 ± 1
	1	13,990	36 ± 5	26 ± 1	10 ± 4
	5	15,600	67 ± 1	57 ± 0	10 ± 1
	10	14,020	78 ± 1	67 ± 0	11 ± 1
D, KH2/pSE380 (−)	0	64	5 ± 2	1 ± 0	4 ± 2
	5	120	42 ± 7	27 ± 1	15 ± 7
	10	116	52 ± 4	49 ± 2	3 ± 3
E, KH2/pHM22 (−)	0	106	4 ± 1	2 ± 1	2 ± 1
	5	373	18 ± 13	13 ± 10	4 ± 4
	10	347	44 ± 15	36 ± 13	8 ± 3
F, KH2/pHM11 (−)	0	136	3 ± 1	2 ± 1	2 ± 1
	5	215	31 ± 11	22 ± 11	9 ± 1
	10	313	56 ± 3	49 ± 4	7 ± 4
G, KH2/pSE380 (+)	0	370	5 ± 0	3 ± 0	2 ± 0
	5	1,030	45 ± 0	30 ± 0	15 ± 0
	10	1,250	29 ± 6	17 ± 3	12 ± 4
H, KH2/pHM22 (+)	0	205	2 ± 1	1 ± 1	1 ± 0
	5	573	17 ± 15	15 ± 14	2 ± 1
	10	620	52 ± 9	36 ± 11	16 ± 3
I, KH2/pHM11 (+)	0	640	77 ± 4	75 ± 4	2 ± 1
	5	418	73 ± 5	70 ± 8	3 ± 3
	10	590	72 ± 4	71 ± 4	1 ± 0
J, KH2R (*recA*)pSE380 (−)	0	84	15 ± 3	2 ± 1	13 ± 3
	5	405	47 ± 3	45 ± 3	2 ± 0
	10	460	46 ± 3	44 ± 4	2 ± 1
K, KH2R (Δ*recA*)pHM22 (−)	0	88	4 ± 3	3 ± 2	1 ± 0
	5	570	24 ± 4	23 ± 5	2 ± 1
	10	330	45 ± 2	41 ± 2	3 ± 3
L, KH2R (Δ*recA*)pHM11 (−)	0	100	5 ± 2	4 ± 2	1 ± 1
	5	195	38 ± 5	35 ± 3	4 ± 3
	10	318	45 ± 13	40 ± 12	5 ± 2
M, KH2R (Δ*recA*)pSE380 (+)	0	345	11 ± 4	3 ± 1	8 ± 4
	5	1,210	28 ± 4	24 ± 2	4 ± 2
	10	1,025	39 ± 4	29 ± 1	10 ± 4
N, KH2R (Δ*recA*)pHM22 (+)	0	228	5 ± 2	3 ± 2	2 ± 1
	5	498	35 ± 11	32 ± 8	3 ± 3
	10	525	55 ± 9	53 ± 7	2 ± 2
P, KH2R (Δ*recA*)pHM11 (+)	0	205	5 ± 1	4 ± 1	1 ± 1
	5	533	22 ± 7	20 ± 7	3 ± 1
	10	560	44 ± 3	41 ± 1	3 ± 2
Q, SR100 (Δ*umuDC*)pHM11 (+)	0	128	69 ± 7	66 ± 8	4 ± 2
	10	145	64 ± 17	62 ± 17	2 ± 1
R, HM105 (Δ*recA mutA*) (−)	0	140	3 ± 1	1 ± 0	1 ± 1
	10	250	70 ± 19	60 ± 28	9 ± 9
S, HM106 (Δ*recA mutC*) (−)	0	148	7 ± 5	6 ± 5	2 ± 1
	10	173	74 ± 4	50 ± 10	24 ± 7

[a] See Table 1 for more complete genotypes. pSE380 is a plasmid vector (control), pHM22 is a plasmid bearing a wild-type *glyV* gene, and pHM11 is a plasmid bearing the mutant *glyV* gene.

[b] Cells were exposed to the indicated concentrations of MNNG for 10 min at 37°C, and then the MNNG was removed as described in Materials and Methods.

[c] Survival of transfected DNA is expressed as the number of infectious centers (ic) per microgram of transfected DNA.

[d] Mutation frequency and specificity were determined as previously described (21). The data are averaged from at least six multiplex sequence elongation assays conducted on pooled progeny phage DNA obtained in two or more separate transfections, except for the following control strains, for which the data are averaged from a single transfection and three elongation assays: CC105 (experiment A), CC105*mutC* (experiment C), KH2/pSE380 (experiment D), KH2/pSE380 (experiment G), KH2R/pSE380 (experiment J), and KH2R/pSE380 (experiment M). Standard deviations were rounded to the nearest integer.

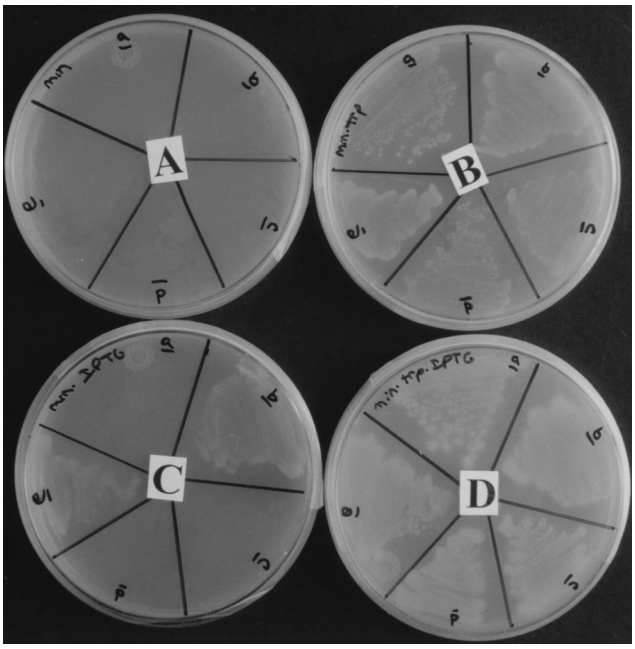

FIG. 4. Suppression of a *trpA* auxotrophic mutation by expression of a mutant *glyV* tRNA gene. *E. coli* FTP5378 *trpA*(GAU234) cells transformed with pHM11, pHM22, or pSE380 were streaked on the following minimal agar plates: A, minimal A medium; B, minimal A medium plus tryptophan (20 μg/ml); C, minimal A medium plus IPTG (1 mM); D, minimal A medium plus tryptophan (20 μg/ml) and IPTG (1 mM). The plates were incubated at 37°C and observed at 24 and 48 h. Growth patterns at 48 h are shown. The five sectors on each plate were streaked with the following strains (starting at the top, going clockwise): a, FTP5378; b, FTP5378/pHM11 (isolate 1); c, FTP5378/pSE380; d, FTP5378/pHM22; e, FTP5378/pHM11 (isolate 2).

aspartates) as playing potentially important roles in the editing activity of the enzyme (3, 8, 10).

Slupska et al. (30) have proposed that the mutator effect of *mutA* or *mutC* cells may be mediated through low-level mistranslation of the ε subunit of the pol-III complex. Because missense suppression efficiency is around 1%, a similar fraction of mutant proteins are expected to form during protein synthesis. Such a mistranslation can create a small number of dominant-negative mutant ε subunit protein molecules in each cell expressing the mutant glycine tRNA. Occasional recruitment of the mutant protein into a pol-III holoenzyme will create a mutator polymerase. Thus, the hypothesis proposes that the tRNA mutation confers a transient mutator phenotype in a small subset of cells; therefore, mutant cells will have a normal wild-type phenotype much of the time but will express a temporary mutator phenotype at time intervals determined by the probability of recruitment of a dominant-negative ε subunit into a pol-III holoenzyme.

Replication of M13 viral DNA proceeds through two stages (14, 18). In the first stage, the viral (plus) ssDNA is converted to a double-stranded replicative form (RF) through the following steps: (i) initiation of replication at a unique site (origin for the minus strand) by the priming action of *E. coli* RNA polymerase (12), (ii) elongation of the primer by pol-III, (iii) primer removal and gap filling by DNA polymerase I, and (iv) nick sealing by ligase, followed by negative supercoiling by DNA gyrase. Mutation fixation in transfected ssDNA is believed to occur during this stage and therefore depends exclusively on host replication machinery. In stage 2, RF→RF, as well as RF→ssDNA, replication occurs through a rolling-circle mechanism in which the phage-specified gp2 protein introduces a nick at a unique site in the plus strand to set off replication, with all other replication functions provided by the host.

Although more complex scenarios can be imagined, it is likely that the UVM-constitutive phenotype displayed by *mutA* or *mutC* cells is mediated by the same mechanism that is responsible for the mutator phenotype. If so, the characteristics of the UVM-constitutive phenotype observed in *mutA* or *mutC* cells and in pHM11-bearing cells (in the presence of IPTG) suggest that mutation fixation must be occurring in a majority of cells. This observation is incompatible with the hypothesis that low-level mistranslation of the ε mRNA gives rise to a transient mutator phenotype when an occasional pol-III complex is assembled from a rare but defective ε subunit. Because missense suppression efficiency is thought to be low (1 to 2%), one anticipates that this mechanism can only be operative in a very small fraction of transfected cells. Thus, this mechanism appears unlikely to account for the UVM-constitutive phenotype we have described here.

However, the involvement of the ε subunit remains an attractive possibility. To retain a role for the ε subunit in the observed phenotypes, one can propose that the critical events in *mutA* cells are upstream of the ε subunit such that the activity of the ε subunit in a majority of pol-III complexes is affected. It is possible that an alteration in the translational rate or accuracy in *mutA* or *-C* cells activates a pathway that ultimately suppresses the editing activity of the ε subunit. Note, however, that mechanisms that do not involve the ε subunit have not been eliminated; indeed, there is no formal proof that mistranslation by the mutant glycine tRNA is required for the

TABLE 3. Effect of mutant glycine tRNA gene expression on spontaneous mutation to rifampin resistance

Expt, strain (relevant genotype)	Plasmid[a]	Presence of 1 mM IPTG	No. of Rif^r mutants/10^8 cells[b]
A, CC105 (*glyV*$^+$ *glyW*$^+$)	None	−	12
B, CC105*mutA*	None	−	92
C, CC105*mutC*	None	−	120
D, KH2 (*recA*$^+$)	pSE380	−	14
		+	11
	pHM22	−	18
		+	12
	pHM11	−	91
		+	585
E, KH2R (Δ*recA*)	pSE380	−	2
		+	15
	pHM22	−	10
		+	2
	pHM11	−	3
		+	6
F, SR100 (Δ*umuDC*)	pSE380	−	4
		+	6
	pHM22	−	10
		+	14
	pHM11	−	43
		+	130
G, HM105 (Δ*recA mutA*)	None	−	4
H, HM106 (Δ*recA mutC*)	None	−	4

[a] pSE380 is the vector control, pHM22 has the wild-type *glyV* gene, and pHM11 has the mutant *glyV* gene.

[b] Rifampin-resistant colonies that grew on LB plates supplemented with rifampin at 100 μg/ml.

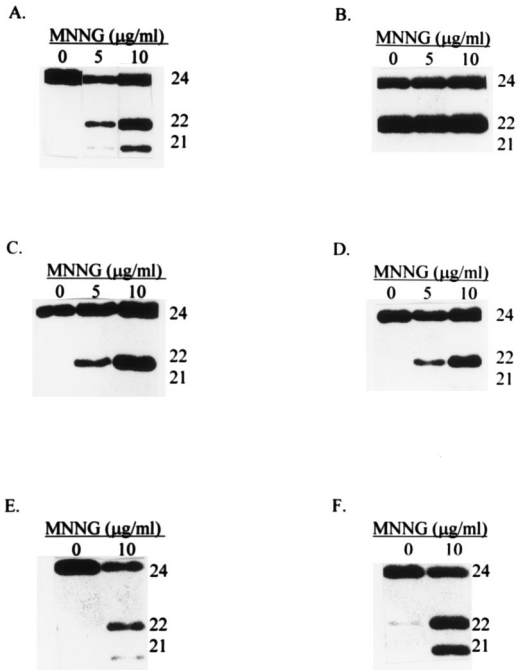

FIG. 5. Examples of multiplex analyses of mutation fixation at an εC residue on M13 ssDNA transfected into cells expressing a mutant glycine tRNA gene. Panels: A, KH2 (wild-type) cells bearing pHM22 (wild-type glycine tRNA gene) grown in the presence of 1 mM IPTG; B, KH2 cells bearing pHM11 (mutant glycine tRNA gene) grown in the presence of 1 mM IPTG; C, KH2R Δ*recA* cells bearing pHM22 (wild-type glycine tRNA gene) grown in the presence of 1 mM IPTG; D, KH2R Δ*recA* cells bearing pHM11 (mutant glycine tRNA gene) grown in the presence of 1 mM IPTG; E, HM105 Δ*recA mutA* cells; F, HM106 Δ*recA mutC* cells. Cells were pretreated with MNNG at 0, 5, or 10 μg/ml for 10 min at 37°C and transfected with ssDNA bearing εC, and the resulting progeny phage DNAs were subjected to multiplex sequence analysis as described in Fig. 2. Expression of the mutant tRNA gene (B), but not that of the wild-type tRNA gene (A), confers a UVM-constitutive phenotype (cf. 0-μg/ml MNNG lanes). However, in a Δ*recA* background, expression of the mutant tRNA gene (D) has little effect. This observation is confirmed by the absence of a UVM-constitutive phenotype in Δ*recA mutA* (E) and Δ*recA mutC* (F) cells. The values to the right of each panel are the lengths in nucleotides of the limit elongation products.

observed mutator phenotypes. For example, the signal that induces the mutator response may be generated by an altered interaction between the mutant glycine tRNA and an aminoacyl-tRNA synthetase.

Our observation that the *mutA* phenotype requires a functional *recA* gene raises a number of interesting issues. Because the RecA protein is known to play several distinct roles in the SOS pathway, the *recA* dependence may indicate that the mutator phenotype is manifested during translesion DNA synthesis at sites of spontaneous DNA damage. However, because the mutator phenotype does not depend on the *umuD* or *umuC* gene, the SOS mutagenesis pathway does not appear to be involved. The second interesting issue is that the UVM response does not require a functional *recA* gene, whereas the UVM-constitutive phenotype in *mutA* cells does. These observations suggest that the *mutA* pathway and the UVM pathway are distinct. However, the striking resemblance in the frequency and specificity of mutations in UVM-induced cells and uninduced *mutA* cells raises the possibility that the two pathways converge at a shared effector molecule regardless of whether the effector is the ε subunit. Thus, one would imagine that increased mistranslation in *mutA* cells generates a constitutive signal that acts in a *recA*-dependent manner to reduce the fidelity of DNA replication, whereas in the UVM pathway, treatment with DNA-damaging agents transiently activates the same mechanism in a manner that bypasses the requirement for *recA*. Despite these intriguing possibilities, whether the same proximate mechanisms underlie elevated mutagenesis in *mutA* cells and in UVM-induced wild-type cells is a question that remains to be addressed.

ACKNOWLEDGMENTS

We thank R. Maurer, J. Miller, E. J. Murgola, M. S. Rahman, and V. A. Palejwala for bacterial strains; L. Samson for suggestions; and anonymous referees for helpful comments.

This work was supported in part by grants from the NIH (CA73026) and the American Cancer Society (CN-113). H.S.M. received partial salary support from a graduate student fellowship awarded by the N.J. Commission on Cancer Research.

REFERENCES

1. **Amann, E., B. Ochs, and K. J. Abel.** 1988. Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene **69:**301–315.
2. **Barbin, A., and H. Bartsch.** 1986. Mutagenic and promutagenic properties of DNA adducts formed by vinyl chloride metabolites, p. 345–358. *In* B. Singer and H. Bartsch (ed.), The role of cyclic nucleic acid adducts in carcinogenesis and mutagenesis, vol. 70. International Agency for Research on Cancer, Lyon, France.
3. **Blanco, L., A. Bernad, and M. Salas.** 1992. Evidence favouring the hypothesis of a conserved 3′-5′ exonuclease active site in DNA-dependent DNA polymerases. Gene **112:**139–144. (Letter.)
4. **Brosius, J.** 1989. Laboratory methods: superlinkers in cloning and expression vectors. DNA **8:**759–777.
5. **Carbon, J., C. Squires, and C. W. Hill.** 1970. Glycine transfer RNA of *Escherichia coli*. II. Impaired GGA-recognition in strains containing a genetically altered transfer RNA; reversal by a secondary suppressor mutation. J. Mol. Biol. **52:**571–584.
6. **Chen, H.-J. C., and F.-L. Chung.** 1994. Formation of etheno adducts in reactions of enals via autooxidation. Chem. Res. Toxicol. **7:**857–860.
7. **Cox, E. C., and D. L. Horner.** 1983. Structure and coding properties of a dominant *Escherichia coli* mutator gene, *mutD*. Proc. Natl. Acad. Sci. USA **80:**2295–2299.
8. **Derbyshire, V., N. D. F. Grindley, and C. M. Joyce.** 1991. The 3′-5′ exonuclease of DNA polymerase I of *Escherichia coli*: contribution of each amino acid at the active site to the reaction. EMBO J. **10:**17–24.
9. **Echols, H., C. Lu, and P. M. J. Burgers.** 1983. Mutator strains of *Escherichia coli*, *mutD* and *dnaQ*, with defective exonucleolytic editing by DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA **80:**2189–2192.
10. **Fijalkowska, I. J., and R. M. Schaaper.** 1996. Mutants in the Exo I motif of *Escherichia coli dnaQ*: defective proofreading and inviability due to error catastrophe. Proc. Natl. Acad. Sci. USA **93:**2856–2861.
11. **Friedberg, E. C., G. C. Walker, and W. Siede.** 1995. DNA repair and mutagenesis. ASM Press, Washington, D.C.
12. **Higashitani, N., A. Higashitani, and K. Horiuchi.** 1993. Nucleotide sequence of the primer RNA for DNA replication of filamentous bacteriophages. J. Virol. **67:**2175–2181.
13. **Kelman, Z., and M. O'Donnell.** 1995. DNA polymerase III holoenzyme: structure and function of a chromosomal replicating machine. Annu. Rev. Biochem. **64:**171–200.
14. **Kornberg, A., and T. Baker.** 1991. DNA replication. W. H. Freeman & Co., New York, N.Y.
15. **Leithauser, M. T., A. Liem, B. C. Stewart, E. C. Miller, and J. A. Miller.** 1990. 1,N^6-Ethenoadenosine formation, mutagenicity and murine tumor induction as indicators of the generation of an electrophilic epoxide metabolite of the closely related carcinogens ethyl carbamate (urethane) and vinyl carbamate. Carcinogenesis **11:**463–473.
16. **Michaels, M. L., C. Cruz, and J. H. Miller.** 1990. *mutA* and *mutC*: two mutator loci in *Escherichia coli* that stimulate transversions. Proc. Natl. Acad. Sci. USA **87:**9211–9215.
17. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
18. **Model, P., and M. Russel.** 1988. Filamentous bacteriophage, p. 375–456. *In* R. Calendar (ed.), The bacteriophages, vol. 2. Plenum Press, New York, N.Y.
19. **Murgola, E. J.** 1985. tRNA, suppression and the code. Annu. Rev. Genet. **19:**57–80.
20. **Murgola, E. J., N. E. Prather, and K. H. Hadley.** 1978. Variations among glyV-derived glycine tRNA suppressors of glutamic acid codons. J. Bacteriol. **134:**801–807.

21. **Murphy, H. S., V. A. Palejwala, M. S. Rahman, P. M. Dunman, G. Wang, and M. Z. Humayun.** 1996. Role of mismatch repair in the *Escherichia coli* UVM response. J. Bacteriol. **178:**6651–6657.
22. **Normanly, J., R. C. Ogden, S. J. Horvath, and J. Abelson.** 1986. Changing the identity of a transfer RNA. Nature **321:**213–219.
23. **Palejwala, V. A., G. A. Pandya, O. S. Bhanot, J. J. Solomon, H. S. Murphy, P. M. Dunman, and M. Z. Humayun.** 1994. UVM, an ultraviolet-inducible RecA-independent mutagenic phenomenon in *Escherichia coli*. J. Biol. Chem. **269:**27433–27440.
24. **Palejwala, V. A., R. W. Rzepka, and M. Z. Humayun.** 1993. UV irradiation of *Escherichia coli* modulates mutagenesis at a site-specific ethenocytosine residue on M13 DNA. Evidence for an inducible recA-independent effect. Biochemistry **32:**4112–4120.
25. **Palejwala, V. A., R. W. Rzepka, D. Simha, and M. Z. Humayun.** 1993. Quantitative multiplex sequence analysis of mutational hot spots. Frequency and specificity of mutations induced by a site-specific ethenocytosine in M13 viral DNA. Biochemistry **32:**4105–4111.
26. **Palejwala, V. A., D. Simha, and M. Z. Humayun.** 1991. Mechanisms of mutagenesis by exocyclic DNA adducts. Transfection of M13 viral DNA bearing a site-specific adduct shows that ethenocytosine is a highly efficient RecA-independent mutagenic noninstructional lesion. Biochemistry **30:** 8736–8743.
27. **Palejwala, V. A., G. Wang, H. S. Murphy, and M. Z. Humayun.** 1995. Functional *recA*, *lexA*, *umuD*, *umuC*, *polA*, and *polB* genes are not required for the *Escherichia coli* UVM response. J. Bacteriol. **177:**6041–6048.
28. **Simha, D., V. A. Palejwala, and M. Z. Humayun.** 1991. Mechanisms of mutagenesis by exocyclic DNA adducts. Construction and in vitro template characteristics of an oligonucleotide bearing a single site-specific ethenocytosine. Biochemistry **30:**8727–8735.
29. **Slater, S. C., M. R. Lifsics, M. O'Donnell, and R. Maurer.** 1994. *holE*, the gene coding for the theta subunit of DNA polymerase III of *Escherichia coli*: characterization of a *holE* mutant and comparison with a *dnaQ* (epsilon-subunit) mutant. J. Bacteriol. **176:**815–821.
30. **Slupska, M. M., C. Baikalov, R. Lloyd, and J. H. Miller.** 1996. Mutator tRNAs are encoded by the *Escherichia coli* mutator genes *mutA* and *mutC*: a novel pathway for mutagenesis. Proc. Natl. Acad. Sci. USA **93:**4380–4385.
31. **Squires, C., and J. Carbon.** 1971. Normal and mutant glycine transfer RNAs. Nat. New Biol. **233:**274–277.
32. **Wang, G., and M. Z. Humayun.** 1996. Induction of the *Escherichia coli* UVM response by oxidative stress. Mol. Gen. Genet. **251:**573–579.
33. **Wang, G., V. A. Palejwala, P. M. Dunman, D. H. Aviv, H. S. Murphy, M. S. Rahman, and M. Z. Humayun.** 1995. Alkylating agents induce UVM, a *recA*-independent inducible mutagenic phenomenon in *Escherichia coli*. Genetics **141:**813–823.

ERRATUM

Escherichia coli Cells Expressing a Mutant *glyV* (Glycine tRNA) Gene Have a UVM-Constitutive Phenotype: Implications for Mechanisms Underlying the *mutA* or *mutC* Mutator Effect

HOLLY S. MURPHY AND M. ZAFRI HUMAYUN

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey 07103-2714

Volume 179, no. 23, p. 7510, column 1, line 24: "per microgram" should read "per 0.05 microgram."
Page 7511, Table 2, column 3 head: "No. of ic/μg" should read "No. of ic/0.05 μg."
Page 7511, Table 2, footnote *c*: "per microgram" should read "per 0.05 microgram."