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Requirement for Homologous Recombination Functions for Expression of the *mutA* Mistranslator tRNA-Induced Mutator Phenotype in *Escherichia coli*

LI REN, ABU AMAR M. AL MAMUN, 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

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Expression of the *Escherichia coli mutA* mutator phenotype requires *recA*, *recB*, *recC*, *ruvA*, and *ruvC* gene, but not *recD*, *recF*, *recO*, or *recR* genes. Thus, the *recBCD*-dependent homologous recombination system is a component of the signal pathway that activates an error-prone DNA polymerase in *mutA* cells.

DNA replication fidelity can be transiently reduced in response to environmental and physiological stimuli. In addition to the well-known *Escherichia coli* SOS system, emerging evidence suggests the existence of a number of such pathways in *E. coli* (7). One of the more intriguing newly recognized mutagenic pathways is the one elicited in *mutA* cells (7, 16, 27), in which the expression of an altered *glyV* glycine tRNA gene results in a strong mutator phenotype (27) characterized by elevation of transversions. In the *mutA* allele, the normal 3′-CCG anticodon is mutated to a 3′-CUG anticodon such that the mutant tRNA misreads the aspartate codon 5′-GAU/C as glycine at a low efficiency.

Expression of the *mutA* phenotype is constitutive and requires the *recA* and *recB* genes, but not *umuD*, *umuC*, *dinB*, or other *lexA*-repressible functions (16, 23), and thus represents a novel inducible mutagenic pathway termed "translational stress-induced mutagenesis" (TSM) (7).

The unexpected requirement for *recA* (in a non-SOS role) and *recB* genes in this pathway suggested that the *mutA* phenotype is homologous recombination dependent, since the RecA protein and RecBCD nuclease are principal components of the major homologous recombination pathway in *E. coli* (10). Whereas *recA* and *recBCD* functions are required for initiation of homologous recombination, *ruvA* and *ruvB* functions act together to catalyze branch migration of the Holliday junction, and *ruvC* encodes a Holliday junction-specific exonuclease (30).

To detect the mutator phenotype as elevated background mutagenesis, a colony papillation assay based on reversion of a *lacZ* mutant allele to *lacZ*$^+$ status, as described in detail by Miller and coworkers (14, 15), was used. The strains and plasmids used in this study are listed in Table 1. In this assay, *lacZ* mutant colonies are grown on minimal A agar plates containing limiting amounts of glucose on which they form colorless (white) colonies. After exhausting glucose as the carbon source in the medium, the colony stops growing. However, the P-Gal (phenyl-β-D-galactoside) in the medium can be utilized as a carbon source by any *lacZ*$^+$ revertant cells present within the *lacZ* mutant colonies. As a result, the *lacZ*$^+$ cells continue to divide to form microcolonies (papillae) within the larger growth-arrested *lacZ* mutant colony. For ease of observation, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), which is hydrolyzed to an insoluble blue dye by β-galactosidase (encoded by the *lacZ*$^+$ gene), is included on the papillation plates so that the papillae stain dark blue and hence become easier to detect (15). This papillation assay was originally used to identify *mutA* and *mutC* cells (14). An example of the effect of the *mutA* allele on colony papillation can be seen in Fig. 1A, sector 1, which shows that a streak of CC105 (wild-type) cells contains only a small number of papillae, reflecting a normal background level of mutagenesis. In contrast, sector 2 shows that a streak of CC105*mutA* cells contains numerous blue papillae, reflecting elevated background mutagenesis. The *mutA* phenotype is abolished in LR600 cells (CC105*mutA recC* [sector 4]) and in LR140 (CC105*mutA ruvC* [sector 8]) cells, but not in LR800 (CC105*mutA recD* [sector 6]) cells. The *mutA* phenotype is restored when complemented for *recBCD* genes on a multicopy plasmid (Fig. 1B, sectors 4 to 6). In contrast, expression of the *mutA* phenotype does not require *recD* (Fig. 1A, sector 6), as expected, because *recD*-defective cells remain recombination proficient (10). Figure 1C shows that the *ruvA* gene is also required (sectors 5 and 6) for the *mutA* phenotype. Figure 1D shows that the *mutA* phenotype is unaffected in cells defective for *recR* (sector 6), *recO* (sector 7), and *recF* (sector 8) genes, suggesting that in contrast to the *recBCD*-dependent homologous recombination pathway, a functional *recFOR*-dependent recombinational repair pathway (10, 28, 29) is not required for the *mutA* phenotype.

The *mutA* phenotype is manifested not only as an elevation in background mutagenesis at apparently undamaged DNA sites, as detected by the papillation assay, but also as a significant elevation in mutagenesis at the mutagenic exocyclic DNA lesion εC (see Fig. 2B for chemical structure) borne on M13 single-stranded DNA (ssDNA) vectors transfected into *E. coli* cells (16, 23). In this assay, M13 ssDNA bearing a single site-specific lesion (εC-ssDNA) is transfected into an appropriate strain, and the resulting progeny phage are analyzed for mutations at the εC site by a quantitative multiplex sequence analysis procedure summarized in Fig. 2C (16, 17, 19, 21). The assay depends on limited elongation of a prelabeled primer to characteristic lengths, depending on the base replacing the lesion upon replication.

An example of the effect of the *mutA* allele on mutation fixation at εC can be seen in Fig. 2D, in which lane 1 shows low mutagenesis (i.e., low intensity of 22- and 21-nucleotide [nt] bands corresponding to C→A and C→T mutants, respectively)

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

TABLE 1. Bacterial and plasmid strains used in this study

E. coli strain or plasmid	Relevant genotype	Source or reference
Strains		
AB1157	*argE3 hisG4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-1 thi-1 ara-1 rpsL31 supE44 tsx-33*	B. Bachman (4)
AK3	*recD1903*::mini-tet	A. Kuzminov (11)
AM115	CC105 *recF322*::Tn*3* (Ap^r)	P1.JAS34 X CC105 to Ap^r; screen for UV^S
AM116	CC105*mutA recF322*::Tn*3 (Ap*^{r}*)*	P1.JAS34 X CC105*mutA* to Ap^r; screen for UV^S
AM117	CC105 *recO1504*::Tn*5* (Kan^r)	P1.JAS20 X CC105 to Kan^r; screen for UV^S
AM118	CC105*mutA recO1504*::Tn*5* (Kan^r)	P1.JAS20 X CC105*mutA* to Kan^r; screen for UV^S
AM119	CC105 *recR252*::Tn*10-9* (Kan^r)	P1.JAS31 X CC105 to Kan^r; screen for UV^S
AM120	CC105*mutA recR252*::Tn*10-9* (Kan^r)	P1.JAS31 X CC105*mutA* to Kan^r; screen for UV^S
CC105	[*ara* Δ(*lac proB*) *xiii*] F′ *lacI Z proB*$^+$	J. Miller (14)
CC105*mutA*	*mutA590C* in CC105	J. Miller (14)
GS1481	Δ*ruvC64*::Kan (Kan^r) in AB1157	R. G. Lloyd (13)
JAS34[a]	*recF322*::Tn*3*(Ap^r)	J.A. Sawitzke (25)
JAS20[a]	*recO1504*::Tn*5* (Kan^r)	J. A. Sawitzke (25)
JAS31[a]	*recR252*::Tn*10-9* (Kan^r)	J. A. Sawitzke (25)
KH2R	Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) in KH2	This laboratory (20)
LR300	*recB268*::Tn*10* (Tet^r) in CC105	This laboratory (23)
LR400	*recB268*::Tn*10* (Tet^r) in CC105*mutA*	This laboratory (23)
LR500	*recC266*::Tn*10* (Tet^r) in CC105	P1.N2103 X CC105 to Tet^r; screen for UV^S
LR600	*recC266*::Tn*10* (Tet^r) in CC105*mutA*	P1.N2103 X CC105*mutA* to Tet^r; screen for UV^S
LR700	*recD1903*::mini-tet (Tet^r) in CC105	P1.AK3 X CC105 to Tet^r; screen for absence of Exo V activity
LR800	*recD1903*::mini-tet (Tet^r) in CC105*mutA*	P1.AK3 X CC105*mutA* to Tet^r; screen for absence of Exo V activity
LR110	*ruvA60*::Tn*10* (Tet^r) in CC105	P1.N2507 X CC105 to Tet^r; screen for UV^S
LR120	*ruvA60*::Tn*10* (Tet^r) in CC105*mutA*	P1.N2507 X CC105*mutA* to Tet^r; screen for UV^S
LR130	Δ*ruvC64*::Kan (Kan^r) in CC105	P1.GS1481 X CC105 to Kan^r; screen for UV^S
LR140	Δ*ruvC64*::Kan (Kan^r) in CC105*mutA*	P1.GS1481 X CC105*mutA* to Kan^r; screen for UV^S
N2103	*recC266*::Tn*10* (Tet^r) in AB1157	R. G. Lloyd (12)
N2507	*ruvA60*::Tn*10* (Tet^r) in AB1157	R. G. Lloyd (26)
Plasmids		
pBR322	Ap^r Tet^r (vector)	R. Brent (2)
pDWS2	pBR322 derivative harboring *recB*, *recC*, and *recD* genes	A. Kuzminov (22)

[a] Other markers used were *recB21 recC22 sbcB15 sbcC201sup0 hsdR ara-14* Δ(*gpt-proA*)*62 lacY1 tsx-33 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1* Rac^- F^- λ^-.

in CC105 (wild-type) cells, whereas lane 2 shows elevated mutagenesis (significantly increased signal in C→A and C→T mutant bands) in CC105*mutA* cells. In quantitative terms, mutagenesis at εC in CC105 (wild-type) cells is about 5% (Table 2), whereas in CC105*mutA* cells, it is about 45% (Table 2). As shown in Fig. 2, in cells defective for *recB* (lane 4), *ruvA* (lane 8), or *ruvC* (lane 10), the *mutA* phenotype is abolished, whereas it is unaffected in cells defective for *recD* (lane 6), in complete agreement with the results obtained with the papillation assay. These observations are quantitatively expressed in Table 2.

The requirement for *recA*, *recB*, *recC*, *ruvA*, and *ruvC* genes (but not the *recD* gene) allows the conclusion that a functional *recBCD*-dependent homologous recombination system is indeed required for the expression of the *mutA* phenotype. While this finding is intriguing, it is not immediately apparent why a functional *recBCD*-mediated recombination system is required for the expression of the *mutA* phenotype. Even though it is tempting to propose that the special features of recombination-mediated initiation of a replication fork on the bacterial chromosome (9) might account for the involvement of recombination in the mutator phenotype, it does not readily account for several observations. (i) An error-prone DNA polymerase is found in cell extracts from *mutA* cells, implying the modification of an existing DNA polymerase or the induction of a normally repressed polymerase (1). (ii) In the in vivo εC mutagenesis assay, mutation fixation occurs during the conversion of the transfected εC-ssDNA to the parental double-stranded replicative form DNA; it is possible that blocked elongation at the lesion site mimics a recombination-mediated initiation event, but this possibility by itself cannot explain mutation elevation at undamaged sites (1, 16). (iii) The requirement not only for recombination-initiation functions, such as *recA*, *recB*, and *recC*, but also for those required for its completion, such as *ruvA* and *ruvC*, suggest that the ability to conclude recombination is as important as the initiation process.

TABLE 2. Effect of *recB*, *recD*, *ruvA*, and *ruvC* defects on the *mutA* phenotype detected as mutation fixation at an εC residue borne on transfected M13 ssDNA

E. coli strain	Mean ± SD survival[a]	Mean ± SD % mutation frequency[b]		
		Total	C→A	C→T
CC105	630 ± 185	5 ± 1	2 ± 0	3 ± 1
CC105*mutA*	800 ± 220	45 ± 3	38 ± 2	7 ± 1
LR300 (CC105 *recB*)	650 ± 132	5 ± 2	2 ± 1	3 ± 1
LR400 (CC105*mutA recB*)	270 ± 65	6 ± 2	3 ± 1	3 ± 1
LR700 (CC105 *recD*)	840 ± 92	6 ± 2	3 ± 1	3 ± 1
LR800 (CC105*mutA recD*)	910 ± 164	47 ± 4	36 ± 2	11 ± 2
LR110 (CC105 *ruvA*)	680 ± 130	5 ± 2	2 ± 1	3 ± 1
LR120 (CC105*mutA ruvA*)	320 ± 40	7 ± 2	2 ± 1	5 ± 1
LR130 (CC105 *ruvC*)	720 ± 190	2 ± 2	1 ± 1	1 ± 1
LR140 (CC105*mutA ruvC*)	350 ± 36	2 ± 2	1 ± 1	1 ± 1

[a] Values represent numbers of infectious centers per transfection (per 50 ng of ssDNA) and are averages of results from three independent transfections of εC-ssDNA.

[b] Multiplex sequence analysis data shown were averaged from three to six independent elongation assays. Numbers are rounded to the nearest integer.

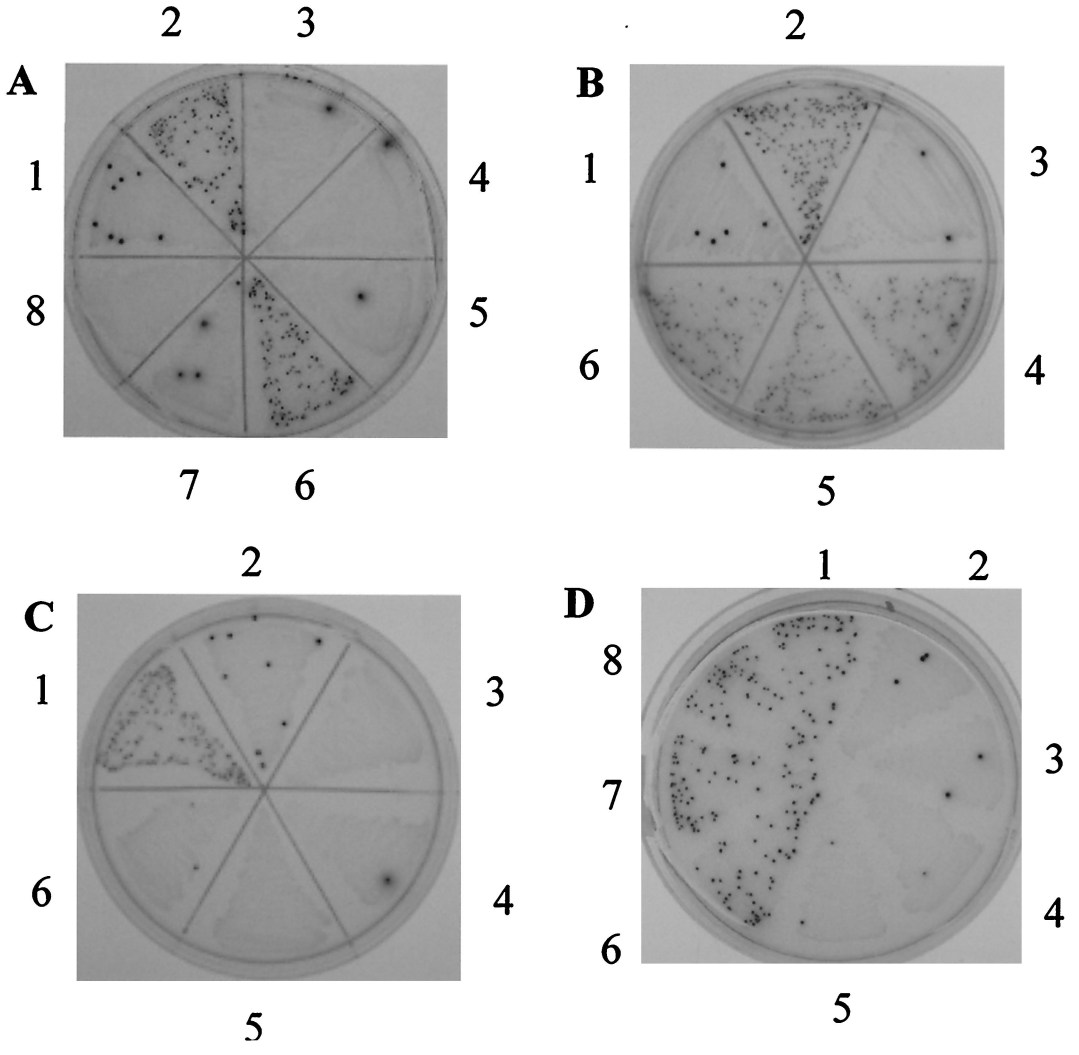

FIG. 1. Requirement for recombination genes required for the *mutA* phenotype detected by a colony papillation assay based on reversion of the *lacZ* mutant to a *lacZ*$^+$ phenotype as described in detail elsewhere (15). Strains were streaked on minimal agar-based indicator plates (papillation plates) and incubated for 5 days at 37°C before observation. (A) Effect of *recC*, *recD*, and *ruvC* mutations on expression of the *mutA* mutator phenotype. Sectors: 1, *E. coli* CC105 (wild-type) control cells; 2, CC105*mutA* cells showing characteristically high papillation; 3, *E. coli* LR500 (CC105 *recC*); 4, *E. coli* LR600 (CC105*mutA recC*); 5, *E. coli* LR700 (CC105 *recD*); 6, *E. coli* LR800 (CC105*mutA recD*); 7, *E. coli* LR130 (CC105 *ruvC*); 8, *E. coli* LR140 (CC105*mutA ruvC*). (B) Overexpression of *recBCD* genes restores the *mutA* phenotype in *mutA recC* cells. Sectors: 1, CC105 control; 2, CC105*mutA*, showing high papillation; 3, LR500 (CC105 *recC*)/pDWS2(*recB*$^+$ -*C*$^+$ -*D*$^+$); 4, 5, and 6, LR600 (CC105*mutA recC* [three isolates])/pDWS2(*recB*$^+$ -*C*$^+$ -*D*$^+$) showing restoration of papillation. (C) Effect of *ruvA* mutation on the expression of the *mutA* mutator phenotype. Sectors: 1, CC105*mutA* showing high papillation characteristic of *mutA* cells; 2, CC105 control with few papillae; 3 and 4, *E. coli* LR110 (CC105 *ruvA* [two isolates]) controls; 5 and 6, *E. coli* LR120 (CC105*mutA ruvA* [two isolates]) showing that the *mutA* phenotype is abolished in *ruvA* cells. (D) Effect of *recF*, *recO*, and *recR* mutations on the expression of the *mutA* mutator phenotype. Sectors: 1, CC105*mutA* showing characteristically high papillation; 2, CC105 control, showing few papillae; 3, *E. coli* AM115 (CC105 *recF*); 4, *E. coli* AM117 (CC105 *recO*) showing few papillae; 5, *E. coli* AM119 (CC105 *recR*) showing few papillae; 6, *E. coli* AM120 (CC105*mutA recR*) showing that the high papillation characteristic of *mutA* cells is unaffected in *recR* cells; 7, *E. coli* AM118 (CC105*mutA recO*) showing that the high papillation characteristic of *mutA* cells is unaffected in *recO* cells; 8, *E. coli* AM116 (CC105*mutA recF*) showing that the high papillation characteristic of *mutA* cells is unaffected in *recF* cells.

Exposed ssDNA regions at the sites of replication arrest are thought to be the signal required for SOS induction. Formation of specific DNA structures during homologous recombination (such as the cross-strand Holliday junction) may similarly act as a signal for TSM induction. However, the requirement for *ruvC*, the Holliday junction resolvase, suggests that the junction by itself probably does not constitute the signal, although other interpretations cannot be ruled out. Rather, the nucleoprotein complex containing the Holliday junction, as well as *ruvA*-, *ruvB*-, and *ruvC*-encoded proteins, may constitute the signal.

It is interesting that the so-called adaptive mutagenesis phenomenon (for recent reviews, see references 3, 5, 6, and 24) is similar to the TSM pathway in its genetic requirements and the fact that mutagenesis is elevated in a *lacZ* marker gene on the F′ episome. In adaptive mutagenesis, −1-bp deletions appear to be increased in the stationary phase, and this increase is partially suppressed by mutations in cells defective for *recA*, *recBC*, and *ruvAB* genes. However, the TSM pathway differs from adaptive mutagenesis in several regards: TSM is manifested in growing cells, mainly induces base substitutions, elevates mutation fixation at a DNA lesion, and increases mutagenesis not only in marker genes carried on the F′ episome, but also on the chromosome, as evidenced by the elevation in

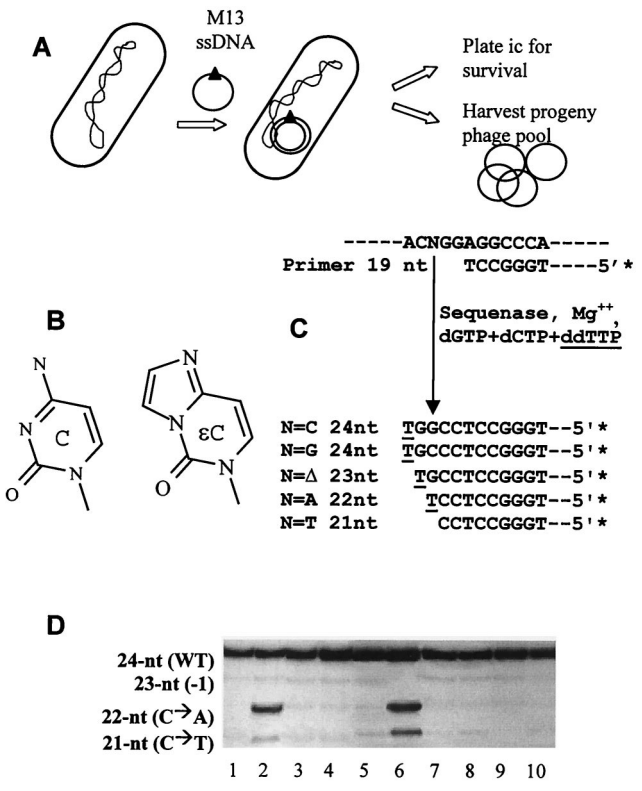

FIG. 2. (A) Summary of methodology used to analyze survival effects and mutagenesis at a site-specific εC residue (solid triangle) borne on M13 ssDNA. Procedures for transfection and measurement of survival (as infectious centers [ic]) and mutagenic effects have been described in detail elsewhere (17, 19–21) and in Materials and Methods. (B) Chemical structure of εC, shown alongside that of normal cytosine for comparison. (C) Principles of multiplex sequence analysis as previously described in detail (17, 21). Five micrograms of pooled progeny phage DNA (~2 pmol) was annealed to ~1 pmol of 5′-^{32}P-end-labeled 19-mer primer. Approximately 0.2 pmol of the annealed template was incubated with approximately 0.5 U of T7 DNA polymerase devoid of 3′-to-5′ exonuclease activity (Sequenase 2.0; U.S. Biochemicals) in the presence of 1 μM (each dCTP and dGTP, 10 μM dideoxythymidine-5′-triphosphate (ddTTP), and 20 mM $MgCl_2$ in buffer (40 mM Tris-HCl [pH 7.6], 50 mM NaCl, 10 mM dithiothreitol). Under these conditions, limited primer extension occurs, such that elongation on each of the four species of template DNA (i.e., wild type, C→T transitions, C→A transversions, and 1-nt deletions) results in a product of a different length. Note that C→G transversions are not induced by εC at significant levels (8, 18, 20) and are therefore not separately measured in the assay. The elongation products were fractionated on high-resolution 16% polyacrylamide–8 M urea gels, and the proportion of each product was determined from densitometric analyses of autoradiographs as described previously (17–19). Every elongation assay was monitored by parallel elongation of standard template DNA mixes containing known proportions of authentic mutant and wild-type DNAs. Mutation frequency was calculated by dividing the signal in each mutant band by the sum of signals in all bands. (D) Examples of multiplex sequence analyses of mutagenesis at the εC lesion. The elongation products are identified to the left of the autoradiograph. WT, wild type. Lanes: 1, *E. coli* CC105 (barely detectable signal in C→A and C→T bands); 2, CC105*mutA* (strong signal in C→A and C→T bands); 3, LR300 (CC105 *recB*); 4, LR400 (CC105*mutA recB*); 5, LR700 (CC105 *recD*); 6, LR800 (CC105*mutA recD*); 7, LR110 (CC105 *ruvA*); 8, LR120 (CC105*mutA ruvA*); 9, LR130 (CC105 *ruvC*); 10, LR140 (CC105*mutA ruvC*).

forward mutagenesis to rifampin resistance in *mutA* cells (16). Furthermore, mutagenesis is also elevated on a transfected M13 viral genome, and, finally, an error-prone DNA polymerase activity is expressed in TSM-induced cells (1).

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REFERENCES

1. **Al Mamun, A. A. M., M. S. Rahman, and M. Z. Humayun.** 1999. *Escherichia* coli cells bearing *mutA*, a mutant *glyV* tRNA gene, express a *recA*-dependent error-prone replication activity. Mol. Microbiol. **33:**732–740.
2. **Brent, R., and M. Ptashne.** 1980. The *lexA* gene product represses its own promoter. Proc. Natl. Acad. Sci. USA **77:**1932–1936.
3. **Cairns, J.** 1998. Mutation and cancer: the antecedents to our studies of adaptive mutation. Genetics **148:**1433–1440.
4. **De Witt, S. K., and E. A. Adelberg.** 1962. The occurrence of a genetic transposition in a strain of *Escherichia coli*. Genetics **47:**577–586.
5. **Foster, P. L.** 1998. Adaptive mutation: has the unicorn landed? Genetics **148:**1453–1459.
6. **Foster, P. L., and W. A. Rosche.** 1999. Increased episomal replication accounts for the high rate of adaptive mutation in *recD* mutants of *Escherichia coli*. Genetics **152:**15–30.
7. **Humayun, M. Z.** 1998. SOS and Mayday: multiple inducible mutagenic pathways in *Escherichia coli*. Mol. Microbiol. **30:**905–910.
8. **Jacobsen, J. S., C. P. Perkins, J. T. Callahan, K. Sambamurti, and M. Z. Humayun.** 1989. Mechanisms of mutagenesis by chloroacetaldehyde. Genetics **121:**213–222.
9. **Kogoma, T.** 1997. Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. Microbiol. Mol. Biol. Rev. **61:**212–238.
10. **Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer.** 1994. Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:**401–465.
11. **Kuzminov, A., and F. W. Stahl.** 1997. Stability of linear DNA in *recA* mutant *Escherichia coli* cells reflects ongoing chromosomal DNA degradation. J. Bacteriol. **179:**880–888.
12. **Lloyd, R. G., C. Buckman, and F. E. Benson.** 1987. Genetic analysis of conjugational recombination in *Escherichia coli* K12 strains deficient in RecBCD enzyme. J. Gen. Microbiol. **133:**2531–2538.
13. **Mandal, T. N., A. A. Mahdi, G. J. Sharples, and R. G. Lloyd.** 1993. Resolution of Holliday intermediates in recombination and DNA repair: indirect suppression of *ruvA*, *ruvB*, and *ruvC* mutations. J. Bacteriol. **175:**4325–4334.
14. **Michaels, M. L., C. Cruz, and J. H. Miller.** 1990. *mutA* and *mutC*: two mutator loci in *Escherichia coli* that stimulate transversions. Proc. Nat. Acad. Sci. USA **87:**9211–9215.
15. **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.
16. **Murphy, H. S., and M. Z. Humayun.** 1997. *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. J. Bacteriol. **179:**7507–7514.
17. **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.
18. **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.
19. **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.
20. **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.
21. **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.
22. **Ponticelli, A. S., D. W. Schultz, A. F. Taylor, and G. R. Smith.** 1985. Chi-dependent DNA strand cleavage by RecBC enzyme. Cell **41:**145–151.
23. **Ren, L., A. A. M. Al Mamun, and M. Z. Humayun.** 1999. The *mutA* mistranslator tRNA-induced mutator phenotype requires *recA* and *recB* genes, but not derepression of *lexA*-regulated functions. Mol. Microbiol. **32:**607–616.
24. **Rosenberg, S. M., C. Thulin, and R. S. Harris.** 1998. Transient and heritable mutators in adaptive evolution in the lab and in nature. Genetics **148:** 1559–1566.
25. **Sawitzke, J. A., and F. W. Stahl.** 1997. Roles for lambda Orf and *Escherichia coli* RecO, RecR and RecF in lambda recombination. Genetics **147:**357–369.
26. **Shurvinton, C. E., R. G. Lloyd, F. E. Benson, and P. V. Attfield.** 1984.

Genetic analysis and molecular cloning of the *Escherichia coli ruv* gene. Mol. Gen. Genet. **194:**322–329.
27. **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.
28. **Tseng, Y. C., J. L. Hung, and T. C. Wang.** 1994. Involvement of RecF pathway recombination genes in postreplication repair in UV-irradiated *Escherichia coli* cells. Mutat. Res. **315:**1–9.
29. **Webb, B. L., M. M. Cox, and R. B. Inman.** 1997. Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. Cell **91:**347–356.
30. **West, S. C.** 1997. Processing of recombination intermediates by the RuvABC proteins. Annu. Rev. Genet. **31:**213–244.