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Functional *recA*, *lexA*, *umuD*, *umuC*, *polA*, and *polB* Genes Are Not Required for the *Escherichia coli* UVM Response

VASEEM A. PALEJWALA, GE WANG, HOLLY S. MURPHY, AND M. ZAFRI HUMAYUN*

Department of Microbiology and Molecular Genetics, UMD-New Jersey Medical School, Newark, New Jersey 07103-2714

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The *Escherichia coli* UVM response is a recently described phenomenon in which pretreatment of cells with DNA-damaging agents such as UV or alkylating agents significantly enhances mutation fixation at a model mutagenic lesion (3,N^4-ethenocytosine; εC) borne on a transfected M13 single-stranded DNA genome. Since UVM is observed in Δ*recA* cells in which SOS induction should not occur, UVM may represent a novel, SOS-independent, inducible response. Here, we have addressed two specific hypothetical mechanisms for UVM: (i) UVM results from a *recA*-independent pathway for the induction of SOS genes thought to play a role in induced mutagenesis, and (ii) UVM results from a polymerase switch in which M13 replication in treated cells is carried out by DNA polymerase I (or DNA polymerase II) instead of DNA polymerase III. To address these hypotheses, *E. coli* cells with known defects in *recA*, *lexA*, *umuDC*, *polA*, or *polB* were treated with UV or 1-methyl-3-nitro-1-nitrosoguanidine before transfection of M13 single-stranded DNA bearing a site-specific ethenocytosine lesion. Survival of the transfected DNA was measured as transfection efficiency, and mutagenesis at the εC residue was analyzed by a quantitative multiplex DNA sequencing technology. Our results show that UVM is observable in Δ*recA* cells, in *lexA3* (noninducible SOS repressor) cells, in LexA-overproducing cells, and in Δ*umuDC* cells. Furthermore, our data show that UVM induction occurs in the absence of detectable induction of *dinD*, an SOS gene. These results make it unlikely that UVM results from a *recA*-independent alternative induction pathway for SOS genes. Similarly, UVM is observed in *polA* (deficient in DNA polymerase I) and *polB* (deficient in DNA polymerase II) cells, suggesting that neither polymerase plays an indispensable role in UVM induction. Furthermore, our data show that the UVM response is accompanied by enhanced survival (UVM reactivation) of M13 DNA bearing εC. The observation of UVM reactivation makes simple repair-suppression models for UVM less attractive and increases the plausibility of mechanisms operating at the level of base insertion. We hypothesize that noncoding lesions fall into two categories. The so-called SOS-dependent (class 1) lesions require SOS functions at the extension (bypass) step, whereas class 2 noncoding lesions do not. It is proposed that UVM, a previously unrecognized damage-inducible response, modulates base insertion at noncoding lesions.

Efficient replication past certain DNA lesions depends on factors not required for the replication of normal template DNA. In *Escherichia coli*, the required additional factors are thought to be encoded by the member genes of the SOS regulon. According to the widely accepted SOS hypothesis, unrepaired DNA damage induces the SOS genes, ultimately resulting in enhanced levels of specific gene products that are proposed to transiently alter the replication machinery.

The major features of the regulation of the SOS regulon are well described (20, 45, 50, 65, 66, 69). Under normal conditions, transcription of the SOS genes is repressed by the LexA repressor protein. Unrepaired DNA damage leads to replication arrest and exposes regions of single-stranded DNA (ssDNA) to which the RecA protein binds and then undergoes a dATP-dependent conformational transition to an activated RecA* (coprotease) form. The RecA* protein promotes autoproteolytic cleavage of the LexA repressor protein, thereby inducing the SOS regulon. The RecA* protein, in addition to its role in SOS induction, is also required for the proteolytic activation of the UmuD protein to UmuD′ and may play a role in recruiting UmuD′C proteins at arrested replication forks (2, 3, 12, 19, 22, 23, 37, 61, 70).

Although the regulation of SOS genes is well understood, the mechanisms of action of SOS functions in achieving translesion DNA synthesis are unknown. According to a recent interpretation of the SOS hypothesis, a nucleoprotein complex that includes the UmuD′C peptides, the RecA* protein, and the normal replication factors including DNA polymerase III (pol III) holoenzyme (a "mutasome") is assembled at the lesion site and is responsible for translesion synthesis (20). This idea is based on genetic evidence for the involvement of the *recA*, *umuD*, and *umuC* genes (45, 65, 66) as well as on the recent observation that a low but detectable level of replicative bypass of an abasic site by the DNA pol III holoenzyme occurs in vitro upon the addition of purified UmuC, UmuD′, and RecA proteins (51). We have argued elsewhere that inducible mechanisms in addition to those provided by the SOS regulon may regulate mutagenesis at certain noncoding DNA lesions (47).

3,N^4-Ethenocytosine (εC), a leading example of a mutagenic exocyclic DNA lesion, is induced by a number of industrial carcinogens such as vinyl chloride and ethyl carbamate (1, 35) and is now thought to also be induced by endogenous mutagens deriving from lipid peroxidation (15, 41, 63). Because of the etheno bridging of two of the three hydrogen-bonding positions, εC is expected to be a noninstructive lesion. The in vitro (56) and in vivo (27, 28, 49) template characteristics of εC

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, UMD-New Jersey Medical School, 185 South Orange Ave., MSB F607, Newark, NJ 07103-2714. Phone: (201) 982-5217. Fax: (201) 982-3644. Electronic mail address: humayun@umdnj.edu.

TABLE 1. *E. coli* strains

Strain	Relevant genotype	Source (reference)
KH2	Δ(*lac-pro*) *trpE4777* (F′ *lacI*^{q} *Z*ΔM15 *pro*$^+$)	This laboratory (54)
KH2R	Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) in KH2	This laboratory (54)
HM100	KH2/pRB160 (pBR322 clone of *lexA*$^+$ gene)	This laboratory
HM200	KH2R/pRB160	This laboratory
JH5	*lac*Δ*U169 dinD1*::MudI(Ap^r *lac*) F′	P. Model (25)
JH5R2	Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) in JH5	This laboratory
MC1061	Δ*polB* F$^-$	R. Moses (14)
CJ251	F′ (pOX38::Cm^r)	C. Joyce (24)
VP100	F′ (pOX38::Cm^r) from CJ251 in MC1061	This laboratory
DM49	F$^-$ *lexA3* in AB1157	D. Mount (44)
VP300	F′ (pOX38::Cm^r) from CJ251 in DM49	This laboratory
CJ231	Δ*polA* Km^r/pCJ102 (pCJ102 = F′ 5′ Exo Cm^r)	C. Joyce (29)
CGSC6451	F1-F10 (F1::Tn*10* Tet^r)	B. Bachmann
GY8347	F$^-$ Δ(*umuD umuC*)	S. Sommer (58)
VHDC	F1-10 (F1::Tn*10* Tet^r) from CGSC6451 in GY8347 Δ(*umuD umuC*)	This laboratory

are indeed those expected for such a lesion. For example, none of the four nucleotides are inserted by *E. coli* DNA pol I Klenow fragment opposite a site-specific εC residue borne on an oligodeoxyribonucleotide template under conditions in which guanine insertion opposite normal cytosine can be readily demonstrated on a control template (56). At high deoxynucleoside triphosphate concentrations, base misinsertion following the adenine rule pattern was observed (A > T > G > C), suggesting that εC has the in vitro template attributes of a noninformational lesion (56). Nevertheless, εC is a *recA*-independent, highly mutagenic lesion in gapped duplex DNA (27, 28, 49) with a mutational specificity (mostly C→T and C→A mutations) indicative of a noninformational lesion. Subsequent work showed that, remarkably, prior exposure of *recA*$^+$ or Δ*recA* *E. coli* cells to UV resulted in significantly enhanced mutagenesis at a site-specific εC residue present on an M13 gapped duplex DNA (47) or M13 ssDNA (46). Since SOS induction requires the *recA* gene, the inducible mutagenesis observed in UV-pretreated cells could represent a novel, SOS-independent phenomenon that we have termed UVM (for UV modulation of mutagenesis). UVM is now known to also be induced by alkylating agents (67) and by other DNA-damaging agents (67a). As discussed elsewhere, a number of hypotheses can account for the observation of inducible mutagenesis in Δ*recA* cells (47) (also see Discussion). Here, we have addressed two specific hypotheses: (i) one or more SOS genes are induced by alternative, *recA*-independent mechanisms, and (ii) UVM results from a polymerase switch from DNA pol III to pol I.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1.

Construction of M13 ssDNA bearing a site-specific εC lesion. M13 ssDNA bearing a site-specific εC residue (εC-DNA) and the corresponding control construct with normal cytosine in place of εC (C-DNA) were constructed as previously described (46) except for the following modification. The 57-nucleotide scaffold oligonucleotide had a single base change (see Fig. 1, underlined base) to differentiate (see Fig. 2) progeny phage arising from the viral plus strand from the occasional progeny that could arise from the elongation of the scaffold that was not completely removed at the denaturation step (see Fig. 1).

UV and 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) treatment of bacterial cells. *E. coli* cells exponentially growing in Luria-Bertani (LB) medium were UV irradiated at 50 J/m^2 (*recA*$^+$) or 10 J/m^2 (Δ*recA*) as described earlier (46, 47) and were processed for transfection. The effect of various UV doses on the survival of strains KH2 and KH2R has been previously described (47). *E. coli* cells exponentially growing in LB medium were exposed to MNNG (0 to 10 μg/ml) for 10 min at 37°C (67). The treated cells were pelleted, washed with fresh LB medium to remove MNNG, and processed for transfection.

Transfection and preparation of pooled progeny phage DNA. The procedures employed for making *E. coli* cells competent for transfection of εC-DNA or C-DNA, for determining survival effects, and for preparing pooled progeny phage DNA have been described in detail elsewhere (46–49, 53).

Quantitative multiplex DNA sequence analysis of pooled progeny phage DNA. The principles and experimental validation of multiplex DNA sequencing technology used to analyze mutation fixation at εC have been described in detail elsewhere (46–48) and are summarized in Fig. 2. Elongation reactions were performed at 37°C for 15 min in a 10-μl final volume and consisted of 0.1 pmol of template DNA (pooled progeny phage DNA) primed with a 5′-^{32}P-end-labeled 19-mer primer, dGTP (1 μM), dCTP (1 μM), ddTTP (10 μM), 20 mM $MgCl_2$, and T7 DNA polymerase (0.5 to 1 vendor unit; Sequenase version 2.0; U. S. Biochemicals) in buffer (40 mM Tris-HCl, 50 mM NaCl, 10 mM dithiothreitol, pH 7.5). As seen in Fig. 2, primer extension on each of the five species of DNA within the progeny DNA pool yields a product of a different length. Fractionation of these elongation products by high resolution 16% denaturing polyacrylamide gel electrophoresis followed by computing densitometry of autoradiographs allows the determination of mutation frequency and specificity (46–48, 56).

RESULTS

The experimental system. Exponentially growing *E. coli* cells were exposed to the inducing agent; this was followed by transfection of M13 ssDNA bearing a site-specific εC residue (εC-DNA) or the corresponding control DNA (C-DNA). The chemical structure of εC, as well as the strategy used for its incorporation into M13 ssDNA, is shown in Fig. 1. Survival of the DNA is determined as transfection efficiency, using a standard plaque assay. Mutation fixation at the lesion site is quantitatively analyzed at the sequence level by a multiplex sequencing technology that we have previously established and validated (an outline is shown in Fig. 2). It is important to recognize that in this experimental system the inducing agent is removed before transfection. Therefore, the inducing agent does not directly act on the transfected DNA.

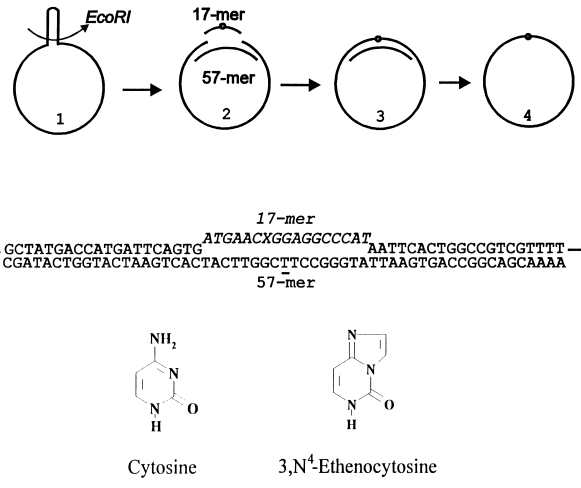

FIG. 1. Construction of M13 ssDNA bearing a site-specific εC residue. The principal steps involved in the construction of the vector have been summarized and have been described previously (46). The DNA sequence of the 3′ and 5′ ends of the linearized ssDNA, of the 17-mer, and of the 57-mer scaffold oligonucleotide are shown. The construct used for the experiment (molecule 4) was obtained by heat denaturing molecule 3 at 90°C for 5 min in the presence of a 10-fold molar excess of a 57-mer anti-scaffold oligonucleotide. Notice that the scaffold contains a mismatched T (underlined) residue so as to create a GT mispair two nucleotides 3′ of the lesion (base X). This mismatch allows the differential detection of occasional progeny phage in which information transfer occurs from scaffold molecules that fail to denature. The chemical structures of cytosine and ethenocytosine are shown at the bottom.

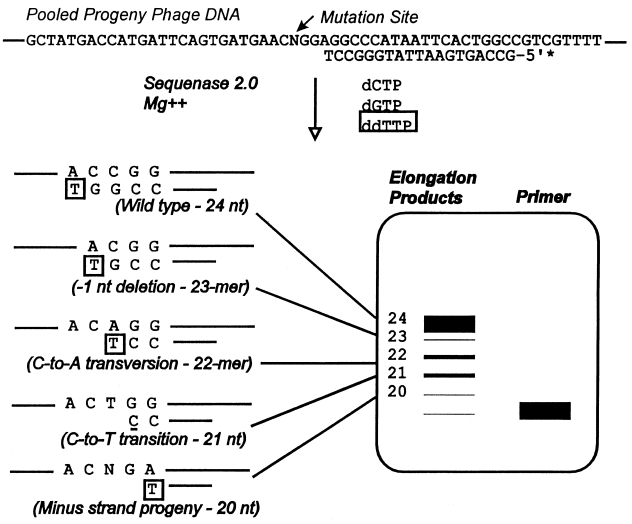

FIG. 2. Outline of procedures used for multiplex sequence analysis of pooled progeny phage DNA. A 5′-γ-^{32}P-end-labeled primer is annealed to the pooled DNA and elongated by a mutant T7 DNA polymerase deficient in 3′→5′ exonuclease activity (Sequenase version 2.0) in the presence of dGTP, dCTP, and ddTTP. Limited primer elongation occurs on each of the five templates present in the progeny pool DNA, giving rise to a product of a different length. Mutation frequency and specificity figures are calculated from the normalized signal in 21-, 22-, and 23-mer products (from mutant templates) and in the 24-mer product (from wild-type template) by computing densitometry of autoradiographs as described in detail elsewhere (46–48). Progeny DNA that arises from the priming of the scaffold molecule gives rise to a 20-mer product as shown above. Any progeny arising by information transfer from unremoved scaffold does not interfere in this assay, as only the signal in the 21-mer through 24-mer bands is analyzed in the assay. It may be noted that the assay does not measure C→G mutations, as εC is not known to induce these mutations at significant levels (27, 28, 48, 49).

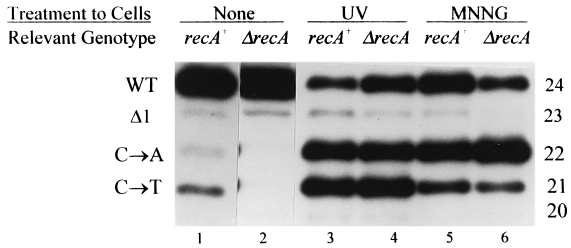

FIG. 3. Multiplex DNA sequence analysis of pooled progeny phage DNA obtained after transfection of M13 ssDNA bearing a site-specific εC residue into wild-type *E. coli* KH2 *recA*$^+$ (lanes 1, 3, and 5) and *E. coli* KH2R Δ*recA* (lanes 2, 4, and 6) cells subjected to the following pretreatments before transfection. Lanes 1 and 2, untreated; lane 3, UV treated (50 J/m^2); lane 4, UV treated (10 J/m^2); lane 5, MNNG treated (10 μg/ml); lane 6, MNNG treated (10 μg/ml). Each lane shows results from an independent transfection and is in principle equivalent to data obtained from sequencing of a large number of individual progeny phage plaques. The procedures used are outlined in Fig. 2 and described in detail elsewhere (46–48). The identity of each elongation product is indicated to the left of lane 1 (Δ1, 1-nucleotide deletion), and the corresponding length in nucleotides is indicated to the right of lane 6. Transfection of εC-containing ssDNA into wild-type *E. coli* KH2 and Δ*recA E. coli* KH2R cells exposed to UV radiation or pretreated with MNNG results in the production of wild-type progeny (24-mer band) as well as mutant progeny (21-mer and 22-mer bands). Pretreatment results in a dramatic increase in mutagenesis, as indicated by the intensity of mutant bands in lanes 3 to 6 compared with those for uninduced cell controls (lanes 1 and 2). As expected, no mutagenesis is observed when a control DNA construct (C in place of εC) is transfected in uninduced or induced cells (data not shown) (see references 46 to 48 for examples). Note that MNNG induces more transversions (22-mer) than transitions (21-mer), whereas UV appears to induce both types equally well. WT, wild type.

UVM induction occurs in *recA*$^+$ and in Δ*recA E. coli* cells. Figure 3 shows that pretreatment of exponentially growing *E. coli* cells with UV or MNNG markedly enhances mutagenesis at εC. In uninduced cells, mutagenesis at εC occurs at low levels, as indicated by the relatively low intensity of the transition (21-mer) and the transversion (22-mer) bands (total mutation frequency, <10%). UV irradiation of *E. coli recA*$^+$ cells (50 J/m^2) or Δ*recA* cells (10 J/m^2) results in a strong induction of transition as well as transversion mutations. Similarly, pretreatment of *recA*$^+$ or Δ*recA* cells with MNNG (10 μg/ml) for 10 min at 37°C results in a strong induction of mutagenesis at εC. Table 2 is a quantitative summary of data from three or four multiplex assays. The data in Fig. 3 and Table 2 make it clear that the induction of enhanced mutagenesis by UV or by MNNG does not require the *recA* gene. It may be noted that even though the overall pattern of UVM induction by MNNG in *recA*$^+$ cells is similar to that for Δ*recA* cells, the induction appears to be stronger in Δ*recA* cells. It is also interesting that C→T transitions and C→A transversions are induced to an equal extent by UV, whereas UVM induction by MNNG results in more transversions than transitions. The significance of these differences is not clear, but they may indicate the involvement of more than one mutational mechanism.

UVM induction occurs in the absence of detectable SOS gene induction. To demonstrate that UVM induction occurs in the absence of significant SOS induction, expression of the SOS gene *dinD* (25) was examined after MNNG treatment under conditions known to induce UVM. *E. coli* JH5 *recA*$^+$ *lac*Δ*U169 dinD1*::MudI(Ap^r *lac*) F′ and JH5R2, a Δ(*srlR-recA*) *306*::Tn*10*(Tet^r) derivative of JH5, were treated with MNNG (10 μg/ml) or mitomycin (10 μg/ml) for 10 min at 37°C, and *dinD* gene expression was measured by assaying for β-galactosidase. Table 3 shows that MNNG treatment that results in strong UVM induction does not induce the *dinD* gene. As expected, mitomycin treatment results in *dinD* induction in JH5 *recA*$^+$ cells.

UVM induction in *lexA3*, in Δ(*umuD umuC*), and in LexA-overproducing *E. coli* cells. Figure 4 shows the effect of MNNG pretreatment of four different SOS-defective strains, and Table 4 provides a quantitative summary of the data represented by Fig. 4. The *lexA3* mutation in *E. coli* VP300 renders the LexA repressor noncleavable and thereby renders the SOS regulon

TABLE 2. The effect of prior UV irradiation or MNNG treatment of *recA*$^+$ and Δ*recA E. coli* cells on mutagenesis at a site-specific εC residue borne on M13 ssDNA

Relevant genotype	Treatment	Mutation frequency (%) (±SD)[a]	Mutation specificity (%) (±SD)[a]		
			C→T	C→A	Δ1[b]
recA$^+$	None	5 (±1)	3 (±1)	2 (±1)	0 (±1)
Δ*recA*	None	3 (±0)	1 (±0)	1 (±0)	1 (±0)
recA$^+$	UV[c]	86 (±2)	43 (±0)	40 (±1)	2 (±1)
Δ*recA*	UV[d]	74 (±1)	40 (±1)	33 (±2)	1 (±1)
recA$^+$	MNNG[e]	57 (±3)	15 (±4)	41 (±1)	1 (±1)
Δ*recA*	MNNG[e]	81 (±4)	10 (±2)	70 (±6)	1 (±1)

[a] Data derived by analyzing progeny DNA pool obtained from a single transfection experiment subjected to three or four multiplex DNA sequencing assays; numbers are rounded to the nearest integer. SD, standard deviation.
[b] Δ1, 1-nucleotide deletion.
[c] 50 J/m^2 as previously described (47).
[d] 10 J/m^2 as previously described (47).
[e] 10 μg of MNNG per ml for 10 min as described in Materials and Methods.

TABLE 3. The SOS gene *dinD* is not induced by MNNG treatment of cells under conditions that induce UVM[a]

Strain	MNNG[b]	Mitomycin[b]	β-Galactosidase (Miller units)[c]	% Mutagenesis at εC[d]		
				C→A	C→T	Total
JH5	−	−	37 (±6)	4 (±1)	4 (±1)	9 (±2)
	+	−	26 (±5)	29 (±5)	13 (±3)	42 (±8)
	−	+	86 (±15)	ND	ND	ND
JH5R2	−	−	6 (±2)	6 (±3)	1 (±0)	7 (±3)
	+	−	3 (±1)	55 (±7)	5 (±2)	60 (±5)
	−	+	4 (±0)	ND	ND	ND

[a] The strain JH5 is a derivative of GW1000 *dinD1*::Mud(Ap^r *lac*) that has been cured of its temperature sensitivity (25). JH5R2 was constructed in our laboratory by P1 transduction of the Δ(*srlR-recA*)*306*::Tn*10* (Tet^r) allele from *E. coli* KH2R into JH5.

[b] Exponential cultures (10^8 cells/ml in LB medium) were exposed to a concentration of 10 μg of MNNG or mitomycin per ml for 10 min at 37°C. The chemicals were removed by pelleting cells by centrifugation and resuspension in LB medium. An aliquot of the resuspended MNNG-treated cells was processed for transfection as previously described (49, 53), and a second aliquot was incubated at 37°C with vigorous aeration for an additional 60 min to allow gene expression. Resuspended mitomycin-treated cells were similarly incubated at 37°C with vigorous aeration for 60 min to allow gene expression. β-Galactosidase assays were carried out as previously described (42).

[c] Average (± standard deviation) of three independent experiments.

[d] Average (± standard deviation) of two independent transfections and six multiplex assays. ND, not determined as a part of this experiment. (Note that mitomycin can induce UVM [45a].)

noninducible by DNA damage (44). Figure 4A and Table 4 show that pretreatment of the VP300 strain with MNNG results in a dose-dependent induction of UVM and suggest that a cleavable LexA repressor is not required for the UVM response. *E. coli* HM100 *recA*$^+$ and HM200 Δ*recA* are LexA-overproducing strains that carry pRB160, a multicopy pBR322-derived plasmid bearing a functional *lexA*$^+$ gene (8). LexA overexpression is expected to block the derepression of the SOS regulon, as reflected by the UV sensitivity of pRB160-bearing cells (8) and as confirmed by us (data not shown). Figure 4B and C and Table 4 show that normal UVM induction occurs in these cells in response to MNNG pretreatment.

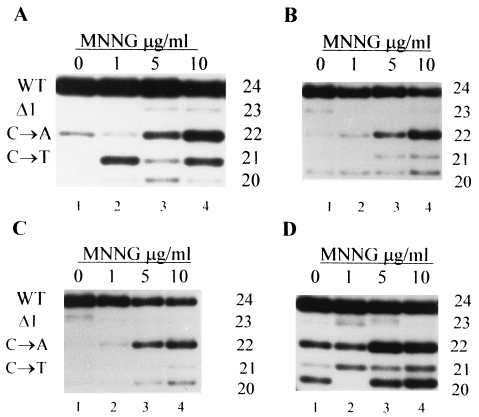

FIG. 4. Multiplex sequence analysis of mutagenesis at an εC residue borne on M13 ssDNA transfected into uninduced or MNNG-induced *E. coli* cells defective in SOS functions. Each strain was treated with 0, 1, 5, or 10 μg of MNNG per ml as previously described prior to transfection with εC-containing ssDNA. Progeny pool DNA was prepared and multiplex DNA sequence analysis was carried out as previously described (46–48) (see the legends to Fig. 2 and 3). (A) *E. coli* VP300 *lexA3*. (B) *E. coli* HM100 *recA*$^+$ (LexA overproducing); (C) *E. coli* HM200 Δ*recA* (LexA overproducing); (D) *E. coli* VHDC Δ(*umuD umuC*). WT, wild type; Δ1, 1-nucleotide deletion.

TABLE 4. Effect of prior MNNG treatment of SOS-defective *E. coli* strains on mutagenesis at an εC residue on transfected M13 ssDNA

E. coli strain and relevant genotype	MNNG (μg/ml)	Mutation frequency (%) (±SD)[a]	Mutation specificity (%) (±SD)[a]		
			C→T	C→A	Δ1[b]
VP300 *lexA3* (Ind$^-$)	0	5 (±2)	1 (±0)	3 (±2)	2 (±1)
	1	15 (±2)	10 (±3)	5 (±2)	1 (±1)
	5	19 (±6)	6 (±3)	13 (±3)	1 (±1)
	10	42 (±5)	9 (±1)	33 (±6)	1 (±1)
HM100 *lexA*$^{++c}$	0	7 (±2)	2 (±1)	2 (±1)	3 (±1)
	1	17 (±9)	2 (±1)	14 (±9)	1 (±1)
	5	19 (±6)	3 (±2)	13 (±6)	4 (±3)
	10	59 (±5)	9 (±4)	48 (±2)	1 (±1)
HM200 Δ*recA lexA*$^{++c}$	0	12 (±5)	1 (±0)	4 (±2)	7 (±3)
	1	16 (±6)	3 (±1)	7 (±1)	5 (±1)
	5	50 (±14)	3 (±2)	44 (±11)	3 (±2)
	10	59 (±6)	7 (±5)	49 (±4)	3 (±2)
VHDC Δ(*umuD umuC*)	0	17 (±4)	1 (±0)	15 (±4)	1 (±0)
	1	22 (±5)	12 (±6)	9 (±2)	1 (±0)
	5	39 (±4)	13 (±11)	25 (±14)	1 (±0)
	10	48 (±8)	7 (±2)	40 (±6)	1 (±1)

[a] Averages derived by analyzing progeny DNA pools obtained from two separate transfection experiments, with each pool subjected to three multiplex DNA sequencing assays; numbers are rounded to the nearest integer. SD, standard deviation.

[b] Δ1, 1-nucleotide deletion.

[c] *lexA*$^{++}$ denotes LexA-overproducing strains bearing the cloned *lexA*$^+$ gene on a multicopy plasmid.

Finally, Fig. 4D shows that the UVM response is detectable in *E. coli* VHDC Δ(*umuD umuC*), in which two genes thought to be indispensable for SOS mutagenesis (59) are deleted. The data in Fig. 3, Fig. 4, Table 3, and Table 4 taken together allow the following conclusions: (i) UVM induction does not require functional *recA*, *lexA*, *umuD*, or *umuC*; (ii) UVM induction is neither dependent upon nor inhibited by SOS induction (e.g., see *E. coli* KH2 data in Fig. 3) (also unpublished data).

Neither *polA* nor *polB* has an indispensable role in UVM. M13 DNA replication is initiated by *E. coli* RNA polymerase-catalyzed priming at a unique site on the viral ssDNA called the minus (or complementary)-strand origin (30). The 30-nucleotide RNA primer is elongated by the *E. coli* DNA pol III holoenzyme. In the last stage of complementary-strand synthesis, the RNA primer is removed and the gap is filled with DNA by *E. coli* DNA pol I. The resulting nicked duplex circular DNA is subsequently ligated by DNA ligase and negatively supercoiled by DNA gyrase to generate the so-called replicative-form (RF) DNA. All further replication (RF DNA to RF DNA, as well as RF DNA to ssDNA) proceeds by a rolling circle mechanism beginning at a nick at a unique site in the plus strand called the plus-strand origin.

A simple hypothesis that can account for UVM is the so-called polymerase switch hypothesis (47), according to which chromosomal DNA damage inflicted by UVM-inducing agents results in a sequestration of pol III such that it becomes unavailable for complementary-strand synthesis on transfected DNA. Instead, in such "induced" cells the complementary-strand synthesis on the incoming M13 ssDNA is carried out by the relatively abundant (~400 copies per cell) pol I. The enhanced mutagenesis, as well as reactivation, induced by the pretreatment of cells simply reflects the properties of pol I.

To test whether pol I is required for the UVM response, *E.*

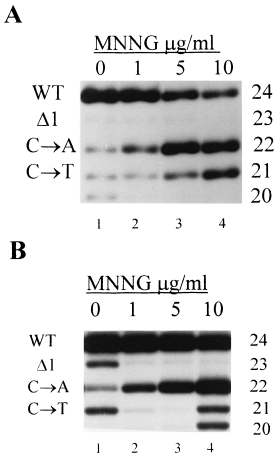

FIG. 5. Multiplex sequence analysis of mutagenesis at an εC residue borne on M13 ssDNA transfected into uninduced or MNNG-induced *E. coli* CJ231 Δ*polA* (A) or *E. coli* VP100 Δ*polB* (B) cells. Each strain was treated with 0, 1, 5, or 10 μg of MNNG per ml as previously described prior to transfection with εC-containing ssDNA. Progeny pool DNA was prepared and multiplex DNA sequence analysis was carried out as previously described (46–48) (see the legends to Fig. 2 and 3). WT, wild type; Δ1, 1-nucleotide deletion.

coli CJ231 Δ*polA* cells were pretreated with MNNG for 10 min at 37°C before transfection with εC-DNA. Figure 5A and Table 5 show that MNNG pretreatment results in a significant enhancement of mutagenesis at the εC residue in transfected DNA, suggesting that pol I is not required for UVM. A curious feature of the pol I data is the observation that constitutive mutagenesis at εC residues (i.e., mutagenesis in uninduced cells) in the standard rich medium (LB) is somewhat higher than that observed for *polA*$^+$ cells. Δ*polA* cells grown in minimal medium show an even greater constitutive mutagenesis at εC residues in uninduced cells, suggesting that the UVM response is partially induced in these cells. Equally interesting is the observation that a low dose of MNNG suppresses the constitutive mutagenesis, whereas higher doses enhance mutagenesis. It is possible that slow gap filling in the lagging strand results in partial induction of UVM. Enhanced background mutagenesis in *polA*-defective cells was previously attributed to chronic partial induction of SOS functions in such cells (4). The data shown in Table 5 raise the possibility that UVM might make a contribution to the enhanced background mutagenesis observed in *polA*-defective cells.

It is possible that the switch responsible for UVM is not a switch from pol III to pol I but a switch from pol III to pol II. Interestingly, *polB*, the gene encoding pol II, is known to be an SOS gene that is induced approximately sevenfold upon SOS induction (7, 14, 26, 55). Even though UVM is a *recA*-independent phenomenon, it is possible that *polB* is induced by an alternative *recA*-independent mechanism and thus accounts for the enhanced mutagenesis and reactivation. To test this possibility, *E. coli* VP100 Δ*polB* cells were treated with MNNG and transfected with εC-DNA. Figure 5 and Table 5 show that UVM is observable in Δ*polB* cells, suggesting a lack of requirement for pol II.

MNNG pretreatment enhances survival of M13 ssDNA bearing a site-specific εC residue. Table 6 summarizes the effect of MNNG pretreatment of various bacterial strains on the survival of M13 ssDNA bearing a site-specific εC residue. These data show that MNNG pretreatment significantly enhances survival in all strains used here and suggest that UVM mutagenesis is accompanied by enhanced survival. In the *E. coli* CJ231 Δ*polA* strain, there is an increase in survival at a low MNNG dose, but the survival is reduced at higher MNNG levels, presumably because of increased MNNG toxicity in *polA* cells. The enhancement in survival (UVM reactivation) is reminiscent of Weigle reactivation (68), a term used to describe enhanced survival of UV-irradiated λ phage in UV-irradiated bacteria compared with that in unirradiated bacteria. However, unlike Weigle reactivation of UV-irradiated phage, UVM reactivation does not require SOS functions, as it is observed in SOS-defective cells.

DISCUSSION

SOS induction is not required for UVM. Experimental results described in this communication demonstrate that none of the SOS functions hypothesized to be essential for inducible error-prone replication are in fact required for UVM. The data also show that UVM is not an alternative pathway triggered only in Δ*recA* cells. Similarly, we have shown elsewhere (67) that even though alkylation damage can induce UVM, the *E. coli* adaptive response (36) to alkylating agents is not required for UVM. The observation that UV-inducible mutagenesis in *recA*$^+$ cells is essentially identical to that observed in Δ*recA* cells (Fig. 3) raises intriguing questions about the relative contributions of the UVM and SOS responses to inducible error-prone replication. In this particular case, SOS functions do not appear to make a significant additional contribution to the observed enhancement of mutagenesis in UV-irradiated *recA*$^+$ cells (cf. UV induction of enhanced mutagenesis in *recA*$^+$ and Δ*recA* cells in Fig. 3). Whether UVM makes a comparable contribution to mutagenesis at other lesions remains to be investigated.

A simple polymerase switch cannot account for UVM. In *E. coli*, the pol III holoenzyme serves as the replicative DNA polymerase responsible for the bulk of DNA synthesis of the bacterial chromosome, as well as most bacteriophage chromosomes. pol I, encoded by the *polA* gene, is required for the

TABLE 5. Effect of prior MNNG treatment of *E. coli* Δ*polA* or Δ*polB* strains on mutagenesis at an εC residue on transfected M13 ssDNA

E. coli strain	MNNG (μg/ml)	Mutation frequency (%) (±SD)[a]	Mutation specificity (%) (±SD)[a]		
			C→T	C→A	Δ1[b]
CJ231 Δ*polA*	0	10 (±3)	2 (±1)	6 (±2)	2 (±1)
	1	19 (±2)	3 (±1)	14 (±2)	2 (±1)
	5	59 (±6)	14 (±5)	43 (±12)	2 (±1)
	10	78 (±1)	29 (±1)	48 (±2)	1 (±1)
CJ231 Δ*polA* (minimal)[c]	0	31 (±2)	22 (±2)	8 (±1)	1 (±1)
	1	12 (±2)	5 (±1)	6 (±2)	1 (±0)
	5	53 (±2)	24 (±2)	29 (±2)	1 (±0)
	10	65 (±3)	19 (±3)	45 (±3)	1 (±1)
VP100 Δ*polB*	0	10 (±6)	4 (±3)	2 (±1)	4 (±2)
	1	10 (±2)	1 (±0)	7 (±2)	1 (±1)
	5	27 (±7)	1 (±1)	24 (±5)	1 (±2)
	10	43 (±5)	4 (±1)	38 (±4)	1 (±2)

[a] Averages derived by analyzing progeny DNA pools obtained from two separate transfection experiments, with each pool subjected to three multiplex DNA sequencing assays; numbers are rounded to the nearest integer. SD, standard deviation.

[b] Δ1, 1-nucleotide deletion.

[c] Cell growth and MNNG treatment were carried out in minimal medium (29) instead of LB medium. All other experimental procedures were identical to those described in Materials and Methods.

TABLE 6. Effect of prior MNNG treatment of *E. coli* strains on survival of transfected M13 ssDNA bearing a site-specific εC residue

Strain and genotype	MNNG (μg/ml)	PFU/50 ng of DNA[a]	Relative survival[b]
KH2 Δ*recA* (control)[c]	0	2,142	1.0
	10	2,693	1.3
KH2 Δ*recA*	0	326	1.0
	10	2,650	8.1
VP300 *lexA3* (Ind$^-$)	0	110	1.0
	1	518	4.7
	5	675	6.1
	10	1,113	10.1
HM100 *lexA*$^{++d}$	0	360	1.0
	1	923	2.6
	5	743	2.0
	10	1,038	2.9
HM200 Δ*recA lexA*$^{++d}$	0	350	1.0
	1	470	1.3
	5	620	1.8
	10	983	2.8
VHDC Δ(*umuD umuC*)	0	111	1.0
	1	783	7.0
	5	812	7.3
	10	840	7.6
CJ231 Δ*polA*	0	395	1.0
	1	1,615	4.1
	5	1,700	4.3
	10	85	0.2
CJ231 Δ*polA* (minimal)[e]	0	1,212	1.0
	1	4,635	4.1
	5	3,283	2.9
	10	98	0.1
VP100 Δ*polB*	0	235	1.0
	1	2,825	12.0
	5	2,910	12.4
	10	3,765	16.0

[a] Data shown are averages from two transfection experiments.
[b] Ratio of PFU/50 ng of DNA in MNNG-pretreated cells over that in untreated cells.
[c] The transfected DNA was a control construct (46) bearing normal cytosine in place of εC.
[d] Strains bearing pRB160 (LexA-overproducing strains).
[e] Cell growth and MNNG treatment were carried out in minimal medium (29) instead of LB medium. All other experimental procedures were identical to those described in Materials and Methods.

completion of lagging-strand synthesis and for gap-filling DNA synthesis during DNA excision repair (30). The existence of *polA* null mutants suggests that other polymerases are able to compensate for the loss of pol I activity. In SOS-induced cells, pol I was reported to acquire a new form suggestive of a role in error-prone replication (33, 34); however, UV mutability appears to be unaffected in *polA* strains (4). pol II, encoded by the SOS gene *polB* (*dinA*) (7, 14, 26, 55), can bypass noninstructive lesions efficiently in vitro (7) but is nevertheless not required for bypass of such lesions in vivo (31). Indeed, *E. coli* cells from which the *polB* gene has been deleted are viable, with UV sensitivity and UV mutability profiles that are indistinguishable from those of *polB*$^+$ strains (55). A recent report has suggested that pol II may be required for the in vivo bypass of abasic sites; however, this requirement can be eliminated by overproduction of the heat shock proteins GroES$^+$L$^+$ (62). At present, the intracellular role of pol II remains unclear.

The experiments carried out with *polA*- and *polB*-defective cells here were designed to address the so-called polymerase switch hypothesis (46, 47), in which pol III is proposed to become transiently inactive or unavailable in treated (UVM-induced) cells. Thus, it is proposed that a second DNA polymerase with markedly reduced fidelity replicates the transfected M13 ssDNA and accounts for UVM. However, the data presented here show that neither pol I nor pol II has an indispensable role in UVM. These data do not rule out a possible nonessential role for pol I or pol II in UVM, and they do not rule out the possibility that in the absence of one polymerase the other could become essential. However, these data do imply that UVM does not depend on native fidelity differences among pol III, pol I, and pol II. We have not yet experimentally addressed the question of whether pol III is involved in UVM.

Enhanced survival of lesion-bearing M13 ssDNA in MNNG-treated cells suggests that a nonexcisive repair mechanism is unlikely to account for UVM. The nonexcisive-repair hypothesis proposes that uninduced cells have a nonexcisive repair enzyme that efficiently converts εC to C by removing the etheno bridge from εC, thus accounting for the relatively low level of mutagenesis in uninduced cells (46). In cells pretreated with UV or MNNG, the hypothetical enzyme is presumed to be depleted because it is needed to repair chromosomal DNA. As a result, the εC residue on the transfected M13 DNA remains largely unrepaired at the time of replication and therefore gives rise to greatly enhanced mutagenesis. Our data show that the survival of M13 ssDNA bearing an εC residue in uninduced cells is 10 to 20% of that of control DNA, implying reduced replicability of the noncoding εC lesion. The repair depletion hypothesis predicts that survival of DNA bearing εC should be further reduced in UVM-induced cells because of the persistence of εC. However, the opposite effect is observed in MNNG-pretreated cells: a consistent and significant enhancement in survival (UVM reactivation) is observed, suggesting that a nonexcisive-repair depletion mechanism is unlikely to account for UVM without additional stipulations.

Possible roles for SOS and UVM functions in replication past noncoding lesions. DNA synthesis past a noninformational lesion requires at least two discrete abnormal replication events. First, base insertion must occur opposite a template site devoid of template instruction. Second, the newly inserted base, which is by definition incorrectly base paired with the template, must nevertheless be extended by the DNA polymerase. Within the context of the SOS hypothesis, attempts have been made to assign specific functions to the proteins RecA, UmuD, and UmuC at each of the above-cited two steps in error-prone replication at noncoding lesions (9–13, 17). Thus, it was proposed that UmuC may not be required in the base misinsertion step (11, 16). The limited homology that UmuD and UmuC share with the accessory proteins of phage T4 DNA polymerase (5) was taken to suggest that these proteins may act as a clamp to tether the polymerase to the lesion site (38), with the end result of facilitating the bypass (extension) step. On the basis of the observation that the RecA protein can inhibit the 3′→5′-editing exonuclease activity of the ε subunit of pol III in vitro (40), a role for RecA* protein in base insertion has been proposed but could not be experimentally supported (2, 9).

We propose that the SOS proteins RecA* and UmuD′C have a role at the bypass step for some, but not all, noninstructive lesions. Thus, we propose the existence of two classes of such lesions. Class 1 noninstructive lesions are the so-called

SOS-dependent mutagenic lesions that require SOS functions at the bypass step. Class 1 lesions do not allow experimental separation of the base insertion step in vivo, because their dependence on SOS functions for the bypass step disallows mutant recovery in *recA*-defective cells. Class 2 lesions such as εC do not require SOS proteins for either the base insertion step or the bypass step and thus allow the experimental observation of the base insertion step in Δ*recA* cells.

Possible mechanisms for error-prone DNA synthesis. UVM reactivation concomitant with increased mutagenesis is consistent with an induced enhancement of base misinsertion through two distinct possible mechanisms that act by inhibiting the proofreading ε subunit of DNA pol III (39, 64, 71). Here, it is important to distinguish between lesion bypass and base misinsertion: even if inhibition of the ε subunit may not be involved in bypass replication (57), it may still be required for enhanced misinsertion. Recent studies have shown that mutation fixation at εC residues is significantly, and constitutively, enhanced in *mutD5*, a *dnaQ* allele defective for proofreading (43, 45a). Therefore, UVM can be accounted for by the induction of a factor that alters the activity of the ε subunit of the pol III holoenzyme. Interestingly, induced nucleotide pool imbalance, a known *recA*-independent cellular response to DNA damage (18, 32, 60), can, in principle, mediate UVM through an effect on the ε subunit of pol III. Treatment of prokaryotic or eukaryotic cells with UV or MNNG elevates dATP and dTTP (but not dCTP or dGTP) levels two- to fourfold (18, 60). Although nucleotide imbalances can enhance base misinsertion presumably through mass action and next-neighbor effects in in vitro experimental systems (39, 52), it is unclear whether the less-than-fourfold pool imbalances observed in vivo can promote mutagenesis of the magnitude observed in UVM by simple mass action and next-neighbor effects. A more intriguing mechanism is suggested by the hypothesis that a phosphorylated thymidine derivative may allosterically alter the editing function of the ε subunit of pol III (6, 21). It is clear that much more needs to be learned about UVM before the mechanistic basis of this interesting phenomenon can be addressed.

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