UV Light Induces IS*10* Transposition in *Escherichia coli*

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Manuscript received September 22, 1997
Accepted for publication March 25, 1998

ABSTRACT

A new mutagenesis assay system based on the phage 434 *cI* gene carried on a low-copy number plasmid was used to investigate the effect of UV light on intermolecular transposition of IS*10*. Inactivation of the target gene by IS*10* insertion was detected by the expression of the *tet* gene from the phage 434 P_R promoter, followed by Southern blot analysis of plasmids isolated from Tet^R colonies. UV irradiation of cells harboring the target plasmid and a donor plasmid carrying an IS*10* element led to an increase of up to 28-fold in IS*10* transposition. Each UV-induced transposition of IS*10* was accompanied by fusion of the donor and acceptor plasmid into a cointegrate structure, due to coupled homologous recombination at the insertion site, similar to the situation in spontaneous IS*10* transposition. UV radiation also induced transposition of IS*10* from the chromosome to the target plasmid, leading almost exclusively to the integration of the target plasmid into the chromosome. UV induction of IS*10* transposition did not depend on the *umuC* and *uvrA* gene product, but it was not observed in *lexA3* and Δ*recA* strains, indicating that the SOS stress response is involved in regulating UV-induced transposition. IS*10* transposition, known to increase the fitness of *Escherichia coli*, may have been recruited under the SOS response to assist in increasing cell survival under hostile environmental conditions. To our knowledge, this is the first report on the induction of transposition by a DNA-damaging agent and the SOS stress response in bacteria.

TRANSPOSABLE elements are widespread among organisms and fulfill an important role in evolution of the genome (Ginzburg *et al.* 1984; Mackay 1986; Finnegan 1989; and Kidwell and Lisch 1997). The rate of transposition is usually very low, and it is tightly regulated by several mechanisms, to prevent genomic chaos and inactivation (Shapiro 1983; Berg and Howe 1989). In several organisms transposition was found to be responsive to environmental agents that cause DNA damage. This includes the Ty transposon in *Saccharomyces cerevisiae* (Rolfe *et al.* 1986; Bradshaw and McEntee 1989), the *copia* element in *Drosophila melanogaster* (Strand and MacDonald 1985), and *Mutator* in maize (McClintock 1984; Walbot 1992). It is therefore somewhat puzzling that the movements of bacterial transposable elements were found in several surveys to be quite insensitive to DNA-damaging agents.

IS*10R*, the right module of the bacterial transposon Tn*10*, can function either as an individual insertion sequence (IS) or it can mediate transposition of the whole Tn*10* element. The transposase, the only protein encoded by IS*10*, catalyzes both Tn*10* and IS*10* transposition in a nonreplicative manner (Foster *et al.* 1981; Halling *et al.* 1982). Transposition of IS*10* is relatively rare (about 10^{-4} per element per generation) and tightly regulated. Several control mechanisms act to reduce expression of the transposase gene, a key factor that determines transposition frequency. However, IS*10* transposition is also influenced by other factors such as the cell cycle: IS*10* transposition is negatively regulated by *dam* methylation and occurs preferentially after DNA replication (Roberts *et al.* 1985). Signals coming from the bacterial host or its environment can also affect IS*10* transposition. Thus, continuous incubation at the stationary phase of growth was found to cause an increase in IS*10* transposition frequency, in a process that was dependent on *lexA* and *recA*, the regulators of the global SOS stress response (Skaliter *et al.* 1992).

We have recently developed a new mutagenesis assay system that monitors the inactivation of the phage 434 *cI* gene carried on a low-copy plasmid (Eichenbaum and Livneh 1995). Using this system we found that intermolecular transposition of IS*10* caused coupled homologous recombination at the insertion site, leading to the formation of cointegrate structures (replicon fusion) (Eichenbaum and Livneh 1995). Here we report that UV irradiation of *Escherichia coli* cells stimulates intermolecular transposition of IS*10*, and that this process is under the control of the SOS stress response.

MATERIALS AND METHODS

Materials: Sources were as follows: restriction endonucleases, New England Biolabs (Beverly, MA); radiolabeled materials, multiprime labeling kit, and Hybond-N nylon membranes, The Radiochemical Center, Amersham (Arlington Heights, IL); antibiotics, Sigma (St. Louis); bacterial media, Difco (Detroit).

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TABLE 1

Bacterial strains

Strain	Genotype	Source
AB1157	*argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44*	Lab stocks
AB2463	AB1157 *recA13*	Lab stocks
DM49	AB1157 *lexA3*	Lab stocks
MC4100	Δ*(argF-lac)205 araD139 rspL150 thiA1 relA1 fib5301 deoC1 ptsF25 rbsR*	C. Gross
RK4936	*araD139 (argF-lac)205 flbB5301 non-9 gyrA219 relA1 rpsL150 metE70 btu::Tn10*	B. Ames
RKZ2	RK4936 Tet^S	Our lab
TK610	AB1157 *ilv-325 uvrA6 umuC36*	T. Kato
TK701	*proA2 his-4 thi-1 lacY1*	T. Kato
TK702	TK701 *umuC36*	T. Kato
WB535	Δ*(lac pro) thi ara met* Δ*(recA-srlR)306::Tn10* (λ200 *imm21 ind*) $F' lacI^q Z^- Y^+ pro^+$	Our lab

Media: The medium used in this study was LB (Luria-Bertani), containing Bacto-trypton 10 g/liter; Bacto-yeast extract 5 g/liter; and NaCl 5 g/liter. Kanamycin (70 mg/liter), ampicillin (100 mg/liter), tetracycline (5 mg/liter), and chloramphenicol (30 mg/liter) were supplemented as required. Dilution and irradiation of bacteria were done in buffer PS (10 mm NaH_2PO_4 and 150 mm NaCl, pH 7.0).

Bacterial strains and plasmids: The strains used in this study are listed in Table 1. Plasmid pZF42 carries the origin of replication of the F episome, the *cat* gene, the *cI(434)* gene, and the *tet* gene fused to the O_RP_R operator-promoter of phage 434 (Eichenbaum and Livneh 1995). Plasmids pMVIS10 and pMV05 were taken from a collection of spontaneous Cro^- mutants that we isolated and characterized previously (Skaliter *et al.* 1992). Both plasmids are derivatives of plasmid pMV2. They carry the phage λ *cro* gene, the *bla* and *kan* genes, and the origin of replication of plasmid pBR322. pMVIS10 contains an IS*10* insertion in *cro*, whereas pMV05 carries a point mutation in *cro*.

Preparation, fractionation, and hybridization of DNA: Rapid preparation of chromosomal DNA was done according to Kempter and Grossbadern (1992), or by further purification on a CsCl gradient according to Weeks *et al.* (1986). Rapid preparation of plasmid DNA from small-volume cultures was done using the boiling method (Holmes and Quigley 1981), and large-scale preparation was done as described by Davis *et al.* (1980). DNA samples were fractionated by agarose gel electrophoresis, after which the DNA was transferred bidirectionally onto Hybond-N nylon membranes and hybridized as described by Smith and Summers (1980). The IS*10* specific DNA was the 0.94-kb *Stu*I-*Nde*I fragment obtained from plasmid pNK290 (Simons and Kleckner 1983). Plasmid pMV2, digested with *Pst*I, *Xho*I and *Bgl* II, and pZF42, digested with *Bgl* II, were radiolabeled and used as probes for the detection of plasmid sequences.

UV-induced survival and mutagenesis: Cells containing plasmid pZF42 were grown to early log at 37° on LB supplemented with chloramphenicol. The cells were concentrated fivefold in buffer PS, after which 4-ml portions were UV irradiated on ice using a low pressure mercury germicidal lamp (254 nm). The dose rate was 0.1 J m^{-2} s^{-1}, as determined by a UV products radiometer equipped with a UVX-25 sensor. UV survival was determined by plating the appropriately diluted cultures on LB plates containing chloramphenicol. In order to determine mutation frequency, UV-irradiated cells were diluted 1:26 in LB, incubated for 90 min at 37°, and then harvested and resuspended in buffer PS. Determination of the total number of cells was done on LB plates containing chloramphenicol and the selection for Tet^R mutant colonies was done on LB plates containing chloramphenicol and tetracycline. Experiments for UV-induced interplasmid transposition of IS*10* were performed in the same way, except that the cells harbored both plasmids pZF42 and pMVIS10, and the growth medium contained both chloramphenicol and kanamycin. Determination of the total number of cells was done on LB plates containing chloramphenicol and kanamycin, and the selection for Tet^R mutant colonies was done on LB plates containing chloramphenicol, kanamycin, and tetracycline. Mutation frequency is defined as the number of Tet^R colonies divided by the total number of colonies.

RESULTS

The experimental system: Our mutagenesis assay system monitors inactivation of the *cI(434)* gene carried on a low-copy number plasmid, containing the F episome origin of replication. This plasmid, termed pZF42, carries the *cat* gene as a selective marker, the *cI(434)* gene, and the *tet* gene fused to the O_RP_R operator-promoter of phage 434 (Eichenbaum and Livneh 1995). Inactivation of the *cI(434)* gene is monitored via loss of the repressor function, which is detected by the activity of the *tet* gene expressed from the phage 434 P_R promoter. In order to study interplasmid transposition we used cells carrying plasmid pZF42 as the acceptor plasmid, and plasmid pMVIS10, a pBR322-derivative carrying an IS*10R* element, as the donor plasmid. Transposition of IS*10* from the donor plasmid into the *cI(434)* reporter gene confers on the cell the ability to grow in the presence of tetracycline, and the type of mutational event was determined by Southern blot hybridization of the plasmids isolated from Tet^R colonies, using both IS*10* and pZF42 DNA probes (Eichenbaum and Livneh 1995).

As the first step we tested the response to UV radiation in the absence of the donor plasmid, of *E. coli* cells

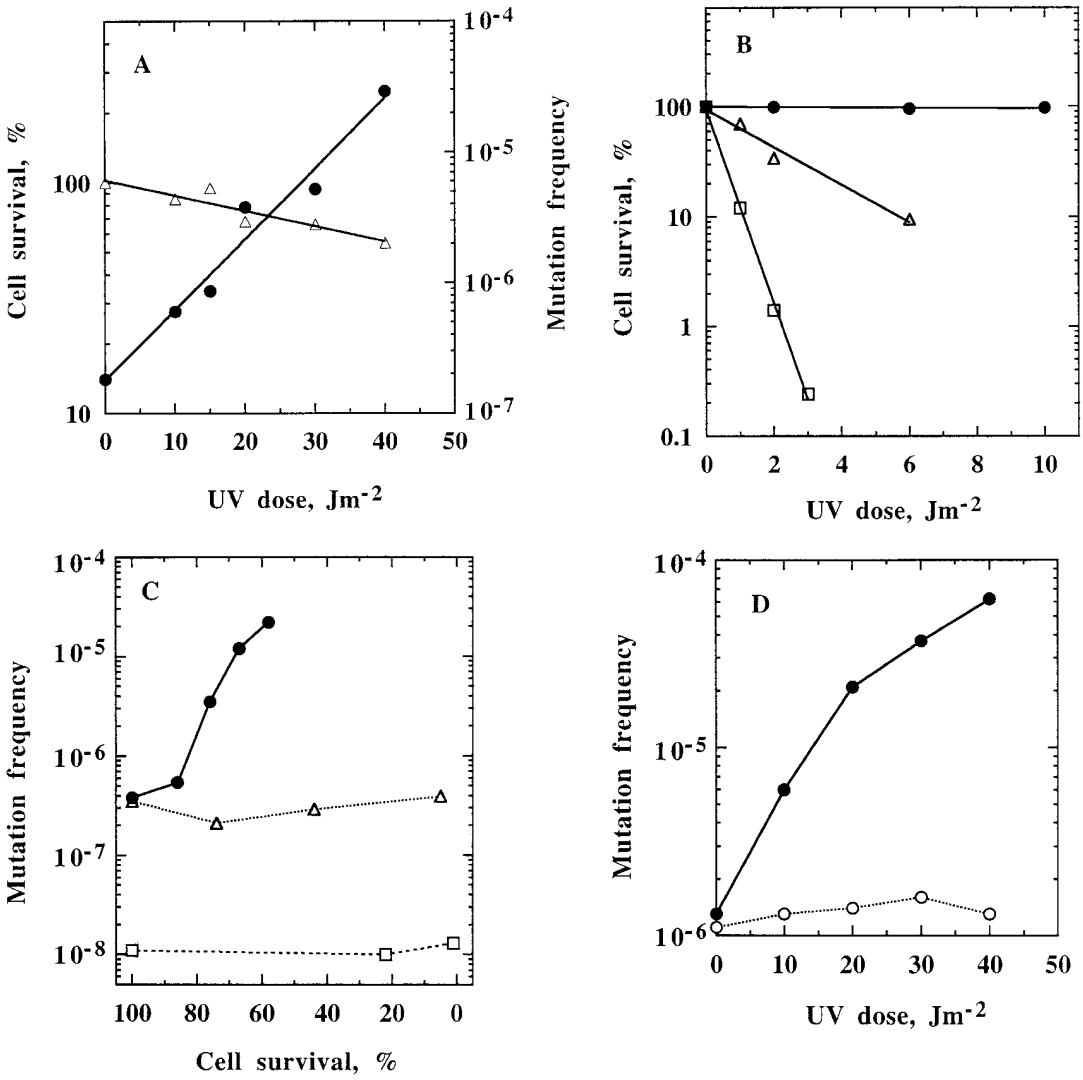

Figure 1.—UV survival and mutagenesis in the *cI(434)* plasmidic system. (A) Strain MC4100(pZF42) was UV irradiated and assayed for cell survival (△) and Tet^R mutagenesis (●). (B and C) The isogenic strains AB1157 (●), DM49(*lexA*3; △) and AB2463 (*recA13*; □) were UV irradiated and assayed for cell survival (B) and for Tet^R mutagenesis (C). (D) Strains TK701 (●) and TK702 (*umuC36* ○), each harboring plasmid pZF42, were UV irradiated and assayed for Tet^R mutagenesis.

harboring the target plasmid. Exponentially growing cells harboring plasmid pZF42 were UV irradiated, after which they were grown up to 180 min without selection in order to enable cell recovery and expression of UV-inducible functions. As expected, UV irradiation caused inhibition of cell division (reviewed in Livneh *et al.* 1993), and no increase in colony count was observed up to 90 min. Subsequently, the cells continued to divide, exhibiting normal exponential growth. UV-irradiated cells were assayed for the formation of Tet^R mutants by plating on LB plates containing tetracycline. The frequency of UV-induced Tet^R mutants depended on the recovery period following irradiation, peaking at 90 min (data not shown). Thus, this period was used as the standard expression/recovery time in subsequent experiments.

UV-induced mutations in *cI(434)* were examined in *E. coli* MC4100, an *E. coli* K-12 derivative (Figure 1). As can be seen, UV irradiation caused an increase of mutation frequency of up to 60-fold over the spontaneous mutation frequency at a dose of 40 J m^{-2}, where survival was still relatively high (60%; Figure 1). UV mutagenesis in *E. coli* depends on the regulators of the SOS stress response, RecA and LexA, and on the UmuD and UmuC gene products (Walker 1985; Friedberg *et al.* 1995). This SOS dependence was examined in the *cI(434)* system using two mutations which render the SOS response noninducible: the *recA13* mutation, encoding a nonfunctional RecA protein, or the *lexA3* mutation, encoding a noncleavable LexA repressor. The UV sensitivity of an isogenic series of cells carrying plasmid pZF42 was found to be as expected, with AB2463-(*recA13*) being more sensitive than DM49(*lexA3*), and the parent AB1157(wild type) being the least sensitive

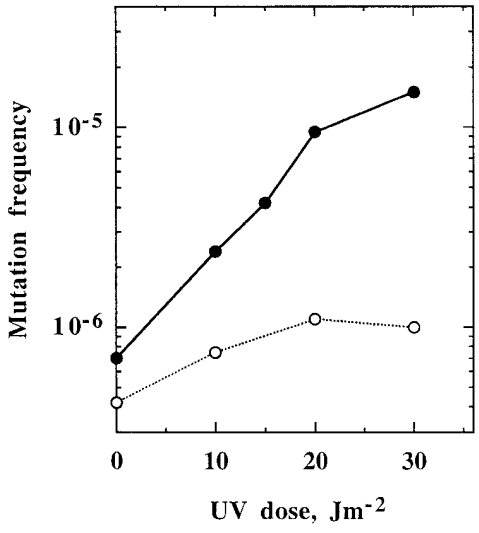

Figure 2.—UV-induced mutagenesis in the *cI(434)* bi-plasmid system. *E. coli* strain TK702 (*umuC36*) harboring plasmids pZF42 and pMVIS10 (●), or pZF42 and pMV05 (○) were UV irradiated and assayed for Tet^R mutagenesis.

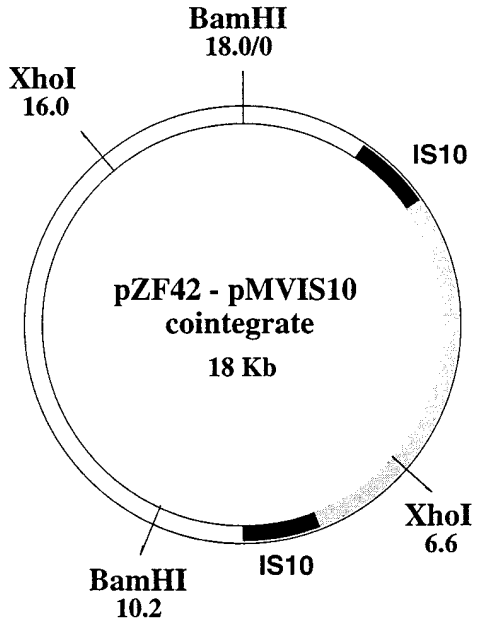

Figure 3.—Schematic structure of a pZF42-pMVIS10 cointegrate. The cointegrate consists of plasmid pZF42 fused to plasmid pMVIS10 via an IS*10*-promoted transposition event. The IS*10* element is duplicated in the process. Restriction sites used for the analysis of cointegrates are indicated. The determination of the structure of cointegrates was described in Eichenbaum and Livneh (1995).

(Figure 1B). When examined for the production of UV-induced Tet^R mutations, the *lexA3* and *recA13* strains were completely nonmutable (Figure 1C). We then examined the effect of the *umuC36* mutation on UV mutagenesis in *cI(434)*. As can be seen in Figure 1D, the UmuC$^+$ strain TK701 showed a 60-fold increase in mutagenesis at 40 J m^{-2}, whereas in the isogenic *umuC36* strain TK702 mutagenesis was drastically reduced, and only a marginal twofold increase of Tet^R mutations was observed (Figure 1D). Thus, UV mutagenesis in *cI(434)* carried on plasmid pZF42 is dependent on RecA, LexA, and UmuC, similarly to chromosomal UV mutagenesis.

UV radiation increases interplasmid IS*10* transposition in a *umuC* strain: The effect of UV radiation on interplasmid transposition of IS*10* was examined using cells harboring the acceptor plasmid pZF42 and the donor plasmid pMVIS10. In order to reduce the "background" of regular *umuC*-dependent UV mutagenesis we utilized a strain carrying the *umuC36* mutation (Figure 1D). UV irradiation of TK702(pZF42; pMVIS10) cells led to an increase of up to 22-fold in the frequency of Tet^R mutants (Figure 2). Control experiments conducted with plasmid pMV05, which contained no IS elements, led to a small increase of only threefold in the mutation frequency, as expected for a *umuC* strain (Figure 2). This suggested that the UV-induced increase of Tet^R mutants in TK702(pZF42; pMVIS10) is related to IS*10*.

In order to examine the type of events that caused the Tet^R mutations, the plasmids were extracted from Tet^R colonies and analyzed by agarose gel electrophoresis followed by Southern blot hybridization. We have previously shown that every IS*10* transposition from pMVIS10 to pZF42 led to the formation of a cointegrate structure, composed of the fused acceptor and donor plasmids, and two copies of IS*10* (Eichenbaum and Livneh 1995; Figure 3). These structures were formed by a two-stage process involving transposition of IS*10* followed by coupled homologous recombination at the transposition site. The cointegrate is easily detected as a high molecular weight DNA maintained at a high copy number, due to the activity of the pBR322 origin of replication (Eichenbaum and Livneh 1995). It is further identified by digestion with restriction nucleases *Bam*HI and *Xho*I. These enzymes, which do not cleave IS*10*, produce two characteristic bands of 6.65 and 3.62 kbp that cohybridize with both pZF42 and IS*10* probes (Eichenbaum and Livneh 1995; Figure 3).

Figure 4 shows the plasmid content of Tet^R colonies obtained after UV irradiation of TK702(pZF42; pMVIS10). Approximately half of the Tet^R mutants contained high molecular weight and high copy number DNA species, typical of cointegrates formed by interplasmid transposition, *e.g.*, lanes 6–9 in Figure 4. Such structures were not observed when UV-induced Tet^R colonies of cells harboring pZF42 and the control plasmid pMV05 were analyzed. Further analysis was performed by digesting the plasmids with *Bam*HI and *Xho*I, and subjecting them to Southern blot hybridization with radiolabeled IS*10* or pZF42 probes. As can be seen in Figure 5, two bands of 6.65 and 3.62 kbp, characteristic of cointegrate structure (Eichenbaum and Livneh

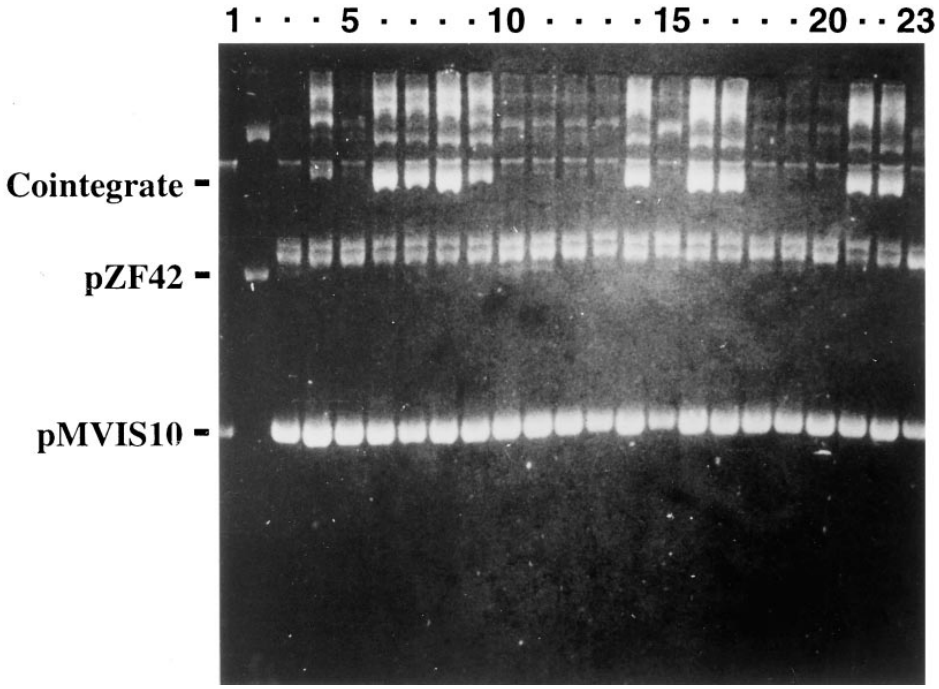

Figure 4.—Plasmid DNA from UV-induced Tet^R colonies of strain TK702(pZF42; pMVIS10). Plasmid DNA from the mutant colonies was extracted and analyzed by gel electrophoresis in ethidium-stained agarose gels. Lanes 1 and 2 contain purified plasmids pMVIS10 and pZF42, respectively, as markers. Lanes 3–23 contain plasmids from 21 independent Tet^R mutant colonies.

1995; Figure 3), were generated, each containing both IS*10* and pZF42 sequences. Analysis of plasmids from spontaneous and UV-induced Tet^R mutants revealed that IS*10* transposition increased ninefold upon UV irradiation at 30 J m^{-2} (Table 2). In addition to the increase in IS*10* transposition, an elevation in small mutations was observed as well. This UV induction of small mutations seems to be an IS*10*-promoted event, since it was not observed when plasmid pMV05 was used instead of pMVIS10. One possible interpretation of this result is that IS*10* suppressed the *umuC36* mutation. Alternatively, IS*10* activation by UV irradiation may have induced a *umuC*-independent mutagenesis pathway.

UV radiation increases interplasmid IS*10* transposition in wild-type and *uvrA* strains, but not in Δ*recA* or *lexA3* strains: Does UV radiation induce IS*10* transposition also in a wild-type strain? We irradiated AB1157 cells harboring both the target plasmid pZF42 and the donor plasmid pMVIS10 and analyzed the Tet^R mutants as before. UV irradiation caused a sharp increase Tet^R mutants (Table 2). Analysis of the plasmids in these mutants by agarose gel electrophoresis and Southern blot hybridization revealed that most of the mutations were point mutations, as expected in a UmuC$^+$ strain. However, along with the increase in point mutations, a 27-fold increase in IS*10* transposition was observed (Table 2). The extent of the increase in IS*10* transposition was threefold higher in the wild-type strain AB1157 (27-fold) as compared to the *umuC36* mutant (ninefold); This may result from strain variation, since TK702 and AB1157 are not isogenic.

We examined the effect of UV radiation on IS*10* transposition in Δ*recA* and *lexA3* strains, in which the SOS stress response cannot be induced. We found no induction of IS*10* transposition in these strains (Table 3), suggesting that UV induction of IS*10* transposition depends on the SOS response. Due to the extreme UV sensitivity of the *recA* and *lexA* mutants (Friedberg *et al.* 1995), the UV dose used for their irradiation was an order of magnitude lower than for the wild-type or *umuC* cells. This raises the possibility that the lack of IS*10* transposition in the *recA* and *lexA* strains was due to insufficient UV damage in DNA, rather than inactivation of SOS regulation. We addressed this possibility by examining UV-induced IS*10* transposition in a *uvrA umuC* strain, which is sensitive to UV radiation due to a defect in nucleotide excision repair, but its SOS regulation is active (Friedberg *et al.* 1995). As can be seen in Table 3, UV irradiation at 3 J m^{-2} of the *uvrA6 umuC36* strain TK610 harboring plasmids pZF42 and pMVIS10 caused a 28-fold increase in IS*10* transposition. Thus, in this strain, IS*10* transposition is induced at the same UV doses that did not induce transposition in *lexA3* and Δ*recA* strains. This strongly suggests that UV-induced transposition of IS*10* is regulated by the SOS stress response.

UV radiation increases chromosome-to-plasmid transposition of IS*10*: We examined whether transposition of chromosomal IS*10* elements is also induced by UV radiation. This was done with *E. coli* RKZ2, which carries two chromosomal IS*10* elements (but not the *tet* gene). UV irradiation of RKZ2 cells harboring pZF42 led to a pronounced increase in the Tet^R mutation frequency. Analysis of Tet^R mutants revealed that transposition of IS*10* from the chromosome to plasmid pZF42 was induced by UV up to 28-fold at a UV dose of 30 J m^{-2}

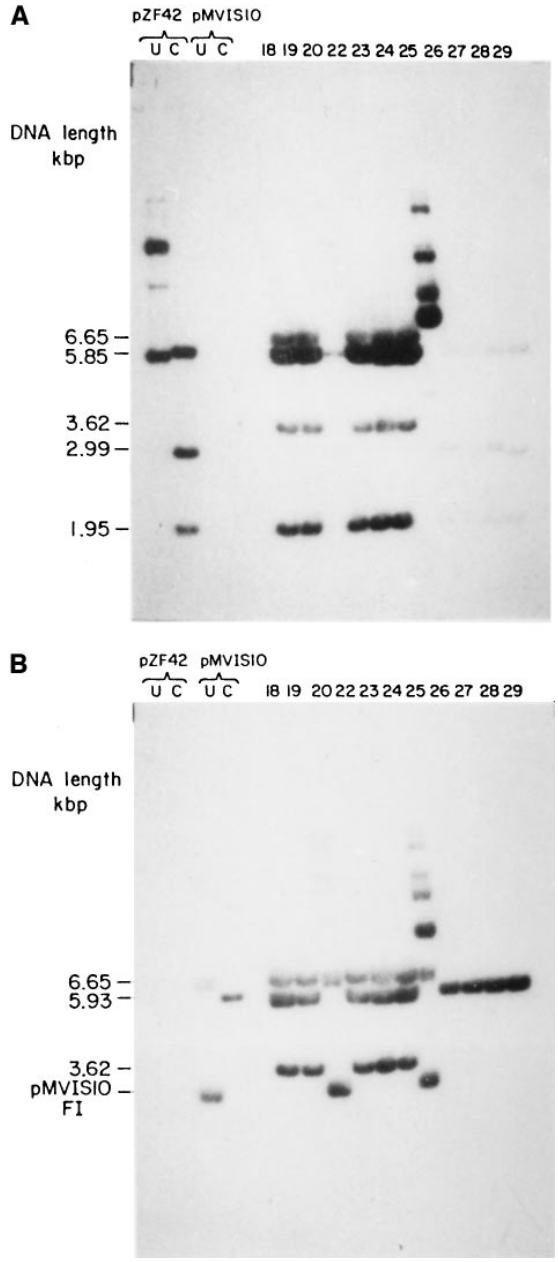

Figure 5.—Hybridization pattern of pZF42 and IS*10* probes to plasmid DNA from UV-induced Tet^R mutants. Plasmid DNA isolated from Tet^R mutants was digested with restriction nucleases *Bam*HI and *Xho*I and fractionated by agarose gel electrophoresis. The DNA was then transferred bidirectionally onto nylon membranes, and probed in parallel with a pZF42-radiolabeled probe (A), or with an IS*10*-radiolabeled probe (B). U, uncut DNA; C, *Bam*HI- and *Xho*I-cleaved plasmid DNA.

(Table 2). Thus, the induction of intermolecular IS*10* transposition is not limited to plasmids only, and occurs also with chromosomal IS*10* elements. As in the spontaneous mutagenesis experiments (Eichenbaum and Livneh 1995), most (95%) of the transposition events of IS*10* from the chromosome to pZF42 resulted in integration of the mutant pZF42 into the bacterial chromosome, as shown by the hybridization of chromosomal DNA to a pZF42 probe. Figure 6 shows an example of Southern blot hybridization of chromosomal DNA extracted from Tet^R colonies. The results were similar to those obtained with spontaneous mutants (Eichenbaum and Livneh 1995). In every one of the mutants the production of a band that cohybridized with both IS*10* and pZF42 specific probes was observed, *e.g.*, arrows A, B. In addition to this band, which gave a strong hybridization signal, pZF42 integration was accompanied by the production of extra IS*10* copies which gave fainter signals, *e.g.*, arrows a, b.

DISCUSSION

A major feature of transposons is their activity as mutagenic agents which are activated under conditions in which fast genetic changes and adjustment to the changing environment are needed (McClintock 1984; Kidwell and Lisch 1997). Indeed, DNA-damaging agents were found to induce transposon-related functions in yeast (Rolfe *et al.* 1986; Bradshaw and McEntee 1989), flies (Strand and MacDonald 1985) and plants (McClintock 1984; Walbot 1992). Moreover, the presence of either Tn*10* (Chao *et al.* 1983) or IS*50* (Hartl *et al.* 1983) was found to increase the fitness of *E. coli* in a chemostat. It was reported that whenever a Tn*10* strain took over, IS*10* transposition was observed. No transposition was detected when the Tn*10* population was overcome by the competitor. It was therefore concluded that the mutagenic properties of this transposon confer an advantage in the same manner as mutator genes, *i.e.*, by increasing the mutation rate of the host bacterium (Chao *et al.* 1983).

In *E. coli* the response to DNA-damaging agents is controlled primarily by the SOS regulatory network, which functions to increase survival under adverse environmental conditions (Little and Mount 1982; Walker 1985). Part of this response is an increase in mutagenesis, which is dependent on the *umuD* and *umuC* genes and represents a DNA-damage dependent inducible mutator (Kato and Shinoura 1977; Echols 1981; Walker 1985). Somewhat surprisingly, experiments designed to examine whether DNA-damaging agents induce transposition in bacteria gave negative results, and thus most of them remain unpublished. In the case of Tn5, a complex relationship between the SOS response and transposition was reported. Thus, Tn5 excision and transposition were found to be enhanced in a *recA* mutant which had constitutive activity of the SOS coprotease. In addition, a sequence homologous to the binding site of LexA, the global repressor of the SOS response, was identified in the region of the transposase gene of IS50. However, Tn5 transposition was not induced by UV light (Kuan *et al.* 1991; Kuan and Tessman 1991). Moreover, according to another report, induction of the SOS response reduced transposition by Tn*5* and IS*50* (Weinreich *et al.* 1991). Tn*10*

TABLE 2

UV light-induced IS*10* transposition with the acceptor plasmid pZF42

Strain	Transposition donor	Mutation frequency × 10^{-7}	
		Point mutation	IS*10* transposition
TK702(pZF42; pMVIS10)			
0 J m^{-2}	pMVIS10	3.0 (1)	10 (1)
30 J m^{-2}	pMVIS10	85 (28)	90 (9)
AB1157(pZF42; pMVIS10)			
0 J m^{-2}	pMVIS10	0.16 (1)	0.16 (1)
20 J m^{-2}	pMVIS10	27 (169)	2.4 (15)
30 J m^{-2}	pMVIS10	53 (331)	4.4 (27)
RKZ2(pZF42)			
0 J m^{-2}	Chromosome	0.11 (1)	0.12 (1)
20 J m^{-2}	Chromosome	10.7 (97)	1.4 (12)
30 J m^{-2}	Chromosome	20.0 (182)	3.4 (28)

Cells harboring plasmids pZF42 and pMVIS10 or pZF42 alone were UV irradiated and assayed for Tet^R mutagenesis. The frequency of plasmid-to-plasmid IS*10* transposition was determined from the fraction of cointegrates among all Tet^R mutants, as revealed by Southern blot analysis. The frequency of chromosome-to-plasmid IS*10* transpositions was determined by Southern blot analysis of chromosomal DNA as described in Figure 6. The values in parentheses represent mutation frequency values relative to unirradiated cells.

excision was found to be activated by UV light (Levy *et al.* 1993). However, unlike transposition, excision is a host-mediated function rather than a transposon-mediated event (Kleckner 1989). Transposition of Tn*10* (Roberts and Kleckner 1988) and IS1 (Lane *et al.* 1994) causes induction of the SOS response. This is most likely due to the DNA cleavage associated with transposition, which provides an inducing signal for the SOS system.

The results presented in this study are, to our knowledge, the first report on the induction of transposition in *E. coli* under the control of the SOS stress response, by a DNA-damaging agent. It should be noted that the UV induction of IS10 transposition was observed with IS*10* residing either on a plasmid or in the chromosome, suggesting that it is not limited to a particular donor. The insertion sites were not analyzed yet, and from the Southern hybridization data it is difficult to estimate whether there are many insertion sites. However, in our previous study with this system we have shown that there were at least two insertion sites in the acceptor plasmid (Eichenbaum and Livneh 1995). In a related study, it was found that the induction of the SOS response by nalidixic acid did not elevate Tn*10* transposition or the transposition of an artificial Kan^R construct of IS*10* (Roberts and Kleckner 1988). This may be due to a difference between IS*10* and Tn*10*. Another possibility is that DNA damage is needed along with SOS induction to enhance IS*10* transposition. UV lesions are known to inhibit DNA replication (reviewed in Livneh *et al.* 1993). This may delay the methylation at *dam* sites, and thus extend the time window during which the DNA is hemi-methylated, a state in which IS*10* transposition occurs (Roberts *et al.* 1985). Alternatively, transposition of IS*10*, which occurs by a "cut and paste" mechanism, may be facilitated by ssDNA gaps formed in the UV-irradiated DNA due to the arrest of replication at DNA lesions, or due to processing of the DNA by repair enzymes (Friedberg *et al.* 1995).

The fact that UV induction of IS*10* was not observed

TABLE 3

IS*10* transposition in Δ*recA*, *lexA3*, and *uvrA6* strains

Genotype	UV dose (J m^{-2})	Survival (%)	Overall mutation frequency × 10^{-7}	IS*10* insertion frequency × 10^{-7}
uvrA6 umuC36	0	100	7.9	3.7 (15/32)
	3	0.24	173.0	105.7 (22/36)
lexA3	0	100	4.5	1.6 (9/26)
	6	6	10.0	3.3 (10/30)
Δ*recA*	0	100	11.5	1.5 (4/30)
	3	0.43	4.3	0.8 (6/34)

The strains used were TK610 (*uvrA6 umuC36*), DM49 (*lexA3*), and WB535 (Δ*recA*). The values in parentheses show the actual numbers of IS*10* transposition mutants out of all the Tet^R mutants that were analyzed.

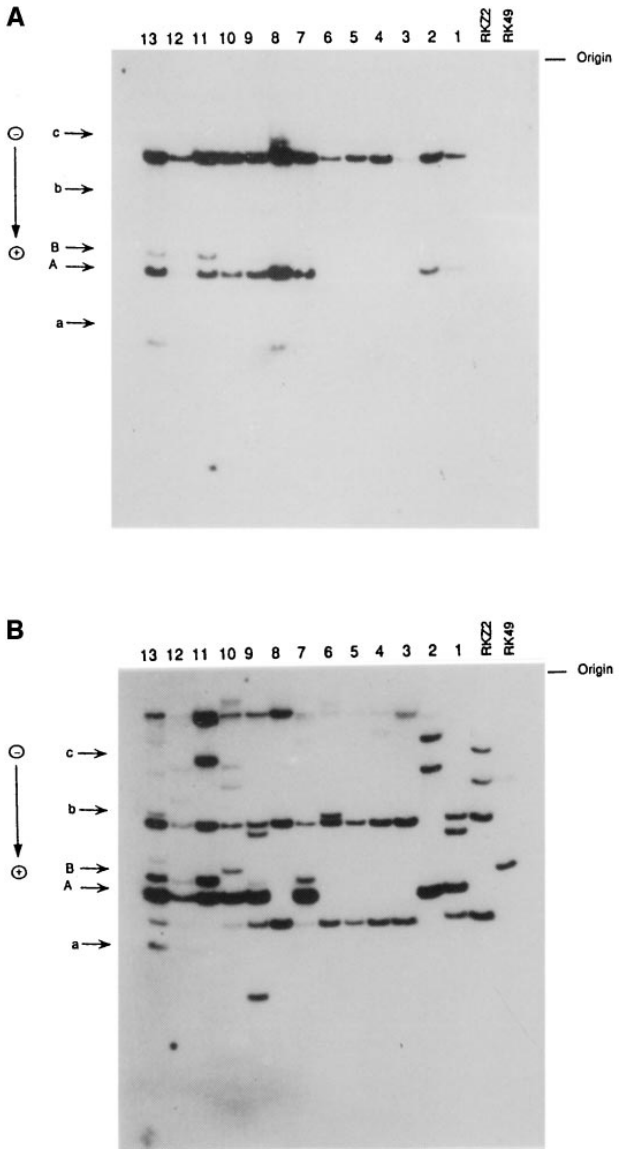

Figure 6.—Southern blot analysis of chromosomal DNA from Tet^R mutants in which pZF42 had integrated into the bacterial chromosome. Chromosomal DNA was isolated from the Tet^R mutants on CsCl gradients, digested with *Eco*RI, and fractionated by agarose gel electrophoresis. The DNA was then bidirectionally transferred onto nylon membranes, and probed with a pZF42-radiolabeled probe (A), or with an IS*10*-radiolabeled probe (B). Arrows A and B mark DNA bands that hybridize to both pZF42 and IS*10* probes. Arrows a–c mark additional copies of IS*10*.

in *recA* and *lexA*(Ind^-) strains suggests that DNA damage alone is not sufficient, and that one or more SOS-regulated proteins are required. Since there is no LexA-binding site in the coding sequence of IS*10*, this dependence on SOS must be indirect. The integration host factor (IHF), known to be involved in a multiplicity of processes in *E. coli* including regulation of gene expression, integration of phage λ, and transposition of IS1 (Friedman 1988; Freundlich *et al.* 1992; Oberto *et al.* 1994), influences IS*10* transposition also (Kleckner 1989). The expression of *himA*, encoding one of the subunits of IHF, was reported to be under the SOS control (Miller *et al.* 1981), and thus, IHF might be one of the host factors involved in UV induction of IS*10* transposition.

As mentioned above, the SOS stress response induces a DNA-dependent mutator activity as part of a multisystem rescue operation for *E. coli* populations challenged by environmental stress (Little and Mount 1982; Walker 1985). Tn*10* is so far the only transposon shown to confer an advantage on its host, in a transposition-dependent manner (Chao *et al.* 1983). This provides a possible rational basis for the recruitment of IS*10* transposition to the UV-induced SOS network. Further studies are required to establish whether other bacterial transposable elements are also inducible by DNA-damaging agents in an SOS-dependent pathway.

This work was supported by the Forchheimer Center for Molecular Genetics. We thank Tamar Paz-Elizur, Yoav Barak and Arie Segal for their help.

LITERATURE CITED

Berg, D. E., and M. M. Howe, 1989 *Mobile DNA.* American Society for Microbiology, Washington, DC.

Bradshaw, V. A., and K. McEntee, 1989 DNA damage activates transcription and transposition of yeast Ty retrotransposon. Mol. Gen. Genet. **218:** 465–474.

Chao, L., C. Vargas, B. B. Spear and E. C. Cox, 1983 Transposable elements as mutator genes in evolution. Nature **303:** 633–635.

Davis, R. W., D. Botstein and J. R. Roth, 1980 *Advanced Bacterial Genetics.* Cold Spring Harbor Press, Cold Spring Harbor, New York.

Echols, H., 1981 SOS functions, cancer, and inducible evolution. Cell **25:** 1–2.

Eichenbaum, Z., and Z. Livneh, 1995 Intermolecular transposition of IS*10* causes coupled homologous recombination at the transposition site. Genetics **140:** 861–874.

Finnegan, D. J., 1989 Eukaryotic transposable elements and genome evolution. Trends Genet. **5:** 103–107.

Foster, T. J., M. Davis, D. E. Roberts, K. Takeshita and N. Kleckner, 1981 Genetic organization of transposon Tn*10*. Cell **23:** 201–213.

Freundlich, M., N. Ramani, E. Mathew, A. Sirko and P. Tsui, 1992 The role of integration host factor in gene expression in *Escherichia coli.* Mol. Microbiol. **6:** 2557–2563.

Friedberg, E. C., G. C. Walker and W. Siede, 1995 *DNA repair and mutagenesis.* ASM Press, Washington, DC.

Friedman, D. I., 1988 Integration host factor: a protein for all reasons. Cell **55:** 545–554.

Ginzburg, L. R., P. M. Bingham and S. Yoo, 1984 On the theory of speciation by transposable elements. Genetics **107:** 331–341.

Halling, S. M., R. W. Simons, J. C. Way, R. B. Walsh and N. Kleckner, 1982 DNA sequence organization of IS*10*-right of Tn*10* and comparison with IS*10*-left. Proc. Natl. Acad. Sci. USA **79:** 2608–2612.

Hartl, D. L., D. E. Dykhuizen, R. D. Miller, L. Green and J. de Framond, 1983 Transposable element IS50 improves growth rate of *E. coli* cells without transposition. Cell **35:** 503–510.

Holmes, D. S., and M. Quigley, 1981 A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:** 193–197.

Kato, T., and Y. Shinoura, 1977 Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutagenesis by ultraviolet light. Mol. Gen. Genet. **156:** 121–131.

Kempter, B., and K. Grossbadern, 1992 Quick preparation of high molecular weight DNA by freezing. Trends Genet. **8:** 226.

Kidwell, M. G., and D. Lisch, 1997 Transposable elements as

sources of variation in animals and plants. Proc. Natl. Acad. Sci. USA **94:** 7704–7711.

Kleckner, N., 1989 Transposon Tn*10*, pp. 227–268 in *Mobile DNA*, edited by D. E. Berg and M. M. Howe. American Society for Microbiology, Washington, DC.

Kuan, C. T., S. K. Liu and I. Tessman, 1991 Excision and transposition of Tn5 as an SOS activity in *Escherichia coli*. Genetics **128:** 45–57.

Kuan, C. T., and I. Tessman, 1991 LexA protein of *Escherichia coli* represses expression of Tn5 transposase gene. J. Bacteriol. **173:** 6406–6410.

Lane, D., J. Cavaille and M. Chandler, 1994 Induction of the SOS response by IS1 transposase. J. Mol. Biol. **242:** 339–350.

Levy, M. S., E. Balbinder and R. Nagel, 1993 Effect of mutations in SOS genes on UV-induced precise excision of Tn*10* in *Escherichia coli*. Mutat. Res. **293:** 241–247.

Little, J. W., and D. W. Mount, 1982 The SOS regulatory system of *Escherichia coli*. Cell **29:** 11–22.

Livneh, Z., O. Cohen-Fix, R. Skaliter and T. Elizur, 1993 Replication of damaged DNA and the molecular mechanism of ultraviolet light mutagenesis. CRC Crit. Rev. Biochem. Mol. Biol. **28:** 465–513.

Mackay, T. F. C., 1986 Transposable element-induced fitness mutations in *Drosophila melanogaster*. Genet. Res. **48:** 77–87.

McClintock, B., 1984 The significance of responses of the genome to challenge. Science **226:** 792–801.

Miller, H. I., M. Kirk and H. Echols, 1981 SOS induction and autoregulation of the *himA* gene for site-specific recombination in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78:** 6754–6758.

Oberto, J., K. Drlica and J. Rouviere-Yaniv, 1994 Histones, HMG, HU, IHF: Meme combat. Biochimie **76:** 901–908.

Roberts, D., B. C. Hoopes, W. R. McClure and N. Kleckner, 1985 IS*10* transposition is regulated by DNA adenine methylation. Cell **43:** 117–130.

Roberts, D., and N. Kleckner, 1988 Tn*10* transposition promotes RecA-dependent induction of lambda prophage. Proc. Natl. Acad. USA **85:** 6607–6041.

Rolfe, M., A. D. Spanos and G. Banks, 1986 Induction of yeast Ty element transcription. Nature **319:** 339–340.

Shapiro, J. A., 1983 *Mobile Genetic Elements*. Academic Press, New York.

Simons, R. W., and N. Kleckner, 1983 Translational control of IS*10* transposition. Cell **34:** 683–691.

Skaliter, R., Z. Eichenbaum, H. Shwartz, R. Ascarelli-Goell and Z. Livneh, 1992 Spontaneous transposition in the bacteriophage λ *cro* gene residing on a plasmid. Mutat. Res. **267:** 139–151.

Smith, G. E., and M. D. Summers, 1980 The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. Anal. Biochem. **109:** 123–129.

Strand, D. J., and J. F. MacDonald, 1985 Copia is transcriptionally responsive to environmental stresses. Nucleic Acids Res. **13:** 4401–4410.

Walbot, V., 1992 Reactivation of mutator transposable elements of maize by ultraviolet radiation. Mol. Gen. Genet. **234:** 353–360.

Walker, G. C., 1985 Inducible DNA repair systems. Annu. Rev. Biochem. **54:** 425–457.

Weeks, D. P., N. Beerman and M. Griffith, 1986 A small-scale five-hour procedure for isolating multiple samples of CsCl-purified DNA: application to isolations from mammalian, insect, higher plant, algae, yeast and bacterial sources. Anal. Biochem. **152:** 376–385.

Weinreich, M. D., J. C. Makris and W. S. Reznikoff, 1991 Induction of the SOS response in *Escherichia coli* inhibits Tn5 and IS50 transposition. J. Bacteriol. **173:** 6910–6918.

Communicating editor: R. Maurer