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Role of the *supX* Gene in Ultraviolet Light-Induced Mutagenesis in *Salmonella typhimurium*

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Salmonella typhimurium strains with *supX* mutations are more sensitive than wild type to killing by ultraviolet (UV) irradiation. Studies with strains bearing the *leuD21* mutation revealed that inactivation of the *supX* locus by a nonsense mutation or a deletion results in a complete lack of ability to produce induced Leu$^+$ reversion mutations after UV irradiation. Suppression of the nonsense *supX* mutation or the presence of an *Escherichia coli* K-12 F′-borne *supX*$^+$ allele restored the capacity for induced reversions and increased cell survival after UV irradiation. Introduction of plasmid pKM101 into *supX* mutant strains also restored their capacity for UV mutagenesis as well as increased survival. The possible nature of the *supX* gene product and mechanisms by which it may affect expression of the inducible SOS error-prone repair system are considered.

Mutations of the *supX* locus (originally designated *su leu 500*), located between *cysB* and the *trp* operon on the *Salmonella typhimurium* linkage map, suppress the leucine auxotrophy imposed by the *leu-500* mutation (26). The *leu-500* promoter mutation (3) causes leucine auxotrophy by reducing expression of the leucine operon to a barely detectable level (2). The *supX* mutations raise the level of *leu* gene expression 6- to 10-fold, sufficient to eliminate the auxotrophy (9, 10, 12). In addition to suppressing the *leu-500* mutation, inactivation of the *supX* locus by point mutations (including nonsense mutations) or deletion mutations also suppresses the *Escherichia coli lac* operon promoter mutations *lacL1*, *lacL8*, and *lacL29*, carried on F′ plasmids in *S. typhimurium* host cells (8, 9). The *supX* mutations were found to be highly pleiotropic, and the mutant strains displayed an array of altered properties: suppression of promoter mutations, low-level antibiotics resistance, raised levels of alkaline phosphatase, a bias toward lysis by the temperate phage P22 (resulting in formation of clear rather than turbid plaques), and an increased cell doubling time (8, 9). The *supX* mutations also result in an increased frequency of excision of certain chromosomal tandem duplications of the *trp* operon after exposure to UV irradiation (Kumar, Lenny, and Margolin, unpublished data). We have confirmed the observation of D. S. Strauss (personal communication) that *supX* strains are unusually sensitive to killing by UV irradiation, although the chromosomal location of the *supX* locus differs from other loci known to affect UV sensitivity. This latter observation prompted us to examine the effect of *supX* mutations on UV mutagenesis. A preliminary report of our results was presented previously (27).

In the present report we will show that UV irradiation does not induce mutations in *S. typhimurium supX* mutant strains. Plasmid pKM101 and its parent R46 (25) have been shown to increase both the resistance to killing by UV irradiation and the response to chemical and UV mutagenesis (7, 22-24, 32). We will demonstrate that plasmid pKM101 restores the capacity for UV-induced mutagenesis and greatly increases cell survival in *supX* mutant strains.

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MATERIALS AND METHODS

Bacterial strains and mutant alleles. All the *S. typhimurium* LT2 strains used in these studies, with or without plasmids pKM101 or *E. coli* K-12 F′123, are listed in Table 1. The highly UV-revertible (21) *leuD21* allele from the Demerec collection resulted from a spontaneous mutation to leucine auxotrophy. The *leuABCD447* mutation is an X ray-induced deletion of the entire leucine operon (19). The *supX* mutations were all selected as suppressors of the leucine auxotrophy imposed by the *leu-500* mutation (26). The spontaneous *supX35* deletion extends from the *supX* locus into the promoter-operator end of the *trp* operon (20). The *supX24* spontaneous deletion extends from the *cysB* locus through both *supX* and the entire *trp* operon. The spontaneous *supX20* mutation to Leu$^+$ is a deletion extending from the *supX* locus into or through the *cysB* gene. The *supX83* nonsense muta-

TABLE 1. *Description of the S. typhimurium LT2 strains*

Strains	Genotype[a]	Mode of origin
PM94	Δ(*leuPOLABCD*)*447 supX20*	Construction by transduction
PM155	*leuD21*	Spontaneous mutation
PM159	Δ(*leuPOLABCD*)*447*	Construction by transduction
PM160	*leuD21* Δ*pyrF146 cysB529*/pKM101	Conjugation: PM764 × TA98[c]
PM161	*leuD21* Δ*pyrF146* Δ*supX35*/pKM101	Conjugation: PM765 × TA98
PM162[b]	*leuD21* Δ*pyrF146* Δ*supX35*/F'123/pKM101	Conjugation: PM766 × TA98
PM163[b]	*leuD21* Δ*pyrF146* Δ*supX35*/F'123 *supX*/pKM101	Conjugation: PM769 × TA98
PM164	*leuD21 trpE50 supX83*/pKM101	Conjugation: PM774 × TA98
PM165	*leuD21 trpE50 supX83 sup*(Am)-1/pKM101	Conjugation: PM777 × TA98
PM166	*leuD21 trpE50 supX83 sup*(Am)-2/pKM101	Conjugation: PM778 × TA98
PM764	*leuD21* Δ*pyrF146 cysB529*	Construction by transduction
PM765	*leuD21* Δ*pyrF146* Δ*supX35*	Construction by transduction
PM766[b]	*leuD21* Δ*pyrF146* Δ*supX35*/F'123	Conjugation: PM765 × KL181/KLF23[c]
PM768[b]	*ara-9 leu-500* Δ*supX24 opp-104*/F'123 *supX*	Spontaneous mutation in PM767[d]
PM769[b]	*leuD21* Δ*pyrF146* Δ*supX35*/F'123 *supX*	Conjugation: PM765 × PM768
PM773	*leuD21* Δ*supX24*	Construction by transduction
PM774	*leuD21 trpE50 supX83*	Construction by transduction
PM777	*leuD21 trpE50 supX83 sup*(Am)-1	Spontaneous mutation in PM774
PM778	*leuD21 trpE50 supX83 sup*(Am)-2	Spontaneous mutation in PM774

[a] The origins and nature of the mutations are described in the text.

[b] *S. typhimurium* LT2 host cells bearing the *E. coli* K-12 F'123 plasmid.

[c] Strains TA98 and KL181/KLF23 were the sources of plasmids pKM101 and F'123, respectively, as described in the text.

[d] The origin of PM767 (*ara-9 leu-500* Δ*supX24 opp-104*/F'123) is described in reference 16.

tion was obtained after treatment with 2-aminopurine (8). The nonsense *trpE50* (formerly *trpA50*) allele from the Demerec collection was obtained as a spontaneous mutation in *S. typhimurium* LT7 and transferred by transduction into an *S. typhimurium* LT2 background. The spontaneous *cysB529* mutation (4, 5) was obtained from the Demerec collection. The *pyrF146* mutation is a nitrous acid-induced deletion (36). The amber suppressor mutations *sup*(Am)-1 and *sup*(Am)-2 were selected as spontaneous suppressors of the *trpE50* nonsense mutation and were found also to suppress the nonsense *supX83* mutation. The method of Berkowitz et al. (1) was used to demonstrate that they are amber suppressors. The *supX* mutation on the F'123 was spontaneous in origin and selected as previously described (16).

The *E. coli* K-12 F'123 was initially introduced into our *S. typhimurium* strains from the *E. coli* KL181/KLF23 strain provided by B. Bachmann. Plasmid F'123 is known to include the *pyrF*, *cysB*, *supX*, *trp*, *tonB*(*chr*), and *opp* genes (16, 17). Plasmid pKM101 was introduced into our strains from the *S. typhimurium* strain TA98 provided by B. Ames. Plasmid pKM101 carries genes for resistance to 25 μg of ampicillin per ml.

Media. Minimal medium in liquid or agar form was prepared as previously described (19). The enriched minimal (EM) agar used for UV mutagenesis and UV survival determinations contained 2.5% (vol/vol) reconstituted Difco nutrient broth. Glucose at 0.2% (wt/vol) served as the carbon source. When required, the following concentrations of supplements were present in the medium: L-tryptophan (40 μg/ml), L-leucine (40 μg/ml), L-cysteine (80 μg/ml), and uracil (40 μg/ml). Medium used for UV mutagenesis and survival of strains bearing pKM101 also contained 25 μg of ampicillin per ml. The bottom agar layer used in the plaque assays was standard reconstituted nutrient agar (BBL Microbiology Systems, Cockeysville, Md.) with an added 0.8% (wt/vol) NaCl. The soft agar used for the overlays consisted of 1.0% (wt/vol) BBL nutrient agar and 0.8% (wt/vol) NaCl in distilled water. Medium used for transductions has been described (19). All cell dilutions were done in saline (0.8%, wt/vol).

Conjugation and transduction methods. The conjugation method for F'123 transfer was as previously described (16). Introduction of plasmid pKM101 was by the method used by Walker (32). Transduction methods used in strain construction were as previously described (19), except that the bacteriophage used was HT101/1 *int* (provided by J. Roth), an integration-negative version of one of Schmieger's high-frequency transducing phage P22 mutants (30).

UV irradiation procedure. Single-colony isolates were inoculated into minimal medium (SSA) and grown overnight at 37°C with aeration. Cultures were diluted to 3×10^8 cells/ml, centrifuged, washed twice, and the cells were suspended in saline. Cell suspensions (15 ml) were exposed to UV radiation in open petri dishes (9 cm in diameter) with constant mild agitation during exposure. The source of UV radiation was a General Electric G15T8 15-W bulb having an intensity of approximately 0.62 J/m^2. Exposure was monitored with a Latarjet dosimeter (15). Illumination during and after UV treatment consisted of yellow light, and the treated cells were incubated in the dark.

Mutation assay procedure and determination of reversion frequencies and percent survival. The basic assay system used was originally developed by Demerec and Cahn (6). Cells of leucine auxotrophic strains (*leuD21*) were UV-irradiated, plated on EM

agar medium, and then scored, after 3 to 5 days incubation at 37°C, for the number of Leu$^+$ revertant colonies representing mutations to prototrophy.

To determine the spontaneous Leu$^+$ reversion frequency, before UV irradiation 0.1-ml samples of various dilutions of the cell suspensions were plated on EM agar and incubated at 37°C for 3 to 5 days, and the revertant colonies (Leu$^+$) were counted. These unirradiated cells were diluted so that the numbers of viable cells plated would approximate the numbers of surviving cells plated from the irradiated cultures. The number of spontaneous Leu$^+$ colonies depends on the spontaneous mutation frequency and the final cell population on each plate. Within a fairly wide range of plate inocula sizes, the same final cell population is reached on each plate, determined by the level of nutrient broth enrichment in the EM agar medium (6).

The number of induced mutations was obtained by subtracting the number of spontaneous Leu$^+$ revertants on plates of unirradiated cells from the total number of Leu$^+$ revertants on plates of irradiated cells. Frequencies of induced reversions were calculated by dividing the number of induced mutations by the number of irradiated viable survivors and then normalized to a population of 1×10^7 surviving cells.

To determine percent survival, appropriate dilutions of irradiated and unirradiated samples were plated on EM plates and incubated at 37°C for 48 h, and the number of colonies was counted.

Test for the SupX phenotype. Replacement of the *leu-500* mutant allele by the *leuD21* mutant allele in the strains used in this study prevented identification of the SupX phenotype by testing for suppression of the leucine auxotrophy. Therefore, before each experiment the SupX phenotype was confirmed by examining the phage P22 plaque morphology produced on the strain in use. SupX$^-$ strains produce clear plaques, whereas SupX$^+$ strains produce turbid plaques (9).

RESULTS

Effects of deletion and nonsense *supX* mutations on UV-induced Leu$^+$ reversion in *S. typhimurium*. To provide a sensitive assay for induced UV mutagenesis, we introduced the *leuD21* mutation (in place of the *leu-500* mutation) into all the strains used in this study. The *leuD21* mutation is not suppressed by *supX* mutations and was shown to be reverted at high frequency by UV irradiation (21).

The *leuD21 supX$^+$* strain (PM155) exhibited a UV-induced Leu$^+$ reversion frequency of 42.5 per 10^7 survivors when exposed to 20 J/m^2 of UV irradiation, whereas the *leuD21 supX35* strain (PM765) produced no induced Leu$^+$ revertants among the 3.8×10^7 surviving cells tested (Table 2). In an equal population of surviving irradiated SupX$^+$ cells, we would have expected more than 160 induced Leu$^+$ revertants. The *supX35* deletion, extending from *supX* into the *trp* operon, would remove any as yet undetected genes located between these two loci. The deletion of such postulated genes, rather than loss of the *supX* locus, could be responsible for the inability to produce UV-induced mutations. To test for this possibility we examined the UV-induced mutagenic response of strain PM774 which contains *leuD21* and the *supX83* amber nonsense mutation. This SupX$^-$ strain also produced zero induced Leu$^+$ revertants after UV exposure (Table 2). In an equivalent surviving population of irradiated SupX$^+$ cells, we would have expected more than 120 induced Leu$^+$ revertants. When SupX$^+$ strains PM777 and PM778, containing amber suppressors of the nonsense *supX83* mutation (see above), were UV-irradiated, we observed a significant frequency of induced Leu$^+$ revertants (Table 2). These results indicate that it is inactivation of the *supX* locus which results in a loss of mutagenic response to UV irradiation.

Effect of the *E. coli supX$^+$* allele. The SupX$^+$ *leuD21* strain, PM155, exposed to 60 J/m^2 of UV irradiation, exhibited an induced Leu$^+$ reversion frequency of 84.5 per 10^7 survivors (Table 3). The SupX$^-$ strain PM765 (*leuD21, supX35*), when exposed to the same UV dose, produced no detectable induced Leu$^+$ revertants. *E. coli* plasmid F′123, bearing a *supX$^+$* allele (16), was introduced by conjugation into the *S. typhimurium* PM765 strain. The resultant hybrid strain (PM766), with the plasmid-borne *E. coli supX$^+$* allele and the chromosomal *leuD21* and *supX35* alleles, displayed a SupX$^+$ phenotype when tested by plaque morphology (see above). UV irradiation resulted in an induced Leu$^+$ reversion frequency of 52.5/10^7 survivors (Table 3). A similar hybrid strain, PM769, in which plasmid F′123 carried a *supX* mutant allele, gave rise to phage P22 clear plaques typical of SupX$^-$ strains and produced no induced Leu$^+$ revertants after UV exposure. Thus, the mutagenic response of PM766 to UV irradiation was due to the plasmid-borne *supX$^+$* allele and not simply the presence of the F′ plasmid.

Effect of the SupX phenotype and the genetic background on sensitivity to killing by UV. The *supX* mutant strains vary in their degree of sensitivity to killing by UV irradiation. Table 4 shows the average percent survival of some SupX$^+$ and SupX$^-$ strains after UV exposure (60 J/m^2). Strain PM765 with *leuD21* and the *supX35* deletion was less sensitive to UV killing than other *supX* mutant strains tested, but was about fivefold more sensitive than the SupX$^+$ strain, PM155 (*leuD21 supX$^+$*). When the *supX35* deletion strain carried the *E. coli* F′123 *supX$^+$* allele (PM766), the survival was increased sixfold. However, when the *supX35* strain carried the *E. coli* F′123 with a *supX*

TABLE 2. *Frequency of induced Leu$^+$ reversion mutations after exposure to 20 J/m^2 of UV irradiation*

Strain	Genotype	SupX phenotype	Total surviving cells tested	Total induced Leu$^+$ revertants observed	Induced reversion frequency per 10^7 survivors[a]
PM155	*leuD21*	+	7.7×10^8	3,269	42.5
PM765	*leuD21 supX35 pyrF146*	−	3.8×10^7	0	0
PM774	*leuD21 supX83 trpE50*	−	2.9×10^7	0	0
PM777	*leuD21 supX83 trpE50 sup*(Am)-1	+	1.5×10^8	1,102	73.5
PM778	*leuD21 supX83 trpE50 sup*(Am)-2	+	1.3×10^8	553	42.5

[a] The induced reversions were calculated by subtracting the spontaneous reversions as described in the text.

TABLE 3. *Frequency of induced Leu$^+$ reversion mutations after exposure to 60 J/m^2 of UV irradiation*

Strain	Genotype	SupX phenotype	Total surviving cells tested	Total induced Leu$^+$ revertants observed	Induced reversion frequency per 10^7 survivors[a]
PM155	*leuD21*	+	4.4×10^7	372	84.5
PM765	*leuD21 supX35 pyrF146*	−	8.7×10^6	0	0
PM766	*leuD21 supX35 pyrF146*/F′123	+	4.4×10^7	231	52.5
PM769	*leuD21 supX35 pyrF146*/F′123 *supX*	−	3.0×10^6	0	0

[a] The induced reversions were calculated by subtracting the spontaneous reversions as described in the text.

TABLE 4. *Percent survival after exposure to 60 J/m^2 of UV irradiation*

Strain	Genotype	SupX phenotype	Titer before UV	Titer after UV	% Survival
PM155	*leuD21*	+	2.8×10^8	7.4×10^6	2.6
PM765	*leuD21 supX35 pyrF146*	−	1.3×10^8	7.1×10^5	0.55
PM766	*leuD21 supX35 pyrF146*/F′123	+	2.3×10^8	7.3×10^6	3.1
PM769	*leuD21 supX35 pyrF146*/F′123 *supX*	−	9.7×10^7	3.8×10^5	0.39
PM774	*leuD21 trpE50 supX83*	−	1.8×10^8	3.2×10^3	0.002
PM777	*leuD21 trpE50 supX83 sup*(Am)-1	+	6.1×10^8	4.0×10^6	0.65
PM778	*leuD21 trpE50 supX83 sup*(Am)-2	+	6.1×10^8	2.7×10^6	0.44
PM773	*leuD21 supX24*	−	8.7×10^7	3.3×10^2	0.0004

mutant allele (PM769), it was as sensitive to killing as the *supX35* strain (PM765) without an F′. This indicates that the increased survival was due to the *supX$^+$* allele and not to the presence of the F′ plasmid, just as we had found for the effect on mutagenic response to UV irradiation.

Strain PM774 had been created by transduction, introducing the *supX83* nonsense mutant allele in place of the *supX24* deletion allele of PM773. The resultant strain, PM774 (*supX83 leuD21*), was much more sensitive to killing by UV irradiation than strain PM765, bearing the *supX35* deletion (Table 4). The added presence of amber suppressor mutations, along with the nonsense *supX83* mutation (strains PM777 and PM778), resulted in a more than 100-fold increase in survival, but it did not reach the survival level of strains PM155 and PM766 with *supX$^+$* alleles (Table 4). Possible factors in the reduced resistance may be an incomplete suppression by the amber suppressors, the presence of different genetic backgrounds, or both. There is evidence that some of our strains differ in their genetic compositions in ways which strongly modify the effect of *supX* mutations on sensitivity to killing by UV irradiation. As shown in Table 4, the original *supX35* strain (PM765) was more than 100-fold more resistant to killing by UV irradiation than strain PM773 bearing the *supX24* deletion. The *supX35* deletion was introduced by transduction into PM773 so that it replaced the *supX24* deletion. The resultant *supX35*-bearing recombinant contained a genome coming primarily from PM773. When this recombinant was exposed to a UV dose of 60 J/m^2, it exhibited the same very low survival (0.0004%) as the *supX24* strain (PM773), in contrast to the 0.55% survival of the original *supX35* strain (PM765).

Tests for the influence of killed cells on the assay for UV mutagenesis. Because of the extensive killing by UV irradiation we substantially diluted the unirradiated controls before plating. This allowed us to inoculate agar

plates with approximately the same number of viable cells per plate from the irradiated $SupX^-$ and $SupX^+$ strains and their unirradiated controls. As a consequence, although the irradiated cells which remained viable were enmeshed in large numbers of killed (non-colony-forming) cells, the control cells, having been diluted with sterile saline solution, were not. We were concerned that the presence of very large numbers of killed cells in the irradiated samples might affect the apparent reversion frequency. For example, the non-growing (killed) cells may still take up and deplete the medium of nutrients in a type of "competitive suppression" (13) or the killed cells might release one or more substances which interfere with the formation or expression of reversion mutations.

To determine whether the presence of large numbers of killed cells could influence the *leuD21* spontaneous reversion frequency, we UV-irradiated a *supX*$^+$ strain (PM159) and a *supX* mutant strain (PM94), each bearing the non-reverting Δ*leu-447* deletion. Suspensions of irradiated cells of these strains, as well as saline, were used as diluents for an unirradiated culture of the $SupX^+$ *leuD21* strain (PM777). Use of the UV-irradiated cells as diluents resulted in approximately 2×10^8 killed cells being combined with the PM777 cells spread on each petri dish of selective medium. This number of introduced killed cells was equal to or greater than the concentrations of killed cells present when undiluted samples of irradiated $SupX^-$ cells were spread on Leu^+ selective agar medium. The frequencies of spontaneous Leu^+ reversions observed in the unirradiated $SupX^+$ culture samples diluted with UV-killed cells were the same as that in the sample diluted with saline (data not shown). Thus, the presence of large numbers of killed cells did not affect the spontaneous reversion frequency of *leuD21* in a $SupX^+$ strain (PM777).

A set of experiments also tested for effects on induced reversions. When strain PM777 was exposed to UV radiation, the frequencies of induced Leu^+ reversions in samples to which 2×10^8 UV-killed cells/plate had been added were the same as that in the sample diluted with saline (data not shown). Therefore, the presence of the added killed cells did not affect the induced reversion frequency of *leuD21*. Furthermore, in the experiments described below with the cells bearing the pKM101 plasmid, extensive dilutions of the irradiated cells before plating did not affect the induced reversion frequencies (data not shown).

In these experiments the number of cells surviving the UV exposure of the Δ*leu-447* cultures used as diluents was too low to affect the reversion frequency of the *leuD21* cells. Previous experiments (data not shown) had demonstrated that the addition of viable non-reverting Δ*leu-447* cells to *leuD21* cells affected the reversion frequency of the latter only when the number of the added viable cells exceeded a certain threshold value. Presumably, at that point the number of viable Δ*leu-447* cells competing for the leucine in the low-level broth enrichment of the selective medium was great enough to significantly reduce the final population reached by the *leuD21* cells. Competitive suppression (13) may also have resulted when enough cells were added to deplete the glucose supply to a level which prevented the growth of some revertants into colonies of visible size. Both of these effects would produce an apparently lowered reversion frequency.

Among further tests for indirect factors affecting reversion frequency, the exchange of the supernatants of UV-irradiated and centrifuged $SupX^+$ and $SupX^-$ strains did not affect the *leuD21* reversion frequencies. In another experiment (data not shown) a 1:1 mixture of $SupX^+$ and $SupX^-$ *leuD21* cells was exposed to UV radiation. The induced reversion frequency of the $SupX^+$ cells (distinguishable from the $SupX^-$ cells by other markers) was not affected by the presence of the large numbers of killed $SupX^-$ cells. These results support the conclusion that differences in the mutagenic responses of UV-irradiated $SupX^+$ and $SupX^-$ strains are not due to the presence in the latter of many more UV-killed cells or of substances released into the medium.

Effect of plasmid pKM101 on UV mutagenesis and survival. The $SupX^+$ strains harboring pKM101 displayed an approximately 100-fold increase in the induced Leu^+ reversion frequency after UV exposure (compare data of Table 5 with those of Tables 2 and 3). Table 5 data also demonstrate that the presence of plasmid pKM101 restores to *supX* mutant strains the capacity for a mutagenic response to UV irradiation (compare the mutagenic responses of strains PM161, PM163, and PM164 to that of PM765). In fact, the *supX* mutant strains with pKM101 exhibited an approximately 10-fold higher reversion response than the $SupX^+$ strains without pKM101. However, in general the number of induced Leu^+ revertants was lower in $SupX^-$ strains with pKM101 than that of $SupX^+$ strains with pKM101. The one exception to this was strain PM163, which carries *supX* mutant alleles on both the F′ plasmid and the chromosome. PM163 displayed an approximately twofold higher induced reversion response than PM162, which carries the *supX*$^+$ allele on the F′ plasmid. This unexpected relationship held up on repetition of the experi-

TABLE 5. *Effect of plasmid pKM101 on the frequency of induced Leu⁺ reversion mutations and survival after exposure to 60 J/m² of UV irradiation*

Strain	Genotype	SupX phenotype	Total surviving cells tested	Total induced Leu⁺ revertants observed[a]	Induced reversion frequency per 10^7 survivors	% Survival
PM160	*leuD21 cysB529 pyrF146*/pKM101	+	9.7×10^5	704	7,257	83.0
PM161	*leuD21 supX35 pyrF146*/pKM101	−	2.1×10^8	4,860	231	58.0
PM162	*leuD21 supX35 pyrF146*/F'123/pKM101	+	1.2×10^8	7,076	589	57.4
PM163	*leuD21 supX35 pyrF146*/F'123 *supX*/pKM101	−	1.7×10^7	2,201	1,294	42.0
PM164	*leuD21 supX83 trpE50*/pKM101	−	6.5×10^7	5,238	805	20.3
PM165	*leuD21 supX83 trpE50 sup*(Am)-1/pKM101	+	6.9×10^6	3,920	5,681	51.0
PM166	*leuD21 supX83 trpE50 sup*(Am)-2/pKM101	+	8.8×10^6	5,675	6,448	50.0
PM764[b]	*leuD21 cysB529 pyrF146*	+	6.2×10^7	610	98	8.9
PM765[b]	*leuD21 supX35 pyrF146*	−	8.7×10^6	0	0	0.55

[a] The induced reversions were calculated by subtracting the spontaneous reversions as described in the text.
[b] Controls: no plasmid pKM101 present.

ments, and we have not yet determined the basis for this effect.

The data of Table 5 also demonstrate that the presence of plasmid pKM101 dramatically increases survival after UV irradiation (60 J/m²). The degree of survival enhancement can be seen by comparing the survival of strains without plasmid pKM101 (Table 4) with the survival of the equivalent strains with pKM101 (Table 5). The presence of pKM101 increased survival of SupX⁺ strains 10- to 100-fold while increasing SupX⁻ strain survival 100- to 10,000-fold.

DISCUSSION

When assayed by the induced reversion frequency of the *leuD21* mutation, strains with *supX* deletion or nonsense mutations were not mutated by UV irradiation. Suppressor mutations in the strain with the nonsense *supX83* mutation restored the mutagenic response to UV irradiation as did the presence of an F′ plasmid bearing an *E. coli supX⁺* allele in the *supX35* deletion strain. The Leu⁺ reversion frequencies of UV-irradiated SupX⁻ strains are the same as the spontaneous reversion frequencies of unirradiated SupX⁺ and *supX* mutant strains (~1.3/10^9 viable cells).

Strain PM777 with the nonsense *supX83* mutation plus an amber suppressor mutation displayed a higher induced reversion response than did strain PM155 (*leuD21 supX⁺*) after UV irradiation. Possibly the particular amino acid substitution inserted by the nonsense suppressor of PM777 restores a modified activity to the *supX* gene product, which results in an increased mutagenic response. Other independently selected amber suppressor mutations in both *supX⁺* and *supX* mutant strains did not enhance the mutagenic response (data not shown).

In an *S. typhimurium* background the F′-borne *E. coli* K-12 *supX⁺* allele appeared weaker than the *S. typhimurium* chromosomal *supX⁺* allele in producing the SupX⁺ phenotype. We noted an incomplete elimination of suppression of the leucine auxotrophy imposed by the *leu-500* mutation (seen as a slightly leaky Leu⁻ phenotype), as well as an incomplete restoration of turbid plaque formation (the plaques were visibly less turbid than the distinctly turbid plaques on *S. typhimurium supX⁺* strains). Furthermore, although the F′-borne *E. coli supX⁺* allele restores a UV-induced mutagenic response to *supX* mutant cells, the data of Tables 3 and 5 suggest that the response is somewhat lower than in cells with the *S. typhimurium supX⁺* allele on the chromosome. We do not know whether the "weaker" *supX⁺* activity reflects a difference in the structure or functioning of the *E. coli* and *S. typhimurium supX* genes or whether it results from the *trans* position on the F′ plasmid.

The apparent influence of the genetic background on the degree of sensitivity to killing by UV irradiation is striking, as can readily be seen in Table 4. The highly pleiotropic *supX* mutations are apparently deleterious, as indicated by their slow growth and the difficulty in maintaining unsuppressed versions of nonsense *supX* mutations (8). There appears to be a very strong selection for a SupX⁺ phenotype. In the case of stable *supX* deletion mutations, there may be selection for modifier mutations that relieve some of the detrimental effects of the *supX* mutant condition. Such modifier mutations may also increase the resistance to killing by UV irradiation. Of all the *supX* mutant strains tested, the *supX35* strain, which displayed the least sensitivity to killing by UV irradiation, was

the most frequently used *supX* mutant strain in the laboratory. It has undergone hundreds of cycles of growth in culture, providing it with extensive opportunities to accumulate such modifier mutations. In contrast, the original *supX24* deletion strain, which showed a very great sensitivity to killing by UV irradiation, was grown out of an 18-year-old soft-agar stab storage vial. Perhaps during the very slow metabolic turnover which must occur in sealed stab cultures over many years, the original sensitivity is retained, or possibly there is selection for mutations which result in an increased sensitivity to killing by UV irradiation. Thus, there is evidence for mutations which alter the sensitivity to killing by UV irradiation without affecting the response to UV-induced mutagenesis. Presumably, these modifier mutations affect error-free repair processes. Regardless, it is clear that the $supX^+$ gene product greatly influences survival in both *supX35* and *supX24* backgrounds. Another possible example of the effect of different genetic backgrounds on sensitivity to UV radiation killing is the approximately threefold greater survival of strain PM764 (Table 5) than of strain PM155 (Table 4).

Plasmid pKM101 restored a greatly enhanced response to UV-induced mutagenesis in *supX* mutant strains. The plasmid does not carry a $supX^+$ allele since its presence did not alter suppression of the *leu-500* mutation nor eliminate the formation of clear plaques by phage P22 on *supX* mutant strains. Plasmid pKM101 has also been shown to suppress the repair deficiencies and lack of UV-induced mutagenesis in *umuC* mutants of *E. coli* (33). The *umuC* locus has been mapped near *hemA* (14) on the opposite side of *trp* from the *supX* locus (29). Plasmid pKM101 does not restore a UV-induced mutagenic response nor enhance the spontaneous mutation rate in *recA* mutant strains of *S. typhimurium* (22–24, 32). The process of mutation enhancement by this plasmid is unclear, but it does appear to involve one or more mechanistic components associated with the *recA* protein itself or its production (32). The quality and quantity of *recA* gene product in *supX* mutant strains is apparently adequate for transduction-mediated recombination (9), as well as for the mutation enhancement by plasmid pKM101 described here.

The greatly increased sensitivity to killing by UV irradiation and the lack of UV-induced mutagenesis in *supX* mutant strains suggest that the inducible error-prone SOS repair system (28, 35) is absent. We do not feel that the *supX* gene product is necessarily directly involved in the error-prone repair process itself, but that it probably affects the expression of one or more genes involved in the generation of this repair system. This would be consistent with the multiple pleiotropy of *supX* mutations and their origins as suppressors of a promoter mutation, all of which suggests that the expression of many genes is affected. This interpretation is supported by the many differences observed in two-dimensional O'Farrell gels of extracts of $SupX^+$ and $SupX^-$ strains (K. M. Overbye and P. Margolin, unpublished data). It seems likely, therefore, that the absence of the *supX* gene product exerts its effect by altering gene expression, perhaps at the level of RNA polymerase-promoter interactions. Although most of the known RNA polymerase components have been mapped at locations far from the position of the *supX* locus, the locations of genes for a number of transcription-associated polypeptides have not yet been precisely determined.

There is now evidence that the *E. coli* gene which codes for topoisomerase I is located on the chromosome between the closely linked *cysB* and *trp* loci (R. Sternglanz, personal communication; M. Trucksis and R. E. Depew, personal communication) as is the *supX* gene (16). Some phenotypic modifications noted in *E. coli* mutants lacking topoisomerase I (R. Sternglanz, personal communication) are similar to those observed in *S. typhimurium supX* mutant strains. The *E. coli* topoisomerase I, which has also been called DNA swivelase and omega (not to be confused with the RNA polymerase-associated omega), removes negative superhelical turns from DNA (34). If, indeed, the *supX* locus should prove to be the structural gene for topoisomerase I, it is interesting to note that in addition to the *recA* gene product, another protein shown to affect DNA helicity, the single-strand DNA binding protein (*ssbA* gene product), has also been shown to play a role in determining sensitivity to killing by UV irradiation (11, 31) and response to UV-induced mutagenesis (H. Lieberman and E. Witkin, personal communication). In *supX* mutant strains, the loss of the mechanism which produces UV-induced mutations is our first example of a phenotype suggesting a turn-down in gene expression as a result of the $SupX^-$ condition. Such a reduction in expression of certain genes could occur, for example, if some repressors have a greater binding affinity for DNA with increased negative superhelicity (18). An alternative possibility is that the effect we observed results from the turn-up in expression of a gene which codes for a repressor.

The functioning of DNA gyrase (which introduces negative superhelical turns into DNA) has been shown to be involved in the expression of catabolite repressible operons, indicating their sensitivity to the helical state of the DNA. This correlation may have significance in interpreting earlier findings. The expression of the leucine operon with a wild-type promoter is not affected by the state of the *supX* gene. The *leu-500* mutation alters the promoter so that expression of the operon is increased in a cell lacking the *supX* gene product. The *leu-500* mutant promoter was found to be capable of undergoing a further mutational step which made the leucine operon catabolite repressible (10). Recent sequencing of the mutant *leu* promoters (R. M. Gemmill and J. M. Calvo, personal communication) has shown that the *leu-500* mutation is located in the −10 base-pair region (Pribnow box) and the mutation conferring catabolite repressibility is in the −35 base-pair region.

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