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Induction by UV Light of the SOS Function *sfiA* in *Escherichia coli* Strains Deficient or Proficient in Excision Repair

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The influence of the nucleotide excision repair system on the induction by UV irradiation of the SOS function *sfiA* has been investigated. The level of *sfiA* expression was monitored by means of a *sfiA*::*lacZ* operon fusion in both the wild-type strain and a *uvrA* mutant. We found that the initial steady rate of *sfiA* expression was proportional to the UV dose and was identical in uvr^+ and *uvrA* backgrounds. This suggests that the initial steady rate of *sfiA* expression is determined by the initial number of lesions and before any effect of excision repair. We confirmed that after 2 h of expression the net synthesis of *sfiA* product is, for the same UV dose, about five times lower in uvr^+ than in *uvrA* strains. We show that this is due to earlier repression of the SOS system in uvr^+ than in *uvrA* strains and not to different initial rates.

The exposure of *Escherichia coli* to UV light induces a group of cellular functions called "SOS functions" (11, 15, 23), including increased repair and mutagenic activities (SOS repair), cell division arrest, and prophage development in lysogens. After DNA damage, induction of all of these functions depends on the activation of the $recA^+$ gene product to a protease able to cleave the $lexA^+$ gene product (10), the general repressor of SOS functions, and the lambda cI^+ gene product (16), the repressor of phage lambda. The SOS functions appear to contribute to the repair or processing of UV-induced DNA damage (11).

Other UV repair systems have been identified. Photoreactivation, which seems to be specific for pyrimidine dimers, a major photoproduct of UV light, involves an enzymatic monomerization of the dimers, using absorbed energy from light in the near-UV and short visible bands (17). The nucleotide excision repair system, which is dependent on the *uvrA,B,C* genes (20, 21), is one of the most powerful defenses of the cell against the biological effects of UV light.

Recently, we presented the "SOS chromotest," a bacterial test for detecting DNA damaging agents (14). It is a colorimetric assay based on the induction by these agents of the SOS function *sfiA* (9), involved in cell division arrest (8). The level of *sfiA* expression is monitored by means of a *sfiA*::*lacZ* operon fusion. We take advantage of the high sensitivity and convenience of the SOS chromotest to further investigate the influence of the nucleotide excision repair system upon the induction of the SOS function *sfiA*.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strains used in this work were derived by standard genetic techniques from a *sfiA*::*lacZ* fusion strain (9). Both strains PQ30 (uvr^+) and PQ33 (*uvrA*) were constitutive for alkaline phosphatase synthesis (14) and deleted for the normal *lac* region so that the beta-galactosidase expression is strictly dependent on the *sfiA* promoter.

Media, buffers, and reagents. Bacteria were cultived in L medium (12) supplemented with 20 μg of ampicillin per ml. Buffers and reagents for alkaline phosphatase and beta-galactosidase assays have been described elsewhere (14).

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UV irradiation. UV irradiation was carried out with a Kodak mineral light lamp having a maximum output at 254 nm. Bacteria were irradiated and diluted in 63 buffer (12). The dose rate was measured with a Latarjet dosimeter.

General experimental procedure. Exponentially growing bacteria at a concentration of 2×10^8 to 5×10^8 cells per ml were centrifuged, washed, suspended in 63 buffer, and then UV irradiated. After irradiation, bacterial suspensions were diluted sixfold in prewarmed L medium, incubated at 37°C for an appropriate period of time, and then assayed for alkaline phosphatase and beta-galactosidase activities as previously described (14). All experiments were carried out under a sodium lamp light to avoid photoreactivation.

Expression of the results. To measure accurately *sfiA* induction after UV exposure, we determined the specific activity of beta-galactosidase by comparing the beta-galactosidase activity (*B*) with the alkaline phosphatase activity (*P*) which is assumed to account for genereal protein synthesis during the incubation period. This activity ratio (*R*) at UV dose *x*, $Rx = Bx/Px$, was normalized to its value in unirradiated cells, $R\hat{0} = B\hat{0}/P\hat{0}$, which was 0.15 in the wild-type strain and 0.18 in the *uvrA* mutant. It was called the induction factor, $Ix = Rx/R\hat{0}$ (14). Under our assumption, the induction factor, *I*, is thus proportional to the specific activity of beta-galactosidase.

RESULTS AND DISCUSSION

We have compared the range of UV fluence in which *sfiA* induction, monitored by a *sfiA*::*lacZ* fusion, can be expressed in either *uvrA* or wild-type strains after 2 h of postirradiation incubation of bacteria (Fig. 1). In both strains, the induction factor, which represents the specific activity of beta-galactosidase (see Materials and Method), increased with the dose and then reached a plateau. Below 0.2 J m^{-2} in the *uvrA* mutant and 0.4 J m^{-2} in the wild-type strain, the induction factor was proportional to the UV dose (Fig. 1, insert). Under the conditions used here, about five times higher UV doses were required in the wild-type strain than in the *uvrA* mutant to reach a similar increase in the induction factor.

Thus, under these experimental conditions, UV induction of the *sfiA* operon appears to be about five times more efficient in the absence of an active nucleotide excision

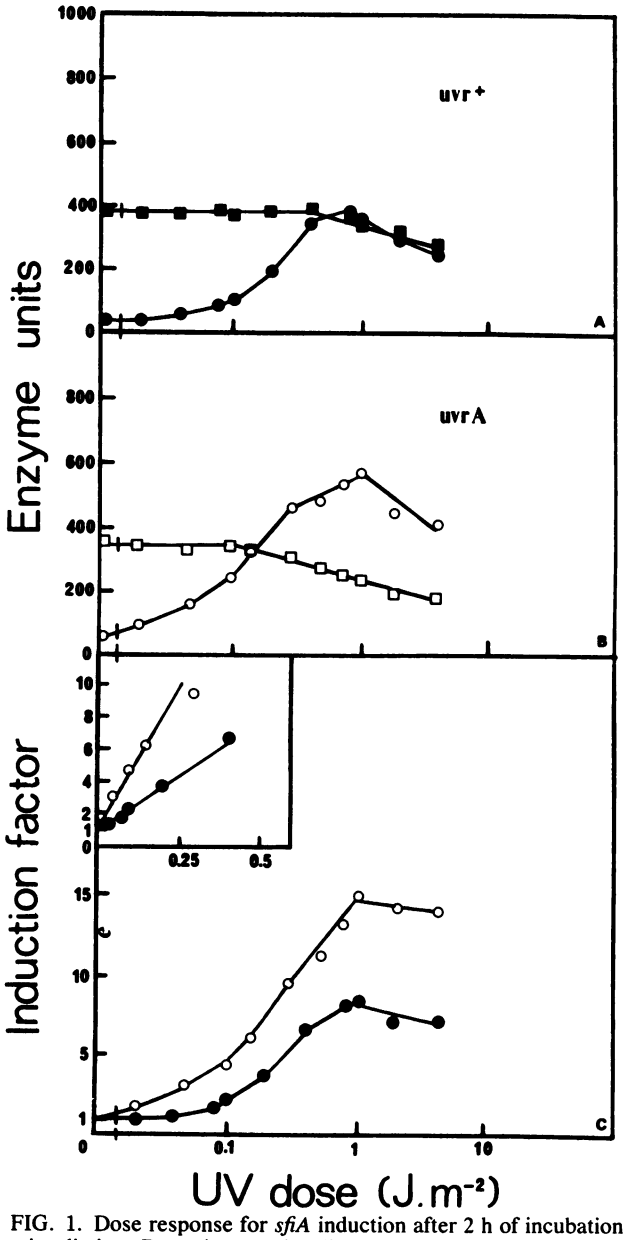


FIG. 1. Dose response for *sfiA* induction after 2 h of incubation postirradiation. Bacteria were irradiated at various UV doses and incubated at 37°C for 2 h. Enzyme levels and induction factors were determined as indicated in the text. Enzyme levels: (A) strain PQ30 *uvr*$^+$—(●) beta-galactosidase, (■) alkaline phosphatase; (B) strain PQ33 *uvrA*—(○) beta-galactosidase, (□) alkaline phosphatase. (c) Induction factor: (● strain PQ30 *uvr*$^+$; (○) strain PQ33 *uvrA*. The main graphic is a semilogarithmic representation. (Insert) Linear representation at low doses.

repair system. This was already described for other SOS functions which were observed at least 1 hour after irradiation, such as prophage induction in lysogens (13), UV reactivation (7), mutagenesis (22), and RecA protein synthesis (18). These results were generally interpreted by assuming that early excision of some of the SOS-inducing UV lesions occurred in the *uvr*$^+$ but not in the *uvrA* strain, resulting in lower induction of SOS functions. More precisely, this interpretation relied on the following assumptions. (i) Some excisable UV lesions are SOS-inducing lesions; i.e., they yield an SOS-inducing signal. (ii) The rate of expression of the SOS genes at time *t* is proportional to the amount of SOS-inducing signal present. (iii) UV lesions which are excised rapidly fail to generate an SOS-inducing signal (18, 23).

However, the SOS response is a transitory process. The turnoff of synthesis of SOS functions can be extremely rapid after disappearance of the SOS-inducing signal and restoration of normal DNA replication (5, 6). So, after a given postirradiation incubation time of bacteria (2 h under our conditions), some turnoff of the *sfiA* operon might have already occurred and the beta-galactosidase activity measured would not then reflect the initial rate of expression of the *sfiA* operon.

Therefore, it was of interest to measure the specific activity of beta-galactosidase in UV-irradiated bacteria versus the postirradiation incubation time. When *uvrA* mutant and wild-type strains were both exposed to the same UV dose, we found that during the first 30 min the induction factor was similar in both strains (Fig. 2). Under these conditions, the induction factor reflects the initial steady rate of *sfiA* expression. After 30 to 40 min of postirradiation incubation, a notable turnoff in *sfiA* expression occurred in the wild-type strain, whereas the rate of *sfiA* expression kept constant in the *uvrA* mutant and a turnoff occurred only after 60 to 80 min. Similar kinetic patterns were observed in different experiments after the UV dose was varied (Fig. 2). The initial steady rate expression was similar in both strains and proportional to the UV dose, suggesting that this steady rate is closely related to the initial number of UV lesions.

It may thus be proposed that, for a given UV dose, the corresponding initial steady rate of the *sfiA* operon is established before elimination by the nucleotide excision repair system of lesions resulting in SOS induction. Assumption (iii) mentioned above must be modified and becomes: (iiia) excision of UV lesions in the *uvr*$^+$ strain results in an earlier disappearance of SOS signal than in the *uvrA* strain.

It was indeed verified (Fig. 3) that, if incubation postirradiation was done for 20 min instead of 2 h, the dose-response curves were identical for *uvr*$^+$ and *uvrA* strains. At doses under 0.5 J m^{-2} the induction factor increased linearly with the UV dose.

In fact, the effect of elimination of SOS-inducing lesions by nucleotide excision repair was not detected before about 30 to 40 min of postirradiation incubation of bacteria (Fig. 2), although the nucleotide excision repair system does not require induction in order to act (1). It is possible, however, that at the low UV doses used, <1 J m^{-2}, the nucleotide excision repair system is not as efficient as at higher UV doses. Under our assumptions, the turnoff of *sfiA* expression observed in the *uvrA* mutant may be accounted for either by dilution of the SOS-inducing lesion at replication during bacterial growth by residual activity of the nucleotide excision repair system or by the action of another repair system.

It has been suggested that a difference in the affinity of the LexA repressor for the operators of individual SOS genes could provide a means for varying the induction kinetics of different SOS functions (3). Those with high-affinity operators would be induced more slowly after DNA damaging treatments and, presumably, turned off more quickly when normal DNA replication is reestablished. Thus, the results obtained by measuring *sfiA* induction may not be the rule for induction of all SOS functions.

On another hand, filamentation in excision-deficient strains occurs at very low UV doses (4), suggesting that very few unexcised UV lesions per chromosome are enough to trigger cell division arrest, one of the SOS functions. We found that a 0.02-J m^{-2} UV exposure was sufficient to

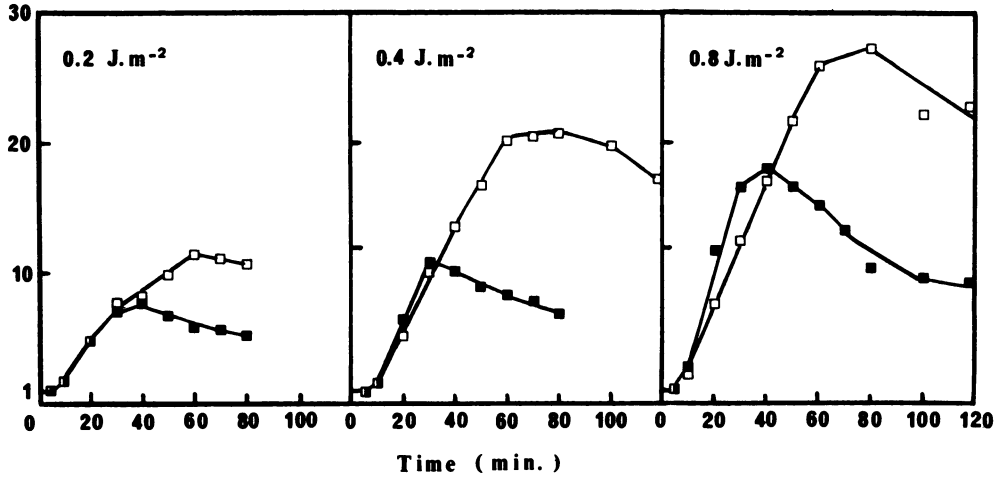
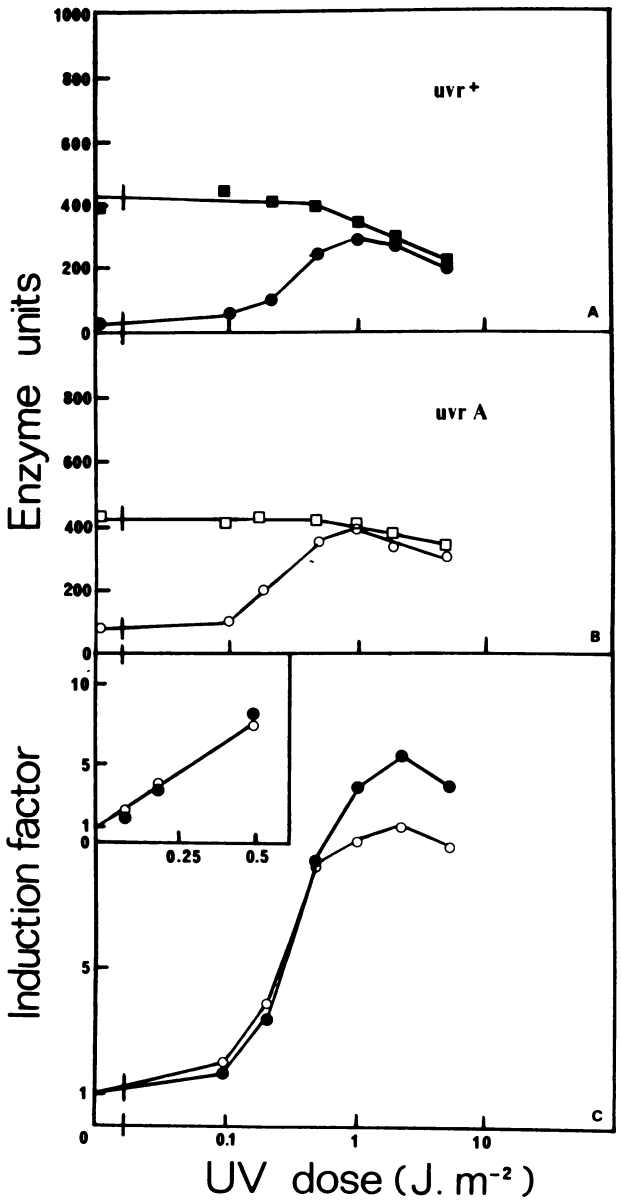


FIG. 2. Kinetics of *sfiA* induction. Bacteria were irradiated and the induction factor was determined, as indicated in the text, versus the postirradiation incubation time. The results are indicated for the three different UV doses shown. Symbols: (■) strain PQ30 *uvr*$^+$; (□) strain PQ33 *uvrA*.



produce a twofold increase in the net synthesis of *sfiA* expression in 2 h in a *uvrA* excision-deficient mutant. Extrapolating the results of Boyle and Setlow (2), a 0.02-J m^{-2} UV exposure would produce about two pyrimidine dimers per *E. coli* genome. Thus, if pyrimidine dimers are responsible for *sfiA* induction, a very small number (if not one) is enough to trigger the response.

It has been suggested that an SOS-inducing lesion may be a daughter strand gap that partially overlaps another daughter strand gap in the sister molecule (19). This type of structure, refractory to nucleotide excision repair, would be formed when two pyrimidine dimers on opposite strands of the parent molecule are close enough so that the daughter strand gaps produced by them overlap, either as they are originally formed or after some DNA degradation. Both the linearity observed between UV doses and increasing *sfiA* expression and the dose range of *sfiA* expression rule out the possibility of overlapping daughter strand gaps being the main inducing lesion.

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FIG. 3. Dose response for *sfiA* induction after 20 min of incubation postirradiation. As in the legend to Fig. 1 except that incubation postirradiation was for 20 min instead of 2 h.

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