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Regulation of the SOS Response Analyzed by RecA Protein Amplification

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A split UV light dose procedure was used in *Escherichia coli* to induce an SOS function, RecA protein amplification, which was measured by an immunoradiometric assay. The SOS system was partially induced after the first UV irradiation, and the inducing effects of subsequent identical UV doses were quantified. Variations in the inducing effects of successive UV doses were related to modulations of the SOS signal level during SOS induction. A reduction in the level of SOS signal was found after 20 min in the wild-type strain, hypothesized to result from negative control of repair functions. A few DNA repair mutants were tested by the same procedure; the *uvrA*, *recF*, and *umuC* genes were involved in SOS induction control, but we found differences in their respective kinetics of expression. On the contrary, in a *recB* mutant, only a slight effect was obtained on this control.

Escherichia coli shows a complex response, the SOS response, after UV irradiation, which mainly induces pyrimidine dimers, a lesion blocking the replicative fork (18, 23). This response consists of the coordinated expression of many cellular functions involved in the process of cell survival. These SOS functions are related to a set of unlinked genes under a common regulatory pathway, which alternates between two states (for reviews, see references 11 and 22). (i) In the repressed state, a common repressor, the LexA protein (LEXA), recognizes a similar operator sequence in front of each SOS gene and allows a low level of expression of the corresponding gene. (ii) In the induced state, provoked by DNA damage, a transiently produced inducing signal triggers LEXA repressor cleavage; this specific cleavage is catalyzed by the RecA protein (RECA), allowing derepression of the SOS genes and consequently the SOS response.

As for the other SOS genes, *lexA* and *recA* are repressed by LEXA. It has been suggested that whatever the nature of the signal, it can gradually be removed from the cell before and during the recovery phase of the SOS response and that at least one SOS function needs to be derepressed for this removal to occur (10). Furthermore, it seemed reasonable to relate the degree of SOS induction to the level of SOS signal produced after DNA damage.

The extent of SOS induction could be approached at a molecular level. Since the in vivo inducing signal(s) has not been biochemically characterized because of its complex and unknown nature, one could measure either LEXA stability or expression of a defined SOS gene.

We chose the latter possibility and measured variations in RECA concentration by an immunoradiometric assay (16) during the uninduced and induced states. To investigate further the evolution of the inducing signal during the SOS response, we used a procedure of split UV doses: we examined the successive inducing effects of repeated UV doses given to the same bacterial population in the exponential phase of growth by measuring the subsequent rate of RECA synthesis. The SOS system was induced after the first irradiation, and the second or third was given at different times. Then the relative inducing effect of a given irradiation could be compared with that of the preceding one, and the variation was related to the persistence of the SOS-inducing signal. Moreover, we characterized a feedback inhibition of SOS induction by the induced functions and the respective involvement of several repair functions in this control.

MATERIALS AND METHODS

Bacterial strains and medium. The *E. coli* K-12 strains used are listed in Table 1. Strain PC1886 was constructed by P1 transduction from strain RH4521 to recipient strain AB1886 by a standard procedure (15) and was checked for defects in UV-induced *his* reversion. Bacteria were grown at 37°C in M63 medium supplemented with 0.2% glucose, 0.5% Casamino Acids (Difco Laboratories), 1 μg of thiamine per ml, 10 μg of thymidine per ml, and 40 μg of tryptophan per ml when needed.

UV irradiation procedure. An overnight culture was diluted (40-fold) with fresh medium in a glass petri dish (25-cm diameter) and grown at 37°C in a controlled-environment incubator shaker (New Brunswick Scientific Co.) without subsequent dilution. The entire experiment (growth and irradiation) was performed at 37°C to avoid temperature effects (data not shown) on the rate of RECA synthesis. Bacteria were UV irradiated (254-nm wavelength) in their culture dish with gentle stirring when the cell concentration was about 3×10^7 to 5×10^7 cells per ml for the first irradiation. UV fluorescence delivered by a UV lamp (Vilbert Lourmat, 4 or 6 W) was measured by a UVX digital radiometer (Ultraviolet Products Inc.). Whatever the bacterial strain, the UV dose allowed at least 90% cell recovery after the first irradiation, as determined by survival curves (data not shown). Bacteria were identically irradiated three times every 20 or 40 min, and after irradiation, they were kept in the dark to avoid photoreactivation. Since there was no dilution, successive irradiations occurred at different cell densities. We established the absence of significant variations (<11%) in RECA synthesis amplification with a culture irradiated at ca. 3×10^7 to 3×10^8 cells per ml (data not shown).

RECA assay. During the experiment, samples (1 to 7 ml) were withdrawn from the culture at the indicated times, centrifuged, and treated for radioimmunometric assay of RECA as previously described (16). The RECA content was

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TABLE 1. Strains of *E. coli* K-12

Strain	Relevant genotype	Source or reference
AB1157	Wild type	2
AB1886	*uvrA6*	2
AB2470	*recB21*	21
RH4521	*umuC122*::Tn*5*	Derived from ES548 (13)
PC1886	*uvrA6 umuC122*::Tn*5*	This work
JC9239	*recF143*	8

standardized to the total soluble protein concentration, and the amplification factor was calculated as the ratio of RECA in irradiated bacteria to that in unirradiated bacteria. The amplification effect of each irradiation on the RECA synthesis rate was quantified by the relative amplification factor (see below).

RESULTS

SOS induction was measured by the extent of RECA amplification. Although UV irradiation doses allowed the same cell survival rate (more than 90%, data not shown) for each mutant, the early kinetics of RECA amplification were different: the initial slope of the RECA synthesis curve was dependent on the genotype of the irradiated strain and on the UV dose (20) (Table 2). For each mutant, the initial slope of RECA amplification was constant, reached a maximum, and then decreased for several hours (20) (Fig. 1). When repeated identical UV irradiations were given to the same culture, variations in the slopes of RECA amplification were found (Fig. 1). We quantified these variations as the relative amplification factor (RAF), which was defined as the ratio between two successive slopes of amplification obtained after each irradiation. We assumed that each RAF value indicated the relative inducing effect of the corresponding irradiation (number 1, 2, or 3). The RAF values could be classified as >1, 1, or <1 corresponding to an amplification effect of the next irradiation that was larger than, identical to, or smaller than that of the preceding irradiation, respectively (the RAF of the first irradiation was standardized to 1). The duration of the experiment was either 60 or 120 min for irradiations delivered every 20 or 40 min, respectively.

The RAF was dependent on three parameters: UV dose given the bacterial population, time delay between two successive irradiations, and repair capacity of the strain. These parameters were tested, and the results are reported in Table 2.

Generally, low UV doses were used and allowed similar bacterial survival. An identical number of DNA lesions gave higher RAF values for irradiations delivered every 20 than every 40 min. This result indicated that the more the SOS system was induced, the less efficient new DNA damage was as an inducer. Thus, the control of the SOS signal level by the induced functions could be examined by our experimental procedure when the kinetics of RECA induction were determined in DNA repair mutants.

In wild-type bacteria, the RAF after the second irradiation was >1 (20 min between radiation doses) or <1 (40-min interval), suggesting that between 20 and 40 min after the beginning of induction, the SOS functions became efficient in handling the inducing DNA damage. The third irradiation had no effect in either case, and the RAF was close to zero. Although this value corresponded to a plateau in RECA content, the latter never reached the maximal RECA amplification (20). This meant that the level of induction of repair functions is sufficient to neutralize completely the potential inducing effect of new DNA damage. The lesions newly produced could be either removed by excision repair or tolerated. Therefore we examined the effect of repair mutations on RAF values.

In the *uvr* mutant irradiated with low UV doses, the RAF was >1 for both irradiation intervals, suggesting a more persistent inducing signal in this mutant than in the wild-type strain. However, the third irradiation gave an RAF of <1, which meant that other functions were efficient. As we pointed out above, UV dose was a parameter that influenced the value of RAF. With high UV doses, it could be assumed that the maximal rate of RECA synthesis would be reached; however, irradiating the *uvr* mutant with 0.7 J/m^2 (data not shown) and 1 J/m^2 (Table 2) gave identical results. There was almost no change in the RAF value, although the maximal rate of RECA synthesis, which is two- to threefold higher, was not obtained (20). Thus, this chronic induction could result from an equilibrium between cell repair processes and the inducing potency of new lesions.

We investigated the potential effect of the *umuC* gene on the control of RECA induction. In *umuC122* and *umuC122 uvrA6* mutants, both the second and third irradiations gave an RAF of >1 (20-min interval between radiation doses). In the double mutant, the RAFs were higher than in the single mutant, showing an additivity of the effects due to each mutation. In addition, late in the induction (third irradiation with the 40-min interval), the inducing effect of the lesions seemed to be controlled, the RAF value becoming close to 1.

The participation of two recombination pathways in the control of RECA induction was checked. The *recB* mutant exhibited control of induction similar to that in the wild-type strain. Only a slight difference could be observed after 80 min of SOS induction. In contrast, the *recF* mutant exhibited a persistence of SOS signal identical to that found in the *umuC* mutant.

DISCUSSION

The lifetime of the SOS signal and the role of SOS genes in the regulation of RECA induction were examined by following RECA amplification after split UV irradiations.

After the first irradiation, RECA induction was almost immediate, a finding which has been confirmed by measuring

TABLE 2. Variations in RAF in multi-UV-irradiated strains

Relevant genotype of irradiated strain	UV dose (J/m^2)	Mean slope[a] ± SD	Mean RAF[b]			
			Second irradiation		Third irradiation	
			20 min[c]	40 min	20 min	40 min
Wild type	6	6.8 ± 1.8	1.9	0.8	0	0.2
	8	8.1 ± 1.8	1.5	0.5	0	0.2
uvrA6	0.3	2.6 ± 0.3	2.8	1.6	0.4	0.5
	0.4	3.2 ± 0.8	2.7	1.5	0.6	0.8
	1	4.4 ± 0.6	1.1	1	0.9	1
umuC122	5	12.2 ± 1.3	1.6	1.2	1.3	0.4
umuC122 uvrA6	0.3	3.9 ± 0.7	3.9	1.4	1.5	0.9
	0.4	4.6 ± 0.8	3.6	1.4	1.4	1
recF143	2	2 ± 0.5	2.3	1.9	1.5	0.7
recB21	2	5 ± 1	1.2	0.6	0.5	0.8

[a] Initial slope of RECA synthesis curve (10^{-2} nanograms of RECA per microgram of total soluble proteins per minute). Each value is the mean of at least two independent experiments for each time between radiation doses.

[b] Each value is the mean of at least two independent experiments, with a coefficient of variation of less than 27%. The RAF of the first UV irradiation was standardized to 1.

[c] Time interval between irradiations.

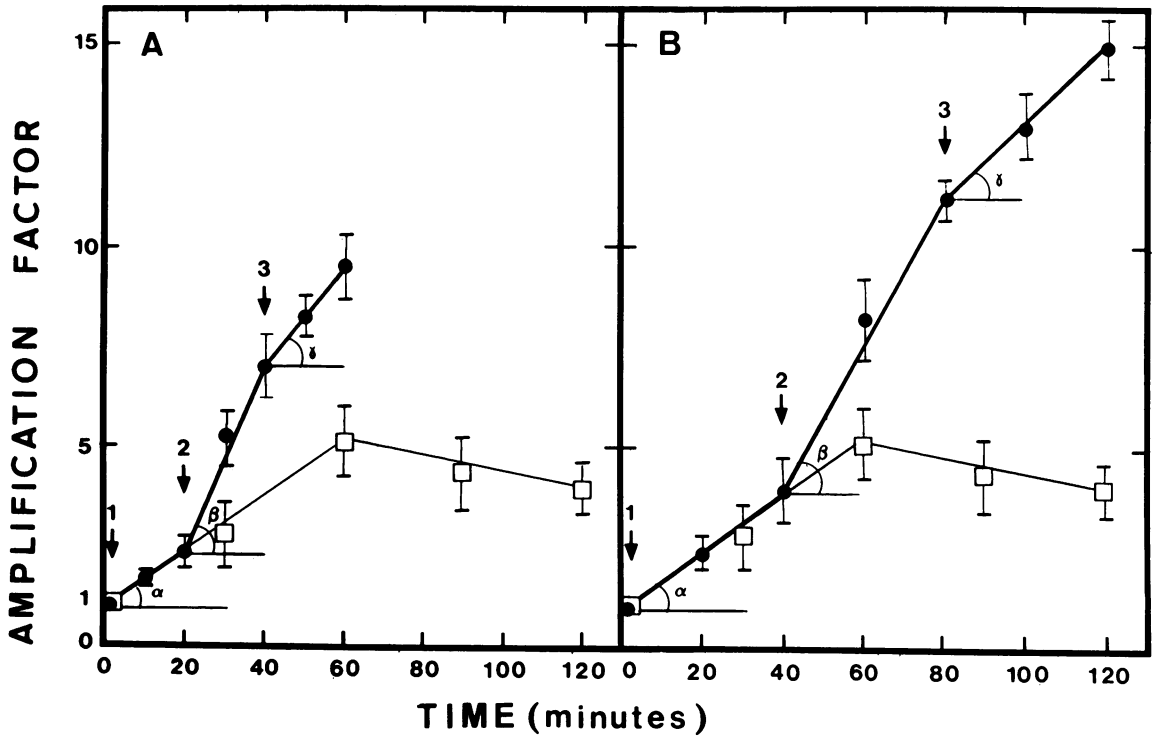

FIG. 1. Kinetics of RECA amplification in strain AB1886. Bacteria were UV irradiated with single (□) or split (●) doses. The arrows show irradiation time, and the UV dose was 0.4 J/m^2 at intervals of 20 (A) or 40 (B) minutes. Each point is the average of three experiments. The amplification factor was calculated as (RECA content in irradiated bacteria)/(RECA content in unirradiated bacteria). The RAF is the ratio between successive slopes, i.e., $RAF_2 = \beta/\alpha$, $RAF_3 = \gamma/\beta$. The RAF of the first irradiation was standardized to 1.

the production of its mRNA (14). RECA amplification was proportional to UV dose (for low doses), and similar amplification was obtained for a ca. eightfold-higher dose in the *uvr*$^+$ than in a *uvr*$^-$ background. *recA* gene induction was dependent on the excision repair pathway, as are most of the other SOS functions (22) except probably *sfiA* (17). The high RECA concentration present in already induced bacteria could interfere with determination of the amplification factor after subsequent irradiation. However, it has been reported that an operator mutation, *recAo*, provoking a high level of intracellular RECA, did not lead to detectable induction of SOS genes (14). Moreover, the kinetics of LEXA cleavage as well as the kinetics of induction of several SOS genes were normal (10, 14). Thus, we assumed that the variations in RAF values corresponded to fluctuations of the SOS signal level, and we studied the influence of two parameters: (i) the persistence and consequently the level of SOS induction and (ii) repair capacities of UV-irradiated strains.

(i) The delay between each irradiation gave two results. First, the inducing effect of new DNA lesions in the wild-type strain was controlled between 20 and 40 min after the beginning of SOS induction. This suggests a drop in SOS signal level and consequent LEXA accumulation after about 20 min of induction. A similar result was found when LEXA stability was analyzed (10). Second, the early control of SOS induction depends at least on *uvrA*, *umuC*, and *recF* repair pathways, since the RAF values (second irradiation) were always >1 for the corresponding mutants. This result could indicate an early derepression of these inducible genes. The rapid derepression of *umuC* (3) and *uvrA* (14) has been reported by workers using different approaches.

By comparing RAF values after the third irradiation (20-min interval), we infer that the control of SOS induction did not last as long in *uvrA* as it did in *umuC* and *recF* mutants; this could be related to the rapid drop in *uvrA* gene transcription (14). On the other hand, the *umuC*- and *recF*-dependent controlling effects persisted similarly. Differences in the timing of induction of each SOS gene could result from the relative affinity of LEXA for corresponding operator sequences (4).

Finally, in each strain tested, the RAF value after the third irradiation (40 min between radiation doses) was usually <1; it might be suggested that compensatory mechanisms are effective in the later phases of SOS induction (120 min in our experimental conditions) to attenuate the SOS response.

(ii) The repair capacities of irradiated strains were able to modulate the SOS response. The way in which bacteria neutralize UV photoproducts consists of either eliminating the lesions by excision repair process or tolerating them by error-prone repair pathways and postreplicative recombination.

Comparison between wild-type and *uvrA* strains emphasized a neutralizing effect of the inducing signal by the excision repair pathway. Even if structures generated by this excision repair process have been reported to induce RECA synthesis (19), this inducing effect seems to be poorly involved in SOS derepression (Table 2).

A mutation in *umuC* interfered with negative control of the SOS signal. The *umuC* gene products involved in induced mutagenesis might permit DNA polymerization through the lesion (5, 7). A defect in this function probably leads to stronger blockage of DNA replication, since a persistent induction was established.

In a double mutant (*umuC uvrA*), the defects in the control of SOS induction due to each mutation were cumulative; hence, this result implies that *umuC* and *uvrA* independently

regulate the inducing signal. This may be related to the independence of their respective DNA repair processes (9).

We examined the participation of two recombination (*recA*)-dependent pathways, referred to as *recBC* and *recF* (6), in the process of attenuation of the inducing signal. The *recB* mutation showed only a slight effect, if any, on the control of SOS induction, at least for the induction time tested. Thus, this control seems to be independent of the major constitutive *recBC* pathway of recombination (6). On the contrary, the effect of the *recF* mutation on the persistence of the signal appeared to be identical to that of *umuC*. The evolution of RAF values during SOS induction in this mutant compared with that in the wild type suggests an inducible process which is *recF* dependent. This result and those of others (1, 12) suggest a *lexA*-dependent *recF* pathway of recombination.

In conclusion, the mechanisms of tolerance and excision repair appear to play an important role in the neutralization of the ability of UV lesions to induce RECA protein.

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