JOURNAL OF BACTERIOLOGY, Nov. 1980, p. 489-498
0021-9193/80/11-0489/10$02.00/0

Vol. 144, No. 2

Synthesis of *recA* Protein and Induction of Bacteriophage Lambda in Single-Strand Deoxyribonucleic Acid-Binding Protein Mutants of *Escherichia coli*

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We investigated the capacity of *Escherichia coli* mutants defective in the single-strand deoxyribonucleic acid (DNA)-binding protein to amplify the synthesis of the *recA* protein, induce prophage λ, and degrade their DNA after treatment with ultraviolet radiation, mitomycin C, or bleomycin. The thermosensitive *ssbA1* strain induced *recA* protein and λ phage normally at 30°C, but no induction was observed at 42°C when ultraviolet radiation or mitomycin C was used. The *lexC113* mutant did not amplify *recA* protein synthesis or induce phage λ at either 30 or 42°C with those agents. Bleomycin was able to elicit induction of *recA* and phage λ in both mutants at any temperature. After induction with ultraviolet radiation at the elevated temperature, no DNA degradation was observed for 40 min, but at later times there was increased degradation in the *lexC113* strain, compared with the wild type, and even greater degradation in the *ssbA1* mutant. We discuss the role of single-strand DNA-binding protein in induction and the possibility that the *lexC* product may exert its influence on *recA* and λ induction at the level of the single-strand DNA gap.

Radiation or agents that damage DNA elicit in bacteria a number of phenotypic responses which are dependent on the *recA* and *lex* genetic background of the cell (27, 39). Examples of such responses (SOS functions) are induction of viruses and colicin, induction of *recA* (protein X), inhibition of septum formation, and inhibition of DNA degradation and inducible error-prone DNA repair. It has been postulated that the processes of induction of these diverse phenomena share a common molecular mechanism (38) which is not completely understood. If such understanding is to be attained, it is fundamental to know precisely the role that the required gene products play in the process of induction. The wild-type *recA* protein is essential for the occurrence of all these phenomena, and its presence has been correlated with the inhibition of the DNA degradation that occurs after treatment with inducing agents (31). *recA* has also been implicated in maintaining the structure of the single-strand gaps formed during induction, postulated to be the site of λ repressor binding (36), and to have endopeptidase activity toward repressors (29). The *lexA* and *lexC* mutations have similar phenotypes, although they map at different positions (14). Both mutants are radiation sensitive and suppress the induction of SOS functions, without loss of recombination ability (3, 7, 25). The *lexA* protein, whose different allelic variants affect the level of *recA* protein synthesis differently, has been postulated to be a repressor of the *recA* gene (4, 11, 24). Recent genetic and biochemical research indicates that the *lexC* gene may code for the *Escherichia coli* single-strand DNA-binding protein (SSB) (20, 21, 30). The evidence stems from the isolation (33) and characterization (6, 20) of a mutant which produces a thermosensitive SSB. The availability of this mutant (*ssbA1*) allowed us to explore the role of the *lexC* gene product in induction. Vales et al. (37) have reported the behavior of *lexC* mutants in response to temperature changes, UV radiation, and induction of phage lambda. This communication is concerned with the expression of the *recA* gene and with the DNA degradation and induction of phage after treatment with three different inducing agents: UV radiation, mitomycin C, and bleomycin. The results obtained establish that active SSB is necessary for the amplified synthesis of *recA* protein normally induced after UV or mitomycin C treatment as well as for λ induction with those agents. DNA degradation in these strains correlated with the level of *recA* synthesis, in agreement with Satta et al. (31), but could also be attributed (at least in part) to inactivation of SSB. Induction with bleomycin seemed to overcome the suppression exhibited by the mutants with UV radiation. We propose

that *lexC113* is not a direct repressor of the *recA* gene, but that it suppresses *recA* induction by competing more effectively for the DNA gaps.

MATERIALS AND METHODS

Bacterial strains (*E. coli* K-12). The strains used are listed in Table 1.

Media and growth conditions. Cells to be labeled were grown in RG medium (5) containing 0.2% glucose, 100 μM $MgSO_4$ and 50 μg of thymine per ml. Unlabeled cultures were grown in above medium supplemented with Casamino Acids to 0.1%. Optical density measurements were made on a Bausch & Lomb Spectronic 20 spectrophotometer.

Exponentially growing cells were labeled with 5 μCi of [^{35}S]methionine per ml (New England Nuclear Corp.). Incorporation was stopped by adding a 1,000-fold excess of unlabeled cysteine and methionine.

Growth of ^{3}H-labeled cells for standardization. An exponentially growing culture of KM1842 in M9 medium (37) was labeled for at least six generations with 50 μCi of ^{3}H-labeled L-amino acid mixture per ml (New England Nuclear).

Preparation of cell extracts for electrophoresis. Labeled cells were collected by centrifugation, washed twice with 10 mM Tris-hydrochloride, pH 8, 5 mM $MgCl_2$, 0.1 mM dithiothreitol, and 0.1 mM EDTA, and resuspended in 0.1 ml of lysis buffer consisting of 9.5 M urea, 2% Nonidet P-40, 5% mercaptoethanol, and 2% ampholines (1.6% pH range 5 to 7, 0.4% pH range 3 to 10). Cells were lysed by five cycles of freezing and thawing, DNase was added to a concentration of 10 μg/ml, and the extracts were stored at −20°C.

Electrophoresis and autoradiography. Cell extracts were subjected to two-dimensional polyacrylamide gel electrophoresis as described by O'Farrell (26). The polyacrylamide gels (10% acrylamide) were dried and exposed to Kodak SB-5 film. The method described by Reeh et al. (28) was used to determine the relative rate of synthesis of the *recA* protein: just prior to lysis of ^{35}S-labeled cells, ^{3}H-labeled cells were added, as an internal standard in determining the relative rate of synthesis. The reliability of this method for quantitation of *recA* protein synthesis has been reported (1).

DNA degradation. Postirradiation DNA degradation was measured as previously described (2).

Phage induction. The production of active phage after treatment with inducing agents was determined as described (37).

TABLE 1. *Bacterial strains*

Strain	Relevant genotype	Origin
KLC436	F⁻ *ssbA1 mel thy rha*	Vales et al. (37)
KLC438	F⁻ *mel thy rha*	Vales et al. (37)
KLC484	F⁻ *lexC113 mel thy rha*	Vales et al. (37)
KLC444	λ⁺ lysogen of KLC436	Vales et al. (37)
KLC445	λ⁺ lysogen of KLC438	Vales et al. (37)
KLC491	λ⁺ lysogen of KLC484	Vales et al. (37)
C600	λ⁺ sensitive	

RESULTS

Synthesis of *recA* protein after UV irradiation in the wild type and *ssbA1* and *lexC113* mutants. To investigate the effect of the *ssbA1* and *lexC113* mutations on the expression of the *recA* gene during induction by UV, we grew the parental and mutant strains in synthetic medium at 30°C to exponential phase. Before the cultures reached 10^8 cells per ml, they were divided into four parts and two of each were placed at 42°C. After 20 min of incubation at either temperature, one part at each temperature was irradiated with 30 J/m^2 and incubation was continued at the original temperature. All cultures were pulse labeled for 10 min with [^{35}S]methionine at a given time. The labeled proteins of these cultures were analyzed by gel electrophoresis. Figures 1 and 2 are densitometric tracings of gels from cells pulse labeled between 30 and 40 min postirradiation. Preliminary experiments with different times of labeling indicated that we were measuring rate of synthesis and not accumulation, and that at 30 min the rate of amplification in the control cells was highest, as also shown by Gudas and Pardee (12). Analysis of the data established the following facts: (i) a temperature shift from 30 to 42°C did not cause increased *recA* synthesis in any of the strains tested; (ii) UV irradiation of the wild type (KLC438) resulted in amplification of *recA* protein synthesis at either 30 or 42°C, as expected (Fig. 1a and b, Fig. 2a and b); (iii) in the *ssbA1* strain (KLC436), UV treatment produced increased synthesis of *recA* protein at 30°C (Fig. 1c and d), but at 42°C no such effect was apparent (Fig. 2c and d); and (iv) the *lexC113* mutant (KLC484) failed to produce increased *recA* synthesis after UV treatment at either 30 or 42°C (Fig. 1e and f, Fig. 2e and f). These experiments were performed several times with similar results. When the label was administered to the mutant strains between 90 and 100 min postirradiation at 42°C, still no increase in *recA* synthesis was observed (data not shown). These results indicate that SSB is necessary for UV-mediated induction of *recA* protein.

Synthesis of *recA* protein after bleomycin treatment. Gudas and Pardee (12) have shown that *dnaA* mutants, unable to induce *recA* protein after nalidixic acid treatment, could do so in response to bleomycin. To determine the behavior of the *ssb* mutants in this respect, we performed experiments like the ones just described for UV, using bleomycin during growth at 30°C and 20 min after the shift to 42°C, and pulse labeling during 30 to 40 min posttreatment.

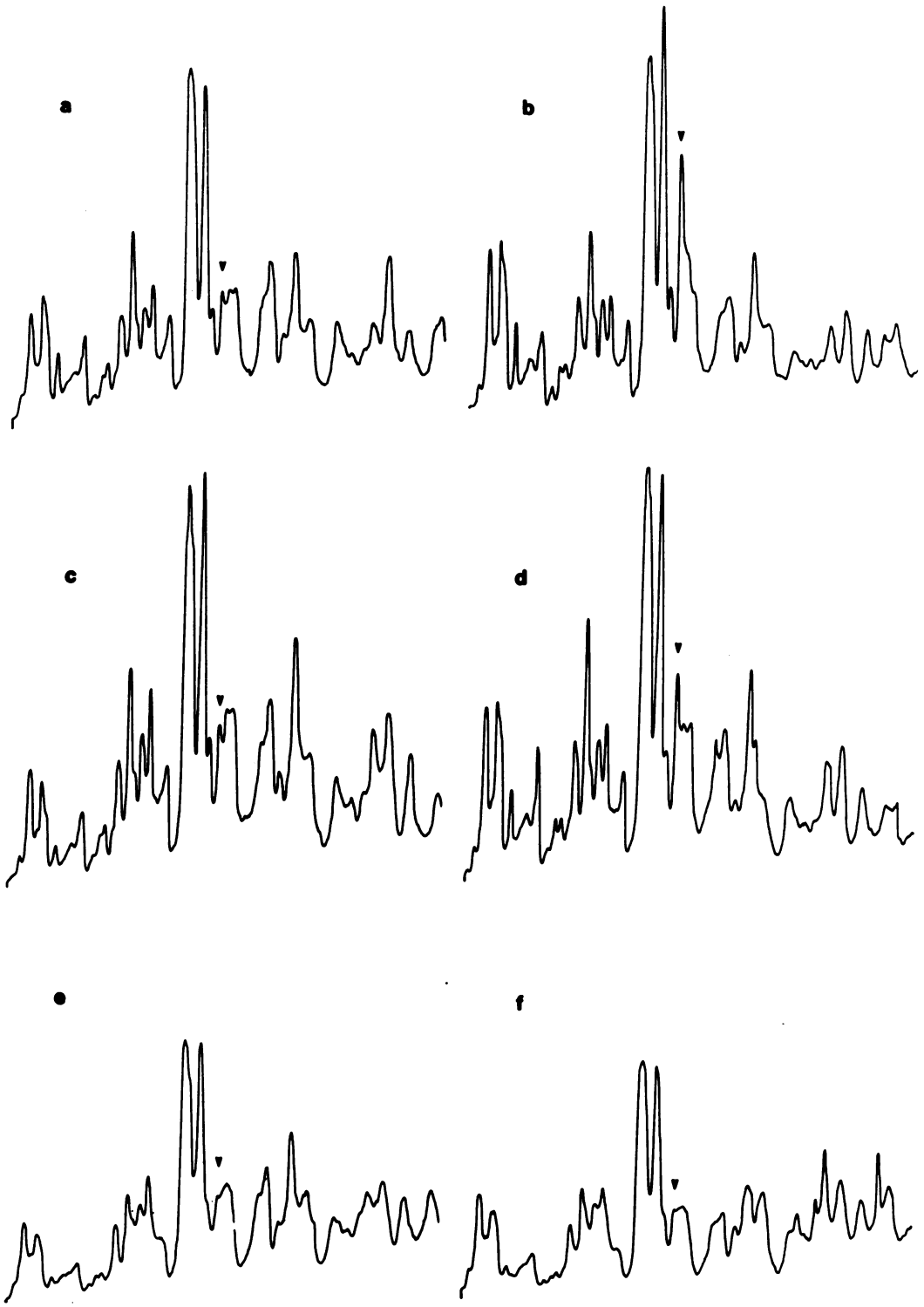

FIG. 1. *Induction of recA protein by UV in wild type and in ssbA1 and lexC113 mutant strains growing at 30°C. Cells growing exponentially in RG glucose were centrifuged and resuspended in 0.25 volume of RG glucose. Incubation at 30°C was continued for 20 min, at which time the cultures were divided into two parts. After another 20 min, one of the parts was irradiated with UV (30 J/m²); the other was left as a control. Thirty minutes after irradiation, the cultures were labeled with 5 μCi of [³⁵S]methionine per ml for a 10-minute period. All UV-treated cells were kept in the dark. The cells were sedimented, washed, lysed, and analyzed by gel electrophoresis as described in Materials and Methods. Shown are densitometric tracings of the autoradiograms obtained: (a) nonirradiated wild type (KLC438); (b) UV-irradiated KLC438; (c) nonirradiated ssbA1 (KLC436); (d) UV-irradiated KLC436; (e) nonirradiated lexC113 (KLC484); (f) UV-irradiated KLC484. Arrows indicate positions where the recA protein migrates.*

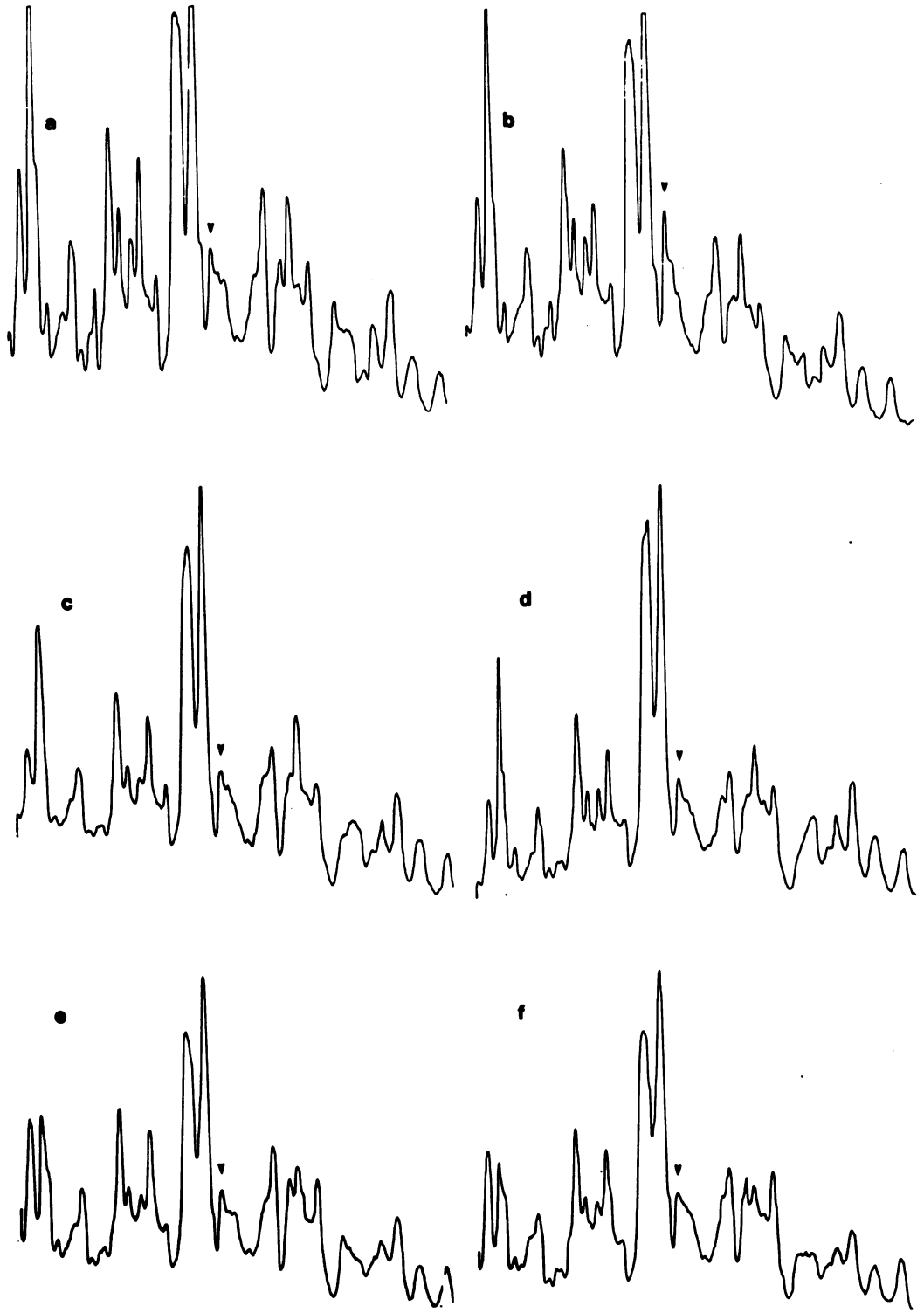

FIG. 2. *Induction of recA protein by UV in wild-type, ssbA1, and lexC113 strains shifted at 42°C. Cells growing exponentially at 30°C in RG glucose were centrifuged and resuspended in 0.25 volume RG glucose. After 20 min at 42°C, they were divided into two parts: one was irradiated with UV (30 J/m²), and the other was left as a control. Both were incubated in the dark at 42°C for 30 min and then labeled and processed as in Fig. 1. Shown are the densitometric tracings of gel autoradiograms: (a) KLC438 control; (b) UV-irradiated KLC438; (c) KLC436 control; (d) UV-irradiated KLC436; (e) KLC484 control; (f) UV-irradiated KLC484. Arrows indicate positions where the recA protein migrates.*

Bleomycin induced *recA* protein synthesis in the wild type as well as in both mutant strains, *ssbA1* and *lexC113* (see below).

Attempts at quantitation of *recA* protein amplification after treatment with UV and bleomycin. The one-dimensional gels are very useful in demonstrating a rather large change in the rate of synthesis or accumulation of proteins. Small changes may go undetected, especially for proteins in the range of 40,000 daltons, since there exist several different molecular species of that size in the cells. For that reason, we used two-dimensional gel electrophoresis, a procedure that allows quantitation of individual proteins (28) and which we proved was applicable to *recA* induction (1). An autoradiogram of a typical two-dimensional gel showing *recA* protein and other proteins used as controls is shown in Fig. 3. It should be noted that *recA* protein is resolved into two spots of apparently different molecular weights. The lower spot, which is the minor component, was also observed by McEntee (17) and seems to belong to a modified or cleaved form of this protein. In our measurements, we cut both spots to determine total radioactivity of *recA* protein. The second observation that should be considered is that, even after separation of the proteins by isoelectric point and size, there are other labeled proteins in the same spot as *recA*. The contaminating proteins are made evident by using the *tif* mutant, whose *recA* protein has a different isoelectric point, as shown by others (4, 10, 17). Examination of two-dimensional gels from labeled *tif-1* strain shows that the mutated protein also gives rise to a modified product that occupies a lower spot than the major component, and that the place in the gel that the wild-type *recA* usually occupies contains a radioactive spot.

Taking into account the factors just described, we attempted to obtain a relative measure of *recA* protein synthesis, using the same protocol as the one described for induction with UV. Prior to making the cell extracts, we mixed the ^{35}S-labeled cells with an equal amount of cells previously labeled with ^{3}H-amino acids, as an internal control, and continued with the procedure for a two-dimensional gel. After autoradiography, the two spots of *recA* protein were cut, as well as other known protein spots used as controls (EF-tu, α-RNA polymerase; Fig. 3), and the double labels were determined. The ^{35}S radioactivity of each protein was normalized to the ^{3}H radioactivity, and the relative rates of synthesis were obtained. These values are shown graphically in Fig. 4 for the wild-type, *ssbA1*,

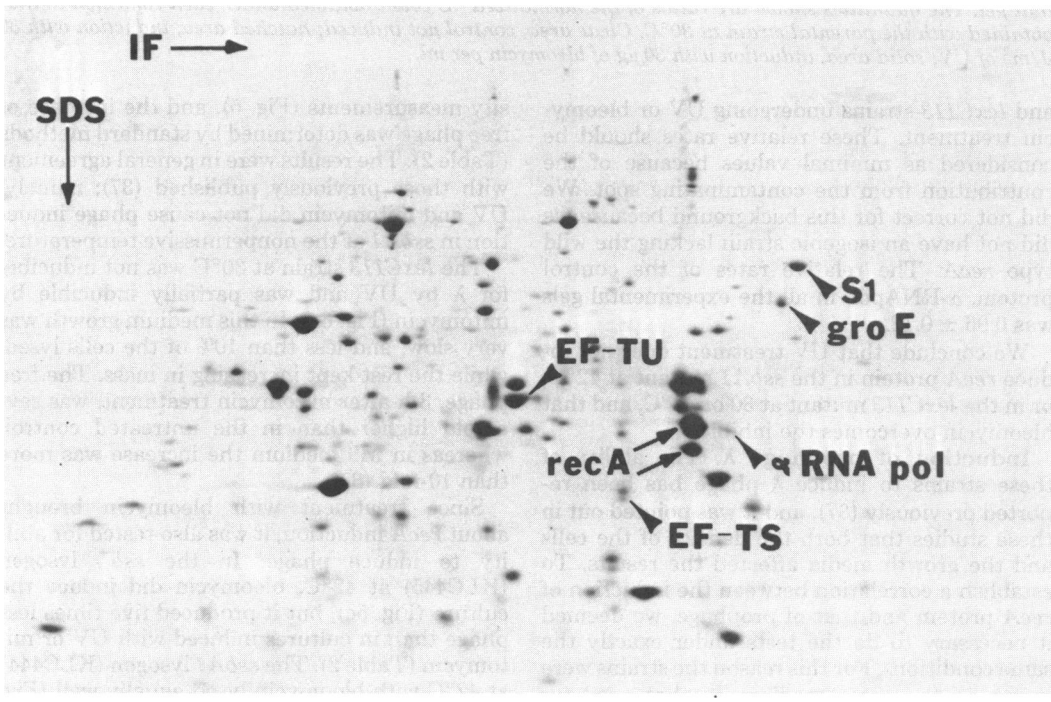

FIG. 3. *Two-dimensional gel electrophoresis of total cell protein of wild-type E. coli after induction. KLC438 cells, growing exponentially in minimal medium, were labeled with [^{35}S]methionine 30 min after treatment with 40 μg of nalidixic acid per ml. Extracts were made and processed as described in Materials and Methods.*

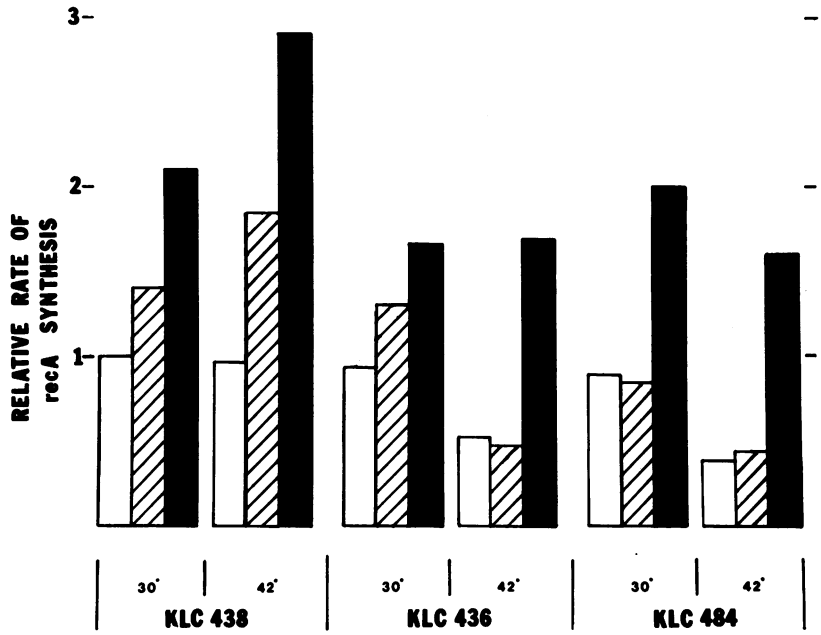

FIG. 4. *Comparison of the relative biosynthetic rate of recA protein in KLC436, KLC438, and KLC484 at 30 and 42°C, with and without induction. Extracts from cultures grown as described in Fig. 1 and 2 were mixed with a standard culture that had been labeled with ^3H-amino acids. The mixed sediment was lysed, and the proteins were separated by two-dimensional gel electrophoresis. The recA spots were then cut out and the radioactivities were determined. The ^{35}S counts from each spot were normalized to the ^3H counts recovered in that gel. The quantities shown are ratios of the normalized ^{35}S count obtained under each condition to that obtained with the parental strain at 30°C. Clear area, control not induced; hatched area, induction with 30 J/m² of UV; solid area, induction with 30 μg of bleomycin per ml.*

and *lexC113* strains undergoing UV or bleomycin treatment. These relative rates should be considered as minimal values because of the contribution from the contaminating spot. We did not correct for this background because we did not have an isogenic strain lacking the wild type *recA*. The relative rates of the control protein, α-RNApol, in all the experimental gels was 0.96 ± 0.12.

We conclude that UV treatment does not induce *recA* protein in the *ssbA1* mutant at 42°C, or in the *lexC113* mutant at 30 or 42°C, and that bleomycin overcomes the inhibition.

Induction of prophage λ. The ability of these strains to induce λ phage has been reported previously (37), and it was pointed out in these studies that both the density of the cells and the growth media affected the results. To establish a correlation between the induction of *recA* protein and that of prophage, we deemed it necessary to do the tests under exactly the same conditions. For this reason the strains were grown in the same medium as that used for labeling *recA* protein, and the inducers were applied at the same cell density.

Phage induction was followed by optical density measurements (Fig. 5), and the increase of free phage was determined by standard methods (Table 2). The results were in general agreement with those previously published (37); namely, UV and mitomycin did not cause phage induction in *ssbA1* at the nonpermissive temperature.

The *lexC113* strain at 30°C was not inducible for λ by UV and was partially inducible by mitomycin (Fig. 6a). In this medium growth was very slow, and less than 10% of the cells lysed, while the rest kept increasing in mass. The free phage, 3 h after mitomycin treatment, was sevenfold higher than in the untreated control, whereas in M9 medium the increase was more than 10-fold (37).

Since treatment with bleomycin brought about *recA* induction, it was also tested for ability to induce phage. In the *ssb*$^+$ lysogen (KLC445) at 42°C, bleomycin did induce the culture (Fig. 5c), but it produced five times less phage than in cultures induced with UV or mitomycin (Table 2). The *ssbA1* lysogen (KLC444) at 42°C with bleomycin lysed equally well (Fig. 5c) but produced almost three orders of magnitude less phage than the induced *ssb*$^+$ (Table 2).

The lysogen of *lexC113* (KLC491) was induced

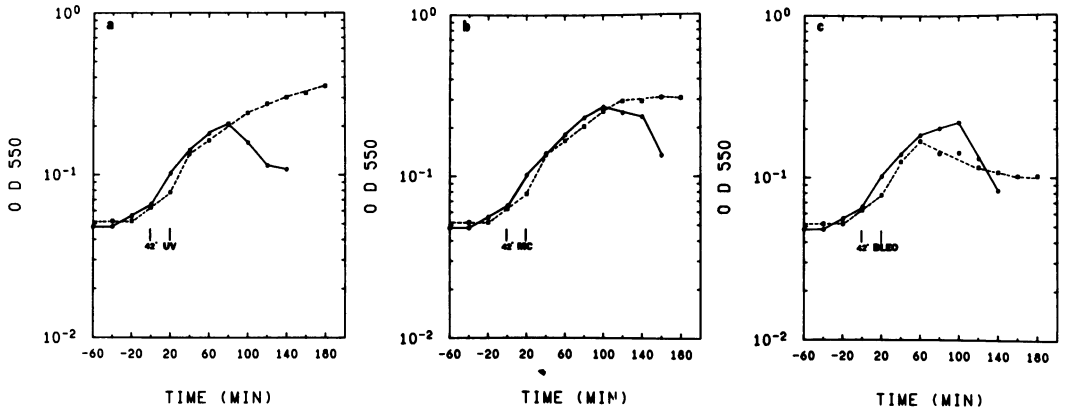

FIG. 5. *Inducibility of prophage lambda in ssb⁺ and ssb mutant lysogens. Optical density measurements of KLC444 (dashed lines) and KLC445 (solid lines). Cultures were growing at 30°C in RG glucose that had been supplemented with Casamino Acids. When the optical densities were between 0.06 and 0.08, the cultures were shifted to 42°C. Twenty minutes after shift up, the inducing agent was added and the cultures were monitored for their change in optical density. At 140 or 180 min after induction, chloroform was added, and the number of phage per milliliter was determined (Table 2). (a) UV-irradiated KLC444 and KLC445 (30 J/m²); (b) mitomycin C-treated KLC444 and KLC445 (4 μg/ml); (c) bleomycin-treated KLC444 and KLC445 (30 μg/ml).*

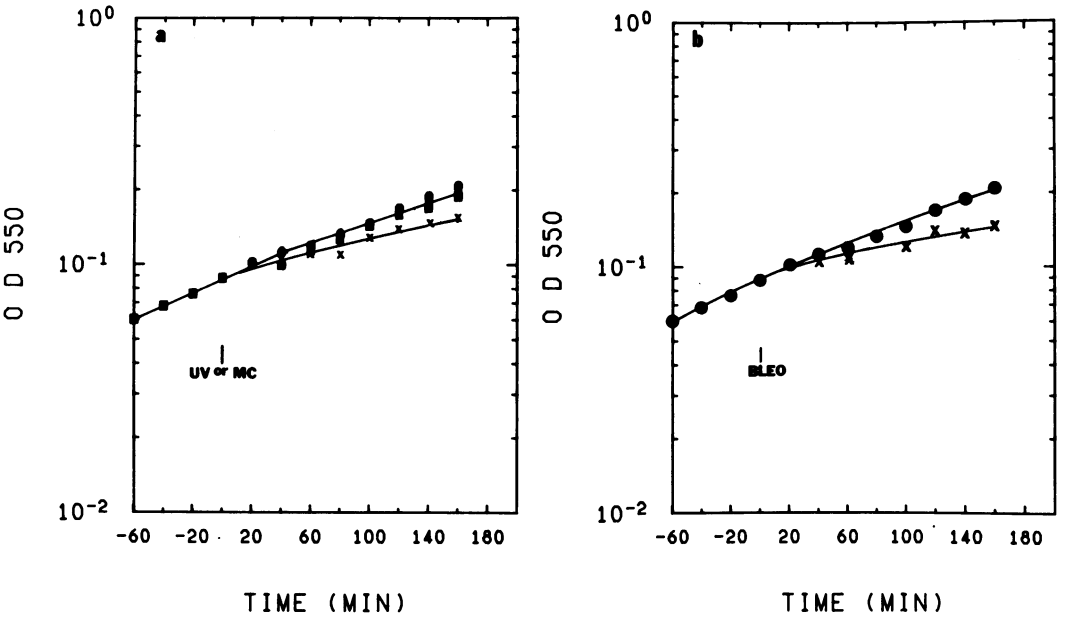

FIG. 6. *Inducibility of prophage lambda in the lexC mutant lysogen. Optical density measurements of KLC491 grown at 30°C in RG glucose supplemented with Casamino Acids. When optical density was between 0.06 and 0.08, the cultures were treated with the inducing agent and then monitored for their change in optical density. At 180 min after treatment, chloroform was added, and the number of phage per milliliter was determined (Table 2). (a) ●, untreated KLC491; ■, UV-irradiated KLC491 (30 J/m²); ×, mitomycin C-treated KLC491 (4 μg/ml). (b) ●, untreated KLC491; ×, bleomycin-treated KLC491.*

to a greater extent with bleomycin than with mitomycin C (Fig. 6 and Table 2) and produced almost as much phage as the bleomycin-induced *ssb⁺* lysogen.

We can conclude that bleomycin overcomes the suppression of induction observed in UV- or mitomycin-treated *lexC* mutant cells.

Postirradiation DNA degradation in wild-type, *ssbA1*, and *lexC113* strains. Previous studies have suggested extensive DNA

degradation in *lexC113* mutant strains in *E. coli* B after UV irradiation (7). To investigate this effect in our *E. coli* K-12 derivatives containing the *lexC113* mutation and compare it directly with the isogenic *ssbA1* derivative, we determined postirradiation DNA degradation in these strains. Figure 7 shows the percentage of acid-insoluble labeled DNA as a function of incubation time at 42°C after irradiation with a dose of 25 J/m^2. These results indicate no significant difference in the amount of postirradiation DNA degradation in any of the strains tested up to at least 40 min after irradiation. After this time, differences in the amount of degradation between the strains became more pronounced. By 120 min after irradiation, approximately 10% of the DNA in the wild-type strain (KLC438), 25% of the DNA in the *lexC113* mutant strain (KLC484), and 55% of the DNA in the *ssbA1* mutant strain (KLC436) was degraded. At the permissive temperature there was no difference between the *ssbA1* mutant and the wild-type strain (data not shown).

TABLE 2. *Induction of prophage lambda*

Strain	Genotype	Temp (°C)	Treatment	Phage/ml[a]	Spontaneous phage[b]
KLC445	Wild type	30	—	3.6×10^4	2.8×10^{-4}
		42	—	5.3×10^4	
		42	UV	2.4×10^8	
		42	Mitomycin C	4.4×10^8	
		42	Bleomycin	2.0×10^8	
KLC444	*ssbA1*	30	—	3.0×10^3	2.8×10^{-5}
		42	—	5.0×10^3	
		42	UV	8.0×10^3	
		42	Mitomycin C	1.6×10^4	
		42	Bleomycin	4.2×10^5	
KLC491	*lexC113*	30	—	6.5×10^5	6.0×10^{-4}
		30	UV	1.7×10^6	
		30	Mitomycin C	4.5×10^6	
		30	Bleomycin	7.5×10^7	

[a] Number of free phage per milliliter after incubation in the presence or absence of the inducer. Lysis of control cells occurred at different times according to the inducer; therefore, the time of addition of chloroform was 180 min for UV and mitomycin and 140 min for bleomycin (see Fig. 5).

[b] Number of free phage per bacterium, determined at a cell density of about 10^8/ml.

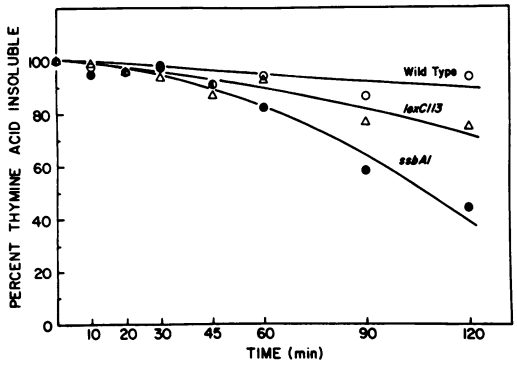

FIG. 7. *DNA degradation after UV irradiation. Exponentially growing cultures labeled with [^{3}H]thymidine in M9 minimal medium were washed and then irradiated with 25 J/m^2. The cultures were warmed to 42°C, and nutrients were added at 0 min. Samples were withdrawn just prior to nutrient addition and again at the indicated times. Acid-insoluble radioactivity was determined as described in the text. Symbols: ○, KLC438 (wild type); ●, KLC436; △, KLC484.*

DISCUSSION

In these studies we analyzed the ability of two mutants of *E. coli* which produce defective SSB proteins to induce SOS functions. Since the mutations confer somewhat different phenotypes, we will discuss them separately.

The *lexC113* strain, isolated by Greenberg et al. (7) in *E. coli* B and termed *exrB*, was considered to be an allele of *lexA* until Johnson mapped it in a separate locus on the right side of *uvrA* (14). It had been logical to assign these two mutations to one gene because they were identical in several respects: (i) both were radiation and methylmethane sulfonate sensitive; (ii) both were dominant in heterozygotes (9, 25); (iii) both degraded DNA extensively after treatment with radiation; (iv) both suppressed the UV induction of λ prophage (3, 25); and (v) the recombination capacities of both were reduced. The only difference observed was that *lexA* mutation suppressed filamentation, whereas the *lexC* mutant was highly filamentous (8). The *lexA* mutation (i.e., *lexA3*) was tested for ability to induce *recA* (protein X) and found to be defective (11). Based on the studies of other alleles of *lexA* (*tsl, spr*), which apparently modify the level of *recA* protein and of *recA* message in the cell, it has been postulated that the product of *lexA* is a repressor of the *recA* gene (4, 11, 19, 24), controlling its transcription. It has also been proposed that *lexA* and *lexC* are both repressors of *recA* and possibly of other operons not shared equally (14).

This study demonstrates again that *lexC113* is indistinguishable from the *lexA* mutation in the suppression of UV-induced *recA* synthesis. Therefore, we might conclude that there are *two* distinct diffusible proteins which are dominant and act as super-repressors. In the case of *lexC*, we know that the gene product is a mutated SSB, and much information has been accumulated on its physicochemical properties and its role in DNA replication. It is difficult to reconcile its high affinity for single-strand DNA and

ability to destabilize duplex DNA (34) with a function as a repressor of an operon(s). Since the *tsl* allele of *lexA* produces *recA* proteins constitutively, the *lexC* protein would have to act in conjunction with *lexA* protein. We propose that the genetic control is caused by competition for the inducer. This can be visualized as follows: SSB is present in limited amounts in the cell (300 molecules per cell), and in actively replicating bacteria, it would be predominantly found complexed with single-strand DNA at the replication forks. After irradiation and concomitant DNA repair, additional single-strand gaps accumulate in the cells. *recA* protein would bind to these DNA regions, become activated, and derepress its own gene by inactivating the *lexA* repressor (15). In the *lexC* strain the mutated SSB either has a much higher affinity for the gaps, preventing *recA* from binding, or is made in excessive amounts, competing successfully against *recA* protein. Preliminary results obtained by J. Chase (unpublished data) with extracts of *lexC113* show that its SSB binds more single-strand DNA. It has also been demonstrated that purified *lexC* protein binds to single-strand DNA and prevents the *recA* protein from destabilizing duplex DNA; therefore, no strand assimilation occurs (18). The same mechanism would explain the failure of this strain to induce λ prophage: it has been demonstrated that λ repressor has affinity for single-strand gaps, and it has been hypothesized that its cleavage occurs at that site (36). Saturation of the single-strand regions with SSB would prevent induction. We report here also that bleomycin was more effective in inducing both λ phage and *recA* in *lexC113*. These results can be explained by the fact that this antibiotic produces a large number of gaps (22; unpublished data) nonenzymatically, providing more binding sites for these DNA-binding proteins. It should be noted that mitomycin is an intermediate inducer of λ in *lexC113* (Table 2; reference 37).

The *ssbA1* strain produces a reversible thermo-unstable SSB. Meyer et al. (21) isolated this mutant protein and showed that it has an altered amino acid sequence. In vitro it is unable to protect single-strand DNA from S1 nuclease attack and to inhibit DNA-dependent adenosine triphosphatases (21). This mutant has provided evidence that SSB is required for DNA replication (20). It interrupts DNA synthesis upon shift to 42°C; therefore, it belongs to the class of elongation mutants. It is known that *dna*(Ts) elongation mutants of the *dnaG*, *dnaB*, and *dnaE* loci spontaneously induce prophage at the nonpermissive temperature (32) and augment *recA* protein synthesis (16). Here we show that *ssbA1* does not. It behaves more like a DNA initiation mutant. *dnaA* does not induce phage or *recA* spontaneously or with nalidixic acid treatment (12), but reacts normally towards UV induction (16). *ssbA1* has been found to be defective in the induction of λ prophage by UV or mitomycin C at 42°C, but not at 30°C (37); this was corroborated here under different conditions. We have also established that the amplification of *recA* protein synthesis in this mutant is inhibited at 42°C with UV or mitomycin as inducer. Treatment with bleomycin caused an increase in *recA* synthesis and promoted phage induction under all conditions. Another example of opposite responses to different inducers was observed with *recB* or *recC* mutants, found to suppress *recA* protein synthesis amplification after nalidixic acid treatment (12) but to respond to bleomycin with increased synthesis (16). These apparently conflicting responses can be reconciled when we consider the different pathways of producing the internal signal for SOS induction for each inducer (35). For example, bleomycin produces nicks and gaps in the DNA nonenzymatically (22), and it leads to a prompt induction. Nalidixic acid acts at the replication fork, and it seems to need *recBC* nuclease to promote gaps (12). On the other hand, UV lesions in DNA can be removed by other nucleases in the absence of *recBC*, leading to formation of gaps. Our work with *ssb* mutants suggests that single-strand binding protein is required for the formation or maintenance, or both, of the gaps after UV treatment. There is in vitro evidence that SSB affects the activity of nucleases (23). The *ssbA1* mutant should prove valuable in demonstrating whether this is also true in vivo. The possibility that SSB plays a role in protecting the single-strand regions is supported by our studies, which show enhanced DNA degradation in the mutants at the nonpermissive temperature. This is not conclusive, since the mutants are defective in *recA* induction, and there is a correlation between the level of this protein in the cell and the extent of DNA degradation (31).

The fact that we found no *recA* induction in the mutants up to 100 min after UV treatment and incubation at 42°C, at a time when there is considerable DNA degradation, indicates that DNA degradation products alone are not sufficient to derepress the *recA* gene.

ACKNOWLEDGMENTS

We thank C. M. Marrow, L. D. Vales, and R. Whittier for excellent technical assistance.

This work was supported by Public Health Service grants AIGM 13415, GM 11301-16, and GM 25451-02 from the National Institutes of Health. J.W.C. is an established investigator of the American Heart Association, grant 78-129.

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