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Overproduction of Single-Stranded-DNA-Binding Protein Specifically Inhibits Recombination of UV-Irradiated Bacteriophage DNA in *Escherichia coli*

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Overproduction of single-stranded DNA (ssDNA)-binding protein (SSB) in *uvr Escherichia coli* mutants results in a wide range of altered phenotypes. (i) Cell survival after UV irradiation is decreased; (ii) expression of the *recA-lexA* regulon is slightly reduced after UV irradiation, whereas it is increased without irradiation; and (iii) recombination of UV-damaged λ DNA is inhibited, whereas recombination of nonirradiated DNA is unaffected. These results are consistent with the idea that in UV-damaged bacteria, SSB is first required to allow the formation of short complexes of RecA protein and ssDNA that mediate cleavage of the LexA protein. However, in a second stage, SSB should be displaced from ssDNA to permit the production of longer RecA-ssDNA nucleoprotein filaments that are required for strand pairing and, hence, recombinational repair. Since bacteria overproducing SSB appear identical in physiological respects to *recF* mutant bacteria, it is suggested that the RecF protein (alone or with other proteins of the RecF pathway) may help RecA protein to release SSB from ssDNA.

The RecA protein of *Escherichia coli* has been shown to play an essential role in genetic recombination and in the induction of at least 17 genes, such as *recA*, *uvrA*, umuC, and *sfiA*, which are negatively controlled by the LexA protein (46, 48). In vitro studies have demonstrated that the RecA protein possesses a variety of activities, including the ATP-dependent assimilation of single-stranded DNA (ssDNA) into homologous duplex DNA and the ATP- and ssDNA-dependent cleavage of LexA protein (12, 36). It has been suggested, therefore, that the same ternary complex of RecA-ssDNA-ATP is involved in strand exchange and in the cleavage of LexA protein (36). However, genetic and biochemical evidence indicates that the complex which is active for cleavage of the LexA protein may not be identical to that which is required for a complete recombinational event. The activity of the RecA-ssDNA-ATP complex might be modulated by proteins such as RecF, UmuC, and ssDNA-binding protein (SSB) that could interact with RecA or ssDNA (5, 8, 22, 25, 37, 44, 47). So far, of these proteins, only SSB has been purified and studied in vitro. SSB is capable of affecting the activity of RecA protein in widely divergent ways (7, 19).

One approach to understanding the function of SSB in RecA-dependent processes in vivo is to study the effects of overproduction of SSB (6). Wild-type bacteria overproducing SSB, owing to the presence of an *ssb*$^+$ multicopy plasmid, have been shown to spontaneously express some SOS genes at high levels. This occurs because RecA protein is spontaneously activated to promote cleavage of the LexA repressor: the steady-state level of LexA protein is reduced twofold in SSB-overproducing cells compared with the level of LexA in bacteria with normal levels of SSB (25). These results are in good agreement with in vitro data indicating that SSB favors the binding of RecA protein to ssDNA and, hence, the cleavage of repressors (19, 26, 35, 36). Survival of bacteria overproducing SSB after UV irradiation is, however, slightly reduced relative to that of bacteria with normal levels of SSB, suggesting that excess SSB may inhibit some DNA repair processes (6, 25). Indeed, evidence that excess SSB inhibits recombinational repair has been obtained by monitoring recombination between a prophage and UV-damaged DNA of a heteroimmune infecting bacteriophage (25). This result, however, might be questioned because it was obtained in *uvr*$^+$ SSB-overproducing bacteria, in which excision repair is probably constitutively overexpressed owing to a partial derepression of the *recA-lexA* regulon (10, 25, 29). Since excision repair may reduce the efficiency of recombination between UV-damaged DNA molecules (11, 20), it could be argued that the inhibiting effect of excess SSB on recombination results not from an inhibition of RecA recombinase activity but from a stimulation of excision repair. The effects of excess SSB on recombination have therefore been reexamined in this study by using *uvr* mutants.

MATERIALS AND METHODS

Bacterial strains, phage strains, and plasmids. The genotypes and sources of the strains used in these experiments are given in Table 1. The method used to select Tet^s derivatives of Tn*10*-carrying strains was as described previously (9). The *uvrB34* mutation was introduced with the use of a HfrH *uvrB34* strain (23). In the experiments based on induction of a *sfiA-lacZ* gene fusion, the fact that approximately 1% of the *uvrA6* bacteria carrying the plasmid pKAC4 (*ssb*$^+$ Δ*uvrA*) gave rise to *uvr*$^+$ bacteria following homogenization of the *uvrA* region (6; data not shown) did not interfere significantly with the results, because induction of the *sfiA* gene is a massive phenomenon and each cell shows a graded response to inducing treatments (39). By contrast, in the experiments monitoring rare events such as recombination, survival after irradiation, or mutagenesis, *uvrB* derivatives should be used to avoid artifacts. Although in a *uvrB* mutant, the UvrA protein is produced, *uvrA* and *uvrB* mutations are expected to have generally the same consequences on DNA repair processes, because only

TABLE 1. Bacterial strains, phage strains, and plasmids

Strain or plasmid	Relevant genotype or description	Reference or source
E. coli K-12		
AB1157	F⁻ *thr leu pro his argE3*(Oc) *thi lac gal ara xyl mtl supE tsx rpsL*	2
GY5069	λ $h^{\phi 80}$ lysogen of AB1157	25
GY5120	As GY5069, but Pro⁺	Cross with GY2526
GY5121	As GY5069, but Pro⁺ *uvrB34*	Cross with GY2526
AB2463	As AB1157, but *recA13*	2
GY1049	λ lysogen of AB2463	25
GY5070	As GY1049, but *lamB*	25
AB1885	As AB1157, but *uvrB5*	2
IC1638	As AB1885, but *sfiB*	4
PQ65	F⁻ *thr leu his thi pyrD galE,Y rpoB* PhoC *lacU169 srl*⁺ *trp*::*Muc*⁺ *sfiA*::Mu d (Ap *lac*)cts	PQ30 (32)
PQ66	As PQ65, but *uvrA6*	PQ33 (32)
KL931	F⁻ *thyA deo rpsL malE*::Tn*10 lacI3 lacZ813*(Oc) (λ *cIind*)	11
GY5122	As KL931, but *malE*::ΔTn*10*	Select Tet^s
KL467	F⁻ *metE his trp rpsL nalA* Δ(*lac-pro*) (λ *cIind*)	K. B. Low
GY5118	As KL467, but Trp⁺	Cross with GY2526
GY5126	As KL467, but Trp⁺ *uvrB34*	Cross with GY2526
KS468	F⁻ *metB pyrE thi rpsL lacMS286* φ80 dII *lacBK1*	18
GY2526	HfrH *uvrB34*	24
Lambda phages		
λ	λ wild type	24
λ $h^{\phi 80}$	Hybrid carrying most of the λ genome, but the host range and the attachment site of φ80	38
λ imm^{434} *int-102 red-3*	Hybrid between λ and 434, which carries most of the λ genome, but the immunity region specific of 434, also recombination deficient	27
λ *lacZ118*	λ p*lac-5-lacZ118* cI857 *S7*	30
λ *lacZ813*	λ p*lac-5-lacZ813*(Oc) cI857 *S7*	30
Plasmids		
pKAC4	pACYC184 *ssb*⁺ Δ*uvrA*	6
pGY5086	As pKAC4 but *ssb*-Def	25

UvrAB and UvrABC complexes have a significant activity with UV-damaged DNA (28, 43).

Media and growth conditions. M90 buffer consists of 0.6% (wt/vol) Na_2HPO_4, 0.3% (wt/vol) KH_2PO_4, 0.1% (wt/vol) NH_4Cl, and 0.5% (wt/vol) NaCl. M90 concentrate (10×) was sterilized by autoclaving and stored at room temperature. M9 buffer is M90 supplemented with 1 mM $MgSO_4$ and 0.1 mM $CaCl_2$. EM9 medium is M9 supplemented with 0.2% (wt/vol) glucose, 0.03 mM thiamine, 0.3 mM thymine, 0.35 mM uracil, and a mixture of 20 amino acids: 0.1 mM cysteine (freshly prepared) and tryptophan; 0.2 mM histidine, methionine, and tyrosine; 0.4 mM arginine, asparagine, aspartate, isoleucine, lysine, phenylalanine, proline, and threonine; 0.6 mM glutamine, glutamate, and valine; 0.8 mM alanine, glycine, and leucine; and 1 mM serine. Amino acid concentrate (5×) was made with M90 buffer, filter sterilized, and stored at 4°C in the dark. SEM9 medium is M9 supplemented with 0.03 mM thiamine, 0.2 mM adenine, 0.2 mM cytosine, 0.2 mM guanine, 0.3 mM thymine, 0.4 mM uracil, and the mixture of amino acids described above. Plasmid-containing bacteria were cultured in the presence of 12 μg of tetracycline hydrochloride per ml. Growth was monitored at 550 nm with a spectronic 1001 spectrophotometer (Bausch & Lomb). An A_{550} of 0.5 corresponds to approximately 1.2×10^8 cells per ml. The selection medium for Lac⁺ colonies was either M9 supplemented with 0.2% (wt/vol) lactose, 0.03 mM thiamine, 0.3 mM thymine, 0.1 mM tryptophan, 0.2 mM tyrosine, 0.4 mM phenylalanine, 12 μg of tetracycline hydrochloride per ml, and 1.5% (wt/vol) Bacto-Agar (Difco Laboratories) (strain GY5122), or M9 supplemented with 0.4% (wt/vol) lactose, 0.03 mM thiamine, 0.4 mM uracil, 0.2 mM methionine, 12 μg of tetracycline per ml, and 1.5% (wt/vol) Bacto-Agar (M9-Lac; strain KS468) (17), or 4% (wt/vol) MacConkey agar base (Difco) supplemented with 1% (wt/vol) lactose and 12 μg of tetracycline per ml (MacConkey-Lac) (M. Dutreix, personal communication). The selection medium for Arg⁺ colonies was M9 supplemented with threonine, leucine, proline, histidine, isoleucine, and valine at 50 μg/ml each, 0.9 μg of arginine per ml, 0.2% (wt/vol) glucose, 0.03 mM thiamine, 12 μg of tetracycline per ml, and 1.5% (wt/vol) Bacto-Agar (4). Phage λ plaques were revealed on λ medium: 0.8% (wt/vol) Bacto-Peptone, 0.5% (wt/vol) Bacto-Tryptone, 0.5% (wt/vol) NaCl, and 1.5% (wt/vol) Bacto-Agar. Phage λ indicator bacteria were grown in LBTU (1% [wt/vol] Bacto-Tryptone, 0.5% [wt/vol] yeast extract [Difco], 1% [wt/vol] NaCl, 0.3 mM thymine, 0.35 mM uracil) supplemented with 0.2% (wt/vol) maltose. Phages were diluted in TMG (10 mM Tris hydrochloride [pH 7.5], 10 mM $MgCl_2$, 0.05% [wt/vol] gelatin). Samples were plated with 3 ml of soft agar (0.75% Bacto-Agar) unless otherwise indicated. Mitomycin C (MC; Sigma Chemical Co.) remained in the samples throughout the experiments. All incubations were performed at 37°C.

UV irradiation. UV light of predominantly 254 nm was obtained from a 15-W germicidal lamp. The fluence rate was measured with a Latarjet meter. The lamp output was 0.05 or 1 J/m² per s. The low dose rate was attained by placing a diaphragm between the lamp and the cell suspension. Separate samples were irradiated for different lengths of time.

Assay of *sfiA-lacZ* gene fusion expression. The method used to assay the *sfiA-lacZ* gene fusion expression was described by Miller (23). Cells were permeabilized with sodium dode-

cyl sulfate and chloroform. Enzyme units were calculated from the following formula:

$$\frac{A_{420} - 1.75A_{550}}{\text{time of reaction}} \times \frac{\text{reaction volume}}{\text{sample volume}} \times 1{,}000$$

Recombination assays. Four systems of recombination were used in the recombination assays. In the first system, lysogens for phage λ $h^{\Phi 80}$ $att^{\Phi 80}$ were used which were infected with heteroimmune phage λ imm^{434} *int red* irradiated prior to infection. A prophage acquiring the imm^{434} region from an infecting phage was no longer susceptible to the repression imposed by the cytoplasmic λ repressor and hence entered lytic growth. The efficiency of recombination was measured by the production of λ imm^{434} $h^{\Phi 80}$ phages (25). In detail, the experiments were performed as follows. The lysogens were grown in EM9-Tet to an A_{550} of 0.5, centrifuged, suspended in the same volume of cold 10 mM $MgSO_4$, infected with UV-irradiated or unirradiated phage λ imm^{434}, incubated for 20 min at 37°C to allow phage adsorption to occur, diluted in 10 mM $MgSO_4$, and assayed for plaque-forming infective centers on indicator strains GY1049 [*recA13*(λ)] and GY5070 [*recA13*(λ) *lamB*]. Strain GY1049 was used to reveal both infecting phages (λ imm^{434}) and recombinant phages (λ imm^{434} $h^{\Phi 80}$), whereas strain GY5070 revealed only recombinant phages.

In the second system, *lacI lacZ813* (λ *cIind*) lysogens were infected with phage λ *lacZ118*. The homoimmune resident prophage prevented replication and expression of phage functions (repressed infection). Since the two mutations in *lacZ* did not complement each other, the formation of Lac^+ colonies was a measure of the recombination proficiency (31). The lysogens were grown in SEM9-Tet medium to an A_{550} of 0.5, centrifuged, suspended in 1/16 volume of 10 mM $MgSO_4$, and mixed in a 1:1 ratio with a suspension of phage λ *lacZ118* that was UV irradiated or unirradiated. These mixtures were incubated for 15 min at 37°C, diluted 1/18 in prewarmed SEM9-Tet medium, and incubated with moderate shaking for 3 h. The cells were then centrifuged, suspended in 1/5 volume of M9 buffer, and plated onto minimal medium selective for lactose-utilizing colonies (Lac^+ recombinants) or onto glucose-minimal medium to measure the titer of viable cells. The recombination frequency was calculated as the ratio of the titer of Lac^+ cells to the titer of total cells.

In the third system, Δ*lac* (λ *cIind*) bacteria were infected simultaneously with the transducing phages λ *lacZ813* and λ *lacZ118* (repressed infection). The efficiency of recombination was measured by the level of Lac^+ enzyme (β-galactosidase) produced (31). The lysogens were grown in SEM9-Tet to an A_{550} of 0.5, centrifuged, suspended in 1/16 volume of 10 mM $MgSO_4$, and mixed (1:1) with phages λ *lacZ813* and λ *lacZ118*. Phage λ *lacZ118* was UV irradiated or not prior to infection. These mixtures were incubated for 15 min at 37°C, diluted 1/18 in prewarmed SEM9-Tet medium, and incubated with moderate shaking. After 3 h, chloramphenicol was added to a final concentration of 100 μg/ml, and the A_{600} of the cultures were recorded. The cell lysates, prepared as described previously (3), were heated for 3 min at 57°C before β-galactosidase activity was assayed (31). Enzyme units were calculated as described above.

In the fourth system, the bacteria carried a specially constructed duplication of partially deleted lactose operons so that Lac^+ recombinants occurred at low frequencies (17). The bacteria were grown in SEM9-Tet or in LBTU-Tet to an A_{550} of 0.5 and diluted 10-fold into fresh growth medium, and 100-μl samples were plated onto media selective for lactose-utilizing colonies.

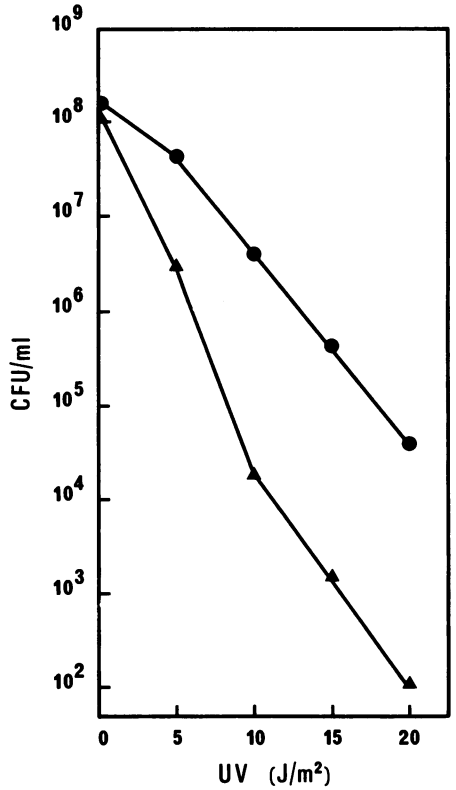

FIG. 1. UV sensitivity of *uvrB sfiB* cells. The bacteria were grown in EM9-Tet to an A_{550} of 0.5 and exposed to increasing doses of UV. CFU were assayed on EM9-Tet-agar medium. Symbols: ●, IC1638(pGY5086); ▲, IC1638(pKAC4).

Mutagenesis assay. Arg^+ revertants of ochre *argE3* strains were scored (4). Bacteria were grown in EM9-Tet medium to an A_{550} of 0.7, harvested, suspended to the same density in M9 buffer, and irradiated. Aliquots (0.1 ml) were plated onto medium selective for arginine-independent colonies (Arg^+). The survival at each UV dose was determined by spreading dilutions onto the same medium, but with arginine present at 50 μg/ml.

RESULTS

Cell survival after UV irradiation. The *recA-lexA* regulon is partly derepressed in bacteria overproducing SSB (25). Therefore, so that any complications arising as a result of secondary effects of excess SSB on *uvr*-dependent excision repair and on *sfi*-dependent inhibition of cell division should be avoided, a *uvrB sfiB* background was used to reexamine the effects of excess SSB on the sensitivity of cells to UV irradiation. *uvrB sfiB* bacteria with amplified levels of SSB (pKAC4 transformants) were indeed much more sensitive to UV than bacteria with normal levels of SSB (pGY5086 transformants) (Fig. 1). Similar results were found for a *uvrB sfiB umuC112*::Tn*5* triple mutant (data not shown).

Induction of the sfiA gene. It has been shown that excess SSB alters the kinetics of induction of the *sfiA* gene in uvr^+ bacteria in different ways: (i) induction curves of the *sfiA* gene are shifted at higher doses of UV or MC, and (ii) initial rates of induction of the *sfiA* gene are slowed (25). These effects were thought to reflect a stimulation of the *uvr*-

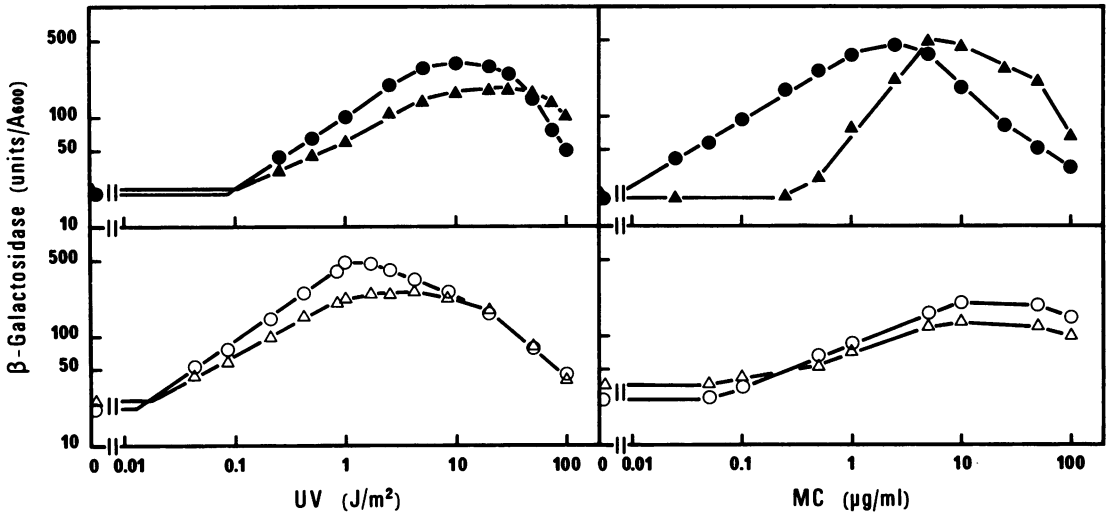

FIG. 2. Induction of the *sfiA* gene following UV irradiation or MC treatment. The bacteria were grown in EM9-Tet to an A_{550} of 0.5. Either portions were UV irradiated in M9 buffer and further incubated in EM9-Tet for 1 h or they were incubated in EM9-Tet with MC for 1 h. The cultures were then kept on ice for 20 min, and the specific activity of β-galactosidase was determined. Values obtained with derivatives of PQ65 have been published (25) and are reproduced here for comparison. Symbols: ●, PQ65(pGY5086); ▲, PQ65(pKAC4); ○, PQ66(pGY5086); △, PQ66(pKAC4); where PQ65 is *uvr*$^+$ and PQ66 is *uvrA6*.

dependent excision repair process by excess SSB (25). To test this hypothesis, the kinetics of induction of a *sfiA-lacZ* gene fusion (measured by β-galactosidase activity) between excision-repair-proficient (*uvr*$^+$) and excision-repair-deficient (*uvrA6*) bacteria with normal or amplified levels of SSB was compared.

As expected, although excess SSB caused a partial derepression of the *recA-lexA* regulon in *uvrA6* bacteria, as indicated by overproduction of RecA protein (data not shown), it no longer changed the doses of UV or MC required to induce the expression of the *sfiA* gene (Fig. 2). In particular, it is noteworthy that the induction of the *sfiA* gene by MC, which was highly sensitive to excess SSB in a *uvr*$^+$ background, became nearly insensitive to this effect in a *uvrA6* background (Fig. 2 and data not shown).

However, the time course of induction of the *sfiA* gene following UV irradiation was still slower in *uvrA6* bacteria overproducing SSB than in *uvrA6* bacteria with normal levels of SSB. Following exposure of *uvrA6*(pGY5086) bacteria to a low UV dose that had minimal effects on cell viability and growth, cellular levels of β-galactosidase increased for 90 min and then decreased, indicating that after a transient induction period, expression of the *sfiA-lacZ* gene fusion was again controlled by the LexA repressor, and accumulated β-galactosidase was diluted among growing bacteria. By contrast, in SSB-overproducing cells the kinetics of accumulation of β-galactosidase was twice as slow and induction lasted for at least 2 h (Fig. 3). These differences disappeared, however, when the cells were exposed to UV doses higher than 10 J/m^2 or when they were induced with MC (Fig. 2 and data not shown); both treatments are known to severely inhibit DNA replication in *uvr* mutants (16; data not shown).

Taken together, these results indicate that excess SSB has a dual effect on the activation of RecA protein to promote cleavage of the LexA protein: it has a stimulating effect in noninduced bacteria, but a weakly inhibiting effect in bacteria whose DNA is damaged but still replicated actively.

Recombination between replicating UV-damaged phage DNA and prophage DNA. The effects of excess SSB on induction of the *recA-lexA* regulon are probably too weak to account for the UV sensitivity of bacteria overproducing SSB. Besides, it has been shown that the *recA* gene, whose derepression is essential for cell survival (33), is induced

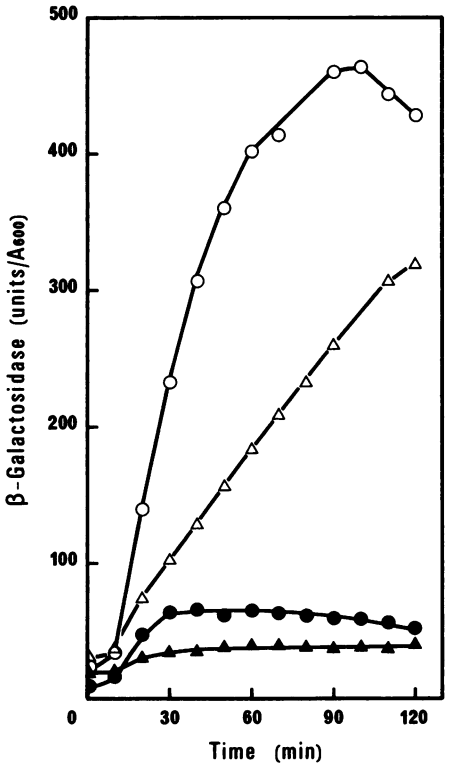

FIG. 3. Time course of induction of the *sfiA* gene after UV irradiation. The bacteria were UV irradiated (0.5 J/m^2) and incubated in EM9-Tet for different lengths of time, and portions of the cultures were assayed for β-galactosidase activity. Symbols: ●, PQ65(pGY5086); ▲, PQ65(pKAC4); ○, PQ66(pGY5086); △, PQ66(pKAC4); where PQ65 is *uvr*$^+$ and PQ66 is *uvrA6*.

TABLE 2. Recombination frequency between prophage $\lambda h^{\phi 80}$ $att^{\phi 80}$ and replicating phage λ imm^{434} UV irradiated prior to infection

Recipient strain[a]	No. of imm^{434} phages/ml		No. of imm^{434} $h^{\phi 80}$ phages/ml[c] + UV (10^4)	Recombination frequency (10^{-2})	
	−UV[b] (10^6)	+UV[b] (10^4)		−UV[d]	+UV[e]
GY5120(pGY5086)	3.0	1.3	1.1 ± 0.1	0.5	85
GY5120(pKAC4)	2.8	0.7	0.3 ± 0.05	0.1	43
GY5121(pGY5086)	3.0	5.9	0.8 ± 0.1	0.3	13
GY5121(pKAC4)	2.8	6.0	0.05 ± 0.03	0.02	0.8

[a] GY5120 is *uvr*$^+$; GY5121 is *uvrB34*.
[b] Phage λ imm^{434} *int red* was left unirradiated (−UV) or exposed to UV doses (+UV) of 200 and 100 J/m^2 when *uvr*$^+$ and *uvrB34* lysogens were used as recipient bacteria, respectively. The multiplicity of infection was 0.05 on the basis of the titer of phage measured prior to irradiation.
[c] Errors indicate the range of values for five samples in a single infection.
[d] The frequency was calculated as the ratio of the titer of UV-induced recombinant phages to the titer of total phages measured prior to irradiation (−UV).
[e] The frequency was calculated as the ratio of the titer of UV-induced recombinant phages to the titer of total phages measured after irradiation (+UV).

much more easily than the *sfiA* gene, and induced levels of RecA protein are similar in *ssb*$^+$ and in SSB-overproducing bacteria (25). Therefore, excess SSB would rather inhibit RecA activities than prevent RecA accumulation.

It has been shown previously that excess SSB reduces the recombination proficiency between UV-irradiated phage DNA (donor DNA) and heteroimmune prophage DNA (recipient DNA) in *uvr*$^+$ bacteria (25). However, since it has been observed that in certain experimental systems, DNA damage that triggers recombination can be removed by the *uvrABC* gene products (the ABC excision nuclease) (11, 20), the inhibition of UV-stimulated recombination by excess SSB could be interpreted as being mediated through an indirect stimulation of excision repair. Results shown in Table 2 do not support this hypothesis, since excess SSB reduced recombination frequencies even more drastically in *uvrB* than in *uvr*$^+$ bacteria.

However, it appears that different recombination pathways operate in *uvr*$^+$ and in *uvrB* strains. In bacteria with normal levels of SSB, although most of the imm^{434} phages produced after irradiation were imm^{434} $h^{\phi 80}$ recombinants in a *uvr*$^+$ background, recombinants were only a minor part of total phages in a *uvrB* background (Table 2).

These results suggest that particularly recombinogenic structures are created through excision repair in replicating λ DNA. One simple interpretation is that λ DNA molecules cut by the ABC excision nuclease are not readily rejoined if replication occurs concurrently with excision. Such persistent lesions probably initiate recombination. Since most phages produced after irradiation in *uvr*$^+$ bacteria were recombinants, any impediment in recombination by excess SSB was therefore reflected in a lower production of total phages (Table 2). By contrast, in *uvrB* bacteria, it appears that repair of UV-induced lesions may generally occur without the concomitant generation of structures prone to recombination. One possible explanation is that postreplication gaps at UV lesions in λ DNA are generally small enough to be repaired by RecA-independent processes, e.g., by the activities of polymerase I and ligase. These processes are not significantly affected by excess SSB, since phage survival was as high in *uvr* mutants overproducing SSB as in *uvr* mutants with normal levels of SSB. However, in the few lesions which were engaged in recombinational processes in *uvr* mutants, excess SSB had a strong inhibitory effect.

Recombination between nonreplicating phage DNA and chromosomal DNA. Does the inhibiting effect of excess SSB on UV-stimulated recombination depend upon replication of the damaged DNA? To answer this question, I used a system similar to that previously used, but prevented expression of phage functions, and thus replication of the infecting phage. Recombination took place between transducing λ *lacZ118* phage DNA (donor DNA) and the homologous region in chromosomal DNA carrying the *lacZ813* mutation (recipient DNA). Since the host strain carried a prophage λ *cIind*, expression of phage functions was prevented and recombination could be assayed by measuring the frequency of Lac$^+$ colony formation (31).

Excess SSB caused a ca. sevenfold reduction in the frequency of recombinants when transducing phages were UV irradiated prior to infection (Table 3). Excess SSB can therefore inhibit UV-stimulated recombination, even though replication is blocked. By contrast, excess SSB did not significantly affect the frequency of Lac$^+$ recombinants when phage DNA was intact.

The contribution of the excision repair has not been evaluated in this system because it was not possible to obtain a *lacI3 lacZ813 uvrB* derivative of strain GY5122 through conjugation with a HfrH *lac*$^+$ *uvrB* strain.

TABLE 3. Formation of Lac$^+$ recombinant colonies after infection of *uvr*$^+$ *lacI lacZ813* (λ *cIind*) lysogens with homoimmune phage λ *lacZ118*

Recipient strain	Phage λ *lacZ118*[a]	Total cells/ml	Lac$^+$ cells/ml[b]	Recombination frequency	UV stimulation factor[c]
GY5122(pGY5086)	−UV	4.9×10^8	6.3×10^4	1.3×10^{-4}	1
	+UV	5.7×10^8	6.0×10^6	1.0×10^{-2}	77
GY5122(pKAC4)	−UV	1.8×10^8	2.0×10^4	1.1×10^{-4}	1
	+UV	2.0×10^8	2.5×10^5	1.3×10^{-3}	12

[a] The multiplicity of infection was 3.5; phage survival after UV irradiation (+UV; 70 J/m^2) was 0.40.
[b] The reversion frequencies of the *lacZ* mutations were $<10^{-7}$.
[c] This factor was calculated as the ratio of the titer of recombinants measured after irradiation to that measured without irradiation.

TABLE 4. Recombination proficiency between two nonreplicating transducing phages

Recipient strain[a]	Phage[b] λ *lacZ813* × λ *lacZ118*	β-Galactosidase units/A_{600} (10^{-2})	UV stimulation factor[e]
GY5118(pGY5086)	Control	3 ± 3[d]	
	− UV	50	1
	+ UV[c]	1,276	26
GY5118(pKAC4)	Control	0 ± 1[d]	
	− UV	83	1
	+ UV	576	7
GY5126(pGY5086)	Control	3 ± 1[d]	
	− UV	33	1
	+ UV	1,308	40
GY5126(pKAC4)	Control	0.5 ± 1[d]	
	− UV	61	1
	+ UV	469	8

[a] GY5118 is *lac uvr*$^+$; GY5126 is *lac uvrB34*.
[b] The multiplicity of infection was 2.5 for each phage.
[c] Survival of phage λ *lacZ118* exposed to 70 J/m^2 (+UV) was 0.48.
[d] The range of values obtained when bacteria were not infected or were infected with only one phage.
[e] For an explanation of this factor, see Table 3, footnote *c*.

Recombination between nonreplicating phage DNA molecules. Does excess SSB cause early inhibition of the recombination process between UV-damaged and undamaged DNA? It is possible to investigate this question by using an assay that detects recombination intermediates without requiring the formation of viable recombinants (31). In the assay used here, recombination took place between two nonreplicating transducing phages, λ *lacZ118* and λ *lacZ813* (30). The efficiency of recombination was measured by monitoring the level of β-galactosidase produced.

In this particular system, excess SSB had weaker effects on UV-stimulated recombination than previously found, but an inhibition of approximately threefold was still observed (Table 4). As expected, recombination of nonreplicating UV-irradiated phage DNA was essentially *uvr* independent (20).

Even in the absence of replication, damage in the DNA can induce changes that stimulate recombination processes. It is not known whether this implicates only a local denaturation of duplex DNA, or whether some nicking and degradative activities by nucleases other than the ABC excision nuclease are also required (11, 20, 21).

On the other hand, these results confirmed that excess SSB had little effect on the recombination frequency of undamaged nonreplicating DNA.

Recombination between two *lac* operons located in the chromosome. The effect of excess SSB on the recombination of undamaged DNA was further investigated by using an assay based on the recombination between two *lac* operons located in the bacterial chromosome (17). The results indicate that excess SSB had little if any effect on spontaneous recombination, whether bacteria grew for a few generations or for numerous generations on the plates (Table 5).

UV-induced mutagenesis. Besides its roles in inactivation of repressors and in recombinational repair, RecA protein is thought to be required for UV-induced mutagenesis (5, 21, 41). Since excess SSB was found to have various inhibiting effects upon processes implicating the RecA protein and UV-damaged DNA, the question arises of whether excess SSB could inhibit UV-induced mutagenesis. The results indicate that excess SSB has a weakly stimulating effect rather than an inhibiting effect on UV-induced mutagenesis (Table 6). Similar results were observed in *uvr*$^+$ bacteria (data not shown).

TABLE 5. Recombination proficiency between two chromosomal *lac* operons in *uvr*$^+$ bacteria

Expt	Growth medium	Selection medium	No. of Lac$^+$ colonies per plate[a]	
			KS468(pGY5086)	KS468(pKAC4)
1	SEM9	M9-Lac	26 ± 2[b]	19 ± 4[b]
			43 ± 4[c]	33 ± 7[c]
2	LBTU	M9-Lac	67 ± 8[b]	75 ± 11[b]
			62 ± 11[c]	85 ± 13[c]
3	SEM9	MacConkey-Lac	2,800 ± 200[b]	3,100 ± 200[b]

[a] Errors indicate the range of values obtained for several samples.
[b] Samples were spread with a bent glass rod.
[c] Samples were plated with soft agar.

DISCUSSION

Effects of excess SSB on spontaneous and on UV-stimulated RecA-dependent processes were previously studied with *uvr*$^+$ bacteria (25). They have been further investigated in this study by using *uvr* mutants to avoid any complications that may arise as a result of overexpression of the excision repair process in SSB-overproducing bacteria (25). Moreover, different recombination assays were used to investigate the effects of excess SSB on the recombination of undamaged DNA molecules.

The results obtained have confirmed that the most dramatic effect of excess SSB is the inhibition of recombination between UV-damaged λ DNA and undamaged DNA. Excess SSB was found to inhibit UV-stimulated recombination in various systems in which replication and excision repair were permitted or prevented. This inhibiting effect appears to be very specific, because excess SSB had little, if any, effect on other processes implicating the RecA protein and UV-damaged DNA, i.e., induction of the *sfiA* gene and UV-induced mutagenesis, or the RecA protein and undamaged DNA, i.e., spontaneous recombination.

TABLE 6. UV-induced mutagenesis

Strain[a]	Amt of UV (J/m^2)	Fraction surviving	Arg$^+$ revertants per 10^7 survivors
AB1885(pGY5086)	0	1	3.9 ± 1.4[b]
	1	0.72	130
	4	0.15	1,200
AB1885(pKAC4)	0	1	8.3 ± 1.4[b]
	1	0.83	190
	4	0.025	2,800
IC1638(pGY5086)	0	1	3.7 ± 1.9[b]
	1	1	190
	4	0.38	1,300
IC1638(pKAC4)	0	1	8.5 ± 1.6[b]
	1	1	210
	4	0.087	2,900

[a] AB1885 is *uvrB5 argE3*; IC1638 is *uvrB5 sfiB argE3*.
[b] Errors indicate the range of values for five samples in a single experiment.

What is the characteristic step in UV-stimulated recombination that may be inhibited by excess SSB? The simplest explanation is that excess SSB inhibits the formation of the nucleoprotein filaments produced by the binding of RecA to ssDNA, which are thought to play a key role in recombination (12).

Early steps in recombination are indeed inhibited by excess SSB, as suggested by the fact that the formation of recombination-transcribable intermediates was inhibited, although less efficiently than that of mature recombinants. However, since excess SSB had little effect on the activation of RecA protein to promote LexA repressor cleavage (as measured indirectly by induction of the *sfiA* gene), this suggests that excess SSB inhibits not the formation of short tracts of RecA protein on ssDNA, which are thought to be active for repressor cleavage (26, 36, 37), but rather that of longer nucleoprotein filaments, which are required for strand pairing or branch migration or both (12). In other words, excess SSB should inhibit the formation of RecA-ssDNA complexes not at the nucleation stage but later, during the elongation stage, even though levels of RecA protein are not a limiting factor.

If excess SSB slows the rate of polymerization of RecA protein on long stretches of ssDNA, the average size of the RecA-ssDNA filaments should be smaller in UV-damaged bacteria overproducing SSB. One would therefore expect the rate of cleavage of the LexA protein to be somewhat reduced, assuming that the RecA-ssDNA filaments are active in promoting cleavage until they invade duplex DNA in a search for homology (37). Indeed, an inhibiting effect of excess SSB on inactivation of LexA could be detected following exposure of bacteria to low UV doses, when the rate of production of gapped DNA through discontinuous DNA replication is probably higher than the rate of filling of such gaps by RecA protein.

On the other hand, the finding that recombination between undamaged DNA molecules was not inhibited by excess SSB may simply reflect the fact that the ssDNA regions, which initiate this type of recombination, are not accessible to SSB. These regions may be too short to bind SSB, or binding of SSB may be prevented by the presence of other proteins, such as the RecBCD enzyme, that could act in concert with the RecA protein to initiate recombinational processes between undamaged DNA molecules (11, 49). The same reasoning may account for the lack of inhibition of UV-induced mutagenesis by excess SSB. In this case, a cofactor for RecA might be the UmuC and UmuD proteins (5, 21, 22). The slight variations observed in the frequencies of mutagenesis in SSB-overproducing bacteria may simply reflect variations in expression of the *recA-lexA* regulon and, hence, variations in the levels of UmuD and UmuC proteins.

The results presented here are in good agreement with in vitro data indicating that under optimal conditions, RecA-promoted cleavage of repressors is stimulated by all concentrations of SSB (26, 35). By contrast, although the rate and extent of formation of D loops are enhanced by lower levels of SSB, they are reduced by higher levels of SSB (13). Taken together, these results support the idea that if SSB is initially required to organize the binding of RecA protein on ssDNA present in large amounts, it must later be displaced to allow the formation of longer RecA-ssDNA filaments (12, 37).

Can the RecA protein alone displace SSB from ssDNA, or is it helped by other proteins? In view of all the characteristics of SSB-overproducing bacteria that have been described here, it is striking to observe that bacteria with high levels of SSB are identical in the following physiological respects to *recF* mutant bacteria. (i) Survival after UV irradiation is decreased. It is noteworthy that both *uvr recF* mutants (44) and *uvr* mutants overproducing SSB show broken survival curves, suggesting that the defects induced in DNA repair occur at low UV doses, when repair is preferentially performed by recombination (34). (ii) The expression of LexA-controlled genes is spontaneously increased (14), whereas it may be reduced following exposure to DNA-damaging agents (1, 42, 47). (iii) UV-induced mutagenesis is not significantly affected (15). (iiii) The level of recombination of UV-damaged phage DNA is reduced (40), whereas that of undamaged DNA is unaffected (50).

The *recF* gene codes for a 40-kilodalton protein, whose function is not known (46). However, *recF* mutants show a wide range of altered phenotypes, indicating that the RecF protein is involved in recombination and repair. Notably, a *recF* mutation produces a major deficiency in the repair of DNA daughter-strand gaps (47). Since there is genetic evidence that the RecA protein and the RecF protein interact with each other (44, 45, 47), I would like to suggest that the role of the RecF protein may be to help RecA protein displace SSB from ssDNA, whether RecF is alone or associated with other proteins that belong to the so-called RecF pathway (46). This view is supported by genetic and biochemical data. First, the *recA441* mutation, which increases the binding affinity for ssDNA of the RecA protein (36, 37), partially suppresses the *recF* mutation (45). Second, Madiraju et al. have found that the mutant RecA803 (Srf) protein overcomes interference by SSB during the formation of joint molecules. They have hypothesized that this property allows *recA803* to partially suppress the deficiency in repair caused by the *recF* mutation in a *uvr* background (M. V. Madiraju, V. S. Templin, and A./A. J. Clark, personal communication).

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