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recA (Srf) Suppression of *recF* Deficiency in the Postreplication Repair of UV-Irradiated *Escherichia coli* K-12

TZU-CHIEN V. WANG* AND KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

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The mechanism by which *recA* (Srf) mutations (*recA2020* and *recA801*) suppress the deficiency in postreplication repair shown by *recF* mutants of *Escherichia coli* was studied in UV-irradiated *uvrB* and *uvrA recB recC sbcB* cells. The *recA* (Srf) mutations partially suppressed the UV radiation sensitivity of *uvrB recF*, *uvrB recF recB*, and *uvrA recB recC sbcB recF* cells, and they partially restored the ability of *uvrB recF* and *uvrA recB recC sbcB recF* cells to repair DNA daughter-strand gaps. In addition, the *recA* (Srf) mutations suppressed the *recF* deficiency in the repair of DNA double-strand breaks in UV-irradiated *uvrA recB recC sbcB recF* cells. The *recA2020* and *recA801* mutations do not appear to affect the synthesis of UV radiation-induced proteins, nor do they appear to produce an altered RecA protein, as detected by two-dimensional gel electrophoresis. These results are consistent with the suggestion (M. R. Volkert and M. A. Hartke, J. Bacteriol. 157:498–506, 1984) that the *recA* (Srf) mutations do not act by affecting the induction of SOS responses; rather, they allow the RecA protein to participate in the *recF*-dependent postreplication repair processes without the need of the RecF protein.

Our current understanding of the major dark repair systems for the processing and repair of UV radiation-damaged DNA in *Escherichia coli* can be summarized as follows. The pyrimidine dimers produced in DNA by UV irradiation can be removed from the DNA by an efficient *uvrABC*-dependent excision repair process (3, 25). If these dimers (primary lesions) are not excised from the DNA and replication proceeds past them, DNA daughter-strand gaps (secondary lesions) will be formed and a dimer will reside in the single-stranded parental DNA that is opposite a gap in the newly synthesized daughter-strand DNA. *E. coli* cells possess efficient recombination systems for the repair of these DNA daughter-strand gaps (19, 20). Finally, unrepaired daughter-strand gaps can be converted to DNA double-strand breaks (tertiary lesions), and these breaks can also be repaired (32, 35).

In excision repair-deficient cells, the repair of DNA daughter-strand gaps is totally dependent on a functional *recA* gene (26, 36), and a major portion of gap-filling repair also requires a functional *recF* gene (8, 10, 18, 32). On the other hand, the repair of DNA double-strand breaks requires both functional *recA* and *recB* genes (32,35), and to a lesser extent it also requires functional *radB* (22) and *recN* genes (16, 24). However, in *recB recC sbcB* cells, a functional *recF* gene is required for the repair of DNA double-strand breaks (34).

The *recF* gene codes for a 40-kilodalton protein (2) whose function is not known. One approach to understanding the function of the *recF* gene in DNA repair is to isolate mutants that suppress the RecF phenotype and to characterize the mechanism by which these suppressor mutations exert their action. Suppressor mutations of *recF* (i.e., *srfA*) have been isolated and shown to map in the *recA* gene (27). These *recA* (Srf) mutations (previously called *srfA*) partially suppress the UV radiation sensitivity of *uvrA recF* cells (27), suggesting that the need for the RecF protein in postreplication repair is circumvented by a *recA* (Srf) mutation. In view of the known repair deficiencies caused by a *recF* mutation (i.e., deficiencies in gap-filling repair and in the repair of DNA double-strand breaks in the *uvrA recB recC sbcB* background), we considered the following possibilities for the effect of a *recA* (Srf) mutation. (i) The ability of the RecA protein to participate directly in the *recF*-dependent postreplication repair processes is normally modulated by the RecF protein, whereas the RecA protein from *recA* (Srf) cells can participate in these repair processes without modulation by the RecF protein. (ii) In some SOS responses, the RecA protein is normally activated by the RecF protein so that it can participate in the induction of other enzymes that are needed in the *recF*-dependent repair processes, whereas the RecA protein from *recA* (Srf) mutants can participate in the induction process without activation by the RecF protein. (iii) The *recA* (Srf) mutation may enhance other repair processes that are *recF* independent. We have investigated these possibilities, and our results support the idea that in *recA* (Srf) mutants the RecA protein can participate in the *recF*-dependent processes of postreplication repair without modulation by the RecF protein.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1. The transduction technique used in strain construction was similar to that described by Miller (14). Strain SR1348 [Δ(*uvrB-chlA*) *recF143 recA2020*] was isolated by selecting UV radiation-resistant clones from UV-irradiated SR305 [Δ(*uvrB-chlA*) *recF143*] cells as follows. Exponential cultures of SR305 in supplemental minimal medium (SMM) (31) were UV irradiated (2 J/m^2) at 2 × 10^8 cells per ml to a survival level of 1%, and the cultures were incubated at 37°C to saturation. After being diluted 10-fold, the cultures were UV irradiated (2 J/m^2) and were allowed to grow to saturation again. This procedure was repeated two more times to increase the proportion of UV radiation-resistant survivors. Approximately 80% of the survivors (24 of 30 clones tested) after this treatment procedure became UV radiation resist-

* Corresponding author.

TABLE 1. *E. coli* K-12 strains

Stanford radiology no.	Genotype[a]	Derivation, source, or reference[b]
KH21 [Δ(*uvrB-chlA*) background][c]		
SR305	*recF143* Srl$^+$	32
SR596	Srl$^+$	32
SR1348	*recF143 recA2020* Srl$^+$	This work (see Materials and Methods)
SR1349	*recF143 recA2020*	SR1348 × P1vira.SR1000, select Tc^r
SR1350	*recF143*	SR305 × P1vira.SR1349, select Tc^r
SR1351	*recF143 recA2020*	SR305 × P1vira.SR1349, select Tc^r
SR1352	+	SR596 × P1vira.SR1349, select Tc^r
SR1353	*recA2020*	SR596 × P1vira.SR1349, select Tc^r
SR1355	*recF143 recA2020 recB21* Thy$^+$	SR1351 × P1vira.SR257, select Thy$^+$
SR1357	*recA2020 recB21* Thy$^+$	SR1353 × P1vira.SR257, select Thy$^+$
SR1372	*recF143 recB21* Thy$^+$	SR1350 × P1vira.SR257, select Thy$^+$
SR1374	*recB21* Thy$^+$	SR1352 × P1vira.SR257, select Thy$^+$
SR1499	*recF143*	SR305 × P1vira.SR1475, select Tc^r
SR1500	*recF143 recA801*	SR305 × P1vira.SR1475, select Tc^r
SR1504	*recF143 recA801 recB21 Thy*$^+$	SR1500 × P1vira.SR257, select Thy$^+$
AB1157 (*uvrA6 recB21 recC22 sbcB15* background)[d]		
SR1419	Srl$^+$	34
SR1620	+	SR1419 × P1vira.SR1349, select Tc^r
SR1621	*recA2020*	SR1419 × P1vira.SR1349, select Tc^r
SR1623	*recA801*	SR1419 × P1vira.SR1475, select Tc^r
SR1626	*recF332*::Tn*3*	SR1620 × P1vira.SR1367, select Ap^r
SR1627	*recA2020 recF332*::Tn*3*	SR1621 × P1vira.SR1367, select Ap^r
SR1629	*recA801 recF332*::Tn*3*	SR1623 × P1vira.SR1367, select Ap^r
Other strains		
SR257	F$^-$ *uvrB5 recB21 leuB19 metE70 rha-5 lacZ53 rpsL151 deo(C2?)* λ$^-$	30
SR669	Hfr KL16 *ilv-318 thr-300 srlA300*::Tn*10 rpsE300* λ$^-$	A. J. Clark (JC10240)
SR859	HfrH *glyA6 thi-1 relA1* λ$^-$	*E. coli* Genetic Stock Center (AT2457)
SR865	F'*lac*$^+$/*lacY thi*	A. J. Clark (JC2625)
SR996	F$^-$ *leuB19 metE70 thyA36 deo(C2?) bioA2 lacZ53 rha-5 rpsL151* λ$^-$	33
SR1000	Same as SR996 except *srlA300*::Tn*10*	SR996 × P1::Tn9 *cts*.SR669, select Tc^r
SR1367	F$^-$ *recF332*::Tn*3 argE3 hisG4 leuB6 proA2 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44 tnaA*::Tn*10* HK19^r φX174^s S13^s	A. J. Clark (JC10990)
SR1475	F$^-$ *uvrA6 recF143 recA801 leuB6 proA2 hisG4 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44 thyA srlA300*::Tn*10*	A. K. Ganesan (SN543)

[a] Genotype symbols are those used by Bachmann (1).
[b] Ap^r, Ampicillin resistance; Tc^r, tetracycline resistance. P1vira is a reisolate of P1 *vir* that was obtained from A. J. Clark.
[c] These strains are F$^-$ and λ$^-$ and carry *leuB19 thyA36 deo(C2?) lacZ53 malB45 rha-5 rpsL151 srlA300*::Tn*10* unless otherwise specified.
[d] These strains are F$^-$ and λ$^-$ and carry *argE3 hisG4 leuB6 proA2 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 srlA300*::Tn*10 tsx-33 rpsL31 supE44* unless otherwise specified. According to Lloyd and Buckman (12), these strains should also contain an *sbcC* mutation, since they are all derivatives of JC7623.

ant. One of the clones (SR1348) was used for further genetic studies.

The evidence that strain SR1348 harbors a suppressor mutation for *recF* in the *recA* locus came from the following genetic data. First, when a P1 phage lysate of strain SR1000 (*recA*$^+$ *srlA300*::Tn*10*) was used to transduce strain SR1348, 35 of 44 tetracycline-resistant (Tc^r) transductants (80%) became as UV radiation sensitive as its parental SR305 cells, indicating that the suppressor mutation is closely linked to the *srlA* locus, as are other *recA* (Srf) mutations (27). Second, when a P1 phage lysate of strain SR1475 [*recA801* (Srf) *srlA300*::Tn*10*] was used to transduce strain SR1348, none of the 192 Tc^r transductants became UV radiation sensitive, suggesting that the suppressor mutation in strain SR1348 lies in the *recA* (Srf) locus. In many strains, a *recA* (Srf) mutation had no phenotypic effect. In such cases, the presence of the *recA* (Srf) mutation was confirmed by backcrossing into a *uvrB recF* (SR305) strain and testing for UV radiation resistance.

Media. SMM, LB medium, and DTM buffer have been described (31). Selection media for resistance to tetracycline and to ampicillin were 0.75% Difco yeast extract (Difco Laboratories) and 2.3% Difco nutrient agar containing tetracycline (15 μg/ml) or ampicillin (40 μg/ml), respectively. SMM agar containing streptomycin at 200 μg/ml was used to select recombinants from a cross of an Hfr strain with an F$^-$ recipient.

UV irradiation. The source (254 nm) and measurement of fluence rate for UV irradiation have been described (30). For survival studies, cultures were grown in SMM and were UV irradiated as previously described (32). Survivors were determined by assaying CFU on SMM agar.

Recombination frequencies. The ability of cells to carry out genetic recombination was tested by conjugation crosses as previously described (30).

DNA repair studies. Cells were grown exponentially at 37°C in SMM. The cultures were UV irradiated and pulse-labeled with [*methyl*-^{3}H]thymidine (64 Ci/mmol; Amersham Corp.) as described previously (32). The fate of DNA newly synthesized after UV irradiation was monitored during repair incubation by sedimentation analysis on both alkaline and neutral sucrose gradients as previously described (32). Sedimentation data obtained from alkaline sucrose gradients were used to determine the number-average molecular weights (M_n) for single-stranded DNA, with ^{14}C-labeled bacteriophage T2 DNA as a molecular-weight marker (23). The M_n of [^{3}H]DNA after 120 min of repair incubation was used to calculate the average number of UV radiation-induced DNA single-strand breaks per genome with the formula: $\{[(M_n)_c/(M_n)_{uv}] - 1\} \cdot [2.8 \times 10^9/(M_n)_c]$, where $(M_n)c$ and $(M_n)_{uv}$ are the number-average molecular weights of DNA from unirradiated control and UV-irradiated samples after 120 min of repair, respectively.

Neutral sucrose gradients were used to study the formation and repair of DNA double-strand breaks as described previously (32). Because the DNA profiles obtained from neutral sucrose gradients during the postreplicational formation and repair of DNA double-strand breaks are very complex (see reference 32), they were analyzed by drawing two vertical lines on the DNA profile for nonirradiated cells, one line corresponding to a relative sedimentation distance of 0.82 and the other line corresponding to a value between 0.50 and 0.55 such that 39% of the total DNA profile lay between the two lines. The percent high-molecular-weight DNA (i.e., percentage of the total DNA that lay between these two lines) was determined for UV-irradiated cells at different repair times to evaluate their ability to repair DNA double-strand breaks (32).

Radioactive labeling of proteins. Cells were grown exponentially at 37°C in SMM to a density of 2×10^8 cells per ml (optical density at 650 nm, 0.25; Zeiss PMQII spectrophotometer) and were UV irradiated (4 J/m^2) while in SMM. After irradiation, the cells were incubated at 37°C for 35 min and were then labeled with a mixture of ^{3}H-amino acids (Amersham) at 10 μCi/ml for 5 min. Unlabeled Casamino Acids were added to the culture at 1% (wt/vol), and incubation was continued for an additional 1 min at 37°C. Cells were harvested by centrifugation, washed once with cold SMM and once with cold 10 mM Tris (pH 6.8) containing 1 mM phenylmethylsulfonyl fluoride, and resuspended in 50 μl of 10 mM Tris (pH 6.8) containing 1 mM phenylmethylsulfonyl fluoride (for one-dimensional gels) or in 50 μl of lysis solution that contained urea at 9.5 M, Nonidet P-40 (Particle Data, Inc.) at 2%, pH 5 to 7 Ampholine (LKB Instruments, Inc.) at 1.6%, pH 3.5 to 10 Ampholine at 0.4%, β-mercaptoethanol at 5%, and phenylmethylsulfonyl fluoride at 1 mM (for two-dimensional gels). Samples were stored at −80°C until used.

Gel electrophoresis and autoradiography. Procedures for the separation of proteins by one- and two-dimensional polyacrylamide gel electrophoresis were essentially those described by Laemmli and Favre (11) and O'Farrell (15). For one-dimensional polyacrylamide gel electrophoresis, samples were lysed in a buffer (Tris [pH 6.8] at 0.0625 M, glycerol at 10%, sodium dodecyl sulfate [SDS] at 1.25%, and β-mercaptoethanol at 5%) by heating in a boiling water bath for 3 to 3.5 min. Samples of cell lysates containing 300,000 cpm of trichloroacetic acid-precipitable material were loaded into each well of the gel. Electrophoresis was performed on a vertical slab gel apparatus (model SE600, Hoefer Scientific Instruments) at 120 V. For two-dimensional polyacrylamide gel electrophoresis, samples in lysis solution were lysed by freezing and thawing five times in the presence of lysozyme at 18 μg/ml. Samples of cell lysates containing 400,000 cpm of trichloroacetic acid-precipitable material were loaded on each tube gel (2×13.5 mm) and were isoelectrically focused for a total of 5,500 to 6,300 Vh. The tube gels were equilibrated in 10% glycerol, 5% β-mercaptoethanol, 2.3% SDS, and 0.0625 M Tris, pH 6.8, as described by O'Farrell (15) before being loaded on an SDS-polyacrylamide gel for electrophoresis in the second dimension at 120 V. The gels were stained with 0.1% Coomassie blue in 45% methanol and 10% acetic acid for 30 min. After destaining, the gels were impregnated with fluor (En^{3}Hance; New England Nuclear Corp.) according to the specifications of the manufacturer and dried. Kodak X-Omat AR XAR-2 film (Eastman Kodak Co.) was exposed to the dried gels at −80°C and developed according to film instructions. The RecA protein (*E. coli*) was purchased from Bethesda Research Laboratories, Inc., and the low-molecular-weight calibration kit for proteins was obtained from Pharmacia Fine Chemicals.

RESULTS

Effect of *recA* (Srf) mutations on the UV radiation sensitivities of *uvrB* and *uvrA recB recC sbcB* cells. To study the mechanism by which *recA* (Srf) mutations (*recA801* and *recA2020*) suppress the *recF* deficiency in postreplication repair, we used excision repair-deficient cells to avoid any complications that might arise as a result of the excision repair process. In the *uvrB* background, the *recA2020* mutation, isolated in this work, had little effect on the UV radiation sensitivities of *uvrB* or *uvrB recB* cells but partially suppressed the UV radiation sensitivities of *uvrB recF* and *uvrB recF recB* cells (Fig. 1), indicating that the *recA2020* suppression of UV radiation sensitivity is *recF* specific. The *recA2020* mutation consistently produced a slightly greater suppression of the UV radiation sensitivity of *recF* cells than did the *recA801* mutation (Fig. 1), which was isolated by Volkert and Hartke (27). In the *uvrA recB recC sbcB* background, the presence of the *recA2020* and *recA801* mutations slightly increased the UV radiation sensitivities of these cells, but they greatly suppressed the UV radiation sensitivity of *uvrA recB recC sbcB recF* cells (data not shown).

Effect of *recA* (Srf) mutations on genetic recombination in *uvrA recB recC sbcB* cells. In the *recB recC sbcB* background, genetic recombination requires a functional *recF* gene and a number of other genes (5, 17, 29). The *recA801* and *recA2020* mutations had no effect on genetic recombination in *uvrA recB recC sbcB* cells. However, both of these *recA* (Srf) mutations suppressed the recombination deficiency in *uvrA recB recC sbcB recF* cells to a similar extent (Table 2). These results are comparable to those obtained with *uvr*$^+$ cells (27), except that several of our strains are considerably more deficient in the inheritance of F′*lac*$^+$ during conjugation crosses.

Effect of *recA* (Srf) mutations on DNA repair. Since a *recF* mutation produces a major deficiency in the repair of DNA daughter-strand gaps (8, 10, 18, 32) and in the repair of DNA double-strand breaks after UV irradiation in the *uvrA recB recC sbcB* background (34), the *recA* (Srf) suppression of the UV radiation sensitivity of *recF* cells may occur either by restoring *recF*-dependent repair processes or by activating

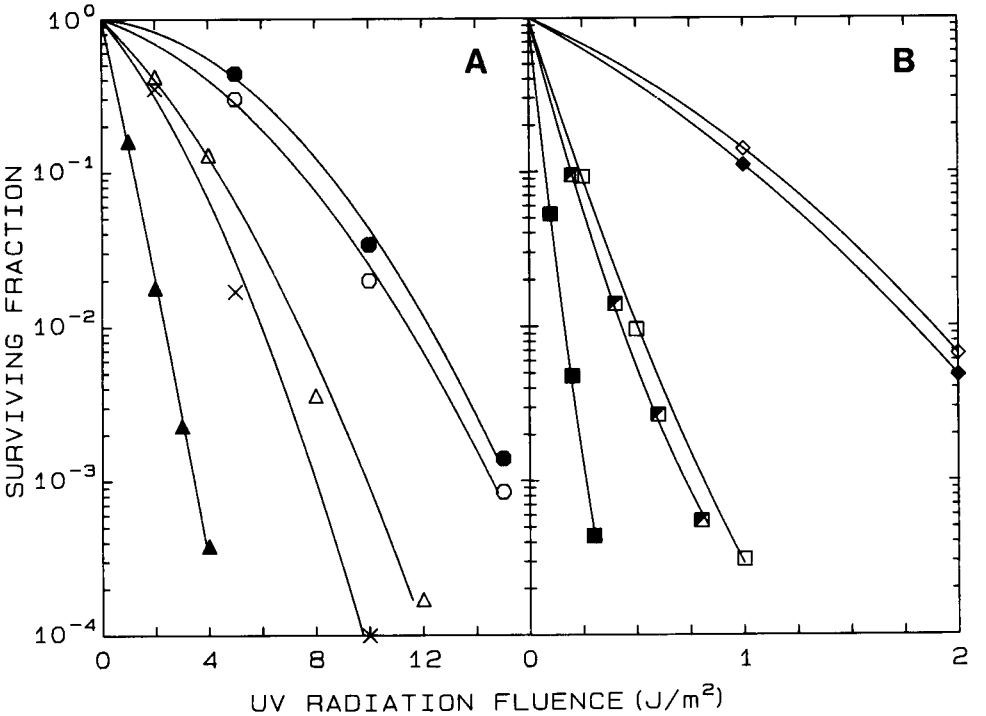

FIG. 1. Effect of *recA* (Srf) mutations on the UV radiation sensitivity of *uvrB* cells. All strains were grown and treated as described in Materials and Methods. CFU were assayed on supplemented minimal medium. Symbols: ●, *uvrB* (SR1352); ○, *uvrB recA2020* (SR1353); ▲, *uvrB recF* (SR1350); △, *uvrB recF recA2020* (SR1351); ×, *uvrB recF recA801* (SR1500); ◆, *uvrB recB* (SR1374); ◇, *uvrB recB recA2020* (SR1357); ■, *uvrB recB recF* (SR1372); □, *uvrB recB recF recA2020* (SR1355); ◩, *uvrB recB recF recA801* (SR1504). Data are the averages of two experiments.

recF-independent repair processes. First, we examined the effect of *recA* (Srf) mutations on the repair of DNA daughter-strand gaps that are produced in nascent DNA after UV irradiation. The cells were UV irradiated, pulse-labeled with [^{3}H]thymidine, and incubated in nonradioactive medium at 37°C for 2 h to allow the maximal repair of DNA daughter-strand gaps. The number of single-strand breaks in the nascent DNA after 2 h of repair incubation was determined and was presumed to reflect the number of DNA daughter-strand gaps that remained unrepaired. In the *uvrB* background, a *recF* mutation produced a large deficiency in gap-filling repair, and a large number of unrepaired DNA daughter-strand gaps accumulated (Fig. 2A). This *recF* deficiency in gap-filling repair was partially suppressed by the *recA801* and *recA2020* mutations; the *recA2020* mutation exerted a much greater suppression than did the *recA801* mutation (Fig. 2A). In the *uvrA recB recC sbcB* background, both the repair of DNA daughter-strand gaps and the repair of DNA double-strand breaks are dependent on a functional *recF* gene (34). The deficiency of *uvrA recB recC sbcB recF* cells in gap-filling repair was partially suppressed by the *recA801* and *recA2020* mutations (Fig. 2B). Similarly, the deficiency of *uvrA recB recC sbcB recF* cells in the postreplicational repair of DNA double-strand breaks was also suppressed by the *recA801* and *recA2020* mutations (Fig. 3).

Synthesis of UV radiation-induced proteins. The *recF* gene has been suggested to play a regulatory role in the SOS

TABLE 2. Influence of *recA* (Srf) mutations on recombination frequencies in *uvrA recB recC sbcB* cells of *E. coli* (AB1157)

Strain	Genotype[a]	Recombination proficiency index[b]	Conjugation proficiency index[c]	Corrected recombination proficiency index[d]
SR1620	+	1	1	1
SR1621	*recA2020*	0.25	0.25	1
SR1623	*recA801*	0.83	0.73	1.1
SR1626	*recF332*::Tn*3*	0.0027	0.14	0.019
SR1627	*recF332*::Tn*3 recA2020*	0.07	0.17	0.41
SR1629	*recF332*::Tn*3 recA801*	0.09	0.20	0.45

[a] All strains carry the *uvrA recB recC sbcB* mutations.
[b] Calculated by dividing the frequency at which recombinants (Leu$^+$ Sm^r) were produced in a conjugational cross of an Hfr (SR859) donor with a tester strain recipient by the frequency obtained with a Rec$^+$ recipient (SR1620). Data are the averages of two experiments.
[c] Calculated by dividing the frequency at which exconjugates (Lac$^+$ Sm^r) were produced in a conjugational cross of an F′*lac*$^+$ (SR865) donor with a tester strain recipient by the frequency obtained with a Rec$^+$ recipient (SR1620). Data are the averages of two experiments.
[d] Recombination proficiency index divided by conjugation proficiency index.

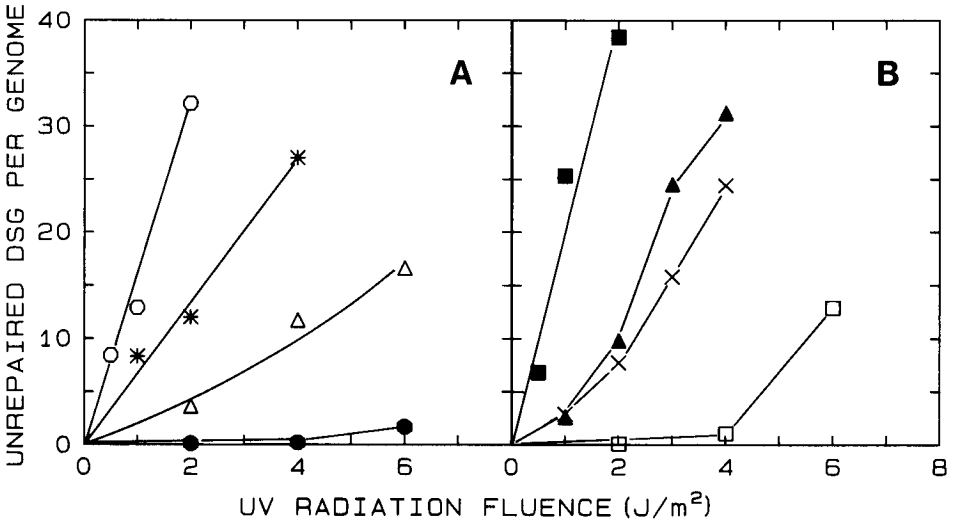

FIG. 2. The *recA* (Srf) suppression of the *recF* deficiency in the repair of DNA daughter-strand gaps (DSG) in UV-irradiated *uvrB recF* (A) and *uvrA recB recC sbcB recF* (B) cells. Cells were UV irradiated, pulse-labeled with [^{3}H]thymidine at 37°C for 5 min, and incubated in nonradioactive SMM for 2 h. The cells were converted to spheroplasts, lysed, and sedimented on alkaline sucrose gradients to determine the number of UV radiation-induced DNA single-strand breaks in the nascent DNA (i.e., unrepaired DSG), as described in Materials and Methods. Symbols: ●, *uvrB* (SR1352); ○, *uvrB recF* (SR1499); △, *uvrB recF recA2020* (SR1351); *, *uvrB recF recA801* (SR1500); □, *uvrA recB recC sbcB* (SR1620); ■, *uvrA recB recC sbcB recF* (SR1626); ×, *uvrA recB recC sbcB recF recA2020* (SR1627); ▲, *uvrA recB recC sbcB recF recA801* (SR1629). Data are the averages of two experiments.

response (6, 9, 13). To test the possibility that the *recA* (Srf) mutations act by circumventing the need for RecF protein in the induction process, we examined the effect of *recA* (Srf) mutations on the synthesis of UV radiation-induced proteins. In one-dimensional gels, UV-irradiated (4 J/m^2) *uvrB* and *uvrB recF* cells both exhibited an enhanced synthesis of the RecA protein (Fig. 4), indicating that *recF* cells have no major deficiency in the induction of the RecA protein. Similar results for *recF* cells have been obtained by others (4, 6, 21), although one report claims a major role for the *recF* gene in the induction of RecA protein after UV irradiation (13). The presence of the *recA2020* mutation in *uvrB recF* cells did not greatly alter the induced synthesis of the RecA protein, nor did it produce any visible change in the position of the RecA protein band in one-dimensional gels (Fig. 4). Similar results were obtained with *uvrB recF recA801* cells (data not shown). To further evaluate whether the *recA* (SrfA) mutations are involved in the induction of non-RecA proteins, and whether the *recA* (Srf) mutations produce an altered RecA protein, we used two-dimensional gel electrophoresis (15) to analyze the synthesis of UV radiation-induced proteins. The proteins were isoelectrically focused in the first dimension and were separated by molecular weight in the second dimension. Preliminary experiments were performed on a 15% polyacrylamide (14.6% acrylamide and 0.4% bisacrylamide) gel to ensure the detection of low-molecular-weight proteins. In UV-irradiated *uvrB* cells, the enhanced synthesis of two proteins was easily observed on two-dimensional gels: one protein corresponded to RecA, and the other (protein N) had a higher pI and a higher molecular weight than those of RecA (data not shown). The identification of protein N is not yet complete, but the protein appears to have similar electrophoretic properties to the RecN protein, recently characterized by Finch et al. (7). The irradiated *uvrB recF* and *uvrB recF recA2020* cells exhibited similar levels of enhanced synthesis of RecA and N proteins, and the RecA protein synthesized in these cells migrated to the same position as did the

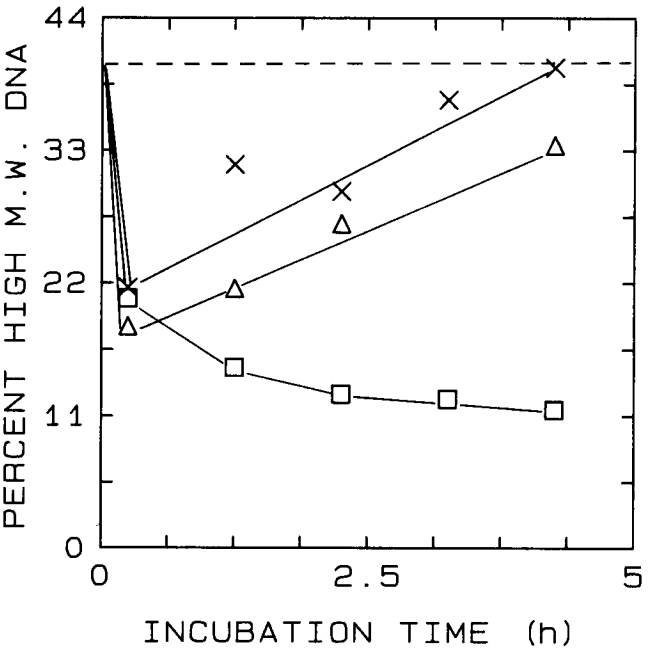

FIG. 3. The *recA* (Srf) suppression of the *recF* deficiency in the repair of DNA double-strand breaks in UV-irradiated *uvrA recB recC sbcB recF* cells. Cells were UV irradiated (0.5 J/m^2), pulse-labeled with [^{3}H]thymidine at 37°C for 5 min, and incubated in nonradioactive SMM at 37°C for different lengths of time. The cells were converted to spheroplasts, lysed, and sedimented on neutral sucrose gradients to follow the formation and repair of DNA double-strand breaks, as described in Materials and Methods. The ability to repair DNA double-strand breaks was monitored by the ability of cells to reform high-molecular-weight (high M. W.) DNA at longer incubation times. The broken line indicates the value obtained from unirradiated control cells and reflects the maximal repair possible. Symbols: □, *uvrA recB recC sbcB recF* (SR1626); △, *uvrA recB recC sbcB recF recA2020* (SR1627); ×, *uvrA recB recC sbcB recF recA801* (SR1629).

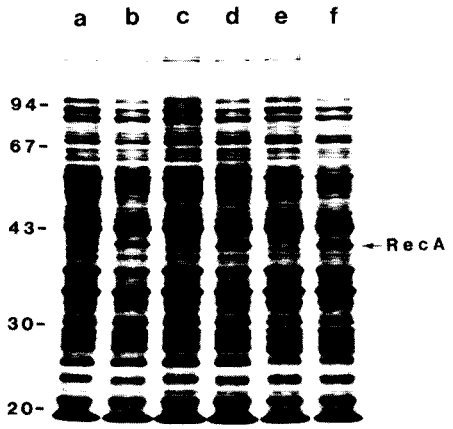

FIG. 4. One-dimensional gel electrophoresis of proteins synthesized in *uvrB recF* (SR1350, lanes a and b), *uvrB recF recA2020* (SR1351, lanes c and d), and *uvrB* (SR1352, lanes e and f) cells. Unirradiated (lanes a, c, and e) and UV-irradiated (4 J/m^2) (lanes b, d, and f) cells were pulse-labeled with a mixture of ^{3}H-amino acids after 35 min of incubation at 37°C, and cell lysates were electrophoresed in a 12% polyacrylamide gel (11.68% acrylamide, 0.32% bisacrylamide) as described in Materials and Methods. Autoradiography time was 20 h. The positions of the RecA protein and of the molecular-weight markers in kilodaltons (identified by Coomassie blue staining) are indicated in the margins.

wild-type RecA protein marker (data not shown), indicating that the *recA2020* mutation does not produce an altered RecA protein, as detected on two-dimensional gels. Similar experiments were also performed on 8% polyacrylamide (7.57% acrylamide and 0.43% bisacrylamide) gels to provide a better separation of proteins with higher molecular weights. A comparison of the synthesis of UV radiation-induced proteins for the *uvrB recF*, *uvrB recF recA2020*, and *uvrB recF recA801* cells is shown in Fig. 5. In all three strains, an enhanced synthesis of RecA and N proteins was easily detected in UV-irradiated cells, and there was no detectable difference in the electrophoretic mobility of the RecA proteins synthesized by these three strains.

DISCUSSION

The *recA* (Srf) mutations partially suppressed the UV radiation sensitivity conferred by a *recF* mutation in different genetic backgrounds (Fig. 1; 27) and acted by partially restoring the postreplication repair proficiency of *recF* cells (Fig. 2 and 3). These results suggest that the *recA* (Srf) suppression of the *recF* deficiency is caused by a partial restoration of *recF*-dependent repair processes rather than by the activation of *recF*-independent repair processes.

Volkert and Hartke (27) suggested that the *recF* gene normally functions to allow the expression of two *recA* activities, one that is required for the RecF pathway of recombination and repair and another that is required for SOS induction. The possibility that the *recA* (Srf) suppression of the *recF* deficiency in postreplication repair acts by circumventing the need of RecF in SOS induction was examined in this work by studying the synthesis of UV radiation-induced proteins. In two-dimensional gels, the synthesis of RecA and N proteins in *uvrB* cells was greatly enhanced after UV irradiation (data not shown). However, the *uvrB recF* cells exhibited similar levels of enhanced synthesis of these two proteins after UV irradiation (Fig. 5), indicating that there is no major deficiency of *recF* cells in the synthesis of these two proteins. Therefore, it does not seem probable that a *recA* (Srf) mutation exerts its suppression by this mechanism.

At present, we favor the idea that the RecF protein may modulate the recombination activity of RecA protein so that it can participate in the *recF*-dependent recombination and repair processes. According to this postulate, *recA* (Srf) mutants produce a modified RecA protein that can participate in at least some of the *recF*-dependent repair processes without the need of RecF protein. The recent observation that the UV radiation sensitivity of a *uvrA recF* cell can also be partially suppressed by a temperature-independent activity of the *recA441* mutation (28) is consistent with such a postulate, and it suggests that the activation of the RecA

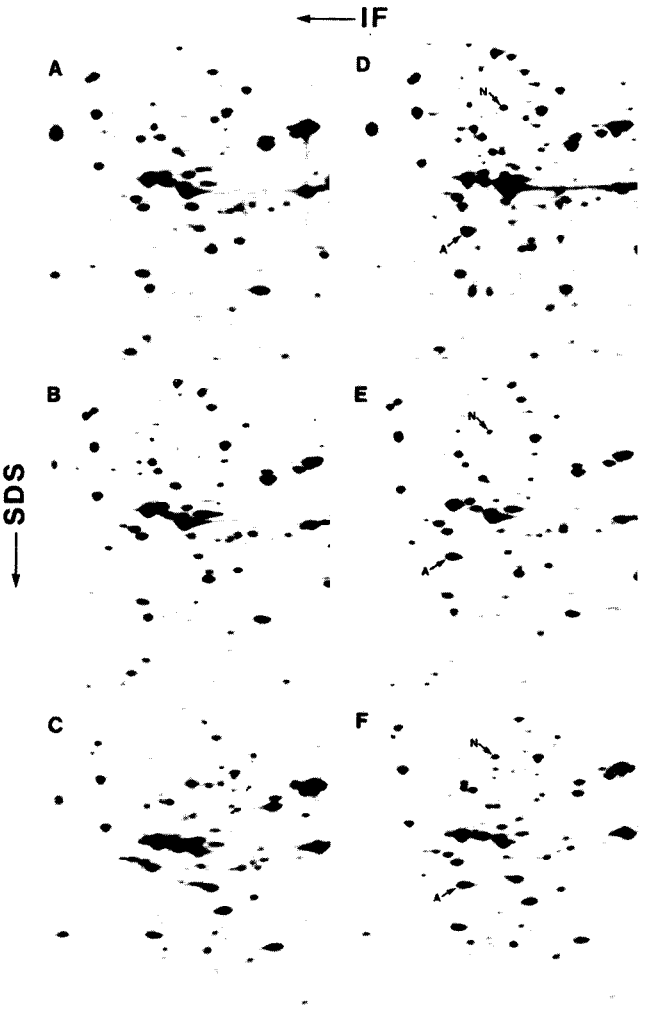

FIG. 5. Two-dimensional gel electrophoresis of proteins synthesized in *uvrB recF* (SR1499, panels A and D), *uvrB recF recA2020* (SR1351, panels B and E), and *uvrB recF recA801* (SR1500, panels C and F) cells. Unirradiated cells (panels A, B, and C) and UV-irradiated cells (panels D, E, and F) were pulse-labeled as described in the legend to Fig. 5, and cell lysates were isoelectrically focused (IF) as described in Materials and Methods. In the second dimension, an 8% polyacrylamide (7.57% acrylamide, 0.43% bisacrylamide)-SDS gel was used (SDS). Autoradiography time was 72 h. The letters A and N indicate the positions of the RecA protein and an unidentified UV radiation-induced protein (possibly RecN protein), respectively.

protease activity plays little role in the suppression of the *recF* deficiency in postreplication repair.

Genetic data indicate that *recA* (Srf) mutations are in the *recA* gene (27). We observed that the two independently isolated *recA2020* and *recA801* mutants produced RecA proteins that migrated to the same spot as did wild-type RecA protein in two-dimensional gels (Fig. 5). These results suggest that the *recA2020* and *recA801* mutations are missense mutations in the *recA* gene and that they produce an altered RecA protein that is unchanged in its net electrostatic charge and molecular weight. A further evaluation of the site of the *recA2020* and *recA801* mutations in the *recA* gene and the identification of the amino acid changes in the RecA protein from *recA2020* and *recA801* cells should help to define the domain of the RecA protein that is putatively involved in the interaction with the RecF protein and in the RecF pathway of recombination and repair.

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