Suppression of the UV-Sensitive Phenotype of Escherichia coli recF Mutants by recA(Srf) and recA(Tif) Mutations Requires recJ+  

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Mutations in recA, such as recA801(Srf) (suppressor of RecF) or recA441(Tif) (temperature-induced filamentation) partially suppress the deficiency in postreplication repair of UV damage conferred by recF mutations. We observed that spontaneous recA(Srf) mutants accumulated in cultures of recB recC sbcB sulA::Mu dX(Ap lac) lexA51 recF cells because they grew faster than the parental strain. We show that in a uvrA recB+ recC+ genetic background there are two prerequisites for the suppression by recA(Srf) of the UV-sensitive phenotype of recF mutants. (i) The recA(Srf) protein must be provided in increased amounts either by SOS derepression or by a recA operator-constitutive mutation in a lexA(Ind) (no induction of SOS functions) genetic background. (ii) The gene recJ, which has been shown previously to be involved in the recF pathway of recombination and repair, must be functional. The level of expression of recJ in a lexA(Ind) strain suffices for full suppression. Suppression by recA441 at 30°C also depends on recJ+. The hampered induction by UV of the SOS gene uvrA seen in a recF mutant was improved by a recA(Srf) mutation. This improvement did not require recJ+. We suggest that recA(Srf) and recA(Tif) mutant proteins can operate in postreplication repair independent of recF by using the recJ+ function.

Escherichia coli has at least two pathways for genetic recombination which both depend on a functional recA gene. One requires the recB and recC genes providing subunits of exonuclease V (34) and constitutes the normal recombination pathway (RecBC pathway [5]). The other is switched on by sbcB mutations in recB recC mutants and requires the gene recF (RecF pathway) plus recJ, recN, recO, recQ, and ruv (11, 16, 19, 21, 23, 31). The genes recN and ruv were shown to be SOS genes (19, 21), and therefore at least some functions of the recF pathway of recombination must be considered part of the SOS system (22). The SOS system of E. coli (18, 40, 46) consists of a set of genes which are coordinately regulated by lexA and recA proteins. Several of these genes have been shown to improve cellular survival after DNA damage. The lexA protein is the common repressor of these genes. It is inactivated by proteolytic cleavage when recA protein is activated by an SOS signal produced during exposure of cells to agents that damage DNA or block replication. The recA and lexA genes are also regulated by the lexA repressor (18).

The exact function of the recF protein (3) is not yet known. From the UV-sensitive phenotype of recF mutants it is clear that recF is involved in DNA repair (11). The recF mutants are defective in the filling of postreplicative gaps in DNA opposite pyrimidine dimers (9, 33, 41) and in the repair of DNA double-strand breaks in a recB recC sbcB genetic background (42). Recently it was observed that certain mutations in the recA gene such as recA(Srf) (previously termed srfA; suppressor of RecF) and recA(Tif) (temperature-induced filamentation) partially suppress the UV-sensitive phenotype of recF, uvrA recF, and recB recC sbcB recF mutants (37, 39). In the last strain a recA(Srf) mutation also partially alleviated the recombination deficiency (37). The suppression of a recF defect by certain recA mutations could indicate a close interaction between these two proteins. Consistent with this assumption, it was observed that recF also aids in the efficient induction of the recA gene (27, 40) and of many other SOS genes by UV irradiation and nalidixic acid treatment, possibly allowing the recA protein to maximize its usage of the SOS signal required for it to mediate proteolytic cleavage of lexA protein (36). The various indications of interactions between recA and recF proteins allow consideration of several ways in which mutant recA protein may circumvent the need for recF in the recovery from UV-induced damage: (i) the recA(Srf) protein no longer requires cooperation with recF protein to efficiently induce SOS repair functions; (ii) the function in DNA repair processes normally supplied by recF is exerted to a large extent by the recA(Srf) protein; and (iii) other repair processes become activated by the mutation in recA.

While this work was in progress, a similar set of hypotheses was examined by Wang and Smith (43), who provided evidence that the recA(Srf) protein participates in postreplicative DNA repair without the need for recF protein (43). We have studied the suppression of the UV-sensitive phenotype of recF mutants by recA mutations by looking for conditions under which the suppression is abolished to find gene functions or other functions necessary for suppression. According to our findings all three hypotheses suggested above on how the deficiency of postreplication repair in recF mutants could be suppressed are correct in part: the recA(Srf) mutation (i) improves recF-independent induction of SOS repair gene(s), (ii) allows recF-independent repair of UV-damaged DNA, and (iii) activates a recJ-dependent repair pathway.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used are listed in Table 1. Alleles recF400::Tn5 (36), recA801 (37), recA441 (14), recJ284::Tn10 (23), lexA51 (29), lexA3 (lexA102 [30], recAa281 (6), and uvrA215::Mu d1(Ap lac) (13) have been described previously. Transductions were done with P1kc or P1vir. Alleles of recA were cotransduced with srlC::Tn10 or srlC+ (8) and alleles of lexA were cotransduced with malE::Tn10. The presence of alleles in strains was identified by their effect on UV sensitivity (recF, recJ, uvrA, recA441,


<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Derivation or source</th>
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<tbody>
<tr>
<td>WA653</td>
<td>recB21 recC22 sbbB15 sulA::Mu dX(Ap lac) argE3 hisG4 proA2 thr-1 leuB6 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 rpsL31 supE44 tss-33</td>
<td>From JC7623 (17) by transduction of sulA::Mu dX(Ap lac) (12) from NO47</td>
</tr>
<tr>
<td>NO47</td>
<td>sulA::Mu dX(Ap lac) Δ(lac pro)XIII hisG4 argE3 thi-1 ara14 xyl-5 mtl-1 rpsL31 tss-33 ivl(Ts)</td>
<td>D. Mount</td>
</tr>
<tr>
<td>WA662</td>
<td>WA663 lexA51 malE::Tn10</td>
<td>From GW1060 (13) by transduction of recA+ srlC300::Tn10</td>
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<tr>
<td>WA663</td>
<td>WA662 recF400::Tn5</td>
<td></td>
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<tr>
<td>WA654</td>
<td>WA653 recF400::Tn5</td>
<td></td>
</tr>
<tr>
<td>BT10</td>
<td>WA665 recA4001(Srf)</td>
<td></td>
</tr>
<tr>
<td>BT24</td>
<td>WA654 recA4001(Srf)</td>
<td></td>
</tr>
<tr>
<td>WA591</td>
<td>uvrA215::Mu dX(Ap lac) recA+ srlC300::Tn10 thr-1 leuB6 hisG3 argE3 galK2 mtl-1 xyl-5 rpsL31 supE44 tss-33 thi-1 ara-14 ivl(Ts) = relA sfiA11 lacΔ(U169)</td>
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</tr>
<tr>
<td>BT12</td>
<td>WA591 recF400::Tn5</td>
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</tr>
<tr>
<td>BT62</td>
<td>As BT12, but uvrA215::Mu dX(Ap lac), also recA801(Srf)</td>
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<td>uvrA155 lexA102 argE3 hisG4 leuB6 proA2 thr-1 thi-1 rpsL31 galK2 lacY1 tss-33 ara-14 xyl-5 mtl-1 supE44</td>
<td>A. J. Clark (MV1131)</td>
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<td>BT85</td>
<td>WA670 pJC763 (rec+) srlC+</td>
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<tr>
<td>BT73</td>
<td>BT12 recJ284::Tn10</td>
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<tr>
<td>BT78</td>
<td>sulA::Mu dX(Ap lac) recF400::Tn5 recA441(Tif) thr-1 leuB6 hisG3 argE3 galK2 mtl-1 xyl-5 rpsL31 supE44 tss-33 thi-1 ara-14 ivl(Ts) = relA lacΔ(U169)</td>
<td>The sulA::Mu d fusion (12) and the recF400 allele (36) were transduced into GW1000 (14)</td>
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<td>BT9</td>
<td>WA591 recA441(Tif) srlC+</td>
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<tr>
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<td>BT12 recJ284::Tn10</td>
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<tr>
<td>BT84</td>
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<td>BT106</td>
<td>BT9 lexA3 malE::Tn10</td>
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</tr>
<tr>
<td>BT107</td>
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<tr>
<td>WA213</td>
<td>HfrH thi-1 rbs-1</td>
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<tr>
<td>WA493</td>
<td>Δ(proB-lac)X111 relA1(?)(?) thi-1 mal24 rpsE2112 (= spoC12) F128 (pro- lac+)</td>
<td>A. L. Taylor (AT715)</td>
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| recA801, recA4001, recA4011, lexA102, and RecA281 | on β-galactosidase synthesis in Mu dX(Ap lac) fusion strains | the recA+ allele by cotransducing it with srlC::Tn10 into strain WA656 (dinD1 recF400::Tn5), in which a recA+ recAo mutation causes significant increases in the UV sensitivity (7; B. Thoms and W. Wackernagel, unpublished results). From BT87 a spontaneous recA(Srf) mutant was chosen [BT88, recA4011(Srf) recA281 srlC::Tn10]. The recA(Srf) mutation was verified by being transduced out with srlC::Tn10 into BT82 (uvrA215 recF400), resulting in about 90% UV-resistance colonies among the tetracycline-resistant transductants. The combination of recA4011 recA281 alleles in BT88 was then cotransduced with srlC::Tn10 into strain BT59 (uvrA155 lexA102 recF400 recA801), which is a srl+ derivative of WA670 (Table 1). The presence of recA(Srf) mutations in donor and recipient cells ensured that transductants would inherit either recA801 or recA4011. The 10 tetracycline-resistant transductants tested were all much more UV-resistant than the parental strain was, indicating that they had inherited a recA(Srf) mutation and the recAo allele. One strain was saved (BT92; Table 1), and we assume that it contains the recA4011 mutation plus the recA281 allele, because of the ca. 90% cotransduction of recA mutations with srlC (8). In most of the Mu d1(Ap lac) fusion strains used, the Mu |
d prophage was stabilized by being converted to Mu dX(Ap lac) (which contains a Tn9 insertion in gene B) by the procedure of Baker et al. (1). With derivatives of JC7623 (recB21 recC22 sbcB15), care was taken not to remove the sbcC mutation present in this strain (20).

Media. Complete (LB) and minimal (M9) media have been described previously (26). Incubation temperatures were generally 30°C, except for transductions and conjugation experiments (37°C).

UV irradiation and SOS induction. UV irradiation was performed as described previously (35, 36). Irradiated cells were immediately plated on complete medium and incubated in the dark. SOS induction took place in minimal medium by UV irradiation.

Assay of β-galactosidase. Preparation of samples, determination of β-galactosidase, and calculation of the optical density of a culture at 600 nm were as reported previously (36).

Bacterial crosses. Bacterial crosses were performed by standard procedures; the ratio of donor cells to recipient cells was 1. Matings lasted 15 min for Hfr and 60 min for F' cells at 37°C. Colonies of recombinants and exconjugates were determined after 2 to 3 days of incubation.

Plasmid transformation. Plasmid pJC763 was isolated as published (2) and transformed by the method of Hanahan (10).

RESULTS

Detection of spontaneous recA(Srf) mutants. In a recB recC sbcB genetic background (probably also containing sbcC [20]) a recF mutation causes a highly UV-sensitive and recombination-deficient phenotype (11). A recF mutation also impairs the induction of SOS genes by UV irradiation and nalidixic acid treatment in this genetic background (Thoms and Wackernagel, unpublished). Since it is known that at least two of the genes of the recF pathway are under lexA-recA control, we asked whether permanent derepression of SOS genes in a recB recC sbcB recF strain by a lexA51 mutation (strain WA663 [Table 1]) would alleviate the defects in recombination and repair. The lexA51 protein has lost its function as a repressor of SOS genes (29). In strain WA663 recombination proficiency was not restored (Table 2) and the repair of UV damage was only somewhat improved (Fig. 1). This shows that the UV-sensitive phenotype of a recF mutant cannot be explained only by the impaired induction of the SOS response. Rather, recF plays also a direct role in postreplication repair (9, 33, 41, 42).

Strain WA663 grew more slowly and made even smaller colonies than its parental recB recC sbcB recF strain did. However, up to 10% fast-growing mutant cells accumulated in overnight cultures grown in medium with ampicillin and kanamycin, to which the strain is resistant (Table 1). The spontaneous mutations in the fast-growing isolates were identified as recA(Srf) mutations (37) by the following crite-
ria. (i) The mutants showed higher UV resistance (Fig. 1) and recombination proficiency (Table 2) than the parental strain did. (ii) When the srlC300::Tn10 allele from a recA+ strain was transduced into the mutants, UV-sensitive, slow-growing transductants indistinguishable from the parental strain were observed at frequencies between 66 and 90%. These cotransduction frequencies are characteristic of the close linkage between srlC and recA (8). (iii) When the recA allele from one of the mutants (recA801) was crossed into a recB recC sbcB recF or a uvrA recF strain, an increase in UV resistance typical of recA(Srf) mutants (37) was observed (Fig. 1; data not shown). Transductants of the recombination-deficient recB recC sbcB recF strain were obtained at an appreciable frequency and showed increased recombination proficiency (Table 2), comparable to published data for a recB recC sbcB recF recA801 strain (37). The results show that recA(Srf) mutations occur spontaneously in WA663. Previously such mutants were obtained on medium containing the mutagen mitomycin C (37) or by four successive UV irradiations of cells (43). Apparently the recA(Srf) mutations also moderate a deleterious effect (slow growth) of full SOS derepression in a recB recC sbcB recF strain.

Suppression of recF deficiency requires increased amounts of recA(Srf) protein. lexA(Ind) mutations (lexA3, lexA102) render the lexA repressor resistant to cleavage by recA protein and thereby block the induction of SOS genes (30). A recF mutation increased the UV sensitivity of a uvrA lexA102 strain considerably (Fig. 2). This UV-sensitive phenotype of recF was not suppressed by a recA801 mutation (Fig. 2). It appears that suppression depends on the inducibility of the SOS response. In one line of experiments we examined whether expression of the recA(Srf) gene at a higher level than in uninduced cells would be needed for suppression. It is known that the recA+ protein is synthe-

![Figure 2](http://jb.asm.org/Downloaded-from) Effect of a recA operator-constitutive mutation and a recF mutation on the suppression of the UV-sensitive phenotype of recF mutants by recA(Srf) in a uvrA155 lexA102(Ind) genetic background: WA603 (parental strain) (A); WA610 (recF400) (B); WA670 (recF400 recA801) (C); BT92 (recF400 recA4011 recA801) (D); BT90 [recF400 recA4011 recA801 pIC763 (recF+)] (E); BT96 (recF400 recA4011 recA801 pIC763 (recF+)) (F); BT94 (recF400 recA801) (G).

![Figure 3](http://jb.asm.org/Downloaded-from) Effect of recJ284::Tn10 on the suppression of the UV-sensitive phenotype of recF mutants by recA801(Srf) in a uvrA215::Mu dAp lac genetic background: WA591 (parental strain) (O); BT12 (recF400) (A); BT62 (recF400 recA801) (B); BT73 (recF400 recJ284) (C); BT74 (recF400 recA801 recJ284) (D). Sizen in lexA(Ind) cells at a level about equal to the level in uninduced lexA+ cells (4). To obtain specific overproduction of recA(Srf) protein in lexA(Ind) cells we constructed a uvrA lexA102 recF strain having a recA4011(Srf) plus a recA801 mutation (see Materials and Methods). This strain was much more UV resistant than the uvrA lexA102 recF recA(Srf) and uvrA lexA102 recF recA+ recA0 mutants (Fig. 2). We conclude that a level of the recA(Srf) protein higher than that in uninduced cells is an important component of the suppressing mechanism.

Requirement of recJ+ for suppression. In another line of experiments we tested whether other genes would also be necessary for the suppression of the UV-sensitive recF phenotype. Mutant alleles [Mu d(Ap lac) fusions] of genes dinA, dinB, dinD, and dinF (14) transduced into the uvrA recF recA801 and recB recC sbcB recF recA801 genetic backgrounds did not decrease the UV resistance of these strains, indicating that these SOS genes were not involved in the suppression (data not shown). We also examined the effect of recN::Mu d(Ap lac) (21) and recJ284::Tn10 mutants (23) in the uvrA recF recA801 strain. The recN mutation slightly sensitized a uvrA recF mutant, but did not abolish the suppressive effect of an additional recA801 mutation (data not shown). However, the recJ mutation completely eliminated the suppression (Fig. 3). Single uvrA or recF mutants or a uvrA recF strain became only slightly more UV sensitive with recJ mutations (Fig. 3; data not shown), and recJ single mutants have full repair capacity for UV damage (23). Furthermore, the relatively high UV resistance of a uvrA recF lexA102 recA801 recA0281 strain was reduced by the recJ mutation to the low resistance of a uvrA lexA102 recF strain (Fig. 2). These results demonstrate an essential role of recJ in the suppression of the repair deficiency of recF mutants by recA(Srf). We could not test whether suppression of the recF recombination deficiency also requires the recJ function, since a recJ mutation blocks recombination in a recB recC sbcB strain (23).

Are increased amounts of the recJ gene product required
for maximum suppression? The recJ+ gene has recently been cloned on a multicopy vector (24). A plasmid with efficient recJ+ expression, pJC763 (24), did not increase the low survival of a uvrA lexA102 recF recA4001 strain after UV irradiation (Fig. 3), which suggests that in the lexA102-repressed state of the SOS system recJ expression is not the limiting factor for suppression. Plasmid pJC763 also did not further improve the UV resistance of a uvrA lexA102 recF recA4001 recAa286 strain (Fig. 3), indicating that the expression of a single recJ gene in these lexA102 cells provides sufficient levels of recJ protein for full suppression.

Effect of recA(Srf) on the induction of a uvrA::lacZ fusion.

Mutations in recF hamper the cellular SOS response. The UV-induced expression of β-galactosidase in a uvrA::Mu d(Ap lac) fusion (13) was determined in different genetic backgrounds. A recA801 mutation restored the poor induction in a recF strain to almost the level observed in a recF+ strain (Fig. 4). The restoration did not depend on recJ+ (Fig. 4). Similar results were also obtained with mitomycin C (data not shown).

Suppression by recA441(Tif) also depends on recJ+. In recA441(Tif) mutants the SOS response is induced by shifting the cells to 42°C in minimal medium with adenine (46). It is thought that this treatment activates the recA protein to become a protease in the absence of DNA damage (18). At 30°C the recA441 allele causes a partial suppression of the recF deficiency during recovery from UV-induced damage (39). A recJ mutation somewhat increased the UV sensitivity of recF and uvrA recF mutants and totally abolished the higher UV resistance of their recA441 derivatives (Fig. 5A and B). We conclude that the recA441-mediated suppression seen at 30°C (39) also depends on the recJ+ function. A lexA3(Ind) mutation abolished the suppression by recA441 (Fig. 5B).

DISCUSSION

The suppression by two different types of mutant recA alleles (Srf and Tif) of the UV-sensitive recF phenotype in excision-deficient mutants was shown to require the recJ+ function (Fig. 2, 3, and 5). Our studies involved the use of the recA441(Tif) mutation (46) and the three Srf alleles recA801 (39) and the newly isolated recA4001 and recA4011. It is not known whether the three Srf alleles have identical phenotypes in all respects, but the dependence on recJ+ for suppression of the UV-sensitive recF phenotype was observed with the two alleles examined for this effect, recA801 and recA4011 (Fig. 3 and 4). Earlier studies by Wang and Smith (43) show that recA(Srf) partially restores the repair of DNA daughter strand gaps opposite unexcised pyrimidine dimers in recF mutants. In a uvrA recB recC sbcB recF genetic background recA(Srf) also partially restores the repair of double-strand breaks which result from cleavage of the parental strand opposite unrepairable daughter strand gaps (43). All these studies were conducted with recJ+ strains. We propose that the suppression results from a new repair pathway that depends on the concerted action of recA(Srf) or recA(Tif) proteins with the recJ+ function. In recF mutants this process would bypass the route of postreplica-

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**FIG. 4.** Induction of β-galactosidase in a uvrA15::Mu d(Ap lac) fusion strain WA591 (V) and its derivatives BT12 (recF400) (□), BT62 (recF400 recA801) (△), and BT74 (recF400 recA801 recJ284) (○). Induction was by UV at t = 0. Uninduced controls are shown by dotted lines. The UV doses applied (in joules per square meter) and the percent survival were as follows: V (2, 22%); □ (0.6, 10%); △ (1.5, 14%); ○ (0.3, 16%). O.D.+600, Optical density at 600 nm.

**FIG. 5.** Effect of recJ284::Tn10 on the suppression of the UV-sensitive phenotype of recF mutants by recA441(Tif) in a uvrA::Mu dX(Ap lac) (panel A) and a uvrA15::Mu d1(Ap lac) (panel B) genetic background. (A) HS112 (recF400) (△); BT78 (recF400 recA441) (■); BT80 (recF400 recJ284) (■); BT79 (recF400 recA441 recJ284) (○). (B) BT12 (recF400) (▲); BT9 (recF400 recA441) (●); BT83 (recF400 recJ284) (●); BT84 (recF400 recA441 recJ284) (○); BT106 (recF400 recA441 lexA3) (△); BT107 (recF400 lexA3) (○).
tion repair that depends on recA⁺ and recF⁺ proteins. It must be noted, however, that recA(Srf) also accomplishes postreplication repair with recF⁺ (37, 43). Under these conditions the repair is not dependent on recJ⁺ (Thoms and Wackernagel, unpublished), indicating that the recF⁺-dependent route is preferred or that the recJ⁺-dependent route becomes active only in the absence of recF. An increase of the recJ⁺ gene dosage did not enhance suppression (Fig. 2). It is not known whether recJ is controlled by the lexA and recA genes (24). The expression of the chromosomal recJ⁺ gene in a lexA(Ind) strain is sufficient for full suppression. In contrast, the recA(Srf) protein must be provided in increased amounts. This conclusion is based on the observations that suppression is absent in a recA(Srf) lexA(Ind) mutant (for comparison, recA⁺ has been shown to be expressed in lexA(Ind) cells at about 80% of the level in uninduced lexA cells (4)) and that suppression can be restored by specific overproduction of recA(Srf) protein but not of recA⁺ protein (Fig. 2). It is conceivable that recJ⁺ protein is present in cells in relatively large amounts or may have a catalytic function in suppression, whereas recA(Srf) protein may have a stoichiometric function and therefore the induced synthesis is required. The observation that suppression by recA (Tif) is blocked by a lexA(Ind) mutation would be consistent with the assumption of a need for recA(Tif) protein overproduction, but may also have other explanations.

The impaired SOS response in recF mutants has been taken as evidence for a positive regulatory function of recF protein in SOS induction (36). This function is restored in recF mutants by recA801, as shown by the improved UV induction of β-galactosidase synthesis in a uvrA: ΔZ recF recA801 strain (Fig. 4). The improved induction does not depend on the recF⁺ gene. Recently it has been shown that recA801 also improves the UV induction of mucB in a recF mutant (38). Previously a facilitated SOS response in recF recA(Srf) was not observed on autoradiographic quantitation of UV-induced proteins (43). Enzymatic determination, although indirect, is more sensitive and can easily detect a twofold increase of expression. An only twofold-larger amount of recA protein was shown to cause a proportional increase in the UV resistance of the cells (32). It is conceivable that the improved SOS induction in recF recA(Srf) contributes to the efficiency of recF suppression by facilitating the induction of the recA(Srf) protein synthesis. Small changes in the amino acid sequences of recA(Srf) and recA441 proteins are essential to bring recF into action, since overproduction of recA⁺ protein did not suppress the repair defect of a uvrA recF mutant. The recA441 allele has been shown to contain two mutations which alter amino acid residues at opposite sites of the polypeptide (15). One mutation, at codon 38 (domain 1), affects binding to single-stranded DNA and makes the recA protein a recombination-proficient constitutive protease (44). The second mutation, at codon 298, is a temperature-dependent suppressor of the other mutation (44) and has been attributed to the domain 3 involved in binding of single-stranded DNA and nucleoside triphosphates (45). Mutants with single mutations in region 3 are recombination deficient and protease constitutive (45). The various recA(Srf) mutants isolated are neither protease constitutive nor recombination deficient (37, 43; Thoms and Wackernagel, unpublished). It seems likely that the determination of the amino acid changes in recA(Srf) proteins would reveal a new functional site, possibly close to region 3. This site could be the one involved in the interaction with recF protein or recJ protein or both, depending on its amino acid sequence.

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LITERATURE CITED


