Use of High and Low Level Overexpression Plasmids to Test Mutant Alleles of the recF Gene of Escherichia coli K-12 for Partial Activity

Steven J. Sandler and Alvin J. Clark

Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, California 94720

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ABSTRACT

We showed that sufficient overexpression of the wild-type recF gene interfered with three normal cell functions: (1) UV induction of transcription from the LexA-protein-repressed sulA promoter, (2) UV resistance and (3) cell viability at 42°C. To show this, we altered a low-level overexpressing recF+ plasmid with a set of structurally neutral mutations that increased the rate of expression of recF. The resulting high-level overexpressing plasmid interfered with UV induction of the sulA promoter, as did the low-level overexpressing plasmid. It also reduced UV resistance more than its low level progenitor and decreased viability at 42°C, an effect not seen with the low-level plasmid. We used the high-level plasmid to test four recF structural mutations for residual activity. The structural alleles consisted of an insertion mutation, two single amino acid substitution mutations and a double amino acid substitution mutation. On the Escherichia coli chromosome the three substitution mutations acted similarly to a recF deletion in reducing UV resistance in a recBZ1 recC22 sbcBl5 sbcC201 genetic background. By this test, therefore, all three appeared to be null alleles. Measurements of conjugal recombination revealed, however, that the three substitution mutations may have residual activity. On the high-level overexpressing plasmid all three substitution mutations definitely showed partial activity. By contrast, the insertion mutation on the high-level overexpressing plasmid showed no partial activity and can be considered a true null mutation. One of the substitutions, recF143, showed a property attributable to a leaky mutation. Another substitution, recF4101, may block selectively two of the three interference phenotypes, thus allowing us to infer a mechanism for them.

The recF gene of Escherichia coli K-12 was first detected as a UV-sensitive and recombination-deficient mutant in a recB recC sbcB triple mutant strain (HORIT and CLARK 1973), which was later found to contain an sbcC mutation also (LLOYD and BUCKMAN 1985). recF single mutants are sensitive to UV irradiation (HORIT and CLARK 1973), are deficient in plasmid recombination (FISHEL, JAMES and KOLODNER 1981; COHEN and LABAN 1983; KOLODNER, FISHEL and HOWARD 1985), are deficient in mutagenesis of single-stranded DNA phages (CIESLA, O'BRIAN and CLARK 1987) and show abnormal induction of the SOS (THOMS and WACKERNAGEL 1987; SASSANFAR and ROBERTS 1990, 1991) and adaptive responses (VOLKERT 1989). This pleiotropic phenotype is consistent with RecF protein's having multiple functions, both enzymatic and regulatory.

To investigate these functions, biochemical and genetic approaches have been fruitful. The biochemical approach led MADIRAJU, TEMPLIN and CLARK (1988) to study an RecA protein that seemed partially to replace RecF protein in vivo. The conclusion of that study was the hypothesis that RecF protein might assist RecA protein to make a recombinationally active nucleoprotein filament on ssDNA occluded by secondary structure or on ssDNA bound to SSB protein. GRIFFIN and KOLODNER (1990) showed that purified RecF protein bound short ssDNA oligonucleotides. Independently, MADIRAJU and CLARK (1991) showed that RecF protein bound long ssDNA molecules cooperatively. In both studies no nucleotide cofactor was required for ssDNA binding. Recently, MADIRAJU and CLARK (1992) showed that both ssDNA and dsDNA stimulated photoaffinity crosslinking of RecF protein and azidoATP and that RecF protein could bind dsDNA in the presence of ATP. They hypothesized that this ATP-dependent binding was important for one or more of RecF protein's functions in vivo.

The genetic approach allows such hypotheses to be tested. Comparison of the recF genes from Escherichia coli, Salmonella typhimurium, Pseudomonas putida, Bacillus subtilis (SANDLER et al. 1992), Proteus mirabilis (SOKVGAARD 1990) and Actinobacillus pleuropneumoniae (LOYNDS, LANGFORD and KROLL 1992) shows that the proteins they encode have a highly conserved putative phosphate binding hole sequence (SCHULTZ 1992). Mutation of this sequence in the E. coli gene to make recF4101 showed that a UV-sensitive phenotype resulted (SANDLER et al. 1992). Since the phosphate binding sequence is normally part of a nucleotide binding fold (SARASTE, SIBBALT and WITTINGH-
bone and cell viability. To do this, we used two levels of overproduction, and consequently two levels of RecF overexpression. One level has been reported previously (SANDLER and CLARK 1990); the second is about fivefold higher (see below). We called these low and high levels, respectively. We achieved the high level by introducing a set of neutral mutations in recF that remove a translational block to expression. Deletion changes the reading frame and is expected to result in a Gly to Arg substitution in codon 36 (Figure 1C) and is expected to make an Arg to Gly substitution (L. SATIN and A. J. CLARK, unpublished data). The deletion changes the reading frame and is expected to encode a truncated partial RecF protein of 57 aa (wild-type RecF protein is 357 aa). We assume that the protein fragment produced has no residual activity and hence the mutation is a null allele.

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We also tested another hypothesis to come out of the biochemical approach. MADRAJU and CLARK (1991) showed that purified RecF protein inhibits in vitro reactions catalyzed by RecA protein. They argued that this inhibition results from the in vivo use of nonphysiological ratios of RecF protein to RecA protein and from the absence of accessory proteins that normally function with RecF protein in vivo. These arguments led to the hypothesis that overproduction of RecF protein in vivo might cause phenotypes similar to those of recA mutants. The same hypothesis might be made on the basis of the suggested interaction in vivo of RecF and SSB proteins (MOREAU 1987, 1988). Here we studied the effects of overexpression of recF, and consequent overproduction of RecF protein, on the recA-dependent UV-induced derepression of a lexA regulon promoter. We also looked at UV resistance and cell viability. To do this, we used two levels of recF overexpression, and consequently two levels of RecF overproduct. One level has been reported previously (SANDLER and CLARK 1990); the second is about fivefold higher (see below). We called these low and high levels, respectively. We achieved the high level by introducing a set of neutral mutations in recF that remove a translational block to expression. Description of the construction of this set of mutations will be reported elsewhere (S. J. SANDLER and A. J. CLARK, unpublished data).

**MATERIALS AND METHODS**

**Bacterial strains:** All bacterial strains used in this work are derivatives of *E. coli* K-12 and are described in Table 1. Unless otherwise noted, all strains containing plasmids were grown in media containing the appropriate drug: either ampicillin (50 µg/ml) or kanamycin (50 µg/ml).

**Site-directed mutagenesis:** Site-directed mutagenesis was done as described by SANDLER et al. (1990). Plasmids used and derived are listed in Table 2. The following oligonucleotides were used in the site-directed mutagenesis:

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Wild type</td>
<td>BACHMANN (1972)</td>
</tr>
<tr>
<td>CAG12204</td>
<td>(Hfr P03) metB1</td>
<td>SINGER et al. (1989)</td>
</tr>
<tr>
<td>DM4000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>sulA:Mu-d(Ap, lac, B::Tn9)</td>
<td>M. VOLKERT</td>
</tr>
<tr>
<td>JC7623</td>
<td>AB1157 except recB21 recC22 sbcB15 sbcC201</td>
<td>KUHNER et al. (1971)</td>
</tr>
<tr>
<td>JC8111</td>
<td>JC7623 except recF143</td>
<td>HORII and CLARK (1973)</td>
</tr>
<tr>
<td>JC11803</td>
<td>AB1157 except recF143</td>
<td>CLARK, VOLKERT and MARGOSSIAN (1979)</td>
</tr>
<tr>
<td>JC15359</td>
<td>AB1157 except recF349 tnaA300::Tn10</td>
<td>BLANAR (1985)</td>
</tr>
<tr>
<td>JC18146</td>
<td>AB1157 except recF4101 tnaA300::Tn10</td>
<td>SANDLER et al. (1992)</td>
</tr>
<tr>
<td>JC18246</td>
<td>AB1157 except recF4104 tnaA300::Tn10</td>
<td>This work</td>
</tr>
<tr>
<td>JC18523</td>
<td>JC7623 except recF349 tnaA300::Tn10</td>
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<tr>
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<td>JC7623 except recF4104 tnaA300::Tn10</td>
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<td>JC18525</td>
<td>JC7623 except recF4104 tnaA300::Tn10</td>
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<td>DM4000 except recF349 tnaA300::Tn10</td>
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<tr>
<td>JC15359</td>
<td>AB1157 except recF143</td>
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</tr>
</tbody>
</table>

<sup>a</sup> thr-1 leu-6 thi-1 lacY1 ara-14 xyl-5 met-1 proA2 argE3 rpsL3I
<sup>b</sup> D(lac-pro)XIII hisG4 argE3 thr-1 ara-14 xyl-5 met-1 rpsL3I

**Table 1**

**E. coli strains**

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
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<tr>
<td>JC18246</td>
<td>AB1157 except recF143</td>
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</tr>
</tbody>
</table>
site-directed mutagenesis as described in SANDLER (1992). Using the mutagenic oligonucleotide prSJS56 and the rate and KASTELEIN (1986; SPANJAARD and DUIN 1988), we added are known to slow translation in certain contexts (DEBOER 1992). Using the mutagenic oligonucleotide prSJS56 and the rate and optimal Arg codons. Since adjacent nonoptimal Arg codons work. Later we discovered that then mixed and treated with T4 DNA ligase. This mixture was then used to transform JC15359. KanR UVs clones were screened.  

### Table 2

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Ancestral plasmid</th>
<th>Primer</th>
<th>Structural mutation</th>
<th>Expression</th>
<th>Drug resistance</th>
<th>Reference</th>
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<td>+</td>
<td>Amp&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>None</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>Kan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>KIRSCHMAN and CRAMER (1988)</td>
</tr>
<tr>
<td>pSE200</td>
<td>None</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>Kan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ELLEDGE and WALKER (1983)</td>
</tr>
<tr>
<td>pSJS159</td>
<td>None</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>Amp&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>None</td>
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<td>+</td>
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<td>+</td>
<td>4105, 4113</td>
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<tr>
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<td>None</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>prSJS61</td>
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<td>None</td>
<td>+</td>
<td>+</td>
<td>Amp&lt;sup&gt;a&lt;/sup&gt;</td>
<td>VIEIRA and MESSING (1987)</td>
</tr>
</tbody>
</table>

<sup>a</sup> pJS237 was constructed by restricting pSJS140 and pJKKmf(−) with EcoRI. The digested plasmids were then mixed and treated with T4 DNA ligase. The mixture was then used to transform JC15359. Kan<sup>a</sup> UV<sup>s</sup> transformants were selected and screened.

<sup>b</sup> The primer and plasmid listed were used to do site-directed mutagenesis as outlined in (SANDLER and CLARK 1990).

<sup>c</sup> The primer and plasmid listed were used to do site-directed mutagenesis as outlined in (SANDLER and CLARK 1990).

<sup>d</sup> pSJS622 was constructed by digesting pSJS530 and pJS609 with BamHI and NcoI. The digested plasmids were then subjected to electrophoresis in 1% low melting agarose gel. The appropriate fragments excised, mixed and then treated with T4 DNA ligase. The mixture was then used to transform JC15359. Amp<sup>a</sup> UV<sup>s</sup> transformants were selected and screened.

<sup>e</sup> pSJS622 was constructed by linearizing pSJS613 with NcoI, treating with DNA pol I Klenow Fragment in the presence of dNTPs to blunt the ends. The mixture was then treated with T4 DNA ligase and used to transform JC15359. Amp<sup>a</sup> UV<sup>s</sup> transformants were selected and screened.

<sup>f</sup> pSJS670 was constructed by restricting pSJS613 and pJKKmf(−) with EcoRI. The digested plasmids were then mixed and treated with T4 DNA ligase. The mixture was then used to transform JC15359. Kan<sup>a</sup> UV<sup>s</sup> transformants were selected and screened.

<sup>g</sup> The primer and plasmid listed were used to do site-directed mutagenesis as outlined in (SANDLER and CLARK 1990).

<sup>h</sup> pSJS670 was constructed by digesting pSJS670 with BamHI and XhoI. The digested plasmids were then subjected to electrophoresis in 1% low melting agarose gel. The appropriate fragments excised, mixed and then treated with T4 DNA ligase. The mixture was then used to transform JC15359. Kan<sup>a</sup> UV<sup>s</sup> transformants were selected and screened.

<sup>i</sup> The primer and plasmid listed were used to do site-directed mutagenesis as outlined in (SANDLER and CLARK 1990).

<sup>j</sup> pSJS659 was constructed in a similar fashion to pSJS613 except that pSJS659 was used as the starting plasmid and Kan<sup>a</sup> transformants were selected instead of Amp<sup>a</sup>.

<sup>k</sup> The primer and plasmid listed were used to do site-directed mutagenesis as outlined in (SANDLER and CLARK 1990).

<sup>l</sup> pSJS687 was constructed by digesting pSJS670 with BamHI and XhoI. The digested plasmids were then subjected to electrophoresis in 1% low melting agarose gel. The appropriate fragments excised, mixed and then treated with T4 DNA ligase. The mixture was then used to transform JC15359. Kan<sup>a</sup> UV<sup>s</sup> transformants were selected and screened.

<sup>m</sup> The primer and plasmid listed were used to do site-directed mutagenesis as outlined in (SANDLER and CLARK 1990).

<sup>n</sup> pSJS688 was constructed in a similar fashion to pSJS670 except that pSJS688 was used instead of pSJS668.

<sup>o</sup> pSJS693 was constructed by restricting pJC876 with BamHI, PstI, and XhoI and pSJS670 with BamHI, PstI, and AflI. The DNAs were then mixed and treated with T4 DNA ligase. This mixture was then used to transform JC15359. Kan<sup>a</sup> UV<sup>s</sup> clones were screened.

<sup>p</sup> pSJS894 was constructed by restricting pSJS893 with NcoI and BamHI and pSJS613 with NcoI BamHI AflI. The DNAs were then mixed, treated with T4 DNA ligase. This mixture was then used to transform JC15359. Kan<sup>a</sup> UV<sup>s</sup> transformants were screened.

<sup>q</sup> pSJS870 DNA was then cut with EcoRI nuclease and the 3.7-kb fragment containing recF4104 was cloned using pBR322 as vector. The resulting plasmid was pSJS714. Using pSJS714 and the method of integrative suppression (SANDLER et al. 1992), we transferred recF4104 to the chromosome. The resulting recF4104 mutant strain was JC18246.

**Hfr matings:** Hfr male bacteria CAG1220 (origin of transfer is PO3 transferring Thr<sup>+</sup> and Leu<sup>−</sup> early and recF<sup>+</sup>). Met<sup>−</sup> and Str<sup>−</sup> late in a counterclockwise fashion) and the F<sup>−</sup> bacteria (JC7623, JC8111, JC18523, JC18524 and JC18525) were incubated at 37° in Luria broth (LURIA and DUIN 1988), we added recF4113 to all the high expression plasmids used in this work. Later we discovered that recF4113 does not increase the rate of translation either by itself or in combination with recF4103 (S. J. SANDLER and A. J. CLARK, unpublished data). recF4113 is a set of four base substitution mutations, in codons 132 and 133, which substitute a pair of optimal Arg codons for a pair of nonoptimal Arg codons. Since adjacent nonoptimal Arg codons are known to slow translation in certain contexts (DEBOER and KASTELEIN 1986; SPANJAARD and DUIN 1988), we added recF4113 to all the high expression plasmids used in this work. Construction of plasmids and a chromosome containing recF4104: Substitution mutation recF4104 was made using site-directed mutagenesis as described in SANDLER et al. (1992). Using the mutagenic oligonucleotide prSJS56 and plasmid pSJS684, we made the mutant plasmid pSJS705.

Hfr matings: Hfr male bacteria CAG12204 (origin of transfer is PO3 transferring Thr<sup>+</sup> and Leu<sup>−</sup> early and recF<sup>+</sup>). Met<sup>−</sup> and Str<sup>−</sup> late in a counterclockwise fashion) and the F<sup>−</sup> bacteria (JC7623, JC8111, JC18523, JC18524 and JC18525) were incubated at 37° in Luria broth (LURIA and DUIN 1988), we added recF4113 to all the high expression plasmids used in this work.
and Low 1969) and the mixture vortexed for 45 sec. Dilutions were then made with 56/2 buffer; 0.1 ml of appropriate dilutions was then added to 3 ml of liquid 0.4% agarose at 57°C and poured onto media (glucose, thiamine, arginine, proline, histidine and 100 μg/ml of streptomycin made in 56/2 solid media) selective for Thr+Leu+[Met+StrR] recombinants. The results shown are the averages of four matings with each F' strain.

**UV survival tests for recB21 recC22 sbeB15 sbeC201 strains:** Strains were incubated at 37°C in Luria broth until they were in log phase, then the cells were centrifuged, washed once in 56/2 buffer, irradiated for various amounts of time at a rate of 0.5 joule/m²/sec and appropriately diluted into 56/2 buffer; 0.1 ml of appropriate dilutions was then spread on agar plates. Cells were incubated at 37°C and colonies counted after 24 hr. All steps after the irradiation took place in a darkened room. The results shown are averages of two experiments with each strain. Survival is relative to unirradiated cultures.

**Quantitation of the amount of RecF protein:** Derivatives of the strain AB1157 containing the plasmids were grown at 30°C in 56/2 minimal media (Willett, Clark and Low 1969), supplemented with the amino acids needed for growth, until the O.D. at A600 was 0.5; 1.0 ml of cell culture was removed at appropriate times. These samples were incubated at 42°C in a shaking water bath for 15 min before 10 μCi of 35S methionine (specific activity was >800 Ci/mmole) was added. The mixtures were allowed to incubate 2 min before the addition of 20 μl of 1% nonradioactive methionine to each mixture. After 1 min of incubation, 0.5 ml of each mixture was removed, centrifuged and the pellet resuspended in 150 μl of final sample buffer (60 mm Tris-HCl pH 6.8, 2% SDS, 0.25 mM β-mercaptoethanol, 20% v/v glycerol, 1 mM EDTA). These mixtures were then boiled for 3 min and centrifuged for 3 min. The remainder of each culture continued to incubate at 42°C for 100 min; 0.5 ml of each culture were then removed, centrifuged and the pellet resuspended, boiled and centrifuged as described above; 50 μl of each boiled extract was then electrophoresed in a 12% polyacrylamide gel (29:1 acrylamide:bis acrylamide). After electrophoresis, the gels were dried onto Whatman 3 MM paper. The amount of radioactivity in the RecF protein band and a chromosomally encoded protein were determined with a Molecular Dynamics phosphor imager.

**Plasmid effects on cell viability, sensitivity to UV irradiation and UV-induction of the sulA promoter:** For cell viability and UV sensitivity studies, the derivatives of the strain AB1157 harboring pJS140, pJS613, pJS622, pJS680, pJS688 and pJS894 were grown at 30°C to O.D. A600 of 0.2. The cultures were then shifted to a 42°C shaking water bath for the appropriate length of time. Then they were centrifuged and washed once in 56/2 buffer. For UV sensitivity tests, samples were irradiated for 40 sec at a rate of 1 joule/m²/sec and diluted into 56/2 buffer. For cell viability tests, unirradiated samples were diluted in 56/2 buffer; 0.1 ml of appropriate dilutions was spread on Luria plates plus ampicillin (50 mg/ml). Plates were incubated at 30°C and colonies counted after 24 hr. All steps after UV irradiation took place in a darkened room.

The studies on UV induction of the sulA promoter were done in a similar manner. Derivatives of the strains DM4000 and JC18536 harboring pJS670, pJS673, pJS687, pJS693 or pJS893 were grown in Luria broth plus kanamycin (50 μg/ml) at 30°C to an O.D. of 0.2 (A600) and then shifted to 42°C. Samples taken at different times after temperature shift were then centrifuged. The pellet was washed once in 56/2 buffer, resuspended in half of the original volume and irradiated at a rate of 0.5 joule/m²/sec; 1 ml of irradiated cells was diluted into 3 ml of Luria Broth and cells were incubated at 30°C. Samples for β-galactosidase assay were taken 120 min after UV irradiation. β-galactosidase assays were done as previously described (Sandler and Clark 1990).

**An explanation of the transcription control system used to overexpress recF alleles:** The recF alleles used in this work are carried by derivatives of pUC118, which has a
very high copy number (Vieira and Messing 1982; Twigg and Sherratt 1980). Regulating transcription of the recF alleles are lambda repressor allele cIa2 and both pL and pR promoters of lambda. Construction of this double promoter system is described by Sandler and Clark (1990). cIa2 encodes a temperature-sensitive repressor, which is active at 30°C and inactive at 42°C (Sandler and Clark 1990). Shifting the temperature, therefore, is expected to derepress transcription of high copy number recF alleles in the plasmid-carrying strains tested.

RESULTS

Characterization of strains carrying recF4104 and other recF alleles on the chromosome rather than on plasmids: An E. coli strain carrying the recF143 (Gly to Arg at codon 286) allele on the chromosome is UV-sensitive if it is otherwise wild-type, i.e., recB+ recC+ sbcB+ sbcC+ or if it is multiply mutant, i.e., recB21 recC22 sbcB15 sbcC201 (Horii and Clark 1973). Figure 2, A and B, shows that recF4104 (the Pro Trp for Leu Asp substitution at codons 308 and 309) also confers UV sensitivity in both genetic backgrounds. To show that recF4104 acts as a null mutation by this test, we have included data on deletion allele recF349 (a partial internal deletion of the recF gene—see MATERIALS AND METHODS) in both backgrounds. In Figure 2B we also show that the Lys to Arg substitution allele recF4101 and prototype allele recF143 confer about the same degree of UV sensitivity as the other mutant alleles in the multiple mutant background. UV sensitivity data on these two alleles in the wild-type background are published by Sandler et al. (1992). All three substitutions, therefore, appear to be null alleles because they all confer essentially the same UV sensitivity as the deletion.

This characterization seems to be contradicted by a test of the recB21 recC22 sbcB15 sbcC201 multiple mutant strains for recombination deficiency. Table 3 shows that strains carrying the recF substitution alleles are 10- to 30-fold more recombination-proficient than the deletion-mutant strain. We cannot be certain how significant this is, however, because the recF strain produces between 2000- to 6000-fold more recombinants than the substitution mutants. To test more conclusively whether the substitution alleles encode proteins with residual activity, we made use of two overexpression systems (low and high) for recF alleles that will be described in the next section.

Overexpression of neutrally mutant recF genes: RecF protein is produced in very small quantities when recF is present on the chromosome (Bianar et al. 1984; Griffin and Kolodner 1990). Sandler and Clark (1990) describe a plasmid that led to overproduction of wild-type RecF protein. Transcription was temperature-dependent and presumably initiated from both pL and pR promoters of lambda. Overproduction of wild-type RecF protein using this plasmid (pJS140) was sufficient for protein purification (Madiraju and Clark 1991). To make a high-level overexpressing plasmid (pJS613), S.J. Sandler and A. J. Clark (unpublished data) altered five recF codons by structurally neutral mutations. Figure 3 shows the difference in the amounts of RecF protein made by pulse-labeling strains carrying the two plasmids (compare lanes 3 and 5). A strain carrying a plasmid with insertion mutant allele recF4114 (this is a null allele—see description in the MATERIALS AND METHODS) is included to show the labeling of proteins comigrating with RecF protein (lane 1). Quantitation of the averages of these data are shown in Table 4.

Figure 2.—UV survival of strains carrying different recF mutations: (A) Survival of (recB+ recC+ sbcB+ sbcC+) strains AB1157 (recF*), JC15359 (recF349) and JC18246 (recF4104) after increasing amounts of UV irradiation. (B) Survival of (recB21 recC22 sbcB15 sbcC201) strains JC7623 (recF*), JC8111 (recF143), JC18523 (recF349), JC18524 (recF4101) and JC18525 (recF4104) after increasing amounts of UV irradiation. Survival is calculated by dividing the number of cells surviving the treatment by the initial number of cells.
TABLE 3

<table>
<thead>
<tr>
<th>Straina</th>
<th>recF allele</th>
<th>Recombinant frequency/100 donorsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC7623</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>JC8111</td>
<td>143</td>
<td>0.0024</td>
</tr>
<tr>
<td>JC18523</td>
<td>349</td>
<td>0.00025</td>
</tr>
<tr>
<td>JC18524</td>
<td>4101</td>
<td>0.0072</td>
</tr>
<tr>
<td>JC18525</td>
<td>4104</td>
<td>0.0049</td>
</tr>
</tbody>
</table>

a The relevant genotype of these strains is recB21 recC22 sbcB15 sbcC201.  
b Thr Leu [Met Str*] recombinants were selected.

We have used the assay in Figure 4B to determine whether structurally mutant recF alleles show residual activity. Table 5 compares the amount of UV-induced β-galactosidase produced after no expression of recF at 42°C with that produced after 15 min of recF* expression at 42°C. The data for recF* and recF4114 in Table 5 are similar to the first two points for each in Figure 4B. recF143 showed no residual activity by this test and allowed approximately the same degree of UV-induction as allowed by recF4114 (Table 5). recF4101 and recF4104 on the other hand acted like recF* and thus showed residual wild-type activity by inhibiting UV induction of sulAp (Table 5).

recF is not associated with another phenotype that we can also use to assay residual activity. Wild-type recF is required for rapid, UV-induction of the lexA regulon (Sassanfar and Roberts 1991). Our UV-induction of sulAp to this point used strains carrying recF* on the chromosome to permit rapid UV-induction when structurally mutant recF alleles were plasmid-borne. In the second part of Table 5, we tested strains with deletion allele recF349 on the chromosome. When recF* was plasmid-borne, 1700 units of β-galactosidase were induced even without exposure to 42°C. This must mean that some expression of recF* took place at 30°C because substituting recF4114 on the plasmid prevented UV-induction (Table 5). The three other structurally mutant alleles showed no residual activity at 30°C. When the strains were incubated for 15 min at 42°C, however, the one carrying recF143 showed a substantial amount (eightfold increase) of UV-induced β-galactosidase. This implies that sufficiently large amounts of recF143 protein can function in rapid UV-induction of the lexA regulon. By this test, therefore, recF143 shows residual activity. The twofold increase in the amount of UV-induced β-galactosidase seen with recF4114 on the plasmid (Table 5) with 42°C incubation may be due to the recA-dependent increase in UV resistance experienced.
Mutant \textit{recF} Alleles

### TABLE 4

Relative rates of expression of \textit{recF} alleles and estimates of half-lives of wild-type and mutant RecF proteins$^a$

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>\textit{recF} allele structural series$^b$</th>
<th>Expression series$^c$</th>
<th>Lanes in Figure 3 providing data</th>
<th>Relative rate of expression$^d$</th>
<th>Half-life in minutes$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSJS140</td>
<td>+</td>
<td>+</td>
<td>3, 4</td>
<td>21 ± 4</td>
<td>ND$^e$</td>
</tr>
<tr>
<td>pSJS613</td>
<td>+</td>
<td>4105, 4113</td>
<td>5, 6</td>
<td>100</td>
<td>122 ± 30</td>
</tr>
<tr>
<td>pSJS680</td>
<td>4101</td>
<td>4105, 4113</td>
<td>7, 8</td>
<td>126 ± 28</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>pSJS688</td>
<td>4104</td>
<td>4105, 4113</td>
<td>9, 10</td>
<td>97 ± 15</td>
<td>48 ± 16</td>
</tr>
<tr>
<td>pSJS894</td>
<td>145</td>
<td>4105, 4113</td>
<td>11, 12</td>
<td>85 ± 11</td>
<td>52 ± 9</td>
</tr>
</tbody>
</table>

$^a$ The gel in Figure 3 was scanned with a phosphorimager. In each lane the number of dpm in the RecF protein band was divided by the number of dpm in the reference band (see Figure 3). These ratios in lanes 1 and 2 were taken to indicate the amount of background label, i.e., the label derived from non-RecF proteins after pulse labeling and chase, respectively. To calculate the relative rates of expression, the background ratio from lane 1 was subtracted from similar ratios derived from lanes 3, 5, 7, 9 and 11. These corrected ratios were then normalized to the corrected ratio from lane 5 taken as 100. To calculate the half-life of RecF proteins, the background ratio from lane 2 was subtracted from similar ratios derived from lanes, 4, 6, 8, 10 and 12. These corrected ratios were then used to calculate a half-life in minutes from the following formula:

$$\text{half-life (min)} = \frac{100 \ln \left( \frac{1}{2} \right)}{\ln \left( \frac{R_{100}}{R_{0}} \right)}$$

where $R_0$ and $R_{100}$ are the cognate corrected ratios at zero time (odd-numbered lanes) and at 100 minutes (even-numbered lanes), respectively.

$^b$ Structural series means mutations that change the amino acid sequence of \textit{recF}. See Figure 1.

$^c$ Expression series means mutations that do not change the amino acid sequence of \textit{recF} but do change the level of expression. See Figure 1.

$^d$ These numbers are the averages and standard deviations of four experiments.

$^e$ ND is not determined.

---

**Figure 4.** Effects of temperature-induced expression of \textit{recF} on UV induction of \textit{lacZ} transcribed from \textit{sulAp}.

Panel A shows the amount of \(\beta\)-galactosidase produced as a function of UV dose. Panel B shows the amount of \(\beta\)-galactosidase produced after a single UV dose (20 J/m\(^2\)) as a function of incubation time at 42\(^\circ\) prior to irradiation. In panels A and B, strains DM4000 carried either of the two high-level overexpressing plasmids pSJS670 (\textit{recF}+) or pSJS673 (\textit{recF}4114). In panel B, results with DM4000 carrying low-level overexpressing \textit{recF} plasmid pSJS140 are shown. The cells were grown at 30\(^\circ\) and incubated at 42\(^\circ\) for 15 min (A) or for 15, 30 and 60 min (B) before UV irradiation (see Materials and Methods). \(\beta\)-galactosidase assays were done after 2 hr of incubation at 30\(^\circ\) in the dark.

---

A. 15 min @ 42\(^\circ\)

![Graph A](image1.png)

B. 20 J/m\(^2\) UV irradiation

![Graph B](image2.png)

by heat shocked cells [see below (GANESAN, HUNT and HANAWALT 1988)].

**Other phenotypes associated with overexpression of the wild-type \textit{recF} gene:** UV-induction of the \textit{lexA} regulon contributes to UV resistance because damage repair genes are induced (PETERSON et al. 1988). Since we had found that UV-induction is inhibited by overexpression of \textit{recF} (Table 5), we expected less UV resistance in a strain carrying \textit{recF}+ on a high-level expressing plasmid than in a similar strain carrying null-mutation \textit{recF}4114. Figure 5A shows that this expectation accorded to the data. We then investigated the effects of \textit{recF} overexpression on UV resistance by looking at the fraction of cells surviving 40 J/m\(^2\) UV light as a function of incubation time at 42\(^\circ\). The results show that the high-level expressing \textit{recF}4114-plasmid strain increased in UV resistance while the high level overexpressing \textit{recF}+ plasmid strain
TABLE 5

UV-induced expression of the sulA promoter* in recF4349 or recF** cells overexpressing recF structural mutant alleles*

<table>
<thead>
<tr>
<th>recF structural allele on plasmid</th>
<th>Chromosomal recF*</th>
<th>Chromosomal recF4349</th>
</tr>
</thead>
<tbody>
<tr>
<td>No recF*</td>
<td>750</td>
<td>ND</td>
</tr>
<tr>
<td>+</td>
<td>650</td>
<td>70</td>
</tr>
<tr>
<td>4114</td>
<td>1000</td>
<td>70</td>
</tr>
<tr>
<td>143</td>
<td>1000</td>
<td>70</td>
</tr>
<tr>
<td>4101</td>
<td>1000</td>
<td>70</td>
</tr>
<tr>
<td>4104</td>
<td>1000</td>
<td>70</td>
</tr>
</tbody>
</table>

* Numbers show the differences in Miller units of β-galactosidase activity per OD600 between the number of units found in irradiated and unirradiated cells incubated for 2 hr at 30°. lacZ is transcribed from UV inducible sulAp; 5 J/m² of UV irradiation were used. Unirradiated cells had a background level of 30–50 units. The two measurements shown in each set are done either with cell cultures grown only at 30° or ones that had been grown at 30° and then incubated at 42° for 15 min before UV treatment (see MATERIALS AND METHODS) to allow for overproduction of the recF protein. The value in parentheses indicates that irradiated cells contained less β-galactosidase than unirradiated cells.

** All plasmids carried expression alleles recF4105 and recF4113. No recF* means that a strain containing the vector only, pUC118, was used.

A. 15 min @ 42° before UV irradiation

B. Fraction surviving after 40 J/m² UV irradiation

** Griffin and Kolodner (1990) found that overexpression of recF* from a recF overexpressing plasmid at 37° decreased cell viability especially of recA- cells. Their expression system differed from ours in that recF is under control of the tac promoter and is induced at 37° by IPTG addition. We compared the rate of RecF protein production from this plasmid after 30 min of IPTG exposure at 42° with rates from our plasmids and found it midway between (data not shown). Figure 6 shows no effect on viability of recA* cells at 42° with our low level overexpressing plasmid. By contrast, using the high level overexpressing plasmid, we do measure a viability loss of recA* cells at 42°. This killing is due to the production of RecF protein because recF4114 prevents it. We have not tested viability loss of recA- cells with either of our plasmids.

We wondered if the inviability caused by plasmid-encoded recF genes carrying the recF* expression set of mutations at 42° played any role in the increase of UV sensitivity produced in these strains at 42°. Comparison of Figure 5B and 6 shows that the maximum degree of UV sensitivity is produced after 15 min of incubation at 42° (Figure 5B) at which time there is virtually no viability loss (Figure 6).

Effect of recF structural mutations on UV resistance and viability at 42°: Figure 7 shows the effects of the structural mutant recF alleles on UV resistance and viability at 42°. With recF349 on the chromosome the three substitution alleles (on plasmids) were similar to insertion allele recF4114 (on plasmid) in that they did not promote UV resistance nor temperature-dependent killing at 42°. With recF* on the chromosome, the mutant alleles also all prevented temperature-dependent killing. In the recF+ background, however, the mutant alleles had different effects on UV...
Mutant recF Alleles

FIGURE 6.—Viability of strains of AB1157 carrying different recF plasmids as a function of incubation time at 42°. Survival is relative to the untreated culture. High-level overexpressing plasmids pSJS670 (recF*) and pSJS673 (recF4114) and low-level overexpressing plasmid pSJS140 (recF) were used. cfu = colony forming units.

A. recF + on chromosome

B. recF349 on chromosome

FIGURE 7.—Viability of plasmid-carrying strains as a function of UV dose and time for expression of plasmid-borne recF alleles at 42°. Fraction survival was determined as in Figure 6. Plasmids are denoted by "p()" where the "p" refers to plasmid and the recF allele is between the parentheses. Strains carrying these plasmids were AB1157 (recF*) and JC15359 (recF349).

resistance. recF143 and rec4114 were similar in that they provoked little UV sensitivity. By contrast, recF4101 provoked a great deal of UV sensitivity and recF4104 is intermediate. The significance of these results is discussed below.

DISCUSSION

RecF protein is necessary for several aspects of recombination and repair of UV damage in E. coli cells. We have examined three missense mutations in recF and found that, when located on the E. coli chromosome, they confer essentially the same phenotype as a deletion mutation when UV resistance was measured. In this respect, all three appear to be null mutations. However, a test of recombination hinted that each might show residual activity.

RecF protein is normally produced in vivo in very small amounts, estimated to be less than 190 molecules per E. coli cell (Madiraju and Clark 1991). We have used two types of plasmid constructs to overexpress the wild-type recF gene. The first was constructed by
Sandler and Clark (1990) to optimize heat-induced transcription and initiation of translation. We constructed the second from the first to optimize the rate of translation. In this paper we show that the rate of RecF protein production from the second plasmid is about fivefold greater than the first. These two levels of overexpression can also be detected as two levels of RecF protein production.

We have used the high level recF overexpressing plasmid to detect three phenotypes putatively associated with abnormally high amounts of wild-type RecF protein: inhibition of UV-induction of sulAp, reduction in UV resistance and decrease in viability at 42 °C. These, in turn, were used to reveal that two alleles of recF mutant in primary structure, recF4101 and recF4104, have residual activity. When overexpressed on our high-level plasmid, both of these alleles still showed the ability to inhibit UV-induction of sulAp-like recF*. In addition, recF4101 can reduce UV survival when overexpressed. The high level overproducing plasmid also allowed us to detect residual activity of recF143, a third structural allele, but by a different test. recF143 showed no residual activity in the test showing reduced UV-induction of sulAp in an recF* genetic background. Instead, it showed residual activity by facilitating UV-induction of sulAp in a recF349 background. Thus, the three mutations affecting primary structure that we tested showed residual activity when combined with our high level overexpression plasmid.

recF is a gene with a multitude of functions (reviewed in Clark 1991). By analogy with other such genes, e.g., recA, we can anticipate that some mutations will selectively affect these functions, inactivating some while leaving others active. In our overexpression tests for residual activities, recF143, recF4101 and recF4104 acted differently and therefore appeared to affect recF functions selectively. We think this appearance is deceptive, however, because it is based on the following two assumptions: (1) all three overexpression tests are equally sensitive and (2) primary structure mutations do not affect the rate of recF expression or the stability of RecF protein. We tested both of these assumptions. First we used the low-level overexpressing plasmid to show differential sensitivity of the three overexpression tests. Viability at 42 °C was not affected by the low overexpression plasmid and hence was the least sensitive test. UV-resistance was affected by the low-level overexpressing plasmid but was less affected than by the high level overexpressing plasmid (Figure 5B). UV-resistance was therefore a more sensitive test than viability at 42 °C. The most sensitive test was UV-induction of sulAp because both high- and low-level overexpressing plasmids affected it nearly equally (Figure 4B). Therefore, the first assumption was disproved. The second assumption was also disproved. We measured the rates of synthesis and half-lives of the three missense mutant proteins (Table 4) and found that the half-lives, but not the rate of synthesis, were significantly less than those for the wild-type protein. Consequently, it seems prudent not to conclude from our tests that the mutations selectively affect one recF function without affecting another. Such a conclusion would require a detailed analysis of each overexpression phenotype, including how it is correlated with increasing amounts of RecF protein, and that analysis requires further work.

Despite these caveats, we think it likely that recF4101 is a mutation that selectively affects the functions of RecF protein. recF4101 had the least effect of the three missense mutations alleles tested on the half-life of RecF protein and was a conservative change substituting arginine for lysine. It affects a sequence of amino acids thought to represent the phosphate binding hole of an ATP binding site (Sandler et al. 1992). Since RecF protein requires ATP to bind to double-strand (ds) DNA but not to bind single-strand (ss) DNA (Madiraju and Clark 1992), it is possible that recF4101 affected functions in which dsDNA binding, but not functions in which ssDNA binding, is important. We intend to test the hypothesis of selective affect on functions with purified RecF4101 protein. We interpret interference with UV-induction of sulAp and the reduction of UV-resistance to reveal ssDNA binding functions of RecF protein in vivo as predicted by Madiraju and Clark (1991) from the in vitro interference of RecF protein with the functions of RecA protein. RecF4101 protein may have also interacted with wild-type RecF protein and inactivated it as suggested by the difference in UV sensitivity of the recF* and recF349 strains with plasmid borne recF4101 (Figure 7).

recF4104 inhibits UV induction of sulAp. By analogy with recF4101, we also predict that recF4104 protein would retain ssDNA binding activity and interfere with the reactions catalyzed by RecA protein tested by Madiraju and Clark (1991).

recF143 differs from the other two amino acid substitution mutant alleles by inactivating both inhibitory activities that they retain. In this case, however, we noted that recF143 was able to complement the chromosomal recF349 deletion in restoring UV-induction of sulAp. Thus, recF143 retains some wild-type activity, which is evident when enough mutant protein is produced. Hence, it also cannot be called a null allele. An obvious prediction from these results would be that purified RecF143 protein has no inhibitory activity on reactions catalyzed by RecA protein and no ability to bind ssDNA. We believe that this prediction may be unwarranted, however, because we have no measure of the amount of functionally active RecF143 protein in cells. Ability to complement recF349 re-
quires only the very small amounts normally produced from chromosomal recF. Thus, only a very small fraction of the RecF143 protein produced may retain functional activity. It is even possible that RecF143 protein is thermally labile at 42° and may be inactivated by the thermal induction required in this situation to overproduce it.

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LITERATURE CITED


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