Control of *recA* dependent activities in *Escherichia coli*: a possible role for the *recF* product

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DNA repair and genetic recombination have been studied in *recF143* mutants of *Escherichia coli* in which expression of inducible, SOS repair activities was altered by additional mutations either in *lexA* or *recA*. *recF143* and *lexA3* appeared to act additively to increase sensitivity to UV irradiation and to reduce recombination proficiency. The *recF* defect was suppressed in strains carrying the *tif-1* allele of *recA* but not in strains carrying mutations that increased synthesis of *recA*⁺ protein or which directly inactivated *lexA* repressor. These and other data are interpreted to suggest that the *recF* defect is related to a reduced activity of *recA* protein. The implications of these findings are discussed in relation to the control of SOS activities and cell division during normal growth.

INTRODUCTION

Cell division in *Escherichia coli* is arrested following damage to DNA in conjunction with an increase in various activities that promote DNA repair (Witkin, 1976). The regulatory basis for this coordinate 'SOS' response is now understood: an inhibitor of division produced by the *sfiA* gene is regulated in parallel with the products of several DNA repair and other genes through a repressor specified by *lexA*. Damage to DNA activates a protease function of *recA* protein, which cleaves this repressor and causes the derepression of all *lexA* regulated genes (Huisman & D'Ari, 1981; Kenyon & Walker, 1980; Little & Harper, 1979; Phizicky & Roberts, 1981; Little *et al.*, 1980).

Although *lexA* and *recA* specify the main regulatory elements that control the SOS response, normal expression of several SOS activities is reduced by mutation of the *recF* gene. The *recF* gene was first identified during the analysis of recombination in *recB/C sbcB* mutants (Horii & Clark, 1973). Subsequent studies revealed many similarities between *recF* mutants and those *lexA* mutants that are unable to induce the SOS response. They are sensitive to UV light and, after irradiation, show reduced amplification of *recA* protein and less cell filamentation than is usual. UV induction of λ prophage is reduced, as is post-replication repair of DNA damage and Weigle-reactivation of irradiated phage (Rothman & Clark, 1977; Armengod & Blanco, 1978; Rothman *et al.*, 1979; Kato & Shinoura, 1977). *lexA* and *recF* mutations also block recombination in *recB/C sbcB* mutants (Clark, 1980) and reduce its efficiency in *recB/C⁺ sbcB⁺* strains (this work).

To gain further insight into the nature of the *recF* defect and its effect on SOS expression we have studied DNA repair and genetic recombination in *recF143* mutants in which expression of SOS genes was altered through mutation of *lexA* or *recA*. The results obtained indicate that the *recF* defect may be related to reduced activity of *recA* protein and we suggest that the *recF* product normally acts to control *lexA* regulated processes in the absence of any damage to DNA.

METHODS

Bacterial strains. The *Escherichia coli* K12 F⁻ strains are listed in Table 1. All are closely related to AB1157 (Bachmann, 1972) (F⁻ *thi-1 his-4 proA2 argE3 thr-1 leuB6 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44*).

JC9239, carrying *recF143*, was obtained from A. J. Clark. Derivatives of AB1157 and JC9239 carrying various alleles of *lexA* or *recA* were constructed by P1 transductions from the appropriate donors into strains previously made *malE::Tn10* or *srl::Tn10*, with selection for *Mal*⁺ and *Srl*⁺, respectively. *sfkB*⁻ strains were constructed by mating with Hfr (Hayes) *sfkB*⁻ (J. George strains GC895) with selection for *Leu*⁺ (*rpsL*) transconjugants. The genotype of all strains was confirmed by the appropriate P1 transductional backcrosses. The multicopy plasmids pJL21 (*lexA*⁺) and pJL26 (*lexA3*) are derivatives of pBR322 (Mount *et al.*, 1980) and were introduced into the appropriate recipients by transformation and selection for tetracycline resistant clones.

Media. LB broth and agar, used for the routine culture of bacterial strains, and 56/2 minimal salts agar for the selection of recombinants and transconjugants from crosses, have been referred to previously (Lloyd & Low, 1976).

Conjugal recombination. The procedure for measuring the efficiency of recombination in Hfr crosses has been described (Lloyd & Low, 1976). F⁻ recipients were mated with Hfr strain KL226 (Hfr Cavalli) for 60 min at 30 °C after which samples were plated on defined agar medium selective for Pro⁺ (*rpsL*) recombinants. Samples of the same F⁻ cultures were also mated with the F-*lac*⁺ donor strain NH4104 (Lloyd & Low, 1976) for 30 min at 30 °C and selection made for Lac⁺ (*rpsL*) F-prime exconjugants. Colonies were scored after 48 h incubation. The yield of recombinants from Hfr crosses expressed as a fraction of that obtained with AB1157 was used as a measure of recombination deficiency. The yield of Lac⁺ exconjugants in the F-prime crosses was used to correct for any reduction in the mating efficiency of the strains relative to AB1157.

UV irradiation. Strains were grown to about 2×10^8 cells ml⁻¹ in LB broth medium and irradiated on the surface of LB agar plates as described previously (Lloyd & Low, 1976; Lloyd & Barbour, 1974). Strains harbouring *lexA* plasmids were grown in the presence of tetracycline (10 µg ml⁻¹) to prevent growth of plasmid-free segregants. Colonies of survivors were scored after 24–36 h at 30 °C.

Nitrofurantoin sensitivity. The procedure used was modified from that described by Ream *et al.* (1980). Strains were grown in LB broth medium to about 2×10^8 cells ml⁻¹ and samples of various dilutions were plated on LB agar containing various concentrations of nitrofurantoin (Sigma). Colonies of survivors were scored after 48 h incubation.

RESULTS

Additivity of lexA3 and recF143 defects

A series of closely related strains carrying various combinations of *lexA* and *recF* alleles were examined for their sensitivity to UV irradiation and for their recombination proficiency in crosses with an Hfr donor. The *lexA3 recF143* double mutant proved more sensitive to UV light and less proficient at recombination than strains carrying either of these mutations alone (Fig. 1a, Table 1a). Mount *et al.* (1980) showed that although the *lexA* gene is autoregulated the level of repressor can be increased, and SOS expression reduced, by introducing multicopy *lexA* plasmids into the cell. The data presented in Fig. 1 (b, c) and Table 1 (b) demonstrate that the additivity of *lexA* and *recF* defects is also observed in strains carrying multicopy *lexA* plasmids. A plasmid carrying the mutant *lexA3* allele had a more extreme effect on UV sensitivity and recombination proficiency than a *lexA*⁺ plasmid, but in both cases the effect was enhanced by a *recF* mutation. The *lexA recF* double mutant carrying the *lexA3* plasmid proved extremely sensitive to UV light, and recombination was reduced to 3–4% of that in a wild-type (plasmid free) strain.

Increased synthesis of recA protein does not alleviate the recF defect

Previous studies (Thomas & Lloyd, 1980) showed that strains carrying *alc-24*, a *recA* regulatory site mutation, synthesize high levels of *recA* protein even in the presence of *lexA3* which normally prevents derepression of *recA*. The UV sensitivity and recombination deficiency of *lexA* and *lexA recF* mutants, particularly those carrying *lexA* plasmids, was alleviated to some extent by *alc-24* (Fig. 1d, Table 1c). However, the *alc-24 lexA*⁺ (*recF*⁺) strain used for comparison proved more proficient at recombination and more resistant to UV light than any of these strains. *alc-24* appeared to have no effect on a *recF* mutant except where the strain was also *lexA*⁻. The *alc-24 recF lexA*⁺ strain proved as sensitive to UV light and as deficient for recombination as the corresponding *alc*⁺ strains (Fig. 1e, Table 1).

tif expression alleviates the DNA repair defect in recF143 strains

The observations described in the previous section demonstrate that both *lexA* and *recF* mutations prevent normal recombination and DNA repair even when the amount of *recA*

Fig. 1. UV survival of *recF* mutants and related strains. Cultures were grown in LB broth at 30 °C and irradiated on the surface of LB agar plates as referred to in Methods. The UV fluence was 1 J per m². (a) ○, AB1157; △, JC9239; ◇, RL201; □, RL202. (b) ●, AB1157/pJL21; ○, AB1157/pJL26; ◆, RL201/pJL21; ◇, RL201/pJL26. (c) ▲, JC9239/pJL21; △, JC9239/pJL26; ■, RL202/pJL21; □, RL202/pJL26. (d) ○, RL203; ▽, RL204; ◆, RL204/pJL21; ◇, RL204/pJL26; ■, RL206/pJL21; □, RL206/pJL26. (e) ○, RL207; ▽, RL205; ■, JC9239; □, RL208; △, RL209; ●, AB2463.

protein is not a limiting factor. The *lexA* defect can be attributed to reduced expression of one or more of the other activities regulated by *lexA* repressor. To investigate whether or not the same could apply to *recF* strains, *recF tif* and *recF tsl* (*lexA*) mutants were constructed and examined for their ability to repair UV damage. The *tif-1* mutation is an allele of *recA* that increases the protease activity of *recA* protein (Phizicky & Roberts, 1981) while *tsl-1* is an intragenic suppressor of *lexA3* which directly inactivates the altered repressor (Mount *et al.*, 1973). Both mutations cause increased expression of *lexA* regulated genes, particularly at high temperature (42 °C). Figure 1 (e) shows that even at 30 °C the UV sensitivity of a *recF* mutant was suppressed by *tif* but not by *tsl*. The survival of *tsl recF*⁺ and *tif recF*⁺ strains did not differ significantly from that of wild-type strains under the conditions used (data not shown). To determine whether or not *tsl* suppresses the *recF* DNA repair defect at 42 °C we made use of the fact that a *recF* mutant is more sensitive to nitrofurantoin than is a *recF*⁺ strain (Ream *et al.*, 1980) (for some unknown reason, a *recF* mutant was slightly more resistant to UV light at 42 °C than at 30 °C, data not

Fig. 2. Nitrofurantoin sensitivity of *recF*, *recF tif* and *recF tsl* strains. Cells were grown and spread on agar plates containing various concentrations of nitrofurantoin. Incubation was at 30 °C (a) or 42 °C (b). The strains used were: ●, AB1157; ○, JC9239; ▲, RL210; □, RL211; △, RL212.

Table 1. *Strains used and recombination efficiency*

Strain	Relevant properties	Recombination deficiency
(a) AB1157	Wild-type (<i>lexA</i> ⁺ <i>recF</i> ⁺ <i>recA</i> ⁺)	1
JC9239	<i>recF143</i>	0.51
RL201	<i>lexA3</i>	0.42
RL202	<i>lexA3 recF143</i>	0.12
(b) AB1157/pJL21	<i>lexA</i> ⁺ / <i>plexA</i> ⁺	0.73
AB1157/pJL26	<i>lexA</i> ⁺ / <i>plexA3</i>	0.17
JC9239/pJL21	<i>recF143/plexA</i> ⁺	0.16
JC9239/pJL26	<i>recF143/plexA3</i>	0.037
RL201/pJL21	<i>lexA3/plexA</i> ⁺	0.34
RL201/pJL26	<i>lexA3/plexA3</i>	0.15
RL202/pJL21	<i>lexA3 recF143/plexA</i> ⁺	0.09
RL202/pJL26	<i>lexA3 recF143/plexA3</i>	0.03
(c) RL203	<i>alc-24</i>	1.00
RL204	<i>lexA3 alc-24</i>	0.55
RL204/pJL21	<i>lexA3 alc-24/plexA</i> ⁺	0.63
RL204/pJL26	<i>lexA3 alc-24/plexA3</i>	0.48
RL205	<i>recF143 alc-24</i>	0.49
RL206	<i>lexA3 recF143 alc-24</i>	ND
RL206/pJL21	<i>lexA3 recF143 alc-24/plexA</i> ⁺	0.35
RL206/pJL26	<i>lexA3 recF143 alc-24/plexA3</i>	0.24
(d) RL207	<i>recF143 tif-1</i>	0.69
RL208	<i>recF143 (lexA3) tsl-1</i>	0.76
RL209	<i>recF143 recA13</i>	ND
AB2463	<i>recA13</i>	ND
RL210	<i>recF143 sfiB</i>	ND
RL211	<i>recF143 tif-1 sfiB</i>	ND
RL212	<i>recF143 (lexA3) tsl-1 sfiB</i>	ND

ND, Not determined.

shown). Figure 2 shows that the *recF tsl (sfiB)* strain remained sensitive to nitrofurantoin at both 30 °C and 42 °C, whereas the *recF tif (sfiB)* strain was resistant at 42 °C and showed increased tolerance to the drug at 30 °C. The presence of the *sfiB* mutation, which is required for division of *tif* and *tsl* strains at high temperature (George *et al.*, 1975), accounts for the slightly increased nitrofurantoin resistance of the *recF tsl* strain at 42 °C (Fig. 2b).

These results demonstrate that the DNA repair defect of a *recF* mutant is suppressible by *tif* but not by *tsl*. This contrasts with the situation found in a *lexA* mutant where the repair defect is suppressed by *tsl* but not by *tif*. A further difference between the *lexA* and *recF* defects is that the former considerably enhances the UV sensitivity of a *recA* strain (data not shown), whereas the latter does not (Fig. 1e).

DISCUSSION

Since transcription of SOS genes appears to be controlled by *lexA* repressor it is difficult to imagine how a *recF* mutation could modify expression of SOS activities other than by affecting the level of repressor. The results presented in this paper are consistent with this view. The phenotype of a *recF* mutant is less severe than that of a *lexA* mutant, but is somewhat similar to that of a *recF*⁺ strain carrying a multicopy *lexA*⁺ plasmid. This, together with the additive effect of *lexA* and *recF* mutations, suggests that the *recF* defect may be due to increased expression of the *lexA*⁺ repressor. We argue, therefore, that the function of the *recF*⁺ product is to reduce the effective level of repressor in the cell. Since a *recF* mutation is suppressed by *tif* and not by *tsl* we further argue that *recF*⁺ product achieves this effect by increasing *recA* protease activity. This is supported by the fact that a *recF* mutation does not enhance the UV sensitivity of a *recA* strain (Fig. 1e). The SOS genes would still be inducible in *recF* mutants, though the response would be retarded due to reduced basal levels of *recA* protease activity. This would account for the normal UV mutability of *recF* mutants (Clark, 1980) and for the fact that the UV sensitivity of *recF* mutants is particularly noticeable at low UV doses (Fig. 1a). Since *recA* protease cannot cleave the mutant *lexA3* repressor (Little *et al.*, 1980), the additivity of *lexA* and *recF* defects would suggest that activated *recA* protein can enhance repair activities in the absence of increased expression of other *lexA* regulated genes. Alternatively, the *recF* product may have an additional role in DNA repair which is independent of *recA* protein, but this is not consistent with the properties of *recF recA* double mutants.

To cleave repressors *in vitro*, *recA* protein must be activated by polynucleotide or single-strand fragments of DNA (Phizicky & Roberts, 1981). The *recF* product could be responsible for such an activator molecule being produced at low levels during normal DNA replication, or it may be a protein which interacts directly with *recA* protein to promote its activity. Alternatively, the *recF*⁺ product could be an RNA molecule that acts as a specific effector of *recA* protein in the absence of DNA damage. McEntee & Weinstock (1981) have shown that *tif-1* protein is activable by polyribonucleotides, and it may be that *recA*⁺ protein is similarly activated *in vivo* by a specific polyribonucleotide sequence.

This interpretation of *recF* function implies that some *recA* protease would be produced during normal growth and provide a means for varying the expression of SOS regulated genes in the absence of any overt damage to DNA. What purpose would be served by the additional level of control? Since *lexA* and *recA* products regulate *sfiA*, and both *lexA* and *recA* mutants divide prematurely to produce smaller cells, many of which contain no DNA (Howe & Mount, 1978), one possibility is that the *recF*⁺ product has a role in controlling the normal cycle of cell division. The location of *recF* near the origin of DNA replication between *dnaA* and *gyrB* (Ream *et al.*, 1980) would be consistent with such a role (Zaritsky & Pritchard, 1973). A link between initiation of DNA replication and *recA* protease activity is suggested by the observation that mutations which inhibit initiation at high temperature (*dnaA46 dnaB252*) prevent *tif* expression (D'Ari *et al.*, 1979).

A further feature of this model for *recF* function is that it provides a simple explanation for the role of the *recF* product in genetic recombination and the different requirements of the so-called RecB/C and RecF pathways (Clark, 1980). In the absence of the *recB/C* product, exonuclease V, the *recF* product could promote the DNA unwinding activity of *recA* protein to provide the

single-stranded regions necessary for initiating recombination events (Cunningham *et al.*, 1979; Radding, 1981).

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