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Genetics

Evidence that *rnmB* is the operator of the *Escherichia coli recA* gene

(*recA* mutations/*recA* mutant plasmids/SOS regulation/DNA repair)

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ABSTRACT ***rnmB281*** **leads to high constitutive levels of recA protein such that no increase after UV-inducing treatment occurs. The mutation maps in or near the portion of *recA* corresponding to the NH_2-terminal end of the protein. Examination of the recA proteins from *rnmB*$^+$ *recA*$^-$/*rnmB281 recA*$^+$ heterozygotes suggests that both *rnmB* alleles are *cis*-acting and codominant. This is the behavior expected from alleles of a regulatory gene such as an operator or promoter of *recA*. The possibility that *rnmB* mutations occur in the promoter of *recA*, though not ruled out, seems unlikely based on the structure of the regulatory region of *recA*. This suggests that *rnmB* mutations are operator constitutive mutations of the *recA* gene and should be called *recAo* mutations. The UV-irradiation responses of *recAo*$^+$ and *recAo281* strains, both *recA*$^+$, are compared and inferences are drawn about the roles of large amounts of recA protein in producing the responses.**

When *Escherichia coli* cells are treated with agents that damage DNA, a pleiotropic set of cellular responses occurs: recA protein is synthesized at a high rate, new DNA repair pathways become functional, induced mutagenesis is expressed, and λ prophage are induced (for review, see ref. 1). These and other responses are collectively termed SOS functions (1, 2). Their expression requires the *recA*$^+$ *lexA*$^+$ genotype. Mutations in either of these genes that block induction of recA protein synthesis also block the expression of SOS functions and cause UV sensitivity (1).

rnmB mutations were originally detected as one class of suppressors of UV sensitivity in a *lexA102 uvrA155* mutant strain of *E. coli* B/r (3, 4). Their action is to suppress lexA-mediated UV sensitivity; when an *rnmB* mutation is introduced, UV sensitivity of *uvrA155* single mutants is not suppressed (4), whereas *lexA* single mutants become more UV resistant (unpublished data).

rnmB mutations are tightly linked to *recA* and result in constitutive synthesis of large amounts of recA protein (4). These results suggest that they may be regulatory mutations in either the operator or promoter of the *recA* gene (4). An alternative explanation is suggested by current models of regulation of recA protein synthesis (5–9), according to which induction of synthesis occurs when the expression of proteolytic activity of recA protein results in cleavage of the *lexA*-encoded repressor of the *recA* gene. Recently, this cleavage reaction has been verified (10). Thus *rnmB* mutations could be *recA* mutations that cause the proteolytic activity of the recA protein to be constitutive and thus could result in continuous cleavage of lexA repressor molecules. These two explanations can easily be distinguished. Mutations in *recA* that alter protease activity should be either dominant to *recA*$^+$ and act in *trans* to cause constitutive recA protein synthesis or recessive to *recA*$^+$. On the other hand, operator-constitutive or up-promoter mutations should be codominant with their wild-type alleles and should act only in *cis*.

To test these possibilities experimentally, merodiploids heterozygous for both *rnmB* and *recA* alleles were constructed. We chose the mutant *recA* alleles, *cis* to *rnmB*$^+$, whose recA proteins could be separated by two-dimensional polyacrylamide gel electrophoresis from the wild-type protein, determined by *recA*$^+$, *cis* to *rnmB281*. Thus, we can distinguish *cis* and *trans* regulatory effects.

Establishment of the genetic nature of the *rnmB281* mutation is necessary for understanding its effects on the expression of SOS functions.

MATERIALS AND METHODS

Bacterial Strains. The bacterial strains used are listed in Table 1. *rnmB281* can be cotransduced with *srl-300*::Tn*10*, whose inheritance can be detected by tetracycline resistance (Tet^R) (11). Inheritance of *rnmB281* can be determined by P1 transductional backcrossing into MV1131, a tetracycline sensitive (Tet^S) *lexA102 uvrA155* mutant strain and testing Tet^R transductants for suppression of UV sensitivity. Donors that gave UV-resistant (UV^R) transductants of MV1131 were presumed to carry the *rnmB281* mutations. These donors were examined for constitutive expression of recA protein synthesis by polyacrylamide gel electrophoresis of their total proteins.

Genetic Methods. P1 transductions were performed as described (4). Transformation with plasmid DNA was performed essentially as described by Moira *et al.* (16) except that (*i*) the cells were washed in 0.9% NaCl, (*ii*) the 42°C heat pulse was reduced from 10 to 2 min, and (*iii*) 5 ml of L broth was added for growth of the transformants. These changes optimized the yields of transformants. Plasmids, pJC602 and pJC603, were constructed by P1 transduction of a *srl*$^-$ derivative of plasmid pLC18-42 (ref. 15; unpublished result).

Cell Viability. Cell viability was determined by comparing both the optical densities and the visible cell titer, obtained by using a Petroff–Hauser counting chamber, with the viable cell titer obtained by plating dilutions on L plates and incubating at 37°C until colonies formed (17).

Phage Methods. UV induction of λ prophage was performed essentially as described (4) except that UV-irradiated lysogenic cells were mixed in LCTG soft agar (17) and plated on LCTG plates containing 2% agar. Procedures for determining survival of λ phage after UV irradiation have been described (17), and similar procedures were used for S13 phage. Methods used to determine cellular ability to perform UV-induced reactivation of UV-irradiated λ and S13 phages have been described (15, 17). λ phage was irradiated with a UV dose of 250 J/m^2 and S13 phage was irradiated with a dose of 75 J/m^2. Host capacity for

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Abbreviations: Tet^R, tetracycline resistant; Tet^S, tetracycline sensitive; UV^R, UV resistant; kb, kilobase(s).

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

Strain	Mutant genotype	Comments, references
	E. coli K-12 strains*	
A74	*alaS3*	(11)
AB1157		(12)
DM49	*lexA3*	(13)
DM1413	*malB32*	Also *arg*$^+$ *supE*$^+$ (7)
JC9937	*srlD50*	(11)
JC9983	*srlD50*	pJC603 (ColE1 *recA35 srl*$^+$) transformant of JC9937
JC9986	*srlD50*	pJC602 (ColE1 *recA13 srl*$^+$) transformant of JC9937
JC10241	*srl-300*::Tn*10*	(14)
JC10287	del(*srlR-recA*)304	Table 5 in ref. 8
JC10522		S13-sensitive derivative of AB1157 (15)
JC10523	*recF143 uvrA6*	(15)
JC11457	*rnmB281*	*srl*$^+$ transductant of MV1138 that retains the *rnmB281* allele
JC11801		S13-sensitive (phage HK19-resistant) derivative of AB1157 (15)
JC11824	*lexA3*	S13-sensitive (phage HK19-resistant) derivative of DM49
JC11867	*srl-300*::Tn*10* *rnmB281 lexA3*	Tet^R Srl$^-$ UV^R transductant of JC11824 obtained by crossing with P1·MV1138
MV1131	*lexA102 uvrA155*	MalB$^+$ UV^S Hcr$^-$ transductant of DM1413 obtained by crossing with P1·WP51
MV1132	*lexA102 uvrA155 srl-300*::Tn*10 rnmB281*	Tet^R Srl$^-$ UV^R transductant of MV1131 obtained by crossing with P1·MV21
MV1138	*srl-300*::Tn*10 rnmB281*	Tet^R Srl$^-$ transductant of JC11801 obtained by crossing with P1·MV1132
MV1148	*srl-300*::Tn*10 rnmB281*	pJC602 (ColE1 *recA13 srl*$^+$) transformant of MV1138
MV1149	*srl-300*::Tn*10 rnmB281*	pJC603 (ColE1 *recA35 srl*$^+$) transformant of MV1138
MV1154	*lexA3 srl-300*::Tn*10*	Tet^R Srl$^-$ transductant of JC11824 obtained by crossing with P1·JC10241
MV1155	*alaS3 lexA3*	Srl$^+$ 42°C sensitive transductant of MV1154
MV1156	del(*srlR-recA*)304 *lexA3*	42°C resistant RecA$^-$ Srl$^-$ transductant of MV1155 obtained by crossing with P1·JC10287
	E. coli B/r strains†	
MV2	*lexA102 rnmB281*	(4)
MV21	*lexA102 srl-300*::Tn*10 rnmB281*	(4)
$WP2_S$		(4)
WP51	*lexA102*	(4)

* *E. coli* K-12 strains are derivatives of AB1157 and contain the following mutations unless noted otherwise: *argE3 his-4 leu-6 proA2 thr-1 rpsL31 galK2 lacY1 tsx-33 ara-14 xyl-5 mtl-1 supE44.*

† All *E. coli* B/r strains listed also contain the following mutations: *uvrA155 trpE65 lon-11 sulA1.* Also, MV2, MV21, and WP51 contain *thyA143* and $WP2_S$ is *thyA*$^+$ and contains *malB15.*

the multiplication of unirradiated phage was determined with cells UV irradiated at doses of 0, 2.5, 5.0, 10, 20, or 30 J/m^2. The titer of infectious centers produced at each dose was divided by the titer produced by unirradiated cells. The following average results (two experiments) were obtained—for JC10522 (*rnmB*$^+$): λvir-1.0, 0.93, 0.88, 0.96, 0.93, 0.96; S13-1.00, 1.02, 1.02, 1.06, 1.02, 0.88; for MV1138 (*rnmB281*): λvir-1.00, 1.02, 1.18, 1.13, 1.22, 1.14; S13-1.00, 1.04, 1.00, 0.92, 0.96, 0.78. These values were used to calculate the percentage of lethal lesions reactivated (17).

Protein Labeling. Cells were grown in minimal medium as described (15), infected with unirradiated phage, washed, and resuspended in 56/2 buffer [0.06 M Na_2HPO_4/0.04 M KH_2PO_4/0.02% $MgSO_4 \cdot 7H_2O$/0.2% $(NH_4)_2SO_4$/0.001% $Ca(NO_3)_2$/0.00005% $FeSO_4 \cdot 7H_2O$]. The cultures were then divided into two aliquots and one was irradiated with UV light (40 J/m^2). Appropriate nutrients were added to each. After 40 min of incubation at 37°C, [^{35}S]methionine (5 μCi/ml; 1 Ci = 3.7×10^{10} becquerels) was added to each sample. The samples were incubated for an additional 5 min, and then cold methionine (100 μg/ml) was added and they were immediately chilled. Cells were harvested as described (15). For one-dimensional electrophoresis, the cells were suspended in final sample buffer (0.05 M Tris base, pH 6.8/1% $NaDodSO_4$/5% 2-mercaptoethanol/1% glycerol/0.002 M EDTA) and treated as described (15). For two-dimensional electrophoresis, the cells were suspended in buffer A (9.5 M urea/2% Noniodet P-40/1.6% ampholytes, pH 5–7/0.4% ampholytes, pH 3–10/5% 2-mercaptoethanol) (18), and protein extracts were prepared by repeated freeze-thaw cycles as described by O'Farrell (18).

Electrophoresis. Two-dimensional polyacrylamide gel electrophoresis techniques were essentially as described by O'Farrell (18). Ampholine mixtures were pH 3–10, 0.4% and pH 5–7, 1.6%. Gel tubes were 3 mm × 130 mm, and electrophoresis was carried out at 400 V for 17 hr. Gels were prefocused at 400 V for 30 min before addition of samples. Gels were extruded from tubes and placed on $NaDodSO_4$/polyacrylamide slabs (12% total acrylamide) as described (18). Electrophoresis was carried out at 0.5 W as determined by a constant power transformer. After the dye front reached the bottom of the gel, the gel was stained and destained as described (15), treated with En^{3}Hance (New England Nuclear) according to manufacturer's specifications, and dried. Fluorography was performed as described (15).

RESULTS

Mapping *rnmB281*. *rnmB281* has been shown to be 90–95% cotransducible with the sorbitol operon (*srl*) (4). It was mapped more precisely by crossing strain MV1156, which carries a deletion mutation designated del(*srlR-recA*) 304 in addition to *lexA3*, with P1 grown on strain JC11457 (*srl*$^+$ *rnmB281*). The deletion del(*srlR-recA*)304 extends from a site in *srlR* to a site between the *Pst I* and *Eco*RI restriction sequences, about 50% of the way into the *recA* gene from the NH_2-terminal end (unpublished results). Srl$^+$ transductants of MV1156 were picked, purified, and tested for UV resistance relative to MV1156 (*lexA3* del(*srlR-recA*)304), JC11824 (*lexA3 rnmB*$^+$), and JC11867 (*lexA3 rnmB281*). For inheritance of the donor *srl*$^+$ genes to occur, recipients must experience one recombinational event on the *srl* side of the deletion and another on the *recA* side of the deletion. This also results in inheritance of *recA*$^+$. Such transductants, which have inherited the *rnmB281* mutation, would be more UV resistant than *rnmB*$^+$ *recA*$^+$ transductants because *lexA3* UV sensitivity would be suppressed. Of 566 Srl$^+$ transductants tested, all were as UV resistant as JC11867 (*rnmB281 lexA3*) and had therefore inherited *rnmB281*. Thus, *rnmB281* is recombinationally separable from the DNA segment deleted by del(*srlR-recA*)304 with a frequency of less than 0.2% and lies either within or very near this region. The maximum distance outside the deleted region can be estimated from the formula of Wu (19) to be 0.001 min (about 40 base pairs).

Because the plasmids used to construct the merodiploids extend 5.5 kilobases (kb) beyond the *srl* end of the deletion and about 2 kb beyond the *recA* end (unpublished results), they must also carry the *rnmB*$^+$ allele and can be used for dominance and recessiveness tests.

Electrophoretic Analysis of recA Protein from *rnmB281* and *recA* Mutant Strains. It has previously been shown that the *rnmB281* mutation results in constitutive synthesis of high levels of recA protein and that no further increase is seen after UV irradiation (4). This is in contrast to wild-type cells, which show low levels of recA protein in nonirradiated cells and a large increase in recA protein synthesis after irradiation. When the proteins from an *rnmB281* mutant strain and wild-type strains (either *E. coli* B/r or *E. coli* K-12) were compared by two-dimensional gel electrophoresis, no differences in isoelectric points of their *recA* proteins were evident (Fig. 1 and unpublished results). On the other hand, recA protein produced by the *recA13* allele is slightly more acidic than wild-type recA protein and is present to its right on two-dimensional gels (Fig. 2). This is also true of the recA protein determined by *recA35* (see Fig. 1). The recA441 (formerly tif-1) protein by contrast is more basic than wild-type recA protein (5, 6, 8) and derepresses its own synthesis conditionally. Because *rnmB281* has no detectable effect on the isoelectric point of recA protein and appears to derepress it unconditionally, it differs from *recA441* both in structural and regulatory properties. In Fig. 2, the proteins in the smaller spots beneath the normal-sized recA proteins have the size of degradation products (8). We have seen these spots only in some extracts (data not shown). Their presence or absence does not affect the relative amounts of different normal-sized recA proteins.

Dominance-Recessiveness of *rnmB* and *recA* Alleles. In a dominance-recessiveness test between *rnmB*$^+$ and *rnmB281*, there are two features of the *rnmB281* phenotype that we can test: high constitutive production of recA protein (derepression) and failure of this production to be increased appreciably by UV irradiation (UV noninducibility). By having *rnmB*$^+$ and *rnmB281* *cis* to *recA* alleles whose proteins are detectably different, we can also determine whether *rnmB* alleles act *cis* or *trans*. A complication is that *recA* mutant alleles are either dominant or recessive to *recA*$^+$ and affect their own regulation. To detect any differences in the dominance-recessiveness of *rnmB*$^+$ and *rnmB281*, we have tested both dominant and recessive *recA*$^-$

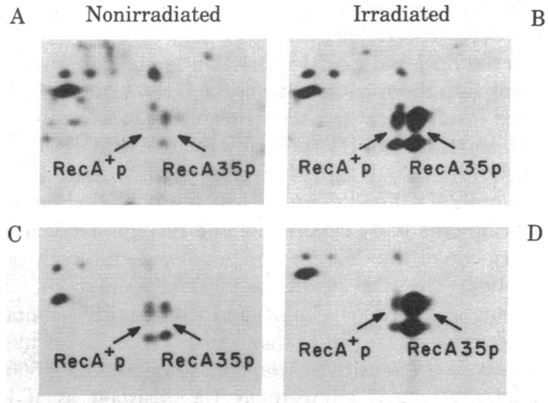

FIG. 1. Portions of autoradiograms after two-dimensional gel electrophoresis of [^{35}S]methionine-labeled proteins from pJC603-containing cells. (*A* and *B*) Strain JC9983 (ColE1 *rnmB*$^+$ *recA35/rnmB*$^+$ *recA*$^+$). (*C* and *D*) Strain MV1148 (ColE1 *rnmB*$^+$ *recA35/rnmB281* *recA*$^+$). Spots corresponding to recA$^+$ (RecA$^+$p) and recA35 (RecA35p) proteins are indicated in each panel. Isoelectric focusing is from left to right and NaDodSO$_4$ dimension is from top to bottom.

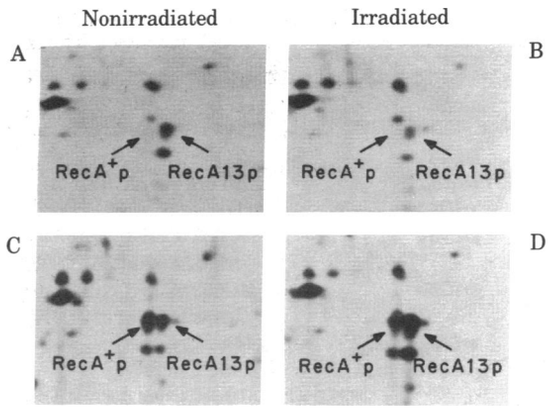

FIG. 2. Portions of autoradiograms after two-dimensional gel electrophoresis of [^{35}S]methionine-labeled proteins from pJC602-containing cells. (*A* and *B*) Strains JC9986 (ColE1 *rnmB*$^+$ *recA13/rnmB*$^+$ *recA*$^+$). (*C* and *D*) Strain MV1149 (ColE1 *rnmB*$^+$ *recA13/rnmB281* *recA*$^+$). Spots corresponding to recA$^+$ (RecA$^+$p) and recA13 (RecA13p) protein are indicated in each panel.

alleles. *recA13* is a dominant mutation when it is present on a high copy-number plasmid in a *recA*$^+$ cell. The dominance appears to be a gene dosage effect; *recA13* becomes recessive to *recA*$^+$ when the wild-type allele is the plasmid allele and *recA13* is the chromosomal allele. The *recA35* mutant allele, on the other hand, is recessive regardless of its presence as the chromosomal or ColE1 plasmid allele (unpublished results).

In the ColE1 *rnmB*$^+$ *recA13/rnmB*$^+$ *recA*$^+$ heterozygote, a low amount of recA$^+$ protein and a larger amount of recA13 protein are seen before irradiation (see Fig. 2*A*). Presumably, the amounts reflect the different gene copy number of each allele due to the high copy-number plasmid carrying *recA13*. After irradiation, no change in the relative amounts of either recA protein is seen (see Fig. 2*B*). This is expected from the dominance of *recA13* to *recA*$^+$. When the ColE1 *rnmB*$^+$ *recA13* plasmid is introduced into the *rnmB281* *recA*$^+$ strain, recA$^+$ protein continues to be synthesized constitutively in high amounts (see Fig. 2*C*) but recA13 protein is not synthesized constitutively in a greater amount than in the *rnmB*$^+$ homozygote. This is the result expected if *rnmB281* acts *cis* and not *trans* and if it is therefore codominant with *rnmB*$^+$, which also acts in *cis* and not in *trans*. After irradiation, no increase in the size of the recA$^+$ protein spot is seen but a slight increase in recA13 protein is evident (see Fig. 2*D*). This implies that *rnmB*$^+$ and *rnmB281* are *cis*-acting codominant alleles with respect to UV inducibility. It is not strong evidence, however, because the transdominant regulatory effect of *recA13* over *recA*$^+$ limits the UV induction of *recA* protein that can occur.

To provide stronger evidence that *rnmB*$^+$ also acts in *cis*, we used a ColE1 *rnmB*$^+$ *recA35* plasmid whose *recA* allele is *trans* recessive to *recA*$^+$ with regard to recombination and recovery from UV damage (unpublished results). In strain JC9983 (ColE1 *rnmB*$^+$ *recA35/rnmB*$^+$ *recA*$^+$), which shows a small spot of recA$^+$ protein and a somewhat larger spot of recA35 protein when unirradiated extracts are examined (see Fig. 1*A*), both proteins increase substantially after irradiation (see Fig. 1*B*). Because a *recA35* homozygous meroploid is incapable of UV induction of recA35 protein, the increase in recA35 protein depends on the presence of recA$^+$ protein (unpublished results). Thus, the *recA*$^+$ gene acts in *trans* to cause UV-induced synthesis of recA35 protein. Strain MV1149 (ColE1 *rnmB*$^+$ *recA35/rnmB281* *recA*$^+$) shows a large spot corresponding to recA$^+$ protein and another large spot corresponding to recA35 protein in the untreated extracts (see Fig. 1*C*). This also shows

that *rnmB281* acts in *cis* to cause constitutive recA protein synthesis. After irradiation, there is little if any increase in the recA$^+$ spot whereas the recA35 spot increases substantially (see Fig. 1*D*). This suggests that *rnmB*$^+$ allows UV inducibility of the *cis* allele *recA35* and that this effect is independent of the presence of the *cis*-acting *rnmB281*. With regard to both regulatory phenomena, *rnmB* alleles act *cis* and not *trans* and therefore are codominant.

Phenotypic Effects of *rnmB281*. An *rnmB281* strain shows no difference in cell viability (viable cell titer as a function of culture turbidity and visible cell titer) when compared with wild-type cells (data not shown), indicating that constitutive synthesis of high amounts of recA$^+$ protein is not deleterious to cell viability.

λCI$^+$ lysogens of an *rnmB281* strain are easily produced, and spontaneous production of λCI$^+$ prophage is as low as that from wild-type lysogens (data not shown). *rnmB281* mutants differ from *spr recA441* (7; *recA441* was formerly called *tif-1*) strains in both properties, although both strains produce large amounts of recA protein constitutively. Moreover, when an *rnmB281* λCI$^+$ lysogen is irradiated, λCI$^+$ is induced with a dose response similar to that of an *rnmB*$^+$ lysogen (data not shown). Because large amounts of recA protein do not circumvent the need for irradiation to induce λ prophage, derepression of recA protein synthesis is insufficient for λ repressor inactivation.

UV-induced reactivation of UV-damaged λ phage (Weigle reactivation) is another phenomenon not expressed on derepression of *recA*$^+$ by *rnmB281*, as shown by the nearly identical survival of irradiated λ phage on *rnmB*$^+$ and *rnmB281* strains (Fig. 3). At a dose of 250 J/m^2 to the phage, the difference in survival is equivalent to a constitutive reactivation of $\approx$4% of the lethal damage. For comparison, Fig. 4 shows the UV-inducible reactivation of UV-damaged λ phage given 250 J/m^2 of UV irradiation. As much as 50% of the lethal damage is reactivated by a UV dose of 30 J/m^2 to the *rnmB*$^+$ strain; the equivalent change in survival is shown in Fig. 3. The *rnmB281* strain shows UV-inducible reactivation of less lethal damage to λ phage (25%) and this equivalent in survival is also shown in Fig. 3. A similarly small amount, if any, of constitutive reactivation of S13 phage is seen as the result of *rnmB281*, as is a reduction in UV-inducible reactivation of S13 (see Fig. 4).

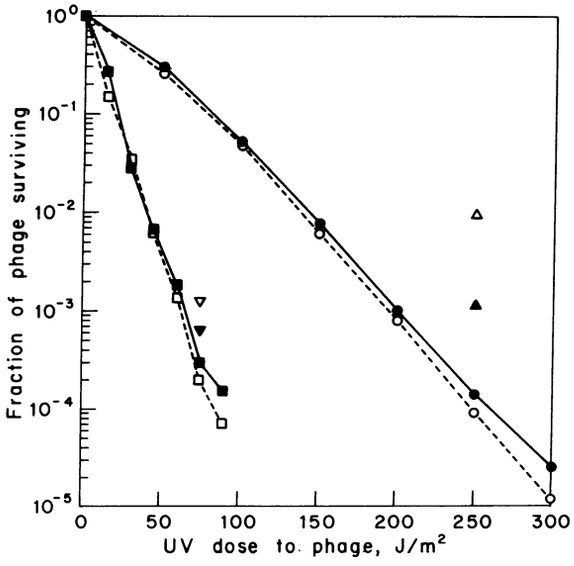

FIG. 3. Survival of UV-irradiated phages [λvir (○ and ●) and S13 (□ and ■)] on unirradiated hosts [JC10522 (*rnmB*$^+$), open symbols; MV1138 (*rnmB281*), closed symbols]. Expected survival of irradiated phage after UV-induced reactivation of a given percentage of lethal lesions is shown for λvir irradiated with 250 J/m^2 (△ and ▲) and S13 irradiated with 75 J/m^2 (▽ and ▼). For UV-induced reactivation, host cells were irradiated with 30 J/m^2: The percentage of lethal lesions reactivated corresponding to the survival is as follows: △, 50%; ▲, 25%; ▽, 21%; ▼, 8.3%.

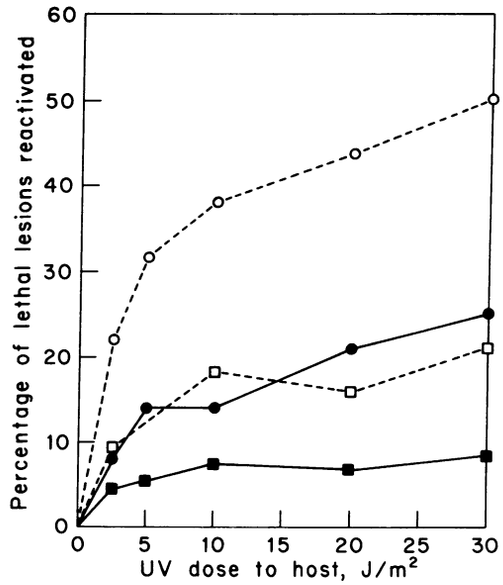

FIG. 4. UV-induced reactivation of phages λvir (○ and ●) and S13 (□ and ■) irradiated with 250 J/m^2 and 75 J/m^2, respectively. Host strains were JC10522 (*rnmB*$^+$) (open symbols) and MV1138 (*rnmB281*) (closed symbols).

Because the lower amount of inducible reactivation in the *rnmB281* strain is not due to high levels of constitutive reactivation (see Fig. 3), we thought it might be due to a decreased capacity of UV-irradiated cells to support phage multiplication or to a deleterious effect of *rnmB281* on the activity of recA protein. Tests show, however, that *rnmB281* does not decrease appreciably the host capacity of irradiated cells. The other alternative was ruled out by using an *rnmB*$^+$ *recA*$^+$ homozygous strain carrying ColE1 *recA*$^+$ plasmid pLC18-42. Amounts of recA$^+$ protein as high as those produced by *rnmB281* are present in this strain. This strain mimics the *rnmB281* strain with respect to negligible amounts of constitutive reactivation of UV-irradiated λ and S13 phages and reduced UV-induced reactivation of the same phages (ref. 15 and unpublished results).

The regulatory and physiological effects of *rnmB281* described above have been confirmed (H. Ginsburg, S. Edmiston, and D. Mount, personal communication) by a study of suppressors of *lexA3*-mediated UV sensitivity similar to *rnmB281* isolated in *E. coli* K-12 strains.

DISCUSSION

rnmB mutations were detected in *E. coli* B/r as suppressors of UV sensitivity in a *lexA102 uvrA155* double mutant strain. In *E. coli* B/r, these mutations suppressed the UV sensitivity but not the UV nonmutability due to *lexA102* (3, 4). In *E. coli* K-12 *lexA102 uvrA155* mutant strains, *rnmB281* also suppressed UV sensitivity but not UV nonmutability (data not shown). In addition, the UV sensitivity of a *lexA3* mutant strain was suppressed (data not shown). Because both *lexA102* and *lexA3* can be suppressed by *rnmB281*, the suppression is probably not due to a specific interaction between mutant *lexA* and *rnmB* products.

rnmB mutations were mapped near the *recA* gene and shown to cause constitutive synthesis of high amounts of recA protein

(4). In both *E. coli* B/r and *E. coli* K-12 derivatives, recA protein synthesis is constitutively derepressed by *rnmB281* regardless of whether the strain is also *lexA*$^+$, *lexA102* or *lexA3*. *rnmB281* was one of three *rnmB* mutations introduced into *E. coli* K-12 and was investigated more thoroughly to determine whether its action is consistent with its being an operator constitutive mutation affecting the *recA* gene.

On the basis of two point crosses (4), *rnmB281* can be calculated (19) to lie within 1.2–2.4 kb of the Tn*10* insertion in *srl-300*::Tn*10*. The *recA* structural gene extends from 3.8 to 4.8 kb from *srl-300*::Tn*10*. Because *rnmB* probably lies within del(*srlR-recA*)304, it must map between the NH_2-terminal end of the *recA* structural gene and *srl-300*::Tn*10*. *rnmB281* does not detectably alter the electrophoretic nor the chromatographic properties of recA protein (unpublished results). These facts are consistent with the hypothesis that the *rnmB281* mutation does not lie within the *recA* structural gene but in the regulatory region controlling *recA*.

This hypothesis is supported by the codominant *cis*-acting behavior of both *rnmB*$^+$ and *rnmB281*. When the *rnmB*$^+$ allele, as a plasmid-borne gene coupled with either *recA13* or *recA35*, is introduced into an *rnmB281 recA*$^+$ strain, high constitutive amounts of the recA$^+$ protein are not reduced. This suggests that *rnmB*$^+$ does not act in *trans* to prevent the action of *rnmB281*. That *rnmB281* also does not act in *trans* cannot be inferred from results with unirradiated cells alone because high plasmid copy number leads to high constitutive amounts of recA mutant protein. To circumvent this problem, we have shown that the *recA35* allele *cis* to *rnmB*$^+$ can be UV induced although the *recA*$^+$ allele *cis* to *rnmB281* cannot be. This result not only demonstrates the *cis*-acting nature of *rnmB* alleles with respect to UV induction but also shows that, in unirradiated cells, the plasmid-borne *recA* mutant genes do not give the maximum amount of recA mutant protein. Therefore, one can infer that, in unirradiated cells, *rnmB281* does not act on *recA* alleles in *trans*. This absence of *trans* regulatory effects makes it unlikely that *rnmB281* is a mutation in the *recA* structural gene. According to current models (5–10) *recA* regulatory effects should be manifest in *trans* as are the dominant effects of *recA13* to *recA*$^+$ and *recA*$^+$ to *recA35* with respect to UV induction of recA protein.

We cannot rule out the possibility that *rnmB281* is an up-promoter mutation; however, the structure of the region presumably regulating the *recA* gene makes this unlikely. A promoter-like sequence (20, 21) and a possible operator sequence (R. Brent and M. Ptashne, personal communication) have been discerned near the NH_2-terminal sequence of *recA*. Unlike the order of promoter and operator relative to *lacZ* (22), the order of these potential *recA* regulatory genes is *recAo recAp recA* [this nomenclature conforms to that suggested by Bachmann and Low (22)]. Even though this order would seem to make it possible for regulation of *recA* to escape repressor activity at *recAo* by an up-promoter mutation, this is probably not possible because *recAo* lies within the RNA polymerase binding site (R. Brent and M. Ptashne, personal communication). Thus, for derepression of *recA* to occur in *lexA*$^+$ cells with a wild-type repressor or in *lexA3* and *lexA102* cells with a mutant, slowly inactivable repressor (10), it seems likely that the operator must be affected. Therefore, we will use *recAo281* in place of *rnmB281*.

The presence of *recAo281* allows us to confirm that the sole effect of UV irradiation in the induction of λ prophage and reactivation of UV-irradiated phage is not the induction of *recA* (4–10). If it were, we would expect inability to be lysogenized by CI$^+$ λ and high constitutive reactivation of UV-irradiated phage to be the phenotype of a *recAo281* strain. UV irradiation may provide at least two possible additional requirements for SOS induction: provision of one or more cofactors to activate recA protein (9) and induction of genes other than *recA* (23). The mutation *recA441* obviates the need for UV irradiation to accomplish the first, possibly because the mutant protein more effectively competes with other proteins for low levels of cofactors (e.g., single-stranded DNA) present in unirradiated cells. Mutations partially inactivating the *lexA* repressor (called *spr*$^-$) accomplish the second result of UV irradiation by derepressing a set of at least six genes (G. Walker, personal communication), including *recA* (5). As a result *spr*$^-$ *recA441* cells cannot easily be lysogenized by wild-type λ phage and show high constitutive reactivation of UV-irradiated λ phage (7, 24).

Reduction in the amount of UV-induced reactivation of UV-irradiated λ and S13 phages by *recAo281* exposes a negative effect of high constitutive amounts of recA protein. This negative effect may stem from inhibition of DNA exonuclease V, the product of *recB* and *recC*, which has both destructive and constructive roles in UV-irradiated cells (3). Alternatively, the negative effect may stem from competition between recA protein and other components of repair systems for substrates or cofactors.

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