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Two-Component Suppression of *recF143* by *recA441* in *Escherichia coli* K-12

MICHAEL R. VOLKERT,[1,2]* LINDA J. MARGOSSIAN,[2]† AND ALVIN J. CLARK[2]

Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709,[1] and Department of Molecular Biology, University of California, Berkeley, California 94720[2]

Received 12 March 1984/Accepted 14 August 1984

Sensitivity to UV irradiation conferred by *recF143* was partially suppressed by *recA441* (also known as *tif-1*). A temperature-conditional component depended on *uvrA* function and is thought to involve thermal induction of excision repair enzymes. In a *uvrA6* mutant, a temperature-independent component of suppression was seen. This is thought to indicate that *recA441* also caused temperature-independent changes in *recA* activity. Two hypotheses are offered to explain how *recA441* produced both thermosensitive and thermoindependent effects.

The *recF* gene in *Escherichia coli* lies between *dnaN* and *gyrB* (3a, 18, 19). *dnaN* and *gyrB* encode, respectively, the β subunit of DNA polymerase III holoenzyme and the novobiocin-sensitive subunit of DNA gyrase. *recF* encodes a 40-kilodalton peptide (3a) whose enzymatic function is not yet known. *recF* mutants, however, show a wide range of altered phenotypes, indicating that the *recF* protein is involved in recombination (5, 10), repair (5, 9, 20–22), and transposition (M. Syvanen, personal communication).

We have sought clues to *recF* function by detecting and characterizing mutations indirectly suppressing *recF143*. A group of three mutations called *srfA* (suppressor of *recF*) has been located in or near *recA* (28). These mutations partially suppress UV sensitivity caused by *recF143*, with a greater efficiency of suppression in the *recB21 recC22 sbcB15* background than in the *recB*$^+$ *recC*$^+$ *sbcB*$^+$ background. Since this correlates with the greater deficiency in conjugational recombination caused by *recF143* in the former than in the latter background and since *srfA* mutations restore conjugational recombination, it was proposed that *srfA* mutations circumvent the need for *recF* protein in recombinational repair of UV damage (28).

In the *recB*$^+$ *recC*$^+$ *sbcB*$^+$ genetic background, *recF143* causes a decrease in the rate of UV induction of *recA* (15, 16, 23) and lambda (1), two aspects of the SOS response to UV damage (14, 30). Since *srfA* mutations do not restore SOS induction in *recF* mutants to any great extent (28; M. R. Volkert and M. A. Hartke, unpublished data), we suspected that the unsuppressed component of *recF143*-caused UV sensitivity was due to a delay in inducing needed gene products. To test whether restoration of SOS induction could also suppress *recF*, we have investigated the suppressive effects of *recA441* (also known as *tif-1*) on *recF143*. In a strain containing *recA441*, the SOS response is induced simply by shifting the temperature from 30 to 42°C (14, 29, 30), provided the cells are incubated in a minimal medium containing adenine (13).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. The genetic nomenclature recommended by Demerec et al. (7) and the gene symbols of Bachmann (3) are followed.

Media. Cells were grown in minimal medium containing E salts (29), glucose (0.4%), Casamino Acids (Difco Laboratories) (0.2%), and thiamine (0.02 μg/ml) at 30°C to a cell density of ca. 10^8/ml. Whenever incubation was at 42°C, adenine was added at a concentration of 75 μg/ml. After irradiation, the cells were plated on DSEM plates (11).

TABLE 1. Bacterial strains

Strain[a]	Genotype				Derivation or reference
	uvrA	*recA*	*recF*	*sfiB*	
recB$^+$ *recC*$^+$ *sbcB*$^+$					
AB1157	+	+	+	+	2
JC3912	*6*	+	+	+	11
JC3913	*6*	+	*143*	+	11
JC7578	+	*441*	*143*	*103*	—[b]
JC7580	+	*441*	+	*103*	—[b]
JC9239	+	+	*143*	+	10
JC10240	+	*56*	+	+	6[c]
MV1163	+	*441*	+	+	—[c,d]
MV1169	*6*	*441*	+	+	—[c,e]
MV1171	*6*	*441*	*143*	+	—[c,f]
recB21 recC22 sbcB15					
JC7597	+	*441*	+	*103*	—[g]
JC7598	+	*441*	*143*	*103*	—[g]
JC8111	+	+	*143*	+	10
JC11830	+	+	+	+	28

[a] All strains are derivatives of AB1157 and carry the following additional mutations unless otherwise noted: *argE3 his-4 leu-6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 rpsL31 supE44 tsx-33*.

[b] JC7578 and JC7580 are derivatives of JM12 (4) which were constructed by mating JM12 with JC9248 (20). *recF*$^+$ (JC8927) and *recF143* (JC8925) derivatives were retained. These were then transduced to *leu*$^+$ *sfiB103* with P1 grown on an *sfiB103 leu*$^+$ strain.

[c] Also carries *srl-300*::Tn*10*.

[d] Tetracycline-resistant (Tet^r) Srl$^-$ *recA441* transductant of JC7580 (P1 · JC10240).

[e] Tet^r Srl$^-$ *recA441* transductant of JC3912 (P1 · MV1163).

[f] Tet^r Srl$^-$ *recA441* tansductant of JC3913 (P1 · MV1163).

[g] Strains JC7597 and JC7598 are derivatives of JC8101 (ilv$^-$) (10) which were constructed by P1 transduction of JC8101 to *leu*$^+$ *sfiB103*; the *sfiB* derivative was then transduced to *srlD*$^-$ (JC7553). P1 grown on JM12 (4) was used to transduce JC7553 to *srl*$^+$ *recA441* (JC7568), and P1 grown on JC9239 (*recF143*) was then used to transduce JC7568 to *ilv*$^+$. Two *ilv*$^+$ derivatives were retained, *recF*$^+$ (JC7597) and *recF143* (JC7598). Thus the two strains are *recF*$^+$ and *recF143* derivatives of a *leu*$^+$ *recA441 sfiB103 recB21 recC22 sbcB15* strain.

* Corresponding author.
† Present address: ARCO Plant Research Institute, Dublin, CA 94566.

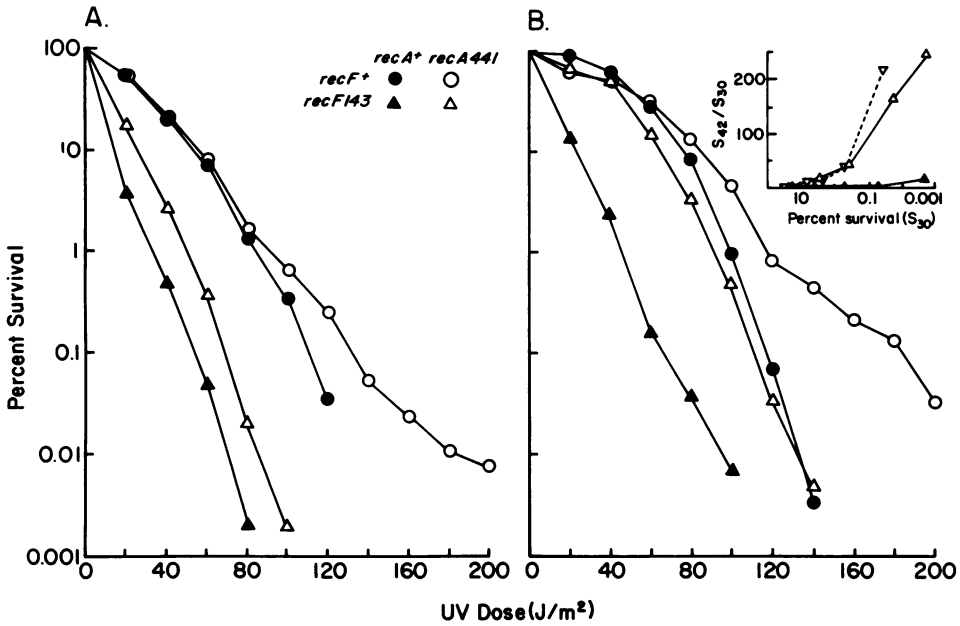

FIG. 1. Effect of *recA441* on UV survival of *uvrA*$^+$ strains carrying *recF*$^+$ or *recF143*. Cultures were grown at 30°C and then divided and incubated at 30 (A) or 42°C (B) 1 h before irradiation. Incubation after irradiation was at 30 and 42°C, respectively. The inset shows the ratio of survival values at 42 and 30°C plotted against survival at 30°C for strains JC9239 *recA*$^+$ *recF143* and JC7578 *recA441 recF143*. Such ratios for strains AB1157 *recA*$^+$ *recF*$^+$ and JC7580 *recA441 recF*$^+$ were similar to those for JC9239 and were omitted. The dotted line in the inset represents data for JC7598 (see Fig. 3).

Incubation conditions. Cultures that had been grown to a cell density of 10^8/ml at 30°C were divided into two equal aliquots. One was incubated at 30°C for 60 min. Adenine was added to the other, and it was incubated at 42°C for 60 min. Cells were then chilled on ice, centrifuged, and suspended in E salts buffer. Cell concentrations were readjusted to ca. 10^8/ml before irradiation.

After irradiation, cells that had been incubated only at 30°C before irradiation were plated at room temperature and incubated at 30°C for 3 days. Cells that had been incubated at 42°C before UV irradiation were incubated at 30°C as described above in some experiments and were incubated at 42°C in other experiments. When postirradiation incubation was at 42°C, the plates were warmed to 42°C before use, and incubation was continued at 42°C for 3 days.

The suppression of *recF143* by *recA441* is equally effective if the preirradiation temperature is elevated (data not shown) or if both the preirradiation and postirradiation incubation temperatures are elevated (see below). This allows the examination of *sfi*$^+$ derivatives, since lethality due to filamentation does not occur in a *recA441* strain if incubation at 42°C is for a short time only (29; data not shown).

Irradiation conditions. Cells were suspended in E salts buffer and irradiated to obtain the desired UV dose. *uvr*$^+$ *recB*$^+$ *recC*$^+$ strains were irradiated at 1 J/m^2 per s. *uvrA* mutants and *recB recC sbcB* mutants were irradiated 0.1 J/m^2 per s. The irradiation source was a champion 15 W germicidal lamp that was calibrated with a germicidal-erythemal radiometer (International Light).

RESULTS

Suppression of *recF143* in *uvrA*$^+$ strains. Figure 1 shows two effects of *recA441* on the survival of *recF*$^+$ and *recF143* strains after UV irradiation. First, it partially suppressed the UV sensitivity caused by *recF143* at both 30 and 42°C, but the suppression was much stronger at the higher temperature and was complete up to doses as high as 40 J/m^2. Thus, the *recA441* mutation allows a *recF143* strain to tolerate, as well as wild type, a considerable amount of DNA damage at 42°C. The strength of this suppression can be seen in the inset of Fig. 1B. Second, *recA441* increased resistance of *recF*$^+$ strains at dose rates of 80 J/m^2 and higher. Hidden by the way in which we graphed the results is a general increase in resistance of all strains due to elevated temperature (cf. Fig. 1A and B). Similar results were obtained with *recF144* strains (data not shown), indicating that the suppressive effects of *recA441* were not allele specific.

Suppression of *recF143* in *uvrA6* strains. The SOS response consists of the induction of several regulons (12), most of which comprise prophage genes such as those of lambda, 434, and φ80 (14, 30). The *lexA* regulon, however, seems to be indigenous to *E. coli*, although some plasmid genes repressed by *lexA* have been found, for example the *cea* gene of ColE1 (27) and the *mucA* and *mucB* genes of pKM101 (26). Among the 15 or so *E. coli* genes repressed by *lexA* are 3 required for excision repair, *uvrA*, *uvrB*, and *uvrD* (8, 17, 24, 25). To see whether temperature induction of these genes contributes to the suppression of *recF143* by *recA441*, we made the necessary *uvrA6* strains and exposed them to UV irradiation. *recA441* still suppressed *recF143* at both 30 and 42°C, but the strength of suppression was similar at the two temperatures (Fig. 2). This indicates that the additional suppression shown at 42°C in Fig. 1 depended on *uvrA*$^+$, whereas the suppression at 30°C did not. The second effect of *recA441*, that of increasing resistance of *recF*$^+$ strains at both 30 and 42°C, was absent in these *uvrA6* derivatives; in fact, *recA441* sensitized the strains at both temperatures, in accord with previously published results (4). The general increase in resistance due to elevated

temperature was not affected by the *uvrA6* mutation (cf. Fig. 2A and B).

Suppression of *recF143* by *recA441* in *recB21 recC22 sbcB15* background. *recF143* increases UV sensitivity to a greater extent in a *recB21 recC22 sbcB15* than in a *recB*$^+$ *recC*$^+$ *sbcB*$^+$ background (10). To see whether *recA441* could suppress this elevated sensitivity, we constructed the appropriate strains and irradiated them. The suppressive effect of *recA441* on *recF143* noted with the wild-type background (Fig. 1) was similarly observed here (Fig. 3). In particular, an increase in resistance was noted at both 30 and 42°C, with a stronger effect at the latter temperature. The magnitude of the 42°C effect was also similar to that observed with the wild-type background (inset to Fig. 1B). We did not carry out measurements at doses sufficient to observe the presence or absence of the second effect of *recA441*, resistance exceeding that of *recA*$^+$ in a *recF*$^+$ strain. The general increase in resistance caused by elevated temperature occurs with *recF143* strains; the data are insufficient to draw a conclusion about the *recF*$^+$ strains.

DISCUSSION

recA441 partially suppressed the UV sensitivity conferred by *recF143* in three different genetic backgrounds. In two genetic backgrounds, this partial suppression consisted of temperature-dependent and -independent components. The temperature-dependent component was absent in a *uvrA6* strain, and we hypothesize that it involves induction of excision repair enzymes. This implies that a defect in excision repair may explain part of the UV sensitivity of a *recF* mutant. In support of this, P. Cooper (personal communication) has found evidence suggesting that long-patch excision repair does not occur in a *recF* mutant and is restored by an additional *recA441* mutation at 42°C in the presence of adenine. One possible explanation of this is that *recF*$^+$ is required for sufficient derepression of the *lexA* regulon to allow long-patch excision repair to occur.

The temperature-independent component of *recA441* suppression, like the suppression due to *srfA* mutations (28), was present in both *uvrA*$^+$ and *uvrA6* strains. We hypothesize that altered *recA* can either substitute for inactive *recF* protein or function independently of *recF* in a recombinational form of repair. This component of suppression was also visible in the *recB21 recC22 sbcB15* background, as judged by the increased UV resistance at 30°C (Fig. 3), indicating that the *recB*, *recC*, and *sbcB* gene products are not necessary for this type of recombinational repair. We did not, however, test whether this suppression at 30°C was *uvrA* independent. Efforts to determine whether *recA441* would suppress conjugational recombination deficiency caused by *recF143* in the same background were equivocal.

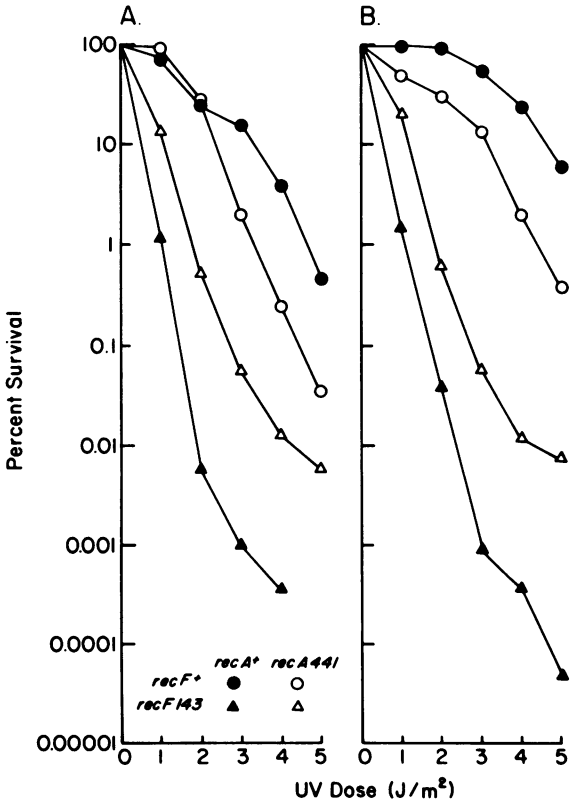

FIG. 2. Effect of *recA441* on UV survival of *uvrA6* strains carrying *recF*$^+$ or *recF143*. Cultures were incubated at 30 (A) and 42°C (B) for 1 h before irradiation. After irradiation, all plates were incubated at 30°C. The following strains were used: JC3912 *recA*$^+$ *recF*$^+$, MV1169 *recA441 recF*$^+$, JC3913 *recA*$^+$ *recF143*, and MV1171 *recA441 recF143*.

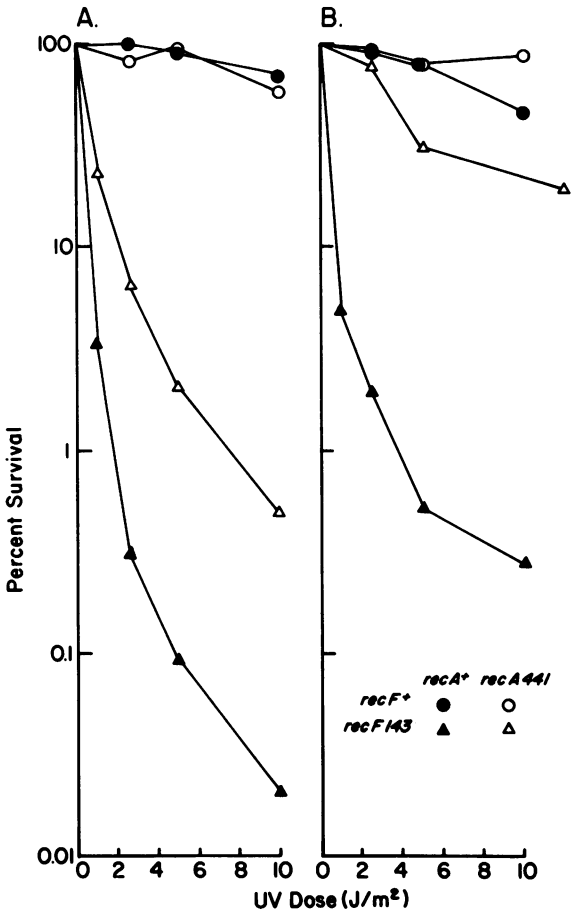

FIG. 3. Effect of *recA441* on UV survival of *uvrA*$^+$ *recB21 recC22 sbcB15* strains carrying *recF*$^+$ or *recF143*. Cultures were incubated at 30 (A) or 42°C (B) as described in the legend to Fig. 1. The following strains were used: JC11830 *recA*$^+$ *recF*$^+$, JC7597 *recA441 recF*$^+$, JC8111 *recA*$^+$ *recF143*, and JC7598 *recA441 recF143*.

Since *recA441* causes both temperature-dependent and -independent phenotypic changes, we offer two hypotheses to account for this. *recA441* may produce a mutant protein that is temperature insensitive but appear to be temperature sensitive because of its ability to take advantage of a thermally produced change in some wild-type component of nucleic acid metabolism. Alternatively, *recA441* may cause the *recA* protein to function independently of temperature in some metabolic processes and in a temperature-dependent fashion in others.

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