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Control of UV induction of recA protein

(*Escherichia coli*/UV irradiation/SOS functions/*recF*/immunoradiometric assay)

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ABSTRACT **The basal level of recA protein in *Escherichia coli* K-12 was estimated by an immunoradiometric assay; it is ≈1,200 molecules per wild-type bacteria in midexponential phase of growth, slightly more in an excision-deficient (*uvrA*) strain, and markedly more in *recF* mutants. Kinetics of induction after UV irradiation showed a rapid increase of recA protein content, which reached a peak level after 60–90 min (20- to 55-fold amplification) and then decreased by dilution of the protein in the growing population. In order to obtain an identical extent of induction of recA protein, a 10-fold higher UV dose was necessary in a wild-type strain compared to the *uvrA* mutant strain. In the *uvrA* strain, the presence of one or only very few pyrimidine dimers on DNA was accompanied by a measurable increase of the constitutive level of recA protein; however, the unexcised dimers were unable to permanently induce the formation of recA protein. The derepressed promoter of *recA* gene is one of the strongest in *E. coli*. Its sequence displays many similarities with that of the strongest early promoters of T5 phage. Mutants (*umuC uvrB* and *recF uvrB*) unable to carry out W-reactivation produced high levels of recA protein after UV irradiation. The data suggested that the *recF* and *umuC* genes negatively control the regulation of recA protein level.**

After DNA damage by radiation, chemical carcinogens, or treatments that inhibit DNA replication, bacteria display a complex set of metabolic reactions, the SOS response, in which a number of cellular functions are coordinately expressed after the damage (1).

In *Escherichia coli*, the SOS response is dependent on expression of the *recA*, *lexA*, and *ssb* genes and includes the following functions: induction of a mutagenic mode of DNA repair, prophage induction, W-reactivation, filamentation, and amplification of recA protein, previously called protein X (2, 3) (for reviews, see refs. 4 and 5).

Recently, *in vitro* studies have begun to elucidate the central role played by the *recA* product, recA protein, in these inducible functions. Thus, highly purified recA protein has been shown to possess a single-strand DNA-dependent protease activity capable of specifically cleaving both the phage λ repressor (6, 7) and the lexA protein (8). Cleavage of the λ repressor accounts for the induction of phage λ, whereas cleavage of the lexA protein can account for the induction of the recA protein itself because the lexA protein acts as a repressor of the *recA* gene and of genes controlling other SOS functions. The sequence of the promoter–operator region of the *recA* gene has been determined, and the lexA protein-binding region has been identified (9, 10). The recA protein also has been shown to be a DNA-dependent ATPase (11) and to catalyze the hybridization *in vitro* of homologous single-stranded DNA, accounting for its central role in homologous recombination (12, 13).

Despite the fact that recA protein plays an essential role in bacterial physiology, the only method to determine the amount of this protein was, until recently, the scanning of polyacrylamide gels (14). Because the intracellular concentration of recA protein might determine its binding ability to DNA and its overall ATPase and proteolytic activities, we have measured it in several *Escherichia coli* strains by using a two-site immunoradiometric assay (IRMA), which we have recently developed (15). Some information also was collected on the role supposedly played by UV-induced pyrimidine dimers in the derepression of the SOS functions. According to the model proposed by Little *et al.* (10), the rate of excision of these dimers controls the rate at which the recA protein decays after its UV induction. Therefore, we have established the kinetics of UV induction of recA protein in a wild-type strain and in excision repair-defective mutants. We also have measured the rate of synthesis of recA protein after derepression of its structural gene to evaluate the strength of the *recA* gene promoter.

Because no specific function has yet been assigned to the excess of recA protein that occurs after the arrest of DNA replication or DNA damages, we finally attempted to establish a link between this amplification and W-reactivation, another SOS function.

MATERIALS AND METHODS

Bacterial Strains. *Escherichia coli* K-12 strains are listed in Table 1.

Medium and Buffers. Bacteria were grown in M63 medium (22) supplemented with 0.2% glucose, 0.5% Casamino acids, and biotin (1 μg/ml) when needed. Their doubling time was 40–45 min. IRMA buffers have been described (15).

UV Irradiation. Bacteria were irradiated in M63 medium in a thin layer at a concentration of 1–2 × 10^8 cells per ml with gentle stirring. The UV lamp (Mineralight lamp; Ultraviolet Products Inc., San Gabriel, CA) emits radiation predominantly at 254 nm. UV fluences were measured with a J2620 Digital radiometer (Ultraviolet Products). After irradiation, bacteria were kept in the dark to avoid photoreactivation.

recA Protein Assay. Samples (3–20 ml) from culture in exponential growth were withdrawn at indicated times and centrifuged, and the pellet was resuspended in 2 ml of IRMA B buffer and kept on ice. Bacteria were sonicated by a Branson sonicator (50 W) three times for 30 sec. The lysate was centrifuged (10,000 rpm for 15 min). Total protein and recA protein concentrations were measured in the supernatant by the Lowry (23) and IRMA (15) procedures. Because bacteria form filaments after UV irradiation, the amount of recA protein could not be

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Abbreviation: IRMA, immunoradiometric assay.

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Table 1. Amplification of recA protein level in *E. coli* mutants after UV irradiation

Strains	Ref.	Relevant genotype	Basal level*	Amplification factor†
AB 1157	16		0.26 ± 0.1	25
AB 1886	16	*uvrA6*	0.30 ± 0.1	27
JC 3890	17	*uvrB301*	0.18 ± 0.06	32
TK 702	18	*umuC36*	0.20 ± 0.09	43
TK 501	19	*umuC36 uvrB301*	0.21 ± 0.07	55
JC 9239	20	*recF143*	0.45 ± 0.1	25
V 5650	21	*recF143 uvrB5*	0.62 ± 0.2	20

* Each value (ng/μg of soluble proteins) represents the mean of at least six independent measurements with different cell concentrations growing in exponential phase (± SEM).

† $\frac{\text{Maximum level after UV irradiation}}{\text{Basal level before UV irradiation}}$.

standardized to the number of cells and was expressed per μg of total soluble proteins.

RESULTS

Basal Level of recA Protein in Wild-Type Bacteria and Repair-Deficient Mutants. The recA protein content in exponentially growing bacteria (strain AB 1157) fluctuated only slightly (data not shown). During the midexponential growth of wild-type bacteria (OD_{578} up to 1), the basal level of recA protein ranged from 1,000 to 1,500 molecules per cell—i.e., 0.2–0.3 ng per μg of total soluble proteins as previously reported (15, 24) (with the assumption that an OD_{578} of 1 corresponds to 2 × 10^8 bacteria per ml). In the early phase of growth, this value was lower by a factor roughly equal to 2, whereas during the stationary phase of culture, it was slightly higher.

This relatively high concentration implies that recA protein is one of the most abundant proteins in *E. coli* (25). By comparison (see Table 1), the basal level of recA protein was slightly decreased in *uvrB*, *umuC*, *umuC uvrB*, possibly slightly increased in *uvrA*, and markedly increased in both *recF* and *recF uvrB* mutants. Identical variations also have been reported by Karu and Belk (24) and by Casaregola *et al.* (26) for *uvrA* mutant.

Induction of recA Protein After UV Irradiation in *uvrA* Bacteria. We followed the kinetics of induction of recA protein after UV irradiation at a fluence of 0.5, 1, and 3 J/m^2 in an excision-deficient strain (AB 1886) in exponential growth. This strain was selected because the pyrimidine dimers, which might be involved either directly or indirectly in the induction process, remain unexcised in the DNA throughout the whole experiment. After UV irradiation, the amount of recA protein increased rapidly and linearly up to 60–90 min, reached a maximum, and then decreased (Fig. 1). No early kinetic studies were performed. The half-time decay was about 2 hr (three generations), and recA protein concentration reached again the same basal level at about eight generations later. This decreased concentration could be due either to a dilution of the protein itself during bacterial growth or to its degradation at a later time, or both. In order to test these hypotheses, we compared the variations of the relative concentration of recA protein in two cultures, one of which was maintained in exponential phase throughout the whole experiment while the other was allowed to reach the stationary phase (Fig. 2). If degradation took place, the relative concentration of recA protein should decrease in both cases. On the other hand, if recA protein were diluted in the progeny, this concentration should stay constant during the stationary phase of growth. The experiment shown in Fig. 2 supports the dilution hypothesis.

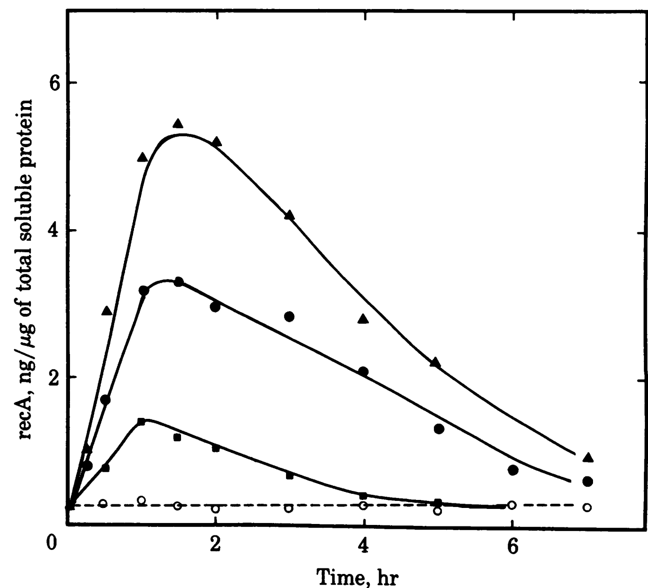

FIG. 1. Kinetics of induction of recA protein in *uvrA* strain (AB 1886). Irradiated bacteria (■, 0.5 J/m^2; ●, 1 J/m^2; and ▲, 3 J/m^2) were grown and diluted when needed to maintain the culture in exponential phase, and the recA protein content was determined. ○, Unirradiated bacteria. Each value was the mean of three independent experiments.

When bacteria were preincubated with chloramphenicol (100 μg/ml; 15 min before irradiation), the UV irradiation (3 J/m^2) did not modify the concentration of recA protein.

The dose–response curve of the recA protein induction was determined (Fig. 3). Irrespective of the UV fluence, the concentration of recA protein reached a peak in a nearly constant time interval.

There is an apparent linear relationship between the amount of UV radiation delivered to the cells up to 2–3 J/m^2 and the peak concentration of recA protein (Fig. 3 *Inset*). Because no excision repair mechanism functions in the *uvrA* strain used

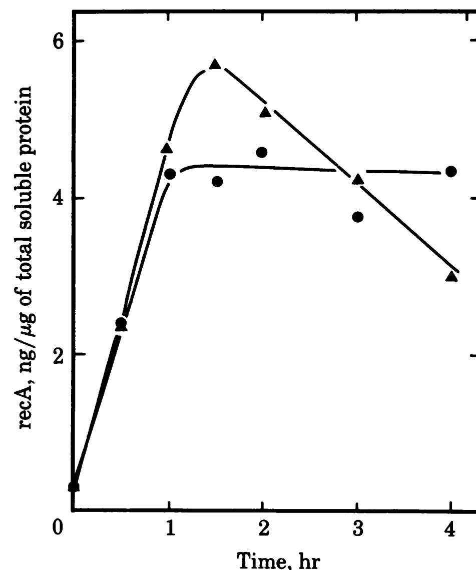

FIG. 2. Kinetics of induction of recA protein in *uvrA* strain (AB 1886). Irradiated bacteria (3 J/m^2) were either diluted 1:10 (▲) and grown exponentially during the experiment or were grown without dilution (●); in the later case, they reached a stationary phase after 1 hr of postirradiation incubation.

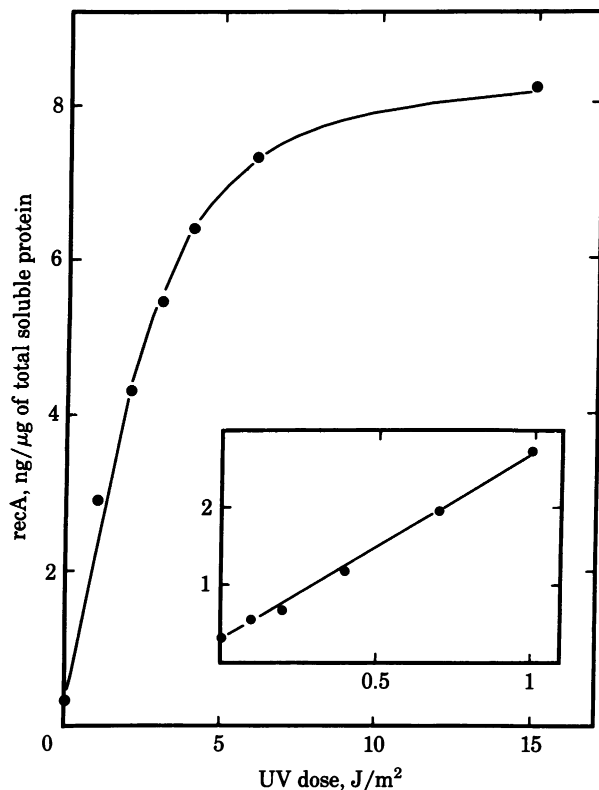

FIG. 3. UV dose–recA protein induction relationship in *uvrA* bacteria. Bacteria (AB 1886) were irradiated and then incubated at 37°C with aeration for 90 min. Each value was the mean of two independent experiments.

here, the increased number of unremoved pyrimidine dimers is paralleled by an increased rate of recA protein synthesis. A dose of 0.2 J/m^2, which induces about 10 dimers per genome (27), provoked a 2-fold increase of the maximal amount of recA protein; the processes that regulate the level of intracellular recA protein are thus highly responsive to UV effects. We did not detect any threshold because an UV dose that generates one or a few dimers was able to increase significantly (≈10%) the level of recA protein above its constitutive value.

The maximal concentration of recA protein formed leveled off at about 15 J/m^2 probably because one or several steps of the inducible process were saturated.

Induction of recA Protein After UV Irradiation in Wild-Type Bacteria. Because we found an apparent linear relationship between the number of lesions in the DNA and the rate of induction of recA protein synthesis (Fig. 3), we compared the amount of recA protein induced in wild-type bacteria, which are able to excise pyrimidine dimers, and *uvr*$^-$ bacteria. Similar kinetics of induction were observed in wild-type bacteria as in *uvrA* (data not shown), and a comparison of the extent of amplification of recA protein synthesis in each strain is shown in Fig. 4*a*. In both cases, the amount of recA protein increased with the UV dose and reached a plateau. On the other hand, in order to observe the same amplification of recA protein, a 8- to 10-fold-higher UV dose was needed in the wild-type than in the excision-deficient strain as has been observed for other SOS functions, such as prophage induction (28, 29), W-reactivation (30), and mutagenesis (31). This result suggests that the presence of pyrimidine dimers in the DNA is necessary for the induction of recA protein to occur.

The maximum amplification corresponded to 30,000–50,000 molecules of recA protein per cell (Fig. 4*a*) (1–2% of total protein

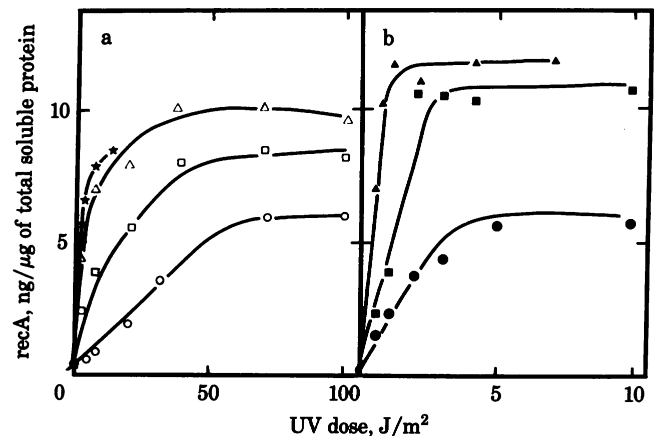

FIG. 4. UV dose–recA protein induction relationship in wild-type and repair-deficient strains. Bacteria were irradiated and then incubated at 37°C with aeration for 2 or 3 hr, depending on the strain (see *Results*). (*a*) ★, *uvrA*; □, *umuC*; △, *recF*; ○, wild type. (*b*) ●, *uvrB*; ■, *umuC uvrB*; ▲, *recF uvrB*.

content), in agreement with previous observations (14); it was repeatedly found to be larger in *uvrA* mutants (Fig. 4*a*) than in the wild-type strain.

Relationship Between recA Protein Induction and W-Reactivation. No role has been ascribed yet to the excess of recA protein in induced *E. coli*. On the contrary, it has been shown that in order to be converted into a protease, as measured by its capacity to inactivate the phage λ repressor (32–35), recA protein concentration does not need to be amplified. On the other hand, a large amplification of recA protein content is not necessarily associated to phage λ induction (2, 7). Here we attempted to establish a correlation between recA protein amplification and W-reactivation.

We used five *E. coli* mutants affecting some of the SOS functions (and particularly W-reactivation of phage λ) but retaining the protease activity of recA protein and, therefore, being completely proficient in prophage induction. Thus, we measured the extent of amplification of recA protein content in *uvrB* (excision deficient), *umuC* (blocked in UV mutagenesis), *recF* (deficient in a minor pathway of recombination), *umuC uvrB* and *recF uvrB* mutants. The first three mutants affect somehow W-reactivation (20) (M. Defais, personnal communication), recF being the most efficient in reducing this phenomenon (36). The last two double mutants completely prevent W-reactivation (36, 37). Kinetics of induction of recA protein were performed after UV irradiation and again showed a maximal induction at about 90 min after irradiation, except for *recF* and *recF uvrB* mutants. In the latter cases, the maximal amplification was delayed to 3 hr (data not shown). A similar delay in prophage induction has been reported for these two strains (21). Maximal recA protein concentrations with increasing doses of UV radiation were determined. The curves are shown in Fig. 4.

The kinetics of recA protein induction in these strains being comparable to those of W-reactivation-proficient strains such as wild type or *uvrA*, it can be concluded that the excess of recA protein that is synthesized in UV-treated W-reactivation-deficient strains is unable to complement the missing functions of *umuC uvrB* and *recF uvrB* mutants that participate in the W-reactivation and mutagenesis. However, it was remarkable that the introduction of mutation within the *umuC* gene, or more markedly within the *recF* gene, improved the capacity of the bacteria to synthesize recA protein by a factor of 2 in the case of *umuC* and by a factor of 6 in the case of *recF* for the lowest UV doses. This result should be taken together with the ob-

servation of the increase of basal recA protein content in uninduced *recF* mutant (Table 1).

DISCUSSION

Our values of the level of recA protein in *E. coli* totally agree with those of Karu and Belk (24), which also have been obtained by an immunometric assay. They significantly differ from the reported results of Casaregola *et al.* (26) who did not note the changes of basic recA protein concentration in mutated strains, especially in *recF* mutants. This failure probably results from artifacts associated with their enzymatic method, which is an indirect one because it relies on the induction of a fused lac operon under the control of the promoter of *recA* gene inserted in phage λ genome. A probable artifact is the occurrence of polar or attenuation effects as suggested by the underestimation of the values of recA protein concentration reported by these authors.

After UV irradiation, a rapid increase of the amount of recA protein is observed in wild-type bacteria and in the pyrimidine dimer-excision-deficient mutants *uvrA* or *uvrB*.

The rate of recA protein synthesis in the *uvrA* mutant is linearly proportional to the UV dose up to 2 J/m^2. It is maintained constant above 15 J/m^2. The maximal concentration is reached 60–90 min after irradiation. This value is in contrast with the time necessary for other SOS functions to reach their maximum (31, 38), which is around 30 min. Such a discrepancy means that those functions (W-reactivation and bacterial and phage mutagenesis) do not require the full level of recA protein to be reached. Moveover, the inducing signal(s) and the subsequent intermediate substrates should be channeled through multiple steps that ensure the controlled buildup of recA protein from the initial unknown signal generated by the pyrimidine dimers or processed from them; the rate at which these signals are processed appears to be independent of UV fluence because the peak of recA protein is reached at identical times (60–90 min) whatever the initial UV dose. When the kinetics and the UV dose–response relationship are compared in wild-type, *uvrA*, and *uvrB* strains, three conclusions can be drawn.

(*i*) The machinery controlling the content of recA protein in *E. coli* is extremely sensitive to the occurrence of the damages generated by UV irradiation, very probably pyrimidine dimers. One such dimer is sufficient to increase the basal level of recA protein by about 10%. This amplification phenomenon provides an opportunity to detect substances acting on DNA, most of them being cytotoxic, mutagenic and carcinogenic. Therefore, we propose to complement the Ames test with an immunoradiometric assay applied to an *E. coli* recA protein-like antigen that has been characterized and purified in *Salmonella typhimurium* (15, 39). The capability to produce recA protein in *E. coli* is 5- to 10-fold more sensitive to an identical dose of UV irradiation than is the mutability of the Ames tester strains of *S. typhimurium* (40).

(*ii*) The rate of recA protein synthesis is roughly 10-fold higher in the UV-treated *uvrA* and *uvrB* mutants than in the control wild type having received the same UV dose. These data can be explained if one assumes that the pyrimidine dimers are, directly or indirectly, involved in the processes that lead to *recA* gene derepression. Most of these dimers are probably excised in *uvr*$^+$ strain by specific endonucleases; consequently, they are not allowed to participate in the processes generating the signals for the induction of recA protein synthesis, which seems to be slower than the excision repair mechanism. However, no quantitative precise conclusions regarding the excision rate can be drawn from these results because the content of recA protein in the wild-type strain is about half that in the *uvrA* mutant at high UV dose. This observation indicates that the product of the *uvrA* gene is not only endowed with endonucleolytic activities but also might exert a negative control on the expression of recA protein, as the DNA-binding properties of the uvrA protein suggests (41).

(*iii*) Pyrimidine dimers can be removed from cellular DNA by two major mechanisms only: photoreactivation and excision repair (4). In our experimental conditions, which do not allow any photoreactivation, there is no removal of dimers from DNA in *uvrA* or *uvrB* mutants. Nevertheless, no persisting induction of recA protein was detected in these mutants, which display similar kinetics of recA protein enhancement and disappearance as those of the wild type; after the initial burst of recA protein synthesis, the remaining dimers are unable to further induce it and to maintain the peak level of recA protein. The exhaustion of some unknown cofactor requested for the induction process or a retroinhibition control of its own synthesis by an excess of recA protein might explain these data. A rapid neutralization of the inducing effects of UV irradiation, for instance a preferential binding of recA protein to gapped DNA regions, is a more plausible hypothesis.

Our results establish also that the derepressed promoter of *recA* gene is one of the strongest ones in *E. coli*. The initial rate of synthesis of recA protein is about 10 molecules per sec for high UV doses. This value approaches the rate of synthesis of the product of *tufA* gene, one of the two constituents of EF-Tu, which is known to have the highest rate in *E. coli*, equal to 34 molecules per sec (25). It is remarkable that the base sequence

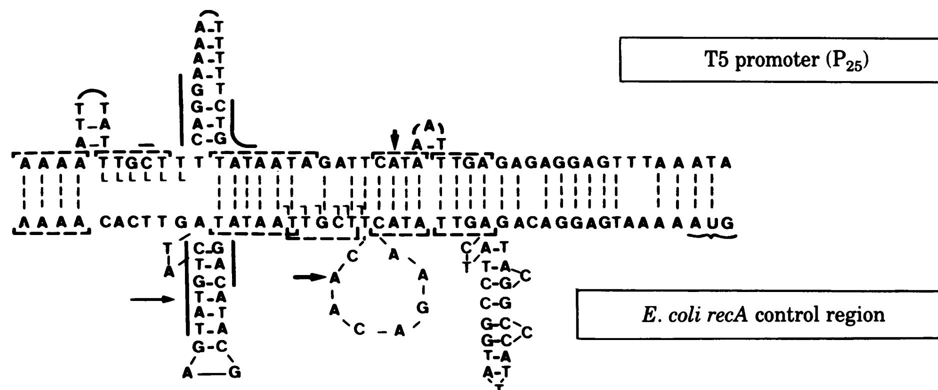

FIG. 5. DNA sequence homology between the promoter–operator region of *E. coli recA* gene and a strong promoter of the early region of T5 phage: P$_{25}$ (42, 43). ——, Consensus sequences of *lexA* binding site in *recA* and *lexA* gene (9, 10); – – –, sequences common to *E. coli recA* gene and T5 early promoters.

of the promoter region of the *recA* gene shows many similarities (Fig. 5) with the sequence of one of the early promoters of T5 phage (P25) (42, 43).

The basal level of recA protein is slightly increased in an unirradiated *recF* mutant (Table 1). For the same UV dose, the concentration of recA protein is 5- to 6-fold higher in *recF* and *recF uvrB* mutants than in the control strains—i.e., wild type and *uvrB* (Fig. 4*a*).

These results are not completely unexpected because the persistence of gaps in the DNA of *recF* strain has been documented (44) and might be responsible for a partially constitutive expression of the recA protein operon.

Furthermore, it has been speculated that the binding of recA protein to single-stranded chromosomal DNA might be favored by recF product (45); it also was proposed that the recF product might compete with other products—namely, ExoI, ExoV, ExoVIII, and its inhibitor—for control of single-stranded DNA oligonucleotides, which possibly regulate the level of activity and, consequently, the amount of recA protein (46). In addition, some phenotypic traits of the *recF* mutants—hyperinducibility (i.e., ability to yield phages at a low dose of UV or mitomycin C), UV hypermutability, and high spontaneous content of the *in vivo* cleavage product of phage λ repressor, R′ (6)—might be accounted for by such a constitutive increase of recA protein. Finally, one hypothesis could be that the *recF* gene negatively controls the expression of the recA gene. This proposition is supported by other published data which suggest that *recF* gene product may prevent the induction of recA protein by nalidixic acid in mutated strains of *recBC* exonuclease V (24).

Our results also suggest that the product(s) of *umuC* also might regulate negatively the level of recA protein.

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