JOURNAL OF BACTERIOLOGY, Aug. 1985, p. 688–695
0021-9193/85/080688-08$02.00/0

Vol. 163, No. 2

Isolation of Protease-Proficient, Recombinase-Deficient *recA* Mutants of *Escherichia coli* K-12

ETHEL S. TESSMAN* AND PATRICIA K. PETERSON

Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

Received 17 December 1984/Accepted 14 May 1985

We isolated *recA* mutants with altered protease activity and then examined recombinase activity to determine whether the protease and recombinase functions of the RecA protein of *Escherichia coli* are separable. We found five mutants that had moderately strong constitutive RecA protease activity but no recombinase activity above the Δ*recA* strain background, the first clear-cut examples of mutants of this class, designated Prt^c Rec$^-$. We also isolated 65 mutants that were protease-defective toward the LexA repressor and found that all of them were also recombinase deficient. Four of these mutants retained both partial recombinase activity and partial inducible protease activity. The recombinase-defective mutants were much more sensitive than the *recA*$^+$ strain to crystal violet, kanamycin, and chloramphenicol, indicating altered membrane permeability. The *recA* (Prt^c Rec$^-$) mutants had a subtle alteration in protease specificity, all being defective in spontaneous induction of phages λ *imm*^{434} and 21. They differed from Prt^c Rec$^+$ mutants of comparable or even weaker constitutive protease strength, all of which showed dramatic spontaneous induction of these prophages. However, treating a Prt^c Rec$^-$ mutant with mitomycin C resulted in significant prophage induction. Thus, the RecA proteins of the Prt^c Rec$^-$ mutants have constitutive protease activity toward the LexA repressor, but have only DNA damage-activable protease activity toward phage repressors. UV-induced mutagenesis from *his* to *his*$^+$ was studied for one Prt^c Rec$^-$ mutant, and induced mutation frequencies as high as those for the *recA*$^+$ strain were found despite the absence of recombinase activity.

Although the RecA protein of *Escherichia coli* has been shown to have multiple functions in cell metabolism under certain conditions (9, 12; T. Kogoma, H. Bialy, N. L. Subia, T. A. Torrey, G. G. Pickett, and K. von Meyenburg, *in* M. Shaechter, F. C. Niedhardt, J. Ingraham, and N. O. Kjelgaard ed., *Molecular Biology of Bacterial Growth*, in press), it appears to have two primary functions: a recombinase function for homologous genetic recombination (3) and for recombinational repair of DNA damage caused by exposure to radiation and to radiomimetic drugs (22, 23, 26), and a protease function in which RecA acts as a protease or a protease cofactor (13) to cleave the LexA repressor protein, inducing the expression of a set of unlinked genes called the SOS system (14, 18, 28, 30), some of which encode proteins involved in repairing damaged DNA. Although it is not yet certain whether RecA is a true protease or merely increases the rate of substrate autodigestion (13), the term RecA protease will be retained for convenience.

The aim of the present work was to determine whether the RecA protease and recombinase functions could be mutationally separated from each other. Separating the functions might permit the assignment of each function to a distinct region of the RecA protein after sequence analysis of DNA from appropriate mutants. We sought mutants that were protease proficient but recombinase deficient and vice versa. Our mutation isolation method required first detecting *recA* mutants with altered RecA protease activity toward the LexA repressor. This isolation method yielded mutants, designated *recA* (Prt^c), that had constitutive protease function and also mutants that were both protease defective and recombinase defective, designated *recA* (Prt$^-$ Rec$^-$) (27). In the *recA* (Prt^c) mutants, RecA protein is always in the protease state without the usual need for DNA damage to activate it. The mutant isolation procedure involved detecting plaques of mutagenized λ *recA* that had altered plaque colors when plated on a strain with a *lac* fusion in a RecA-inducible gene (27). After screening *recA* (Prt^c) mutants, we found five mutants that had substantial constitutive protease function but lacked any RecA recombinase activity; they were designated *recA* (Prt^c Rec$^-$). Until now the only somewhat similar mutant known has been a *recA142* strain (2, 8, 21) which has very slight constitutive protease activity and a low level of DNA damage-activable protease activity and is recombinase deficient. We describe here both the Prt^c Rec$^-$ mutants and the Prt$^-$ Rec$^-$ mutants. We show that the two RecA functions are separable but only to a limited extent.

MATERIALS AND METHODS

Procedures and media not mentioned here are described in the accompanying paper (27).

Phenotype symbols. Phenotype abbreviations used are as follows: Prt^c, constitutive protease activity; Prt$^+$, wild-type level of DNA damage-activable protease activity; Prt$^\pm$, partial damage-activable protease activity; Prt$^-$, absence of protease activity; Rec$^+$, at least 50% of the wild-type recombination frequency as measured in an Hfr liquid culture mating; Rec$^-$, less than 1% of the wild-type recombination frequency; Rec$^\pm$, 1 to 50% of the wild-type recombination frequency.

Bacterial strains. Most of the bacterial strains used in this work and also the parental λ *recA* phage are described in the accompanying paper (27). The *recA* mutants described here, except for a few listed in Table 4, were all constructed by using λ *recA* cI *ind* mutant phages to lysogenize strain EST1515 [Δ*recA sfiA dinD*::Mu d(Ap *lac*)]. The wild-type strain was EST1450 [EST1515(λ *recA*$^+$ cI *ind*)]. Other strains used are EST1544 [EST1515(λ *imm*^{434})]; EST1625 [EST1515(φ21)]; EST1584 [EST1515(λ *imm*^{434})(λ *recA*$^+$)]; EST1583 [EST1515(λ *imm*^{434})(λ *recA1203*) Prt^c Rec$^-$]; and

* Corresponding author.

EST1694 [EST1515(λ *recA1203*) Prt^c Rec$^-$]. Strain EST1663 is the *lexA71* (Def) derivative of the Δ*recA* strain EST1515. Another *lexA71* (Def) strain used here was EST1664 [EST1663(λ *recA1203*)]. Strains containing the *sulA*::*lac* fusion were constructed by lysogenizing strain EST1855 (27), which is Δ*recA sulA*::Mu d(Ap *lac*), with the desired λ *recA* mutant phage or with λ *recA*$^+$.

UV irradiation. To obtain survival curves, log-phase cells grown in M9-CAA medium (15) were centrifuged twice, suspended in M9 salts (15) at a concentration of 2×10^7 cells per ml, and shaken while being irradiated 45 cm from a 15-W germicidal lamp. To obtain a low UV fluence rate, the lamp was shielded by a cardboard sheet in which a slot was cut that was calibrated by using UV survival curves of λ *vir*. UV-sensitive strains were irradiated at a fluence rate of 0.07 J/m^2 per s. Plating was done on M9-CAA plates (27), followed by incubation at 35°C.

Measurement of inducible mutagenesis. The frequency of UV-inducible mutagenesis from *his* to *his*$^+$ was measured by irradiating cell suspensions spread on limiting histidine plates (27). Cultures were grown in M9-CAA medium to a concentration of about 10^9 cells per ml and centrifuged twice, each time being resuspended in M9 salts. Wild-type cells were not concentrated after resuspension, whereas the *recA* (Prt^c Rec$^-$) strain was concentrated 20-fold. Samples (0.1 ml) of resuspended cells were spread on limiting histidine plates and irradiated at a UV fluence rate of 0.07 J/m^2 per s. Plates were incubated at 35°C for 3 to 5 days, and the number of *his*$^+$ colonies per plate was counted. The 20-fold concentration of the cells and irradiation on the plate were necessary because the *recA* (Prt^c Rec$^-$) strain had a low efficiency of plating (EOP) (10%) and a high spontaneous mutation rate. Therefore, large numbers of irradiated cells had to be examined to detect mutagenesis above the spontaneous level. For several different *recA* (Prt^c) alleles, including *recA1203*, *recA1211*, and *recA1212*, the number of spontaneous mutants was independent of the initial number of cells spread over the range from 10^6 to 10^9 cells per plate, depending only on the final number of cells in the film on the plate, which was 2×10^8. In contrast, the number of UV-induced mutants per plate depended only on the initial number of surviving cells spread. The number of surviving cells at each UV dose was determined in a manner similar to that described by Witkin (29), by washing the cells off a duplicate set of plates after irradiation and plating the cell washings on limiting histidine plates for colony counting after suitable dilution. Plate washes were performed by pipetting 2 ml of M9 salts onto each plate, dislodging the cells with a spreader, and then removing the wash liquid and weighing it to determine the volume so as to calculate the total cell number.

Measurement of recombination frequencies. Spot tests for recombination were carried out as described by Clark and Margulies (3) by spotting each mutant on a lawn of HfrH (strain CSH62) on selective plates. Recombination to Thr$^+$ Leu$^+$ Str^r was scored by formation of spots of growth.

Liquid culture Hfr matings were performed by growing the donor strain CSH62 and the F$^-$ recipient strain in M9-CAA medium to about 2×10^8 cells per ml at 37°C and then mixing them at a ratio of 1 donor cell to 10 recipient cells. The mixture was gently shaken for 1 h at 37°C and then vortexed, diluted through M9 salts, plated on selective plates, and scored for recombination to Thr$^+$ Leu$^+$ Str^r after 48 h of incubation at 35°C.

Spot test for ability of λ *recA* (Prt^c) phages to spontaneously cleave phage 434 and 21 repressors. If a Δ*recA* cell is lysogenic for a lambdoid phage having a wild-type repressor, introduction of a *recA* (Prt^c) allele should result in spontaneous cleavage of the repressor followed by induction of the resident prophage and lysis of the cell because of the constitutive RecA protease activity. We used this principle for a spot test to determine whether *recA* (Prt^c Rec$^+$) and *recA* (Prt^c Rec$^-$) alleles present on λ *recA* phages could give efficient spontaneous cleavage of the repressors of resident prophages 434 and 21 in indicator bacteria. The two indicator strains used were EST1544 Δ*recA dinD sfiA*(λ *imm*^{434}) and EST1625 Δ*recA dinD sfiA*(φ21). These indicator strains were plated in top agar on lambda plates (27), and then plaque pickings of a series of mutant λ *recA* (Prt^c) *cI ind* phages were spotted in duplicate on the seeded top agar together with the λ *recA*$^+$ *cI ind* control phage. All the phages were also spotted on strain EST1515 to eliminate those which formed clear spots because of mutations in phage genes. After incubation overnight at 37°C, the plates were scored for formation of clear or turbid spots.

RESULTS

Protease activity and UV sensitivity of *recA* (Prt^c Rec$^-$ mutants. Screening 26 dark-blue *recA* mutant colonies for UV sensitivity revealed six novel Prt^c mutants that were very UV sensitive in spot tests, suggesting that they might be Rec$^-$. Liquid-culture mating tests with an Hfr strain showed that five of these mutants were indeed as recombinase defective as the Δ*recA* control strain, whereas the sixth mutant showed some residual recombinase activity (Table 1). These *recA* (Prt^c Rec$^-$) mutants had about 35% of the β-galactosidase specific activity (expressed from *dinD*::*lac*) of the strongest *recA* (Prt^c Rec$^-$) mutants (27). Since Prt^c Rec$^+$ mutants of this same protease strength form medium-blue, not dark-blue, colonies, we assume that the dark-blue colony color of the Prt^c Rec$^-$ mutants is due in part to their altered membrane permeability (see below). All six Prt^c Rec$^-$ mutants had their β-galactosidase specific activity increased 1.5- to 2.0-fold by mitomycin C. The increase in specific activity caused by mitomycin C was due to an absolute increase in the β-galactosidase concentration and was not an artifact of a decrease in growth rate (as measured

TABLE 1. Properties of *recA* (Prt^c Rec$^-$) mutants

Strain	Allele no.	β-Galactosidase sp act[a] (U)	Relative recombination frequency[b]	Inhibition by C+G[c]
EST1550	*lexA71* (Def) *recA*$^+$	200	NT	−
EST1450	*recA*$^+$	10	1.0	−
EST1472	*recA1205*	59	1×10^{-2}	−
EST1446	*recA1201*	50	1×10^{-4}	+ + +
EST1694	*recA1203*	69	2×10^{-4}	−
EST1683	*recA1204*	67	5×10^{-4}	−
EST1454	*recA1206*	52	2×10^{-4}	−
EST1115	*recA1207*	58	1×10^{-4}	−
EST1515	Δ*recA306*	7	3×10^{-4}	−

[a] β-Galactosidase activity expressed constitutively from the SOS gene *dinD*::*lac* was measured as described previously (15).

[b] Recombination frequencies were measured in liquid culture matings with an HfrH strain and plating for Thr$^+$ Leu$^+$ Str^r recombinants on selective plates. Reversion of the recipients to Thr$^+$ Leu$^+$ was not observed. Therefore, recombination frequencies on the order of that obtained for the Δ*recA* strain, ca. 5×10^{-4}, reflect RecA-independent recombination. NT, Not tested.

[c] Measured as described in reference 27. Symbols: −, not inhibited by C+G; + + +, inhibited by C+G down to the *recA*$^+$ strain basal level.

by the optical density at 600 nm [OD_{600}]) in the presence of mitomycin C. Thus, these mutants showed damage-activable protease activity as well as constitutive protease activity. The enhancement of protease activity is shown for one Prt^c Rec^- mutant in Fig. 1.

Five of the six Prt^c Rec^- mutants were not inhibited by a combination of cytidine plus guanosine (C+G), which appear to be precursors of negative effectors of constitutive protease activity (27). The sixth Prt^c Rec^- mutant (*recA1201*) was severely inhibited by C+G but gave an anomalous response to effectors. It differed from all the Prt^c Rec^+ mutants tested in that it did not respond to DNA damage in the presence of C+G (27). In contrast, Prt^c Rec^+ mutants that were inhibited by C+G had damage-activable protease activity like the wild-type in the presence of C+G. We showed previously for Prt^c Rec^+ mutants (27) that the absence of C+G inhibition always correlated with very high constitutive protease strength. This correlation did not apply to the noninhibitable Prt^c Rec^- mutants, which had only about one-third of the constitutive protease strength (as measured by induction of *dinD*) that might have been expected on the basis of their noninhibitable Prt^c phenotype.

One Prt^c Rec^- mutant (*recA1203*) was studied for UV sensitivity. It was very UV sensitive compared with the *recA*$^+$ strain, although somewhat less sensitive than the isogenic Δ*recA* control strain (Fig. 2). The UV survival curves of *lexA* (Def) derivatives of the *recA1203* and Δ*recA* strains are also shown in Fig. 2. These data permit the contribution of the inducible components of excision repair and mutagenic repair to the survival of recombination-deficient cells to be estimated. Only constitutive levels of the repair proteins should be present in the *lexA*$^+$ Δ*recA* strain; in contrast, nearly maximal or maximal inducible levels of the repair proteins should be present in three derepressed strains carrying *recA1203* (Prt^c Rec^-), *lexA* (Def) *recA1203*, and *lexA* (Def) Δ*recA*. These derepressed strains were only

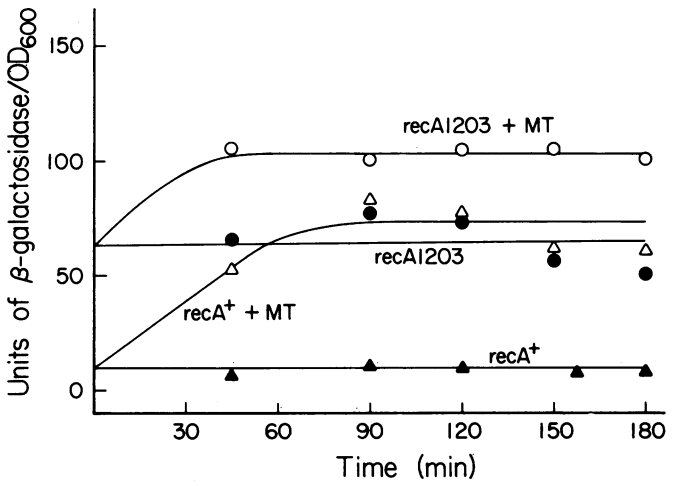

FIG. 1. Enhancement by mitomycin C (MT) of RecA constitutive protease activity of a Prt^c Rec^- mutant, *recA1203*, indicated by the specific activity of β-galactosidase expressed from *dinD*::*lac* as a function of growth time at 35°C. The *recA1203* (Prt^c Rec^-) strain (●, ○) and the isogenic *recA*$^+$ control strain (▲, △) were grown overnight in M9-CAA medium and then diluted 50-fold in the same medium and grown to an OD_{600} of about 0.1 (zero time). Mitomycin C (0.5 μg/ml) was then added (○, △). Incubation at 35°C was continued, and samples were removed at the times indicated for assay of β-galactosidase specific activity.

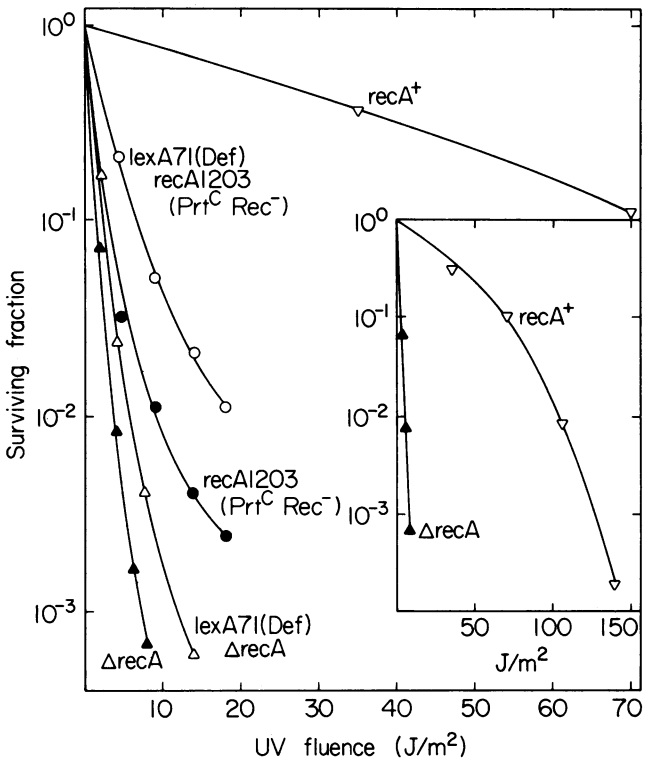

FIG. 2. Effect of UV irradiation on survival of a Prt^c Rec^- mutant, *recA1203* (●), the isogenic control strains, Δ*recA* (▲) and *recA*$^+$ (▽), *lexA71* (Def) Δ*recA* (△), and *lexA71* (Def) *recA1203* (○). Cells were grown in M9-CAA medium, centrifuged twice, suspended in M9 salts, and irradiated with a 15-W germicidal lamp at a fluence rate of 0.07 J/m^2 per s while being stirred. The irradiated cells were plated on M9-CAA plates and incubated at 35°C.

a little more UV resistant than the *lexA*$^+$ Δ*recA* strain, with the *lexA* (Def) *recA1203* strain being the most resistant.

UV-induced mutagenesis of a Prt^c Rec^- mutant. We studied UV-induced mutagenesis of a Prt^c Rec^- strain because we wanted to determine whether recombinase activity of the RecA protein was needed for mutagenesis of the bacterium. We determined mutation frequency as a function of UV fluence for the *recA1203* (Prt^c Rec^-) and *recA*$^+$ strains (Table 2). The frequency of UV-induced mutagenesis was as high for the Prt^c Rec^- strain as for the *recA*$^+$ strain. The finding of UV-induced mutagenesis for the *recA1203* strain indicates that recombinase activity is not needed for UV-induced mutagenesis.

Prt^c Rec^- mutants are defective in constitutive induction of lambdoid prophages. The six Prt^c Rec^- mutants found here were isolated as Prt^c mutants on the basis of their ability to spontaneously cleave the LexA repressor. However, these mutants all appeared to be defective in spontaneous cleavage in vivo of the repressors of φ21 and λ *imm*^{434}. This was observed by spotting all the λ *recA* (Prt^c) phages on lawns of two lysogenic strains, EST1625 [Δ*recA*(φ21)] and EST1544 [Δ*recA*(λ *imm*^{434})], to see whether the phages formed clear spots. We found that the parental phage, λ *recA*$^+$ *cI ind*, formed turbid spots on these lysogenic indicators, whereas the phages corresponding to the Prt^c Rec^+ mutants, even weak Prt^c Rec^+ mutants, formed clear spots due to induction of the resident prophages and lysis of the cells. In contrast to the formation of clear spots by the λ *recA* (Prt^c Rec^+) phages, all six λ *recA* (Prt^c Rec^-) phages formed turbid spots

TABLE 2. UV-induced mutation in the Prt^c Rec$^-$ mutant strain *recA1203* and in the *recA*$^+$ strain

recA allele	UV fluence (J/m^2)	Avg no. of *his*$^+$ colonies per plate ± SEM[a]	No. of surviving cells per plate[b]	Frequency[c] of *his*$^+$ mutants (10^{-8})
recA1203 (Prt^c Rec$^-$)	0.0	12 ± 1	1.7×10^8	6.0
	0.35	59 ± 4	NM	
	0.70	92 ± 10	7.0×10^7	114
	1.05	91 ± 4	2.7×10^7	298
	1.40	89 ± 1	1.7×10^7	456
	1.75	93 ± 5	2.4×10^7	337
	2.1	85 ± 6	7.4×10^6	1,150
recA$^+$	1.1	1 ± 0.2	8.0×10^7	0.5
	0.7	11 ± 3	NM	13
	1.4	48	NM	60
	2.1	99 ± 11	NM	124
	2.8	179 ± 23	8.4×10^7	212
	17.5	254	8.1×10^7	318
	35.0	430	7.8×10^7	550

[a] In most cases three plates were UV irradiated at each fluence immediately after being spread and were then incubated for 4 days at 35°C. The error is the standard error of the mean; no error indicates that only one plate was counted.

[b] Duplicate plates were spread and irradiated and then were immediately washed with M9 salts, and the cells were dislodged with a spreader. The cells washed off each plate were weighed to determine the volume of liquid for calculation of total cells per plate. These cells were then suitably diluted and plated on limiting histidine plates to determine their concentrations. NM, Not measured.

[c] The spontaneous mutation frequency was independent of the initial number of cells spread over a 1,000-fold range, depending only on the final number of cells in the film, 2×10^8. In contrast, the UV-induced mutation frequency depended only on the initial number of surviving cells spread. The number of spontaneous *his*$^+$ colonies was subtracted from the total number before the induced frequencies were calculated. When the number of surviving cells was not counted, the mutation frequency was calculated on the assumption that cell survival was 100%.

on the lysogenic indicators, showing that they failed to spontaneously induce the resident prophages of the indicator strains. The ability of a λ *recA* (Prt^c) phage to form clear or turbid spots on indicator strains lysogenic for λ *imm*^{434} or φ21 was not related to the constitutive protease strength of the corresponding Prt^c Rec$^+$ or Prt^c Rec$^-$ strain (Table 3). As a control, almost all the λ *recA* (Prt^c Rec$^+$) mutant phages used were found to form turbid spots on the isogenic strain EST1515, which lacked any resident inducible prophage.

Since the λ *recA* (Prt^c Rec$^-$) phages formed turbid spots on the lysogenic indicators, it seemed likely that the cells in the spot would include stable double lysogens. This turned out to be the case. Cells picked from the turbid spots were immune to both the λ *recA* (Prt^c Rec$^-$) phage and to λ *imm*^{434} and φ21, depending on which lysogenic indicator was used for the spot test. As to the possibility that the ability of λ *recA* (Prt^c Rec$^-$) phages to form stable double lysogens on lambda plates might be due to inhibition of constitutive protease activity by C+G in the tryptone broth plating medium, it should be noted that five of the six Prt^c Rec$^-$ mutants, including the *recA1203* strain, were not inhibited by C+G, whereas most of the Prt^c Rec$^+$ mutants, which gave clear spots, were greatly inhibited (27).

In the Δ*recA* strain (EST1583) that was lysogenic for both λ *imm*^{434} and λ *recA1203* (Prt^c Rec$^-$), there was a wild-type spontaneous level of prophage induction (Fig. 3), with the majority of the phage yielded being λ *imm*^{434}. We wished to see whether this spontaneous induction could be enhanced by DNA damage produced by mitomycin C. The comparison strain, EST1584, was lysogenic for λ *recA*$^+$ as well as for λ *imm*^{434}. Mitomycin C did increase the phage yield of the Prt^c Rec$^-$ strain 10-fold and of the *recA*$^+$ strain 100-fold (Fig. 3).

TABLE 3. Ability of prophages to be spontaneously induced by λ *recA* (Prt^c) phages versus the constitutive protease strength of the corresponding lysogens

Phage	Phenotype	Spot clarity[a]	β-Galactosidase sp act (U) of corresponding lysogen[b]
λ *recA1217*	Prt^c Rec$^+$	Clear	194
λ *recA1230*	Prt^c Rec$^+$	Clear	57
λ *recA1203*	Prt^c Rec$^-$	Turbid	69
λ *recA1204*	Prt^c Rec$^-$	Turbid	67
λ *recA1207*	Prt^c Rec$^-$	Turbid	58
λ *recA1237*	Prt^c Rec$^+$	Clear	33
λ *recA1231*	Prt^c Rec$^+$	Clear	23
λ *recA1232*	Prt^c Rec$^+$	Clear	23

[a] Clarity of spots formed by λ *recA* (Prt^c) cI *ind* phage on a Δ*recA* strain lysogenic for prophage λ *imm*^{434} or φ21.

[b] Each value is for specific activity expressed from *dinD*::*lac* and is the average of at least three determinations (15).

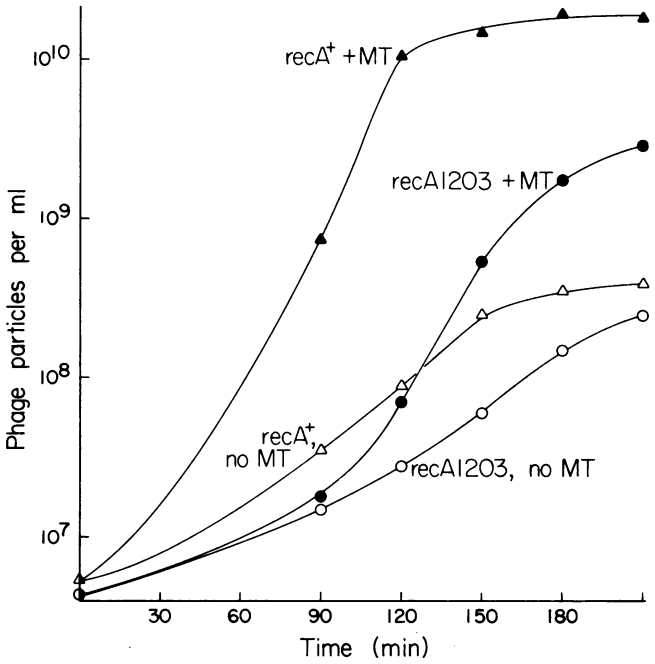

FIG. 3. Spontaneous and mitomycin C-induced phage production in a *recA1203* (Prt^c Rec$^-$) strain lysogenic for λ *imm*^{434} (○, ●) and in a *recA*$^+$ isogenic control strain (△, ▲). The Prt^c Rec$^-$ strain EST1583 was Δ*recA*(λ *recA1203*)(λ *imm*^{434}); the Prt^c Rec$^+$ control strain EST1584 was Δ*recA*(λ *recA*$^+$)(λ *imm*^{434}). For each strain two cultures were grown to an OD_{600} of 0.1 (time zero), and then mitomycin C (MT) was added to one flask (▲, ●) but omitted from the second (△, ○). Mitomycin C was added at 5.0 and 1.0 μg/ml for the *recA*$^+$ and *recA1203* (Prt^c Rec$^-$) strains, respectively. Incubation at 35°C was continued, and samples were removed at the indicated times, shaken with chloroform, and assayed for phage concentration. The final phage concentration in strain EST1544, an isogenic Δ*recA* control strain lysogenic only for λ *imm*^{434}, was 10^2 particles per ml.

TABLE 4. RecA protease activity and recombinase activity of partially defective *recA* mutants[a]

recA allele	Color on XGal-MT plates	Mitomycin C-induced β-galactosidase sp act (U)[b] in background:		Recombination frequency[c]
		dinD::*lac*	*sulA*::*lac*	
recA$^+$	Dark blue	1.0	1.0	1.0
recA1244	Light blue	<0.05	<0.02	0.0002
recA1242	Dark blue	0.15	NM	0.05
recA1243	Dark blue	0.15	1.0	0.03
recA1245	Dark blue	0.17	NM	0.01
recA1246	Dark blue	<0.05	0.13	0.01
recA1247	Dark blue	<0.05	<0.02	0.03
recA1248	Dark blue	<0.05	<0.02	0.0002
recA1250	Dark blue	<0.05	<0.02	0.0002

[a] Strains containing mutations *recA1242* through *recA1250* were chosen for measurement of possible protease activity because they gave dark-blue streaks on M9-CAA-XGal plates containing mitomycin C (MT, 0.2 μg/ml), implying some induction of *dinD*::*lac*. The alleles that gave no measurable induction of *dinD*::*lac* in liquid culture were then tested in the *sulA*::*lac* background in liquid culture.

[b] Induction was tested in liquid culture at 0.5 μg of mitomycin C per ml. Each value is the average of the induced plateau values obtained between 90 and 180 min minus the uninduced value. NM, Not measured.

[c] Measured in matings with an HfrH strain. The values of 0.0002 are at the level of RecA-independent recombination.

The main point is that mitomycin C did induce the λ *recA* (Prt^c Rec$^-$)/λ *imm*^{434} double lysogen. Because of the sensitivity of Rec$^-$ cells to mitomycin C, this experiment required the use of a fivefold-lower mitomycin C concentration for the λ *recA* (Prt^c Rec$^-$) lysogen than for the λ *recA*$^+$ control (1.0 versus 5.0 μg/ml) to detect any increase in phage yield over the spontaneous level. Decreasing the mitomycin C dose for the Prt^c Rec$^-$ strain further, to 0.5 or 0.25 μg/ml, gave the same phage yield as 1.0 μg/ml, although lower growth times were required. The present results suggest that the RecA proteins of the Prt^c Rec$^-$ mutants have altered repressor recognition, because they behave like wild-type protease with respect to phage repressor in the sense that they require activation by DNA damage, even though they are constitutive proteases with respect to the LexA repressor.

Search for *recA* mutants with damage-activable rather than constitutive protease activity. To search for recombinase-deficient *recA* mutants that had wild-type rather than constitutive protease activity, i.e., Prt$^+$ Rec$^-$ mutants, 300 plaques with the wild-type color (medium blue) were picked from the *sulA*::*lac* indicator strain, and the cells in the centers of the plaques were streaked for mitomycin C sensitivity. Only 1 of 300 medium-blue plaques contained mitomycin C-sensitive cells. The lysogen resulting from this blue plaque, the *recA1242* mutant, was only partly protease proficient, retaining 15% of the wild-type protease activity as measured by *dinD* induction with mitomycin C (Table 4). The recombination frequency of the *recA1242* strain was 5% of that of the wild type. Thus, the *recA1242* strain was not the Prt$^+$ Rec$^-$ mutant type sought; instead it was Prt$^\pm$ Rec$^\pm$. If the type sought, Prt$^+$ Rec$^-$, does in fact exist, it occurs at a frequency of less than 0.003.

Protease-deficient *recA* mutants were always Rec$^-$. Wild-type λ *recA* forms bright, medium-blue plaques on the *sulA*::*lac* indicator strain EST995 on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XGal). The intense color formed on the *sulA*::*lac* indicator may be due to the formation, during phage replication in the plaque, of some DNA structure that provides a signal for activation of RecA to the protease state. Activated RecA would then turn on the *sulA*::*lac* gene, and the cells in the plaque center would turn blue. We predicted that if gray plaques were observed after mutagenesis of λ *recA*$^+$, it would be because the cells in the plaque centers contained protease-defective RecA proteins. As described below, this turned out to be the case; in addition, however, all of these mutants were also recombinase defective.

About 70 gray plaques were picked from XGal plates seeded with the *sulA*::*lac* indicator. Purified phage from each of the gray-plaque mutants was used to lysogenize the Δ*recA* *dinD*::*lac* strain, EST1515. To determine whether the 65 lysogens derived from gray plaques were in fact protease defective, the lysogens were first streaked onto XGal plates with and without mitomycin C at 0.1 and 0.2 μg/ml. A higher mitomycin C concentration, 0.5 μg/ml, killed all the mutant strains but allowed good growth of the wild type. The wild-type control strain EST1450 (*recA*$^+$ *dinD*) formed a light-blue streak on XGal plates without mitomycin C and a dark-blue streak with mitomycin C. In contrast, 59 lysogens derived from gray plaques showed no color change on mitomycin C plates but continued to form light-blue streaks. Six lysogens derived from gray plaques showed dark-blue streak color in the presence of mitomycin C, suggesting that they might have some damage-activable protease activity. The six lysogens that gave dark-blue streaks were tested quantitatively for damage-activable protease activity by measuring β-galactosidase specific activity from the difficult-to-induce *dinD*::*lac* (11) fusion in cultures containing mitomycin C (0.25 or 0.5 μ/ml); four of these lysogens were also tested for induction of the easily inducible *sulA*::*lac* fusion (10). Only three of the six mutants that formed dark-blue streaks in response to mitomycin C showed measurable induction of either *dinD* or *sulA*, and these three had considerably reduced protease activity (Table 4). Thus, even the six lysogens that appeared to retain some damage-activable protease activity as measured in the streak test turned out to be appreciably protease defective. Also, 12 lysogens that showed no enhanced blue color on mitomycin C streak plates were tested for mitomycin C-inducible β-galactosidase synthesis from *dinD*. All 12 showed no damage-activable protease activity. We conclude that the *recA* lysogens derived from gray plaques were all protease defective, with most being severely defective.

Because of their sensitivity to mitomycin C and their extreme sensitivity to UV in spot tests, the 65 *recA* lysogens derived from the gray plaques were tested for defects in recombinase activity. They were first screened by recombination spot tests on a lawn of an Hfr strain (3). Sixty-one of the mutants showed no recombination in the spot tests above the background found for the Δ*recA* strain, which in liquid culture matings was about 0.01% of the wild-type recombination frequency. Four mutants gave spot tests results that showed recombination far above the background found for the Δ*recA* strain, and liquid culture Hfr matings were then carried out for these four. These mutants with partial recombinase activity had recombination frequencies that varied from 3 to 1% of that of the wild type, i.e. about 100 times above the background of the Δ*recA* strain. The four mutants that showed this partial recombinase activity were members of the group which showed some degree of protease activity as measured by formation of blue streaks on XGal-mitomycin C plates (Table 4). The correspondence between protease activity and recombinase activity of the partially defective mutants derived from gray plaques is shown in Table 4, which also includes the values for the

recA1242 strain described above, which was derived from a blue plaque although it was partially defective in both functions. Four of the five mutants with partial recombinase activity showed some measurable protease activity. No protease-deficient, recombinase-proficient mutants have been found so far by our screening method.

Rec⁻ mutants have altered membrane permeability. All the *recA* (Prt⁻ Rec⁻) mutants as well as the Δ*recA* parent strain were streaked on MacConkey-lactose plates at room temperature (23°C) to test for growth inhibition by crystal violet, as was done for the *recA* (Prt^c Rec⁺) mutants (27). The Δ*recA* control (strain EST1515) did not grow at all, although it grew well at 30°C on the same plates. The Δ*recA* mutation confers sensitivity to the crystal violet (1.0 mg/ml) present in MacConkey plates, and this sensitivity is independent of the *dinD*, *sulA*, and *sulC* (6) mutations as shown by the fact that two Δ*recA* strains, JC10289 and W3110 Δ*recA*, which do not contain these mutations, were just as sensitive to crystal violet as strain EST1515. The 65 *recA* (Prt⁻ Rec⁻) lysogens obtained here were all defective in growth when streaked on MacConkey plates at 23°C, but the degree of residual growth on these plates varied considerably among mutants that appeared to be equally defective in protease and recombinase activity.

To confirm the apparent altered membrane permeability of *recA* (Rec⁻) mutants inferred from the results with MacConkey plates, the mutants were tested on M9-CAA plates (27) containing crystal violet (1.5 mg/ml), kanamycin (9.3 μg/ml), or chloramphenicol (1.6 μg/ml), and the plates were incubated for 48 h at 30°C. Evidence that sensitivity to these agents is an indication of altered permeability is given in reference 27. All 65 lysogens were streaked on the crystal violet plates at 30°C and all failed to grow, whereas the *recA*⁺ strain grew well. The EOP on crystal violet was less than 10^{-3} for several mutants tested. Both the Δ*recA* strain and nine isogenic *recA* (Prt⁻ Rec⁻) mutants tested were also more sensitive to chloramphenicol and kanamycin than was the *recA*⁺ strain. The EOP on the chloramphenicol plates for the nine Prt⁻ Rec⁻ mutants and the Δ*recA* strain was 0.01 or less, and on kanamycin plates it was about 0.1, with the colonies being very small, whereas the *recA*⁺ strain formed large colonies, with an EOP of 1.0 on these plates.

The Prt^c Rec⁻ mutants were even more sensitive to chloramphenicol and kanamycin than the Prt⁻ Rec⁻ mutants, with the EOP on each agent being less than 0.002 for three Prt^c Rec⁻ mutants. The greater sensitivity of the Prt^c Rec⁻ mutants than of the Prt⁻ Rec⁻ mutants is probably due to a contribution of each of the two mutations in the Prt^c Rec⁻ mutants to increased permeability. The unexpected dark-blue colony color of the Prt^c Rec⁻ mutants could be accounted for by greatly increased permeability for XGal. This might be expected to increase the rate of entry of XGal into the cells, where the high constitutive level of β-galactosidase would cleave it, yielding a higher concentration of the blue cleavage product. The resulting blue colonies would therefore be darker than the colonies from cells that had no alteration in permeability but produced the same amount of β-galactosidase.

DISCUSSION

Our finding *recA* mutants that have constitutive protease activity but entirely lack recombinase activity, i.e., *recA* (Prt^c Rec⁻) mutants, indicates that the RecA protease function does not require an intact recombinase function. Such a separation of functions was previously suggested by Yarranton and Sedgwick (24, 31) on the basis of strains they constructed that presumably contained hybrid RecA proteins and showed protease activity but not recombinase activity. Their evidence was indirect and subject to other interpretations (31). Our results based on analysis of the new *recA* mutants show that although protease activity can be observed in the absence of recombinase activity, subtle alterations of protease function accompany recombinase defects. For example, we found that all the *recA* (Prt^c Rec⁻) mutants had altered RecA protease substrate specificity and that one of these mutants had an unusual type of conditional RecA protease activity (27).

Two general questions are raised by the existence of Prt^c Rec⁻ mutants: are they single or double mutants, and in what stages of recombination are they blocked? The DNA of one Prt^c Rec⁻ mutant, the *recA1201* strain, has been sequenced (W.-B. Wang and E. S. Tessman, unpublished data). The *recA1201* strain was found to be a double mutant that contained two widely separated mutations on the *recA* gene. Resolution of the double mutant into its two single-mutant components showed that each single mutant was Prt^c Rec⁻. One of the single mutants obtained from the *recA1201* strain had the same constitutive protease strength and recombination defectiveness as the double mutant. The second single mutant from the *recA1201* strain was a weaker Prt^c mutant than the double mutant and was partially Rec⁻. The finding that a single base change can simultaneously confer constitutive protease activity and recombinase deficiency indicates that the RecA protein is unlikely to have separate domains for protease activity and recombinase activity. Elucidation of the functional organization of the RecA protein awaits DNA sequence analysis of other Prt^c Rec⁻ mutants isolated in this work and also of other types of selectively altered *recA* mutants.

To determine the stage at which the recombinase is blocked in the Prt^c Rec⁻ mutants, we must take into account our finding that all of the 65 *recA* mutants selected for being protease defective turned out to be recombinase defective as well. The degree of defectiveness in one function was correlated with the degree of defectiveness in the other function. This result strengthens the belief that the same ternary complex of RecA protein, single-stranded DNA, and ATP is required for both protease activation and the early stages of recombinase function (4, 5, 20, 25). If this is indeed the case, then our Prt^c Rec⁻ mutants should be able to initiate recombination since they have protease activity. Their recombinase blocks should affect later stages of recombination, including hydrolysis of ATP and branch migration.

Mutants of the reverse phenotype, Prt⁻ Rec⁺, were not found by us. One mutant of this reverse phenotype, the *recA430* strain, was isolated by others with a different selection procedure and has been intensively studied (7, 8, 16, 19). The *recA430* mutant shows reduced but still significant protease activity toward the LexA repressor in vivo (7, 19). RecA430 protease is altered in specificity of repressor recognition (7), being unable to cleave lambda repressor but able to cleave the repressor of phage φ80 as well as LexA. Thus, there are still no known mutants which are recombinase proficient but lack protease activity for all of the commonly tested substrates of RecA protease.

Although the Prt^c Rec⁻ mutants were selected for having constitutive protease activity toward the LexA repressor, they had no constitutive protease activity toward the repressors of phages 434 and 21; however, they did have damage-activable protease activity for these phage repressors. In contrast, even very weak Prt^c Rec⁺ mutants showed efficient

constitutive protease activity for these phage repressors. Thus, the Prt^c Rec$^-$ mutants are partly altered in the substrate specificity of their RecA proteins.

Previously one mutant was known that might be called Prt^c Rec$^-$. This is strain *recA142*, which is recombinase deficient (3), shows slight spontaneous induction cleavage of lambda prophage (21), and is very defective in damage-activable protease activity as measured by induction of lambda prophage (8, 21). Recently the *recA142* mutant has been shown to have substantial damage-activable protease activity toward an easily inducible lambda repressor mutant called λ *c*I *ind*^{s}-1 (8). The in vivo protease activity of the *recA142* mutant toward the LexA repressor has not been measured, so it is not yet known to what extent the *recA142* mutant resembles the Prt^c Rec$^-$ mutants discussed here.

The UV sensitivity of a *recA* (Prt^c Rec$^-$) mutant was compared with that of an isogenic Δ*recA* strain. Also compared were the UV sensitivities of *lexA* (Def) derivatives of the Δ*recA* strain and the Prt^c Rec$^-$ strain. These experiments provided an estimate of the contribution of inducible repair processes to survival of recombinase-deficient cells. We conclude that inducible excision repair and inducible mutagenic repair contribute little to cell survival when the RecA recombinase function is defective. For one or more of the various inducible repair proteins involved, the constitutive level may account for a considerable portion of the repair. With respect to the relative importance of the different RecA-inducible repair processes, it was shown previously (17) that amplification of the RecA protein alone without induction of other SOS functions accounts for the major part of resistance to UV.

Study of one Prt^c Rec$^-$ mutant, *recA1203*, showed that it gave UV-induced mutagenesis of *E. coli* at frequencies comparable to that of the wild type. Also, this mutant was found to give UV reactivation and increased mutagenesis of UV-irradiated phage S13 (I. Tessman, personal communication). Thus, the recombinase activity of RecA is not needed for mutagenic repair. Previously Blanco et al. (1) had reported that for the *recA142* mutant, which has partial protease activity but is recombinase defective, UV-induced cell mutagenesis was observed, although at a frequency lower than that for the wild type.

Recombinase-defective *recA* mutants, whether protease proficient or deficient, all showed increased sensitivity to crystal violet, kanamycin, and chloramphenicol, low-molecular-weight solutes that are not structurally related. Sensitivity to these agents was greatest in the Δ*recA* strains and varied in severity for the different point *recA* mutations isolated here. This enhanced sensitivity indicates that Rec$^-$ cells have altered membrane permeability. We conclude that RecA is essential for the maintenance of cell membrane integrity, although its role may be indirect. Previously we found that *recA* (Prt^c Rec$^+$) mutants also had striking alterations in membrane permeability, with permeability increasing with the protease strength of the *recA* (Prt^c Rec$^+$) alleles (27). The membrane alteration of Prt^c Rec$^+$ mutants is due to the strong induction of some SOS gene, possibly *recA* itself, whereas for Prt$^-$ Rec$^-$ mutants the altered permeability must have a different cause. It is possible that both excess and insufficient RecA protein can indirectly alter the cell membrane.

The finding of altered membrane permeability for both the Prt^c Rec$^+$ and the Prt$^-$ Rec$^-$ mutants shows the importance of obtaining a number of mutants of a given class to reveal a physiological defect. Our mutant isolation procedure makes such mutants readily available and should facilitate the study of the role of the RecA protein in DNA replication and transcription.

ACKNOWLEDGMENTS

We thank Irwin Tessman and Laszlo Csonka for reading the manuscript and Margie Miles and Patty Borders for expert secretarial assistance.

This work was supported by Public Health Service grant AI 17566 to E.S.T. from the National Institutes of Health.

LITERATURE CITED

1. **Blanco, M, G. Herrera, P. Collado, J. Rebollo, and L. M. Botella.** 1982. Influence of RecA protein on induced mutagenesis. Biochimie **64**:633–636.
2. **Clark, A. J.** 1973. Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7**:67–86.
3. **Clark, A. J., and A. D. Margulies.** 1965. Isolation and characterization of recombination deficient mutants of *E. coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **53**:451–459.
4. **Cox, M., and I. R. Lehman.** 1981. *recA* protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange. Proc. Natl. Acad. Sci. U.S.A. **78**:3433–3437.
5. **Craig, N. L., and J. W. Roberts.** 1981. Function of nucleoside triphosphate and polynucleotide in *Escherichia coli recA* protein-directed cleavage of phage lambda repressor. J. Biol. Chem. **256**:8039–8044.
6. **d'Ari, R., and O. Huisman.** 1983. Novel mechanism of cell division inhibition associated with the SOS response in *Escherichia coli*. J. Bacteriol. **156**:243–250.
7. **Devoret, R., M. Pierre, and P. L. Moreau.** 1983. Prophage φ80 is induced in *Escherichia coli* K-12 *recA430*. Mol. Gen. Genet. **189**:199–206.
8. **Dutreix, M., A. Bailone, and R. Devoret.** 1985. Efficiency of induction of prophage λ mutants as a function of *recA* alleles. J. Bacteriol. **161**:1080–1085.
9. **Froehlich, B., and W. Epstein.** 1981. *Escherichia coli* mutants in which transcription is dependent on *recA* function. J. Bacteriol. **147**:1117–1120.
10. **Huisman, O., and R. d'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
11. **Kenyon, C. J., and G. C. Walker.** 1980. DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:2819–2823.
12. **Lark, K. G., and C. A. Lark.** 1980. *recA* dependent DNA replication in *E. coli*. A new mutation affecting the ability of *recA* to regulate DNA replication. ICN-UCLA Symp. Mol. Cell. Biol. **14**:881–889.
13. **Little, J. W.** 1984. Autodigestion of lexA and phage λ repressors. Proc. Natl. Acad. Sci. U.S.A. **81**:1375–1379.
14. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
15. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Morand, P., M. Blanco, and R. Devoret.** 1977. Characterization of *lexB* mutations in *Escherichia coli* K-12. J. Bacteriol. **131**:572–582.
17. **Quillardet, P., P. L. Moreau, H. Ginsburg, D. W. Mount, and R. Devoret.** 1982. Cell survival, UV-reactivation and induction of prophage lambda in *Escherichia coli* K12 overproducing *recA* protein. Mol. Gen. Genet. **188**:37–43.
18. **Radman, M.** 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis, p. 355–367. *In* P. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for repair of DNA, part A. Plenum Publishing Corp., New York.
19. **Rebollo, J. E., P. L. Moreau, M. Blanco, and R. Devoret.** 1984. Restoration of RecA protein activity by genetic complementation. Mol. Gen. Genet. **195**:83–89.
20. **Roberts, J. W., and R. Devoret.** 1983. Lysogenic induction, p.

123–144. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

21. **Roberts, J. W., and C. W. Roberts.** 1981. Two mutations that alter the regulatory activity of *E. coli* protein. Nature (London) **290**:422–424.
22. **Rupp, W. D., and P. Howard-Flanders.** 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**:291–304.
23. **Rupp, W. D., C. E. Wilde III, D. L. Reno, and P. Howard-Flanders.** 1971. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J. Mol. Biol. **61**:25–44.
24. **Sedgwick, S. G., and G. Y. Yarranton.** 1983. Negative complementation of the *Escherichia coli recA*$^+$ gene: its use in separating inducible DNA repair and mutagenesis, p. 437–445. *In* E. C. Friedberg and B. A. Bridges (ed.), Cellular responses to DNA damage. Alan R. Liss, Inc., New York.
25. **Shibata, T., C. Das Gupta, R. P. Cunningham, J. K. Williams, L. Osber, and C. M. Radding.** 1981. Homologous pairing in genetic recombination. The pairing reaction catalyzed by *Escherichia coli* recA protein. J. Biol. Chem. **256**:7565–7572.
26. **Smith, K. C., and D. H. C. Meun.** 1970. Repair of radiation-induced damage in *Escherichia coli*. I. Effect of *rec* mutations on postreplication repair of damage due to ultraviolet radiation. J. Mol. Biol. **51**:459–472.
27. **Tessman, E. S., and P. K. Peterson.** 1985. Plaque color method for rapid isolation of novel *recA* mutants of *Escherichia coli* K-12: new classes of protease-constitutive *recA* mutants. J. Bacteriol. **163**:677–687.
28. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damages in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
29. **Witkin, E. M.** 1975. Persistence and decay of thermo-inducible error-prone repair activity in nonfilamentous derivatives of *tif-1 Escherichia coli* B/r: the timing of some critical events in ultraviolet mutagenesis. Mol. Gen. Genet. **142**:87–103.
30. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.
31. **Yarranton, G. T., and S. G. Sedgwick.** 1982. Cloned truncated *recA* genes in *E. coli*. II. Effects of truncated gene products on *in vivo recA*$^+$ protein activity. Mol. Gen. Genet. **185**:99–104.