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# Plaque Color Method for Rapid Isolation of Novel *recA* Mutants of *Escherichia coli* K-12: New Classes of Protease-Constitutive *recA* Mutants

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**As a prerequisite to mutational analysis of functional sites on the RecA protein of *Escherichia coli*, a method was developed for rapid isolation of *recA* mutants with altered RecA protease function. The method involves plating mutagenized λ *recA*$^+$ cI *ind* on strains deleted for *recA* and containing, as indicators of RecA protease activity, Mu d(Ap *lac*) fusions in RecA-inducible genes. The λ *recA* phages were recognized by their altered plaque colors, and the RecA protease activity of the λ *recA* mutant lysogens was measured by expression of β-galactosidase from *dinD*::*lac*. One class of *recA* mutants had constitutive protease activity and was designated Prt$^c$; in these cells the RecA protein was always in the protease form without the usual need for DNA damage to activate it. Some Prt$^c$ mutants were recombinase negative and were designated Prt$^c$ Rec$^-$. Another class of 65 *recA* mutants isolated as being protease defective were all also recombinase defective. Unlike the original temperature-dependent Prt$^c$ Rec$^+$ mutant (*recA441*), the new Prt$^c$ Rec$^+$ mutants showed constitutive protease activity at any growth temperature, with some having considerably greater activity than the *recA441* strain. Study of these strong Prt$^c$ Rec$^+$ mutants revealed a new SOS phenomenon, increased permeability to drugs. Use of this new SOS phenomenon as an index of protease strength clearly distinguished 5 Prt$^c$ mutants as the strongest among 150. These five strongest Prt$^c$ mutants showed the greatest increase in spontaneous mutation frequency and were not inhibited by cytidine plus guanosine, which inhibited the constitutive protease activity of the *recA441* strain and of all the other new Prt$^c$ mutants. Strong Prt$^c$ Rec$^+$ mutants were more UV resistant than *recA*$^+$ strains and showed indications of having RecA proteins whose specific activity of recombinase function was higher than that of wild-type RecA. A Prt$^+$ Rec$^-$ mutant with an anomalous response to effectors is described.**

The RecA protein of *Escherichia coli* is a multifunctional enzyme which can act as both a protease and a recombinase and which in certain mutant strains plays a role in DNA replication (23; T. Kogoma, H. Bialy, N. L. Subia, T. A. Torrey, G. G. Pickett, and K. von Meyenburg, *in* M. Schaechter, F. C. Neidhardt, J. Ingraham, and N. O. Kjelgaard, ed., *Molecular Biology of Bacterial Growth*, in press) and in transcription (12). It is also required for maintenance of membrane integrity (40). In the presence of damaged DNA, RecA protein is activated to the protease state in which it cleaves a repressor protein, LexA, thereby inducing the synthesis of numerous unlinked genes comprising the SOS system, many of whose products are required for repairing DNA damage (26, 41, 44). To determine what sites on the RecA protein must be intact for each of these functions, we have begun a mutational dissection of the RecA protein, using a new method that permits rapid isolation of mutants with altered RecA protease activity.

The question whether RecA is a true protease or a protease cofactor has been raised by the work of Little (25), who demonstrated self-proteolysis of LexA repressor and lambda repressor proteins in vitro. Since RecA appears to be essential for proteolysis in vivo and greatly enhances proteolysis even in vitro, it is appropriate and convenient to retain the term RecA protease until more definite information is obtained about how RecA functions.

The immediate objective of this work was to isolate protease-constitutive (Prt$^c$) *recA* mutants, i.e., mutants in which the RecA protein is always in the protease state without the need for DNA-damaging agents to activate it. We sought such mutants because it was likely that their RecA proteins would have alterations in the effector-binding sites that are involved in the activation of RecA by damaged DNA. More specifically, we sought to identify the Prt$^c$ mutants with the greatest protease strength, since these would be expected to have the most striking changes in the effector-binding sites and would therefore be the most suitable mutants for biochemical analysis of the activation process. This paper describes the strategies required to identify the strongest Prt$^c$ mutants and reports how these mutants differ from the weaker ones.

The activation of RecA to the protease form requires the simultaneous binding to RecA of two effector species, namely single-stranded (ss) DNA and a nucleoside triphosphate (NTP), preferably dATP (35), resulting in a ternary complex (7, 8, 28, 35). Much of what we know about the protease function of RecA was learned from the original Prt$^c$ mutant strain *tif-1* (*recA441*), which shows strong protease activity in the absence of any DNA damage when the cells are shifted to high temperature (15). RecA441 protein binds both effectors much more tightly than wild-type RecA and is converted to the protease state by much smaller amounts and shorter fragments of ssDNA (28, 35). It was therefore proposed that RecA441 is activated by the small ss gaps in the DNA of undamaged cells, perhaps at the replication fork. The temperature-dependent Prt$^c$ activity of the *recA441* strain is enhanced by adding adenine to the culture medium and is inhibited by adding a combination of the nucleosides cytidine plus guanosine (C+G) (15, 20, 39, 43).

Before the present work, *recA* mutants were usually

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isolated on the basis of altered recombinase activity (5). Exceptions are *recA441* (15), *recA430* (30), and also several *recA* (Prt$^c$) mutants. The last type were isolated on a λ *recA* phage by a detection method devised by Mount (32), but these λ *recA* (Prt$^c$) mutants were not amenable to rapid quantitative characterization in vivo since the method for detecting them depended on their inability to form lysogens. Another *recA* (Prt$^c$) mutant was obtained after introducing *recA441* from *E. coli* K-12 into *E. coli* B/r by mating (45), but since *recA441* is now known to be a double mutation (21), this Prt$^c$ mutant strain, *recA730*, may represent a single-mutation component of *recA441*.

In the present work we also isolated *recA* mutations on a λ *recA* phage, but we used a detection method which yields λ *recA* (Prt$^c$) mutants that form lysogens readily. The ability to insert the mutated *recA* allele into the cell chromosome by lysogenization was essential for determining RecA protease strength and recombinase activity. Isolating *recA* mutations on a λ *recA* phage has the advantage of avoiding irrelevant mutants that might be caught if colonies rather than phage plaques were screened; such unwanted mutants could include colonies in which the Mu d(*lac*) phage had transposed or which had mutated to *lexA* (Def). Also, the plaque method has no bias against mutants such as Rec$^-$ strains whose colonies have reduced efficiencies of plating (EOPs).

## MATERIALS AND METHODS

**Phenotype symbols.** Phenotype symbols are as follows: Prt$^c$, constitutive RecA protease activity; Prt$^+$, wild-type level of DNA damage-activable protease activity; Prt$^-$, defective protease activity; Rec$^+$, wild-type recombination frequency in a liquid Hfr culture mating; Rec$^-$, recombination frequency 100 times lower than the wild-type frequency or less; Ap$^r$, ampicillin resistance.

**Bacterial strains.** The strains used are listed in Table 1. The plating indicators for detection of λ *recA* mutant plaques were derivatives of either strain GW1040 (19) or AM103, each of which has a Mu d(Ap *lac*) fusion (4) in a different RecA-inducible gene. For detection of λ *recA* (Prt$^c$) phages, a derivative of strain GW1040 was used. The features of strain GW1040 essential to the present work are a *dinD*::*lac* fusion and also its *sulA* and SulC$^-$ characters (10, 13), which are required to prevent the lethal filamentation caused by *recA* (Prt$^c$) mutations. A temperature-resistant derivative was obtained by selecting, on ampicillin plates at 42°C, a colony that gave dark-blue streaks on 5-bromo-4-chloro-3-indolyl-β-D-galactoside(XGal)-mitomycin C agar. This strain, EST1130, was found to be stable against Mu lysis and Mu d(*lac*) transposition. Transposition of Mu d(*lac*) in strain EST1130 can be estimated to occur at a frequency of about $10^{-4}$ if the frequency of blue colonies is used as an index. Only after at least 72 h of incubation on XGal agar at 35°C could transposition be detected on confluent streaks, appearing as characteristic pale and dark sectors. These transposition sectors were not detected in single colonies of strain EST1130 or its derivatives. Mutation to *lexA* (Def), which was observable in many of the new *recA* (Prt$^c$) mutants described here, showed up after 24 h of incubation as discrete dark-blue sectors on confluent streaks. These sectors appeared only in the *recA* (Prt$^c$) mutants and not in *recA*$^+$ strains. Single colonies of the Prt$^c$ mutants showed no sectors. Since *recA* mutants were detected as phage plaques rather than as colonies, transposition or mutation within cells in the indicator bacteria could not influence the mutant isolation procedure. A Δ*recA* derivative of strain EST1130, EST1515, was the *dinD*::*lac* plating indicator and was also the parent strain of all the λ *recA* mutant lysogens. All strains were stored in 5% glycerol at −70°C.

λ *recA* (Prt$^-$ Rec$^-$) phages were detected on strain EST995, which contains a *sulA*::Mu d(*lac*) fusion derived from strain AM103 (A. McPartland, J. Yamashita, and M. Villarejo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981,

TABLE 1. Bacterial strains

| Strain | Relevant genotype or phenotype[a] | Source or reference[b] |
|---|---|---|
| GW1000 | *recA441 sulA11* Δ*lacU169 thr-1 leu-6 his-4 thi-1 argE3 ilv*(Ts) *galK2 rpsL31* | 19 |
| GW1040 | As GW1000, but *dinD1*::Mu d(Ap *lac*) *c*ts | 19 |
| EST1130 | As GW1040, but Ts$^+$ | Spontaneous mutation |
| EST1515 | As EST1130, but Δ*recA306 srl*::Tn*10* | P1, EST945 × EST1130 |
| EST945 | JC10289 (λ *recA*$^+$ *att*$^+$ *c*I$^+$) | This work |
| JC10289 | As AB1157, but Δ*recA306 srl*::Tn*10* | 9 |
| EST1018 | As EST1515, but with *recA441* from λ *recA441 c*I857 | This work |
| AB1157 | *thr-1 leuB6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44* | A. J. Clark |
| AB1899 | AB1157 *lon*, nonmucoid | A. McPartland |
| AM103 | As AB1899, but *sulA*::Mu d(Ap *lac*) *c*ts | A. McPartland |
| EST955 | As AM103, but Ts$^+$ | This work |
| EST1855 | As EST955, but Δ*recA306 srl*::Tn*10* | P1, EST945 × EST955 |
| EST913 | W3110 Δ*lacU169* | Our collection |
| EST965 | As EST913, but *sulA*::Mu d(Ap *lac*)(Ts$^+$) | P1, EST955 × EST913 |
| EST995 | As EST965, but Δ*recA306 srl*::Tn*10* | P1, EST945 × EST965 |
| EST1450 | EST1515(λ *recA*$^+$ *att*$^+$ c*I ind*) | This work |
| GW2707 | *dinD1*::Mu d(Ap *lac*) *lexA71* (Def)(pGW600) | 22 |
| EST1619 | As EST1130, but *lexA71*::Tn*5* (Def) | P1, GW2707 × EST1130 |
| EST1663 | As EST1619, but Δ*recA306 srl*::Tn*10* | P1, EST945 × EST1619 |
| EST1550 | EST1663(λ *recA*$^+$ c*I ind*) | This work |
| DM49 | *lexA3* (Ind$^-$) | 33 |
| JGC175 | *ssb*$^+$ *zjb-1*::Tn*10* | 14 |
| EST901 | *lexA3* (Ind$^-$) *zjb-1*::Tn*10* | P1, JGC175 × DM49 |
| EST1128 | As EST1130, but *lexA* (Ind$^-$) *zjb-1*::Tn*10* | P1, EST901 × EST1130 |
| EST1138 | As EST1128, but Tet$^s$ | 27 |
| EST1142 | As EST1130, but *lexA3* (Ind$^-$) Δ*recA srl*::Tn*10* | P1, EST945 × EST1138 |
| EST1944 | EST1142(λ *recA*$^+$ c*I ind*) | This work |
| EST1308 | CSH62, HfrH | 29 |
| SA820 | Δ*att*$^\lambda$ | S. Adhya |
| EST1555 | As GW1000, but Δ*recA306 srl*::Tn*10* | P1, EST945 × GW1000 |
| EST1926 | EST1555(λ *recA*$^+$ c*I ind*) | This work |
| IT1819 | As EST1130, but S13 sensitive | I. Tessman |
| GW2100 | *umuC122*::Tn*5* | 11 |
| IT1823 | As IT1819, but *umuC122*::Tn*5* | I. Tessman |
| SP577 | Δ*att*$^\lambda$ *gal bio nadA*::Tn*10* | R. Somerville |
| IT1874 | As IT1819, but Δ*recA306 srl*::Tn*10* *umuC122*::Tn*5* | This work |

[a] Ts$^+$, Non-temperature sensitive, Tet$^s$, tetracycline sensitive.
[b] P1, P1 transduction.

K58, p. 147). Of the two Δ*recA sulA*::*lac* strains used here, EST1855, derived from strain AB1157, gave negligible transposition of Mu d(*lac*) and was SulC$^-$; thus, it could be used for lysogenization but did not show sufficient plaque color contrast to be used as an indicator. Strain EST995, derived from W3110, showed transposition of Mu d(*lac*) as sectors in single colonies and was also SulC$^+$; thus, it could not be used for lysogenization but gave excellent plaque color contrast.

**Phage strains.** The λ *recA* phages used to obtain *recA* mutants were constructed by Sara Cohen and David Mount. These workers used as the vector λNM426 (34), which is *c*I$^+$ *att*$^+$ *int*$^+$ *xis*$^+$ and is deleted of all *Eco*RI sites except one in the b2 region. Into this site was cloned *recA* DNA from a λ *recA*$^+$ specialized transducing phage. The resulting λ *recA*$^+$ *c*I$^+$ *att*$^+$ phage was crossed by Cohen and Mount against λ *c*I857 and λ *c*I *ind*. The λ *recA* mutants described here were all isolated from λ *recA*$^+$ *c*I *ind*. Lytic lysates of this phage were made in strain SA820. To release λ *c*I *ind* phages from lysogens, a heteroimmune phage is plated on a lawn of the lysogen. The plaques contain λ *c*I *ind* as well as the heteroimmune phage (M. Lieb, personal communication). Other phages used were λ *imm*$^{434}$ from the National Institutes of Health collection, φ21 from Norman Melechen, and λ *recA441* *c*I857 from Kevin McEntee. The transducing phages used for strain construction were P1 Cm *clr-100* and P1 *vir*$^s$, UV irradiated at a fluence of 40 J/m$^2$.

**Media.** M9-CAA medium was M9 salts (29) supplemented with 2 g of glucose, 5 g of Casamino Acids, 2 mg of thiamine, 10 μmol of $FeCl_3$, and 1 mmol of $MgSO_4$ per liter. M9-CAA plates contained in addition 15 g of agar per liter. XGal-M9-CAA agar contained in addition 40 mg of XGal per liter. XGal-Mal plates were XGal-M9-CAA plates with 2 g of maltose per liter substituted for glucose. XGal-MT plates contained XGal-M9-CAA supplemented with mitomycin C.

The media used for growing and plating phage lambda, including lambda plates, were as described by Gottesman and Yarmolinsky (16), except for top agar, which contained (per liter) 10 g of nutrient agar, 1 mmol of $CaCl_2$, and 10 mmol of $MgSO_4$.

LB plates contained (per liter) 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 12 g agar. TB plates contained (per liter) 10 g of tryptone, 5 g of NaCl, and 12 g of agar. Limiting histidine agar medium was similar to M9-CAA agar except that Casamino Acids, the source of histidine, was reduced to 40 mg/liter and threonine, leucine, arginine, isoleucine, and valine were each added to 40 mg/liter.

Tetracycline was used at a concentration of 25 μg/ml, except for work with *lexA* (Def) strains, for which 5 μg/ml was used. Kanamycin was used at 30 μg/ml, ampicillin at 50 μg/ml, and chloramphenicol at 12.5 μg/ml. For testing drug sensitivity, crystal violet, mitomycin C, chloramphenicol, and kanamycin were used as indicated below.

**Phage mutagenesis.** λ *recA*$^+$ *c*I *ind* phage were mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine essentially by the procedure of Auerbach and Howard-Flanders (1), except that the UV fluence to the cells was 15 J/m$^2$, the concentration of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine during phage growth was 25 μg/ml, and the host strain was SA820.

**Lysogenization.** Mutant λ *recA* phages were plaque purified, spotted on an XGal-M9-CAA plate seeded with the Δ*recA dinD* strain EST1515, and incubated at 35°C. Cells were picked from the center of the spot and streaked on scavenging agar to eliminate nonlysogens. The scavenging plates used were XGal-CAA-Mal medium seeded with 5 × 10$^9$ particles of phage λ *b*2 *c* in top agar. After 20 h of incubation at 35°C the resulting streaks consisted almost entirely of lysogens. The streak colors on the scavenging agar were a good index of the phenotypes of the mutant strains. Lysogeny of single colonies obtained from the streaks on the scavenging plates was verified by cross-streaking on agar streaked with λ *b*2 *c* and λ *vir*.

**Measurement of β-galactosidase synthesis.** Cultures were grown overnight at 30°C in M9-CAA medium. The overnight cultures were diluted 50-fold in the same medium and grown in most cases at 35°C. The zero point was taken when the optical density at 600 nm ($OD_{600}$) reached 0.1 to 0.2. The β-galactosidase assay was that described by Miller (29), except that all samples were centrifuged to remove debris before the $OD_{420}$ was read. Specific activity was calculated as described previously (29). For determining C+G inhibition, cultures of each strain were grown overnight in M9-CAA medium with no additions and in M9-CAA medium containing C (300 μg/ml) and G (350 μg/ml) and then diluted 50-fold in the same medium, and β-galactosidase activity was measured (29) at different times during a 2.5-h growth period at 35°C. Curves of the type shown in Fig. 1 were obtained, and the average specific activities with and without C+G were determined. At least two lysogens of each mutant phage were tested, with three being tested for each class 1 mutant.

**UV irradiation.** To obtain UV survival curves, log-phase cells grown in M9-CAA medium were centrifuged and suspended in M9 salts at a concentration of 2 × 10$^7$ cells per ml, and 3 ml was irradiated with a 15-W germicidal lamp while being stirred. Plating was done on M9-CAA agar, followed by incubation at 35°C.

**Measurement of spontaneous mutagenesis.** To assay mutation from *his* to *his*$^+$, cells were grown to late log phase in M9-CAA medium, centrifuged twice, and suspended in M9 salts, after which 0.1-ml amounts were spread on limiting histidine agar and incubated at 35°C for 3 days. The *recA441* and *lexA71 recA441* strains were spread on limiting histidine plates supplemented with adenine (100 μg/ml) and incubated at 41.5°C. The number of spontaneous mutants which arose was independent of the number of initial cells over the range from 10$^6$ to 10$^9$ cells per plate. The values obtained are expressed as number of mutants per plate but are equivalent to mutation frequencies, since the final number of cells in the background film on the limiting histidine plates was a constant, 2 × 10$^8$ cells per plate.

**Measurement of recombination frequencies.** Liquid culture Hfr matings were performed by growing the HfrH donor strain CSH62 and the F$^-$ recipient strain to be tested 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 in M9 salts, plated on plates containing streptomycin (100 μg/ml), and scored for recombination to Thr$^+$ Leu$^+$ after 48 h of incubation at 35°C.

## RESULTS

**Isolation of λ *recA* mutant phages.** The λ *recA* mutant phages were detected by their plaque colors on XGal agar by using as plating indicators two strains that were deleted for *recA* and also carried fusions of Mu d(Ap *lac*) in RecA-inducible genes. One indicator strain (EST1515) had its *lac* fusion in *dinD*, which is difficult to induce (19), whereas the other indicator strain (EST995) had its *lac* fusion in *sulA*, which is easily induced (17). Difficulty of induction refers to the greater lag time required for induction of *dinD*::*lac* than

for *sulA*::*lac* (17, 19) and to the greater UV fluences required to induce *dinD* than *sulA* (unpublished data).

The plaque colors of parental and mutant λ *recA* phages on the two strains are given in Table 2. Detection of Prt$^c$ plaques, which were dark blue on both indicators, required the use of the *dinD*::*lac* indicator to provide sufficient contrast to wild-type plaques. Conversely, detection of Prt$^-$ plaques, which were gray on both indicators, required the use of the *sulA*::*lac* indicator to provide sufficient contrast to wild-type plaques.

The frequency of dark-blue plaques produced by the mutagenized phage on the *dinD* strain was about 0.2%, and the frequency of gray plaques on both strains was about 5%; by comparison, the frequency of clear mutants was about 3%. Blue plaques were easily detectable on a background of 2,000 parental gray-blue plaques, so that hundreds of mutant phages could be found quickly. Significantly, an attempt to find a thermoinducible *recA* (Prt$^c$) mutant like the *recA441* strain failed. Fifty dark-blue plaques were picked from *dinD*::*lac* plates that had been incubated at 40°C, but all gave rise to *recA* (Prt$^c$) mutants that were constitutive even at 30°C, like the rest of the *recA* (Prt$^c$) mutants isolated here. Thus, the *recA441* strain is an atypical *recA* (Prt$^c$) mutant, as DNA sequence analysis has recently shown (21, 42).

**Classes of *recA* mutants.** Most of the *recA* (Prt$^c$) mutants showed a wild-type recombination frequency in liquid culture Hfr matings, but 6 of 26 mutants that formed dark-blue colonies were recombinase defective and UV sensitive. The designation Prt$^c$ Rec$^+$ is used here for Prt$^c$ mutants with recombination frequencies at least 50% of that of the wild-type in liquid culture Hfr matings. In contrast, the designation Prt$^c$ Rec$^-$ is used for Prt$^c$ mutants whose recombination frequencies were less than 1% of that of the wild-type; mutants with intermediate recombination frequencies are designated Rec$^\pm$. The properties of the Prt$^c$ Rec$^-$ mutants are described in the accompanying paper (40). The λ *recA* phages that formed gray plaques on the *sulA*::*lac* indicator strain EST995 gave lysogens that were defective in damage-activable protease activity, as expected; in addition, all were recombinase defective to some degree. These mutants were either Prt$^-$ Rec$^-$ or Prt$^\pm$ Rec$^\pm$ (40).

The protease-constitutive mutants, Prt$^c$ Rec$^+$, differed in the following properties: (i) specific activity of β-galactosidase expressed constitutively from the *dinD*::*lac* fusion gene; (ii) efficiency of spontaneous cleavage of the repressor of λ *imm*$^{434}$; (iii) sensitivity to mitomycin C, crystal violet, antibiotics, and broth; (iv) frequency of spontaneous mutagenesis; and (v) ability of constitutive protease activity to be inhibited by C+G. Only the first two properties were known to be indexes of constitutive protease strength before this work. In the course of characterizing the mutants, the third and fourth properties also proved to be useful indexes of protease strength. The first four properties served to classify the Prt$^c$ mutants into the classes shown in Table 3.

The *recA* (Prt$^c$) alleles fell into four classes by the degree of RecA constitutive protease strength, with class 1 being the strongest. Protease strength was determined by the degree of induction of the following SOS functions: β-galactosidase expression from the *dinD*::*lac* gene, plaque clarity of λ *imm*$^{434}$ *c*I$^+$ plated on the Prt$^c$ mutant, and degree of sensitivity to drugs (a composite of the sensitivity to mitomycin C, chloramphenicol, crystal violet, and kanamycin). Using the degree of sensitivity to drugs as an index of protease strength permitted the separation of the Prt$^c$ mutants into the four classes listed in Table 3; the detailed data are given in Table 4. This index was the only SOS induction phenomenon that could distinguish class 1 from class 2. Class 2 broke down into two subclasses that differed in drug sensitivity, but the distinction between 2A and 2B was not as sharp as the distinction between classes. Plaque clarity served to distinguish mutants of class 3 from the two stronger classes. Class 4 was clearly distinguished from class 3 by the degree of *dinD*::*lac* expression and plaque clarity. Spontaneous mutation frequency from *his* to *his*$^+$ was found to distinguish the same four classes that were distinguished by the three SOS induction indexes. Mutants of classes 4A and 4B were separated from each other by an arbitrarily chosen level of β-galactosidase activity. Classes 1, 2A, 2B, and 3 were also resolved by a different criterion, the degree of inhibition of constitutive protease activity by C+G.

TABLE 2. Plaque color of λ *recA* mutants

| Phenotype of λ *recA* lysogen | | Phenotype symbols | Color on indicator strain carrying: | |
|---|---|---|---|---|
| Protease | Recombinase | | *dinD*::*lac* | *sulA*::*lac* |
| Wild type | Wild type | Prt$^+$ Rec$^+$ | Gray-Blue | Medium blue |
| Constitutive | Wild type | Prt$^c$ Rec$^+$ | Dark blue | Dark blue |
| Constitutive | Defective | Prt$^c$ Rec$^-$ | Dark blue | Dark blue |
| Defective | Defective | Prt$^-$ Rec$^-$ | Gray | Gray |

In particular, the property of sensitivity to drugs, which we proved to be an SOS phenomenon, permitted the two strongest classes of mutants to be distinguished from each other and from the rest. The separation into classes provided a guide for choosing mutants to be studied in further exploration of the RecA function.

**Protease strength of the Prt$^c$ Rec$^+$ mutants as measured by expression of *dinD*::*lac*.** To identify the strongest Prt$^c$ mutants, a large number were characterized by degree of constitutive protease strength. It was necessary to use different indexes of protease strength in the search for one with sufficient resolving power. The first index of protease strength used was expression of β-galactosidase from the SOS fusion gene *dinD*::*lac*. This quantitative assay identified new Prt$^c$ mutants that had greater protease strength than the *recA441* strain but did not identify the strongest Prt$^c$ mutants.

Of 15 Prt$^c$ mutants (classes 1 through 3), all gave approximately the same high β-galactosidase specific activity, about 85% of the value obtained for the maximally derepressed *lexA* (Def) reference strains (Table 3). The 15 Prt$^c$ mutants that gave this high value were termed strong, whereas the other Prt$^c$ mutants (classes 4A and 4B) were arbitrarily termed moderate and weak, respectively. The β-galactosidase specific activity of strong, moderate, and weak Prt$^c$ Rec$^+$ mutants were compared with that of the derepressed strain *lexA71* (Def) as a function of time at 35°C (Fig. 1).

Since 15 Prt$^c$ mutants had the same high level of expression from *dinD*::*lac* yet differed in other properties, it seemed likely that the assay was being saturated. The *dinD*::*lac* assay did however serve to distinguish the *recA441* mutant from stronger Prt$^c$ mutants. The protease strength of three strong Prt$^c$ Rec$^+$ mutants was compared with that of the original Prt$^c$ mutant strain *recA441* at 41°C, a temperature at which the *recA441* strain has constitutive protease activity. The strains were *recA1213*, a mutant of class 1, and *recA1211* and *recA1215*, mutants of class 2. When the strains were compared at 41°C, the new Prt$^c$ mutants were found to have about 1.9-fold higher specific activity than the

TABLE 3. Properties of *recA* (Prt$^c$ Rec$^+$) mutants

| Mutant class and strains | Allele no.[a] | β-Galactosidase sp act[b] (U) | λ *imm*$^{434}$ plaques | Spontaneous mutants[c] (no. per plate ± SEM) | Sensitivity to drugs[d] | Inhibition by C+G[e] |
|---|---|---|---|---|---|---|
| Reference strains | | | | | | |
| EST1450 | *recA*$^+$ | 10 | Turbid | 1.4 ± 0.2 | − | − |
| EST1450 | *recA*$^+$ plus MT | 90 | NM | NM | NM | NM |
| EST1550 | *lexA71* (Def) *recA*$^+$ | 200 | Turbid | 5.6 ± 1.0 | ++++ | − |
| EST1663 | *lexA71* (Def) Δ*recA306* | 210 | Turbid | 1.2 ± 0.9 | NT | − |
| EST1695 | *lexA71* (Def) *recA1211* | 200 | Clear | 282 ± 5 | NT | − |
| EST1619 | *lexA71* (Def) *recA441* | 205 | Clear | 49 ± 7 | NT | − |
| EST1130 | *recA441* | 103 | Turbid | 56 ± 2 | NT | +++ |
| EST1018 | *recA441* | 83 | Turbid | 50 ± 3 | NT | +++ |
| Class 1 | | | | | | |
| EST1813 | *recA1202* | 184 | Clear | 475 ± 16 | ++++ | − |
| EST1822 | *recA1212* | 165 | Clear | 360 ± 34 | ++++ | − |
| EST1816 | *recA1213* | 158 | Clear | 256 ± 8 | ++++ | − |
| EST1818 | *recA1217* | 194 | Clear | 317 ± 15 | ++++ | − |
| EST1787 | *recA1220* | 169 | Clear | 286 ± 14 | ++++ | − |
| Class 2A | | | | | | |
| EST1888 | *recA1218* | 183 | Clear | 181 ± 6 | +++ | + |
| EST1826 | *recA1219* | 150 | Clear | 190 ± 37 | +++ | + |
| Class 2B | | | | | | |
| EST1799 | *recA1211* | 174 | Clear | 194 ± 10 | ++ | ++ |
| EST1819 | *recA1214* | 184 | Clear | 166 ± 5 | ++ | ++ |
| EST1821 | *recA1215* | 177 | Clear | 203 ± 13 | ++ | ++ |
| EST1729 | *recA1234* | 176 | Clear | 181 ± 12 | NT | ++ |
| Class 3 | | | | | | |
| EST1629 | *recA1221* | 191 | Semiturbid | 97 ± 7 | + | +++ |
| EST1630 | *recA1222* | 170 | Semiturbid | 96 ± 5 | + | +++ |
| EST1632 | *recA1224* | 162 | Semiturbid | 66 ± 7 | + | +++ |
| EST1185 | *recA1227* | 160 | Semiturbid | 121 ± 7 | + | +++ |
| Class 4A | | | | | | |
| EST1117 | *recA1229* | 83 | Turbid | 51 ± 8 | − | +++ |
| EST1118 | *recA1230* | 57 | Turbid | 12 ± 4 | − | +++ |
| EST1701 | *recA1235* | 71 | Turbid | 13 ± 4 | − | +++ |
| EST1168 | *recA1236* | 82 | Turbid | 63 ± 6 | NT | +++ |
| Class 4B | | | | | | |
| EST1113 | *recA1231* | 23 | Turbid | 9 ± 1 | NT | NM |
| EST1174 | *recA1232* | 23 | Turbid | 6.1 ± 0.9 | NT | NM |
| EST1178 | *recA1237* | 33 | Turbid | 6.2 ± 0.2 | NT | +++ |
| EST1840 | *recA1241* | 15 | Turbid | 4.0 ± 0.2 | NT | NM |

[a] MT, Mitomycin C; added at 2 μg/ml.
[b] Values are averages for samples taken over a 2.5-h period (see the legend to Fig. 1) and were calculated as described by Miller (29). For strain EST1450 induced with mitomycin C, the average plateau value is shown.
[c] Each value is the average for six plates (12 or more plates for mutants in classes 1 through 3).
[d] Composite sensitivity (see the text), scored from maximum (++++) to wild-type level (−). NT, Not tested; NM, not measurable.
[e] Scored as follows: +++, inhibited to the *recA*$^+$ strain basal level; ++ and +, inhibited to 2.5 and 4 times the basal level, respectively; −, no inhibition.

*recA441* strain. However, because of the complex regulation of the SOS system, it is unlikely that β-galactosidase specific activity is linearly related to RecA protease strength. Adenine (100 μg/ml) increased the specific activity of the *recA441* strain by 25%, whereas it had no effect on the new Prt$^c$ mutants at either 34 or 41°C.

**Clear-plaque formation by λ *imm*$^{434}$ as an index of RecA constitutive protease strength.** An index of protease strength with better resolving power at high values than expression of *dinD*::*lac* was the ability of phage λ *imm*$^{434}$ to form clear plaques on a lawn of the Prt$^c$ mutant. This index enabled us to distinguish class 3 mutants as being weaker than classes 1 and 2, although the three classes could not be distinguished by the *dinD*::*lac* assay.

The fact that a strain with strong constitutive RecA protease activity can give clear plaques when lambda is plated on it was shown by Mount (31) for his original *lexA* (Def) mutant strain DM1187 [*lexA51* (Def) *recA441*]. Many of the new strong *recA* (Prt$^c$) alleles, unlike *lexA*$^+$ *recA441* mutations, permitted clear-plaque formation by λ *imm*$^{434}$ even in a *lexA*$^+$ background.

Formation of turbid plaques of λ *imm*$^{434}$ on a lawn of a given Prt$^c$ mutant might also occur if the Prt$^c$ protease could not recognize or cleave the phage 434 repressor rather than simply having weak constitutive activity. Lack of recognition was ruled out, however, by a different type of plating test, described in the accompanying paper (40), in which it was found that all the Prt$^c$ Rec$^+$ mutants listed in Table 3 could recognize and cleave repressors of phages 434 and 21. Thus, mutations that confer constitutive protease activity

TABLE 4. Sensitivity of *recA* (Prt$^c$ Rec$^+$) mutant colony growth[a] to various agents

| Mutant class | Allele no. | Inhibition by C+G[b] | Colony growth with: | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Chloramphenicol (1.6 μg/ml) | Mitomycin C 1.5 μg/ml | | Mitomycin C 2.0 μg/ml | Crystal violet (2.0 mg/ml) | | Kanamycin (9.3 μg/ml) |
| | | | *dinD* | *dinD* | *dinD*$^+$ | *dinD* | *dinD* | *dinD*$^+$ | *dinD* |
| Reference strains | *recA*$^+$ | − | 1.0 | ++++ | ++++ | +++ | ++++ | ++++ | +++ |
| | *lexA* (Def) *recA*$^+$ | − | <0.01 | − | − | − | − | − | |
| Class 1 | *recA1202* | − | <0.01 | − | − | | − | − | − |
| | *recA1212* | − | <0.01 | − | − | | − | − | − |
| | *recA1213* | − | <0.01 | − | − | − | − | − | − |
| | *recA1217* | − | <0.01 | − | − | − | − | − | − |
| | *recA1220* | − | <0.01 | − | − | | − | − | − |
| Class 2A | *recA1218* | + | 0.04 | + | | | + | | |
| | *recA1219* | + | | + | | | + | | |
| Class 2B | *recA1211* | ++ | 0.08 | ++ | ++ | | ++ | ++ | + |
| | *recA1214* | ++ | 0.10 | ++ | ++ | + | +++ | | + |
| | *recA1215* | ++ | 0.32 | ++ | ++ | | ++ | | + |
| Class 3 | *recA1221* | +++ | 0.39 | | | + | ++++ | | ++ |
| | *recA1222* | +++ | 0.47 | +++ | +++ | + | ++++ | | ++ |
| | *recA1224* | +++ | 0.44 | +++ | +++ | + | ++++ | | ++ |
| | *recA1227* | | | +++ | | + | ++++ | | ++ |
| Class 4A | *recA1229* | +++ | 1.3 | +++ | | ++ | | | +++ |
| | *recA1230* | +++ | 1.2 | +++ | | ++ | | | ++++ |
| | *recA1235* | +++ | 1.0 | +++ | | ++ | | | +++ |

[a] The term colony growth includes both EOP and colony size. The scoring system covers both these properties. However, for the test of sensitivity to chloramphenicol, only EOP data are presented. Scoring: −, no or very few colonies on the test plate versus about 500 on the control plate (i.e., an EOP of 0.01 or less); +, very small, thin colonies compared with the large dense control colonies, EOP of 0.1 to 0.3; ++, small thin colonies, EOP of 0.3 to 1.0; +++, medium-sized colonies, EOP of about 1.0; ++++, colonies same large size as the *recA*$^+$ control strain, EOP of 1.0. Incubation was at 30°C.

[b] See Table 3, footnote *e*.

appear not to alter the substrate recognition of the RecA protease.

**Enhanced permeability to drugs as an index of protease strength.** An SOS phenomenon with greater resolving power for strong mutants than the two assays described above was found from the unexpected observation that strong Prt$^c$ Rec$^+$ mutants were much more sensitive to mitomycin C than were *recA*$^+$ strains. Since the Prt$^c$ Rec$^+$ mutants were more UV resistant than the *recA*$^+$ strains (see below), it seemed likely that their mitomycin C sensitivity was due to increased permeability for this agent. Enhanced sensitivity to mitomycin C and several other substances turned out to be an SOS phenomenon which could be used to discriminate between different high levels of constitutive protease strength.

EOP and colony size of Prt$^c$ Rec$^+$ mutants were scored on agar that contained different concentrations of mitomycin C but lacked C+G (M9-CAA plates). Some Prt$^c$ mutants were found to be extremely sensitive, with sensitivity to mitomycin C and the other agents tested increasing sharply with decreasing temperature (Table 4). The *lexA71* (Def) *recA*$^+$ strains showed the same high mitomycin C sensitivity as some of the strongest Prt$^c$ mutants.

The Prt$^c$ Rec$^+$ mutants as well as *lexA71* (Def) *recA*$^+$ strains were also tested for sensitivity to crystal violet, chloramphenicol, and kanamycin, all on agar lacking C+G. The *lexA71* (Def) strains were as sensitive as the most sensitive Prt$^c$ mutants. They were even more sensitive than the most sensitive Prt$^c$ mutants on MacConkey-lactose plates at 23°C. The most sensitive Prt$^c$ mutants (class 1 in Table 4) were easily distinguished from the other Prt$^c$ mutants by their failure to grow on MacConkey-lactose agar at room temperature. This class also showed severe growth retardation on TB and LB agar at 30°C, suggesting that some agent in broth can be deleterious to their growth.

The greater sensitivity to drugs was not due to the lack of *dinD* function since λ *recA* (Prt$^c$ Rec$^+$) derivatives of a strain with an intact *dinD*$^+$ gene (EST1555) showed the same drug sensitivity as the strains containing *dinD::lac* (Table 4). Also, the enhanced permeability was probably not due to mutations elsewhere in the λ *recA* prophage genome that might affect host membrane proteins, because drug sensitivity was similar for all mutants assigned to a given class by the other criteria.

Proof that increased permeability to drugs is an SOS phenomenon came from reversal of the phenomenon by the *lexA3* (Ind$^-$) mutation (33) (Table 5). A set of *lexA3* (Ind$^-$) derivatives of the class 1 mutants were constructed by lysogenizing strain EST1142 [*lexA3* (Ind$^-$) Δ*recA dinD*] with the λ *recA* (Prt$^c$) phages corresponding to the class 1 mutants. The *lexA3* (Ind$^-$) mutation reversed the increased sensitivity to chloramphenicol and kanamycin observed for the *recA* (Prt$^c$) alleles in the *lexA*$^+$ background (Table 5). (Reversal of sensitivity to mitomycin C and crystal violet could not be measured because *lexA3* alone confers sensitivity to these agents). The growth retardation of class 1 mutants on TB and LB plates at 30°C was also completely reversed by the *lexA3* mutation (data not shown). We

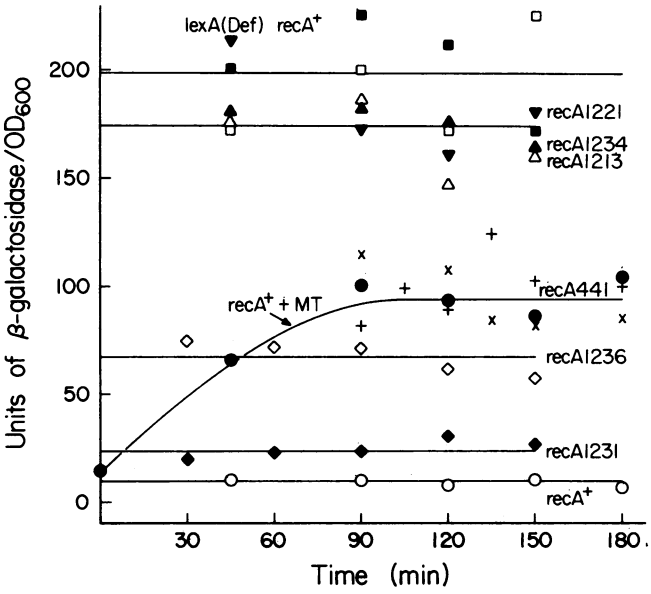


FIG. 1. Specific activity of β-galactosidase expressed from the RecA-inducible gene *dinD*::*lac* in different *recA* (Prt$^c$ Rec$^+$) mutant strains as a function of time. The experiments were performed at 35°C, except for the two *recA441* strains, which were grown at 30°C and shifted to 41°C at time zero. All the strains used except for the *recA441* strains and the *lexA* (Def) control were lysogens of strain EST1515 [Δ*recA dinD*::Mu d(Ap *lac*)], whose only source for the *recA* gene was the prophage λ *recA* cI *ind att*$^+$. The wild-type control was lysogenic for λ *recA*$^+$, and each mutant strain was lysogenic for a different λ *recA* (Prt$^c$ Rec$^+$) mutant phage. The maximally derepressed control strain carried the mutation *lexA71*::Tn*5* (Def) (22) and was lysogenic for λ *recA*$^+$. The strongest *recA* (Prt$^c$ Rec$^+$) mutants shown here (*recA1213*, *recA1221*, and *recA1234*) all had the same protease strength as measured by *dinD*::*lac* expression but were members of three different mutant classes by the other criteria. Symbols: strain EST1550 [*lexA71* (Def) *recA*$^+$], two experiments (□, ■); strain EST1450 (*recA*$^+$), uninduced (○) and induced with mitomycin C at 2.0 μg/ml (●); ▲, *recA1213*; ▼, *recA1221*; △, *recA1234*; ◇, *recA1236*; ◆, *recA1231*; +, strain EST1130 (*recA441*); × strain EST1018 (*recA441*).

conclude that increased permeability to drugs created by *recA* (Prt$^c$) mutations is due to induction of an SOS function and thus is an index of constitutive protease strength.

The Prt$^c$ mutants are listed in Table 4 by degree of sensitivity to drugs, with the most sensitive first. One group of five Prt$^c$ mutants were much more sensitive than the rest and thus were similar to the *lexA* (Def) *recA*$^+$ strain, maximally induced for all SOS functions; this defined class 1 as the strongest Prt$^c$ class. The degree of drug sensitivity also resolved the other Prt$^c$ mutants into separate classes. For the strong mutants, the same class assignments were obtained below by two other criteria, spontaneous mutation frequency and resistance of protease activity to C+G inhibition.

**Spontaneous mutation frequency as an index of protease strength.** At the outset of this work, spontaneous mutation frequency was not known to be an index of protease strength. However, after all the Prt$^c$ mutants had been characterized by protease strength by using the degree of induction of three SOS functions, namely *dinD*::*lac* expression, plaque clarity, and permeability to drugs, it was seen that spontaneous mutation frequency was a useful index of protease strength, distinguishing the same classes as the other criteria (Table 3). This index was of particular value for discriminating the weaker mutants from each other. It also identified the class of strongest mutants (class 1), although the distinction between classes 1 and 2 was not as great as that obtained by measuring permeability to drugs.

We examined spontaneous mutations of our strains because the original Prt$^c$ mutant *recA441* strain has a greatly increased rate of spotaneous mutagenesis at 41°C (13, 43). This increase was absolutely dependent on the presence of an intact *umuC* gene, because introduction of the *umuC122*::Tn*5* allele into the *recA441* strain IT1819, yielding strain IT1823, reduced the mutation frequency to the level of the *recA*$^+$ strain. The same dependence on the *umuC* gene was also found when three mutants of the strongest class were examined. The required *umuC* mutant derivatives were obtained by lysogenizing strain IT1874 with λ *recA1213*, λ *recA1217*, and λ *recA1220*. We also found that inducing the *umuDC* genes by turning on the SOS system with a *lexA* (Def) mutation was not sufficient to increase spontaneous mutation (strain EST1550, Table 3); constitutive protease activity of RecA was also required (strain EST1695), which supports the idea of a second role for RecA in mutagenesis (3).

**Allele-specific inhibition of RecA constitutive protease activity by C+G.** To further distinguish strong Prt$^c$ mutants from each other, the degree of inhibition of their constitutive protease activity by C+G was tested. These nucleosides totally eliminate the constitutive protease activity of *recA441* strains (15). The effect of C+G was measured by the degree of inhibition of constitutive expression of β-galactosidase from the *dinD*::*lac* gene (Fig. 2). Four groups of Prt$^c$ Rec$^+$ mutants were distinguished on the basis of their response to C+G, which corresponded to the classes in Table 3: class 1, noninhibitable by C+G, with specific activity 10 times the *recA*$^+$ strain basal level; class 2A, inhibitable down to about 4 times the basal level; class 2B, inhibitable by C+G down to about 2.5 times the basal level; class 3, inhibitable down to the basal level. Thus, the mutants with the greatest protease strength were the only ones noninhibitable by C+G.

The noninhibitable β-galactosidase synthesis found for class 1 mutants was due only to the *recA* (Prt$^c$) mutations in these strains and not to spontaneous mutation to *lexA* (Def); this was proven by curing each class 1 mutant of its λ *recA* (Prt$^c$) phage. Curing was accomplished by transducing Δ*att*$^\lambda$ into a tetracycline-sensitive version of each class 1 mutant, using strain SP577 as the donor. The resulting Δ*recA* nonlysogens showed about half the low basal level of β-galactosidase specific activity found for the uninduced *recA*$^+$ control strain (Table 3).

TABLE 5. Reversal by the *lexA3* (Ind$^-$) allele of the drug sensitivity of class 1 *recA* (Prt$^c$) mutants[a]

| *recA* (Prt$^c$) allele | Relative EOP[b] on M9-CAA plates containing: | | | |
|---|---|---|---|---|
| | Chloramphenicol (1.6 μg/ml) | | Kanamycin (9.3 μg/ml) | |
| | *lexA*$^+$ | *lexA3* | *lexA*$^+$ | *lexA3* |
| *recA*$^+$ | 0.80 | 0.60 | 0.68 | 0.70 |
| *recA1202* | 0.01 | 0.55 | 0.007 | 0.60 |
| *recA1212* | 0.01 | 0.64 | 0.01 | 0.74 |
| *recA1213* | <0.002 | 0.52 | 0.002 | 0.69 |
| *recA1217* | 0.002 | 0.55 | 0.003 | 0.72 |
| *recA1220* | 0.008 | 0.51 | <0.002 | 0.60 |

[a] Each *lexA3* strain was a λ *recA* lysogen of strain EST1142 (*lexA3* Δ*recA dinD sulA*); each *lexA*$^+$ strain was a λ *recA* lysogen of strain EST1515, (*lexA*$^+$ Δ*recA dinD sulA*).

[b] Relative to that on a drug-free plate at 30°C for 48 h.

It should be noted that the *lexA71* (Def) *recA*$^+$ control strain EST1550 itself showed a nonspecific 40% reduction of β-galactosidase specific activity in the presence of C+G. This reduction appeared to be due to the fact that at a given growth time the cell mass, as measured by the $OD_{600}$, was greater in the presence than in the absence of C+G, whereas β-galactosidase activity, as measured by the $OD_{420}$, was about the same whether or not C+G was present. This reduction was considered nonspecific because the *lexA71* (Def) mutant is maximally derepressed for *dinD* (22) and therefore any change in its β-galactosidase specific activity is independent of the *recA* allele (Table 3). The Prt$^c$ mutants that were termed noninhibitable by C+G (class 1) showed the 40% decrease in the presence of C+G seen for the *lexA* (Def) strain for the same reason.

**Further characterization of Prt$^c$ Rec$^+$ mutants by response to effectors.** For the original Prt$^c$ mutant strain *recA441*, inhibition by C+G is reversed by DNA damage (15). It was important to test whether reversal of C+G inhibition by DNA damage occurred for Prt$^c$ Rec$^+$ mutants other than the *recA441* strain because one Prt$^c$ Rec$^-$ mutant, the *recA1201* strain, showed no reversal. Using mitomycin C as a DNA-damaging agent, we tested the four strong mutants of class 3 and also three weaker Prt$^c$ Rec$^+$ mutants of class 4A, all of which were severely inhibited by C+G. We found that a given dose of mitomycin C in the presence of C+G induced the wild-type level of β-galactosidase specific activity for all the Prt$^c$ Rec$^+$ mutants, i.e., the C+G inhibition was reversed. This is shown for a class 3 mutant in Fig. 2.

In previous work we showed that activation of the RecA441 protein in vivo involved competition between derivatives of adenine and of C+G (39). In the present work we found only about 20% restoration by adenine (at 100 to 300 μg/ml) of the constitutive protease activity of several

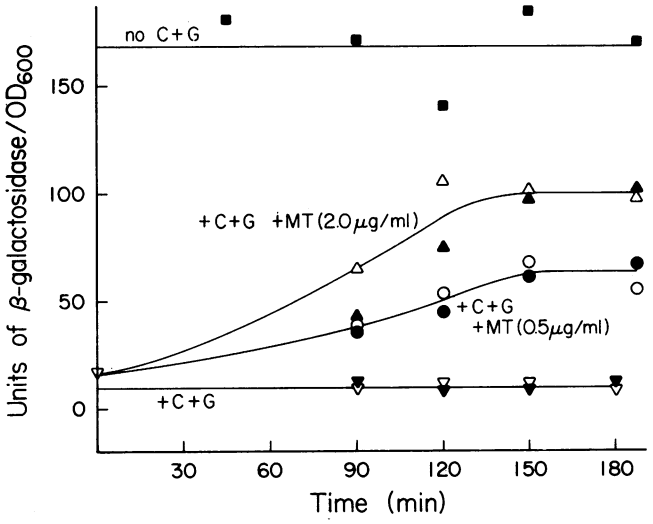


FIG. 2. Inhibition by C+G of the constitutive RecA protease activity of a *recA* (Prt$^c$ Rec$^+$) *dinD*::*lac* class 3 mutant strain, *recA1221*, and reversal of this inhibition by mitomycin C (MT) as indicated by the increase in specific activity of β-galactosidase expressed from *dinD*::*lac* at different times after addition of mitomycin C. At time zero mitomycin C at 0.5 or 2.0 μg/ml was added to cultures of the *recA1221* (Prt$^c$ Rec$^+$) (solid symbols) or the *recA*$^+$ (open symbols) strain. Growth was continued at 35°C, and samples were assayed for β-galactosidase specific activity at the indicated times. Symbols: ■, no C+G; ▼, ▽, with C+G; ●, ○, C+G plus MT (0.5 μg/ml); ▲, △, with C+G plus MT (2.0 μg/ml).

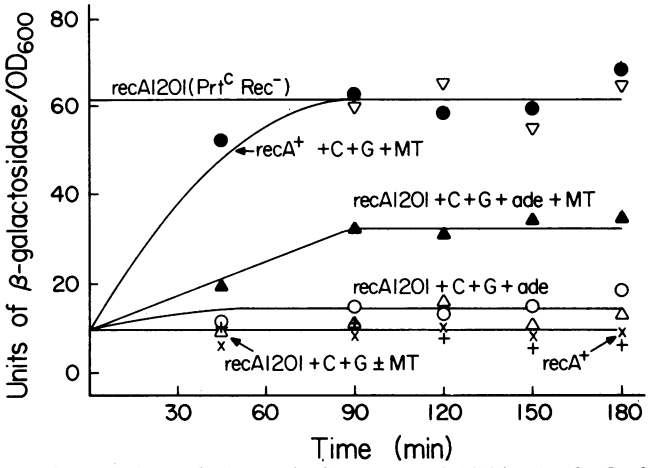


FIG. 3. Failure of mitomycin C to reverse inhibition by C+G of the constitutive protease activity of the *recA1201* (Prt$^c$ Rec$^-$) strain. The *recA1201* culture grown in M9-CAA medium with C+G was divided into three parts. To one part was added mitomycin C (MT, 0.5 μg/ml), to a second was added mitomycin C (0.5 μg/ml) plus adenine (ade, 200 μg/ml), and to a third no additions were made. Mitomycin C (0.5 μg/ml) was added to the *recA*$^+$ culture with C+G at time zero. Symbols: ●, *recA1201*, no additions; +, *recA1201* with C+G; △, *recA1201* with C+G and mitomycin C; ○, *recA1201* with C+G and adenine; ▲, *recA1201* with C+G, adenine, and mitomycin C; ▽, *recA*$^+$ with C+G and mitomycin C; ×, *recA*$^+$, no additions.

C+G-inhibited Prt$^c$ *dinD* strains, including the *recA441* strain. This low degree of reversal by adenine compared with the strong reversal found previously may be due to the greater difficulty of induction of *dinD* than of the SOS phenomena used previously as assays.

**A *recA* (Prt$^c$ Rec$^-$) mutant with an anomalous response to effectors.** Although Prt$^c$ Rec$^-$ mutants are described in detail elsewhere (40), discussion of one mutant is pertinent here because it failed to show the reversal of C+G inhibition by mitomycin C described above for the Prt$^c$ Rec$^+$ mutants. A mutant with an anomalous response of this type is likely to have an unusual alteration of the effector-binding sites on the RecA protein. The anomalous mutant strain, *recA1201* (Prt$^c$ Rec$^-$), showed moderate constitutive protease activity that was inhibitable by C+G to the *recA*$^+$ strain basal level (Fig. 3). In the absence of C+G, the *recA1201* strain showed the same response to mitomycin C as other moderate Prt$^c$ mutants, i.e., an increase in β-galactosidase activity of 1.5 to 2.0 times the constitutive level (data not shown). However, in the presence of C+G, mitomycin C gave no induction of β-galactosidase above the *recA*$^+$ strain basal level, i.e., the C+G inhibition of protease activity was not reversed (Fig. 3). Thus, the response of this Prt$^c$ Rec$^-$ mutant to mitomycin C in the presence of C+G was completely different from the response of seven new Prt$^c$ Rec$^+$ mutants and of the *recA441* strain, all of which had the C+G inhibition reversed by mitomycin C.

**Superresistance of certain *recA* (Prt$^c$) mutants to UV; evidence for enhanced specific activity of recombinase function.** UV survival curves were obtained for two Prt$^c$ Rec$^+$ mutants of class 1 and one class 2 mutant. DNA sequence analysis of two of the mutants, carrying *recA1211* and *recA1213*, respectively, showed that they were different alleles (W.-B. Wang and E. S. Tessman, unpublished data). These three Prt$^c$ mutants were considerably more UV resistant than the *recA*$^+$ strain (Fig. 4A). A similar result was found previously (24) for the constitutive protease mutant (*recA730*) derived from the *recA441* strain.

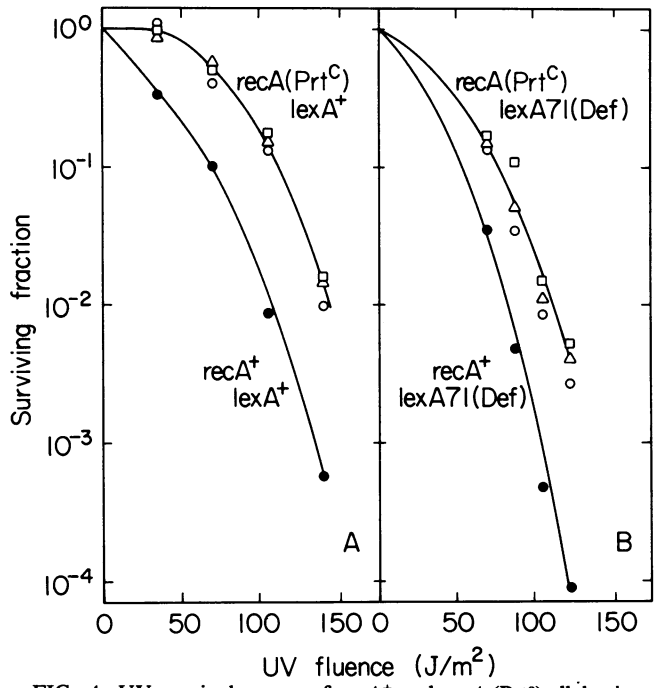


FIG. 4. UV survival curves of *recA*$^+$ and *recA* (Prt$^c$) alleles in a *lexA*$^+$ background (A) and an isogenic *lexA* (Def) background (B). (A) Strains: ○, *lexA*$^+$ *recA*$^+$ (EST1450); ●, *lexA*$^+$ *recA1211*; △, *lexA*$^+$ *recA1213*; □, *lexA*$^+$ *recA1212*. (B) Strains: ●, *lexA71* (Def) *recA*$^+$ (EST1550); ○, *lexA71* (Def) *recA1211* (EST1678); △, *lexA71* (Def) *recA1213* (EST1665); □, *lexA71* (Def) *recA1212* (EST1696).

To test whether the greater UV resistance might be due in part to an increased specific activity of the recombinase function of the RecA (Prt$^c$) proteins rather than simply to an increase in the amount of RecA protein, we compared the same *recA* (Prt$^c$) alleles with *recA*$^+$ under conditions of maximum induction of the SOS system, i.e., in a *lexA71* (Def) background (Fig. 4B), and found that the strong *recA* (Prt$^c$) alleles were again more UV resistant than was *recA*$^+$. In the *lexA71* (Def) background, RecA and all other SOS proteins are assumed to be maximally induced in all strains (22). Therefore, any differences in UV resistance should be due to a difference in the *recA* allele. UV resistance is used as a rough index of recombinase function (36). The fact that the *recA* (Prt$^c$) alleles were more UV resistant than *recA*$^+$ in the *lexA* (Def) background suggests that the corresponding RecA (Prt$^c$) proteins have enhanced specific recombinase activity.

## DISCUSSION

We have isolated two general classes of *E. coli recA* mutants with altered RecA protease activity by using a new isolation method: one class has constitutive protease activity, and the second class has lower protease activity than wild-type RecA, undetectably low in most cases. In mutants with constitutive protease activity, denoted *recA* (Prt$^c$), RecA protein is in the protease state at all times without the usual need for damaged DNA to activate it. For many years only one *recA* (Prt$^c$) mutant was available, namely the *recA441* (*tif-1*) strain (15), and most of what we know about RecA protease activity comes from the study of the RecA441 protein (8, 28, 35). However, the *recA441* strain is an atypical Prt$^c$ mutant. It is a double mutant (21), with one of the mutations appearing to be a thermosensitive suppressor of constitutive protease activity (42).

Although several more *recA* (Prt$^c$) mutants have been isolated recently (32), including some derivatives of the *recA441* strain (45), there was a need for a rapid method for isolating and characterizing Prt$^c$ mutants so that a large number of mutants with different phenotypes would be available for study. Our isolation of about 150 Prt$^c$ mutants, most of which were recombination proficient and UV resistant, made possible the detection of a new class of mutants, Prt$^c$ Rec$^-$, that had substantial constitutive protease activity but were completely recombination deficient and very UV sensitive (40).

The main aim of the present work was to identify Prt$^c$ mutants with very strong constitutive protease activity, since these would be the most interesting for biochemical analysis of the RecA protease function. It was therefore necessary to discriminate among mutants that differed widely in protease strength. To identify the strongest Prt$^c$ mutants, an assay with good resolution at high protease strength was sought when it became apparent that the assays used first were being saturated. Of the three assays used here, the one that gave the poorest resolution of the strong protease mutants was expression of the difficult-to-induce SOS gene *dinD::lac*. The assay with the best resolution at high protease strength depended on a new SOS phenomenon, increased permeability to drugs. This was proven to be an SOS phenomenon because the greater sensitivity to drugs was totally reversed by the *lexA3* (Ind$^-$) mutation, which blocks expression of the SOS system (33). The new assay provided an index of protease strength which clearly distinguished the five strongest mutants from the other 150 mutants tested. After the strongest protease mutants were identified by their greatly increased permeability to drugs, it was seen that they responded differently from all the others to C+G, which inhibits the constitutive protease activity of most Prt$^c$ mutants but does not affect the strongest.

The new SOS phenomenon was detected by the extreme sensitivity of certain Prt$^c$ mutants to mitomycin C, an unexpected finding in view of the superresistance of these same mutants to UV. The Prt$^c$ mutants also showed hypersensitivity to crystal violet, chloramphenicol, kanamycin, and some unknown component of broth. This increased permeability to drugs may result from induction of a still unidentified SOS gene or from one that is already known.

The strongest Prt$^c$ mutants also had the highest spontaneous mutation frequencies. We found here that spontaneous mutation frequency can be used as an index of constitutive protease strength; it distinguished the same mutant classes that were distinguished by proven indicators of protease strength. This result could not have been predicted in advance because spontaneous mutation frequency is a complex parameter, doubly dependent on the constitutive protease activity of RecA. In this work we found that spontaneous mutation of even the strongest Prt$^c$ mutants required an intact *umuC* gene. We also found that derepression of *umuDC* by a *lexA* (Def) mutation is not sufficient for enhanced spontaneous mutagenesis; a RecA protein with constitutive protease activity is also required, in confirmation of the results of Blanco et al. (3). Thus, increased spontaneous mutagenesis requires the constitutive protease activity of RecA both for induction of *umuDC* and also for a noninducing second role of RecA.

In view of the enhanced binding of effectors to the RecA441 protein (7, 8, 28, 35), we expected the new Prt$^c$ mutants also to be altered in the effector-binding sites of

RecA protease. The following argument shows how the strong correlation that we found between constitutive protease activity and resistance of this activity to C+G inhibition can be interpreted to mean that the new mutants do indeed exhibit enhanced binding of the effectors.

Activation in vitro of either RecA441 or wild-type RecA protein to the protease state involves the formation of a ternary complex in which two different effectors must bind concurrently to the RecA protein (7, 8, 35). These two effectors are ssDNA and an NTP, dATP being the most effective of the latter (35). To explain the allele-specific differences in degree of C+G inhibition of protease activity in terms of the in vitro requirement for an NTP effector of RecA protease activity (8, 35), we assume that adenine is the precursor of the positive NTP effector (36) and that C and G are precursors of negative NTP effectors. Previously we reported evidence for competition in vivo between adenine and C+G in activating the protease function of *recA441* (39). Because of this competition we expect that the stronger the affinity of a given type of RecA protein for ATP-like effectors, the less will be the binding of derivatives of C+G. Accordingly, we expect a Prt$^c$ mutant that is not inhibitable by C+G to have a RecA protein with a stronger affinity for ATP-like effectors than a RecA protein from a Prt$^c$ mutant that is inhibitable.

We assume that all Prt$^c$ mutants are altered in effector binding to some degree. However, we would expect the mutations that cause the altered binding affinities to be located not only in the regions of the *recA* gene that code for the ATP-binding site but also in the regions that code for the ssDNA-binding site, since binding of the ATP-like effector is dependent on binding of ssDNA (8).

The ability of DNA-damaging agents such as mitomycin C to reverse C+G inhibition of Prt$^c$ mutants probably reflects an enhanced binding of the ATP-like effector of protease function caused by binding of damaged DNA, which provides the ssDNA effector. A mutant that responds differently from the others to DNA-damaging agents is therefore likely to be altered in its effector-binding properties. Such a mutant was the *recA1201* (Prt$^c$ Rec$^-$) strain, which differed from Prt$^c$ Rec$^+$ mutants in that its inhibition by C+G could not be reversed by mitomycin C, i.e., in the presence of C+G plus mitomycin C, no β-galactosidase was induced from *dinD*::*lac*. In contrast, Prt$^+$ Rec$^+$ mutants treated with mitomycin C in the presence of C+G gave the wild-type induced level of β-galactosidase activity. The *recA1201* mutant is unusual because it shows constitutive and damage-activable protease activity in the absence of C+G, but fails to show the expected damage-activable protease activity in the presence of C+G. RecA1201 protein may be altered in effector preference so that binding of negative NTP effectors is strongly favored over binding of the positive NTP effector.

RecA (Prt$^c$) proteins, because of their apparent increased affinity for the positive effectors of protease function, might be expected to have enhanced recombinase function, since initiation of recombination, like activation to the protease state, requires binding of ssDNA and an ATP-like effector (6, 38), and the same effector-binding sites on RecA may be used for both functions (37). We were able to use UV resistance as a rough index of recombination frequency because recombinational repair appears to be the most important component in repair of UV-irradiated DNA (36). To determine relative specific recombinase activity, UV resistance was measured in a *lexA* (Def) strain so that all *recA* mutants would be maximally and therefore equally derepressed for RecA protein synthesis. Comparison of the UV survival of several strong *recA* (Prt$^c$) mutants with that of a *recA*$^+$ strain in a *lexA* (Def) background showed that the *recA* (Prt$^c$) mutants were more UV resistant than the *recA*$^+$ strain (Fig. 4B). The increased UV resistance found for the Prt$^c$ mutants supports the idea that increased protease activity is accompanied by increased specific activity of recombinase function.

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