Roles of RecA Protease and Recombinase Activities of *Escherichia coli* in Spontaneous and UV-Induced Mutagenesis and in Weigle Repair

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The RecA protein has a second, direct role in the mutagenesis of *Escherichia coli* and bacteriophage lambda in addition to its first, indirect role of inducing the SOS system by enhancing the proteolytic cleavage of the LexA repressor protein. The need for RecA protease and recombinase functions in the direct role was examined in cells containing split-phenotype RecA mutations, in the absence of LexA protein. Spontaneous mutation of *E. coli* (*his* → *his*⁺) required both the protease and recombinase activities. The mutation frequency increased with increasing RecA protease strength. In contrast, UV-induced mutation of *E. coli* required only the RecA protease activity. Weigle repair and mutation of UV-irradiated phage S13 required only RecA protease activity, and even weak activity was highly effective; RecA recombinase activity was not required. RecA⁺ protein inhibited RecA (Prt^c [protease constitutive] Rec⁺) protein in effecting spontaneous mutation of *E. coli*. We discuss the nature of the direct role of the RecA protein in spontaneous mutation and in repair and mutagenesis of UV-damaged DNA and also the implications of our results for the theory that SOS-mutable cryptic lesions might be responsible for the enhanced spontaneous mutation in Prt^c Rec⁺ strains.

The RecA protein of *Escherichia coli* responds to DNA damage by activation to a so-called protease state in which it enhances the proteolytic cleavage of the LexA repressor protein, thus inducing the SOS gene system, including some genes involved in repair of damaged DNA (10). The RecA protein can also be activated to this protease state by mutations, *recA* (Prt^c), which confer constitutive protease activity on the protein even in the absence of DNA-damaging treatments (6–8, 15, 22). For the three *recA* (Prt^c) mutants studied, it has been suggested that their RecA (Prt^c) proteins are in the activated state without DNA damage because they bind the regions of single-stranded (ss) DNA present in normal cells (11, 13). Many additional *recA* (Prt^c) mutants are now available (15, 16), and it is conceivable that some of these may not even require any binding of ssDNAs to activate the protein to the protease state. The terms protease state and protease activity are not meant to imply that the RecA protein is a true protease in contrast to the possibility that it may be a cofactor in the self-proteolysis of the LexA repressor (9).

Mutagenesis of *E. coli* and of bacteriophage lambda has been shown to require activated RecA protein for induction of the *umuDC* genes of the SOS system. Intact *umu* genes are essential for both spontaneous (4, 15, 21) and UV-induced (1, 7, 14) mutagenesis. But even when the *umu* genes are fully induced by a *lexA* (Def) mutation, activated RecA is required for a second, direct role in mutagenesis (2, 4, 16, 21) in addition to its first, indirect role. This second role of activated RecA protein does not involve induction of any non-SOS genes (21), a finding that justifies the assumption that the second role is a direct one.

In the present work the direct role of RecA function in mutagenesis was dissected with the aid of a large variety of *recA* mutants (15, 16), particularly those with the phenotypes Prt^c Rec⁺, Prt^c Rec⁻, and Prt⁻ Rec⁻. The protease and recombinase activities of RecA are interrelated in a complex

way (19). Fortunately, the *recA* mutants provide a means to treat these activities as separate to a first approximation and to correlate them with mutagenesis and repair. We show that some aspect of the RecA protease activity that is required for cleavage of the LexA repressor was required for the direct role in mutagenesis of UV-irradiated *E. coli* and also in Weigle repair and mutagenesis of UV-irradiated phage S13. Some aspect of RecA recombinase activity, as well as protease activity, was needed for spontaneous mutagenesis of *E. coli*.

MATERIALS AND METHODS

Bacterial strains. All the *lexA*⁺ strains were lysogens of strain EST1515 $\Delta recA306$ *sulA211* *dinD1::Mu* d(Ap *lac*) *his-4*, a temperature-resistant strain whose derivation was described previously (15). All the λ *recA* phages used to form the lysogens were obtained by mutagenesis of λ *recA*⁺ *cl* *ind* *att*⁺, a phage containing a cloned *recA* gene constructed by Sara Cohen and David Mount; the properties of the *recA* alleles were described previously (15) except for *recA1603* and *recA1604*, which were found to be class 4 Prt^c Rec⁺ mutants by the criteria of Tessman and Peterson (15). All the *lexA* (Def) strains were constructed by lysogenizing strain EST1663, which is *lexA71* (Def) $\Delta recA306$ *sulA211* *dinD1::Mu* d(Ap *lac*) *his-4* (15), or its S13-sensitive derivative, IT1865. The *recA*⁺/*recA* (Prt^c Rec⁺) diploid strain (EST2214) was constructed by lysogenizing *lexA* (Def) *recA*⁺ *sulA211* *dinD1::Mu* d(Ap *lac*) *his-4* (EST2076) with λ *recA1217*.

Media. The M9-CAA medium and the limiting histidine plates have been described (15). When cytidine (C) and guanosine (G) were added to limiting histidine plates, C was added to 300 μ g/ml and G was added to 350 μ g/ml.

Phenotype symbols. A *recA* mutant with constitutive ability to greatly enhance the proteolytic cleavage of LexA repressor protein is designated Prt^c. The Rec⁻ designation indicates reduced recombination in bacterial matings and greatly increased UV sensitivity (15).

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TABLE 1. Effect of *recA* (Prt^c Rec⁺) and *recA* (Prt^c Rec⁻) alleles on spontaneous mutagenesis of *E. coli* (*his-4* → *his*⁺)

Mutant class ^a	<i>recA</i> allele	No. of <i>his</i> ⁺ colonies per plate (mean ± SEM) ^b			
		Without C plus G		With C plus G	
		<i>lexA</i> ⁺ ^c	<i>lexA</i> (Def)	<i>lexA</i> ⁺	<i>lexA</i> (Def)
Prt ⁺ Rec ⁺	<i>recA</i> ⁺	1.4 ± 0.2	5.6 ± 1.0	1.3 ± 0.2	5.0 ± 1.7
Prt ^c Rec ⁺	1				
	<i>recA1202</i>	475 ± 16	447 ± 18	232 ± 27	232 ± 5
	<i>recA1212</i>	360 ± 34	318 ± 20	ND	131 ± 9
	<i>recA1213</i>	256 ± 8	328 ± 13	ND	124 ± 6
	<i>recA1217</i>	317 ± 15	336 ± 46	ND	125 ± 10
	<i>recA1220</i>	286 ± 14	358 ± 13	ND	104 ± 11
	2				
	<i>recA1211</i>	194 ± 10	311 ± 11	40 ± 5	98 ± 7
	<i>recA1215</i>	203 ± 13	298 ± 11	33 ± 4	71 ± 5
	3				
	<i>recA1221</i>	97 ± 7	117 ± 10	2.0 ± 1.2	37 ± 1
	<i>recA1222</i>	96 ± 5	139 ± 10	4.3 ± 1.1	ND
	<i>recA1224</i>	66 ± 7	113 ± 7	ND	45 ± 4
	4				
	<i>recA1231</i>	9.0 ± 1.0	109 ± 9	2.7 ± 0.6	42 ± 3
	<i>recA1232</i>	6.1 ± 0.9	120 ± 4	1.7 ± 0.7	33 ± 4
	<i>recA1237</i>	6.2 ± 0.2	102 ± 11	1.7 ± 0.7	39 ± 10
	<i>recA1238</i>	5.5 ± 0.7	94 ± 5	1.3 ± 0.3	12 ± 1
	<i>recA1603</i>	1.5 ± 0.5	63 ± 8	ND	8 ± 2
	<i>recA1604</i>	2.0 ± 2.0	66 ± 5	ND	9 ± 1
Prt ^c Rec ⁻	<i>recA1201</i>	4.8 ± 1.1	7.0 ± 2.3	ND	ND
	<i>recA1203</i>	11.0 ± 1.0	9.0 ± 1.2	ND	ND
	<i>recA1204</i>	10.0 ± 1.5	5.7 ± 2.2	ND	ND
	<i>recA1205</i>	6.0 ± 2.3	8.3 ± 3.0	ND	ND
	<i>recA1206</i>	6.5 ± 0.5	4.7 ± 1.2	ND	ND
	<i>recA1207</i>	4.5 ± 0.5	11.3 ± 0.7	ND	ND
Prt ^c Rec ⁻ (avg for all strains)		7.1 ± 1.1	7.1 ± 1.0		

^a The protease strengths of the Prt^c Rec⁺ mutants decrease from classes 1 to 4 (15). The protease strengths of the Prt^c Rec⁻ mutants fall between those of the class 3 and 4 mutants based on the induction of *dinD* (16).

^b Each value is the average for at least six plates. ND, Not determined.

^c Reproduced from previous findings of Tessman and Peterson (15) except original data for *recA* alleles 1238, 1603, and 1604 and all the Prt^c Rec⁻ mutants.

Determination of spontaneous and UV-induced mutagenesis of *E. coli* from *his*⁻ to *his*⁺. Spontaneous mutagenesis was measured as described previously (15). To determine the frequency of UV-induced mutagenesis, cultures of *recA* (Prt^c Rec⁻) mutants were grown in M9-CAA medium to the late log phase and centrifuged and concentrated 10-fold in M9 salts, after which 0.1-ml portions were spread on limiting histidine plates and UV irradiated. The plates were incubated for 4 days at 37°C. For each strain, the survival at each UV dose was determined by spreading 10⁴- to 10⁶-fold dilutions of the cultures on limiting histidine plates and then irradiating the plates. This procedure gave more consistent results than did a previously described procedure (16) in which undiluted cells are spread and irradiated, the plates are washed, and the cells are then diluted before respreading for assay of survivors. The procedure used for determining the frequency of UV-induced mutagenesis of Prt^c Rec⁻ mutants was also used for the study of Prt^c Rec⁺ mutants and the wild-type strain except that cells were not concentrated when centrifuged and suspended in M9 salts.

Determination of total number of cells in the background film on limiting histidine plates. Cells of the *lexA* (Def) *recA*⁺ *sulA211 dinD1 his-4* strain, EST1550, were grown to about 10⁹ cells per ml in M9-CAA medium, centrifuged twice, and

suspended in the same volume of M9 salts, after which 0.1 ml was spread on each of two plates containing limiting histidine agar that was either supplemented with C plus G or was unsupplemented. The plates were incubated at 35°C for 24 h, by which time the films had all reached maximum turbidity; incubation for a second day did not increase the number of cells per plate. Each plate was washed with 2 ml of M9 salts, and the cells were dislodged with a spreader; the plate washings were weighed to determine the volume recovered. The cells from each plate were diluted in M9 salts and plated in duplicate on limiting histidine plates for cell counts.

Derivatives of *E. coli* K-12 sensitive to phage S13. Phages S13 and St-1 are closely related (5) but have a distinctive difference: most strains of *E. coli* K-12 normally adsorb St-1 but not S13. Among St-1⁺ K-12 mutants, approximately 50% adsorb S13. We used this property to select S13^s derivatives of *E. coli* K-12. Weigle repair and mutagenesis were measured as previously described (17).

RESULTS

Spontaneous mutation of *E. coli* requires RecA protease activity. The effect of *recA* (Prt^c) alleles (Table 1) on the

frequency of the spontaneously occurring mutation *his-4* → *his*⁺ was determined in a *lexA* (Def) background to separate the second role of RecA from its better known role in cleavage of the LexA repressor. Five indices of protease strength that were previously evaluated (15) were used to relate the spontaneous mutation frequency to protease strength; these indices give semiquantitative estimates of the proteolytic activity of the RecA protein for cleavage of the LexA repressor because they were studied in a *lexA*⁺ strain. These indices are (i) induction of the SOS gene *dinD::lac*, which distinguishes mutants of moderate and low protease strength (class 4) from all the rest; (ii) inhibition by C plus G of the induction of *dinD::lac*, which distinguishes the strong protease mutants (classes 1 and 2) from each other and from classes 3 and 4; (iii) induction in the *lexA*⁺ background of one or more unknown SOS genes that cause enhanced permeability for various drugs (this index distinguishes all four classes of mutants); (iv) induction of λ *imm*⁴³⁴, which distinguishes classes 1 and 2 from the weaker classes 3 and 4; and (v) the spontaneous mutation frequency in the *lexA*⁺ background, which reflects in part the degree of induction of the SOS genes *umuDC* and also in part the second role of the RecA protein (this index distinguishes the four classes of mutants from one another). The last index is reproduced in Table 1 for comparison with the spontaneous mutation frequencies that were obtained in the *lexA* (Def) background.

In the *lexA* (Def) strain, the second role of the RecA protein was immediately evident. In a strain containing a *recA*⁺ allele, derepression of the SOS genes produced only a small increase (ca. fourfold) in the spontaneous mutation frequency; the value of 5.6 ± 1.0 mutants per plate was the basal level in the absence of substantial activated RecA protein. A large increase (57- to 80-fold) occurred with the strongest (class 1) protease-constitutive mutants. The weaker the protease activity the less effective was the *recA* mutant in its second role, but even the weakest *Prt*^c mutants (class 4) showed a substantial increase (12- to 21-fold) in mutation frequency if the LexA protein was inactive.

A comparison of spontaneous mutation frequencies between the *lexA*⁺ and *lexA* (Def) strains revealed the relative importance of protease activity in the direct and indirect roles of the RecA protein in the formation of spontaneous mutations (Table 1). For the strongest protease-constitutive *recA* alleles (class 1), there was little difference. This was because the class 1 mutants were presumably strong enough in their ability to derepress the SOS genes (the primary role of RecA) so that the *lexA*⁺ strain was almost equivalent to a *lexA* (Def) strain in expression of the *umuDC* genes. But for the weaker classes of *recA* (*Prt*^c) mutants, the spontaneous mutation frequency was substantially higher in the *lexA* (Def) strain than in the *lexA*⁺ strain. This was most striking among the class 4 mutants. For the same *recA* allele, the difference between the *lexA*⁺ and *lexA* (Def) strains must have been largely due to the ability of RecA protease to effect cleavage of the LexA repressor. Therefore, the striking differences seen in the two backgrounds for the weaker classes of *Prt*^c mutants must have been due to the inability of their RecA proteins to fully induce the SOS genes; the indirect role appears to be more dependent than the direct role on the degree of protease activity.

Inhibition of the spontaneous mutation frequency by C plus G. In liquid culture, addition of the combination of C and G, a precursor of negative effectors, reduces the protease activity for *dinD::lac* expression in class 2, 3, and 4 *Prt*^c mutants, but not in class 1 mutants (15). Derivatives of C plus G interact in vivo directly with RecA protein, rather

FIG. 1. Frequency of *his-4* → *his*⁺ mutations and cell survival as a function of UV fluence. All strains were λ *precA* lysogens of the *lexA*⁺ strain EST1515: EST1454 *recA1206*, EST1822 *recA1212*, and EST1450 *recA*⁺ (15, 16). The off-scale value of the spontaneous mutation frequency of the *recA*⁺ strain was 6×10^{-9} .

than producing some nonspecific effect on metabolism (19). In the plate test for spontaneous mutants (Table 1), addition of C plus G reduced the mutation frequency for all classes of *recA* (*Prt*^c) alleles in both *lexA*⁺ and *lexA* (Def) backgrounds. The reduction in mutation frequency by C plus G was not a trivial effect due to a reduced number of cells in the growth film on the limiting histidine plates, which could account for the reduced number of spontaneous mutants, since the number of spontaneous *his*⁺ colonies depends on the total number of cells in the film. The number of cells in the film was measured for the *recA*⁺ allele in the *lexA* (Def) background by washing the plate as described in Materials and Methods. The number of cells was $(1.5 \pm 0.1) \times 10^8$ in the absence of C plus G and $(1.1 \pm 0.1) \times 10^8$ in the presence of 300 μ g of C per ml plus 350 μ g of G per ml; the difference could not have accounted for the two- to threefold reduction in the number of mutant colonies.

Therefore, a semiquantitative correlation between the spontaneous mutation frequency and RecA protease activity is demonstrated in Table 1, which indicates that the second role of RecA in spontaneous mutation depends on its protease function.

Spontaneous mutation of *E. coli* requires RecA recombinase activity. The existence of *Prt*^c *Rec*⁻ mutants (15) enabled us to determine whether the recombinase function was also required for the second role of RecA protein in spontaneous mutation (Table 1). The *Prt*^c *Rec*⁻ mutants had considerably more protease activity than do the weakest *Prt*^c *Rec*⁺ strains (15, 16), but in contrast to the weakest *Prt*^c *Rec*⁺ mutants, in the *lexA* (Def) background they showed no significant increase in spontaneous mutation frequency over the *recA*⁺ value. Thus, we see that the second role of the RecA protein in spontaneous mutation requires not only the protease function but also some aspect of the recombinase function. The five *Prt*^c *Rec*⁻ mutants have been found by DNA sequence analysis to represent alterations at four different sites (19).

UV-induced mutation of *E. coli* does not require RecA recombinase activity. It has been shown that the *recA1203* (*Prt*^c *Rec*⁻) allele, which is devoid of recombinase activity, gives a frequency of UV-induced mutagenesis for *his-4* → *his*⁺ in a *lexA*⁺ background that is as high as that obtained

TABLE 2. Dependence of Weigle mutagenesis of UV-irradiated phage S13 on RecA protease and recombinase activity^a

Test strain ^b	Surviving fraction (S) ± 10%	Weigle repair sector (W) ^c ± 10%	Mutation frequency (M)	Specific mutation frequency (M/h _r) ^d
IT1865 <i>lexA</i> (Def) <i>ΔrecA</i> (Prt ⁻ Rec ⁻)	9.2×10^{-6}	<0.01	0.012 ± 0.005	NM
EST1640 <i>lexA</i> (Def) <i>recA441</i> (Prt ^c Rec ⁺)	3.0×10^{-8}	ND	0.017 ± 0.005	ND
EST1529 <i>lexA</i> ⁺ <i>ΔrecA</i> (λ <i>recA1203</i> [Prt ^c Rec ⁻])	9.2×10^{-6}	0.17	0.140 ± 0.020	0.071 ± 0.010
	1.0×10^{-5}	0.165	0.125 ± 0.020	0.067 ± 0.010

^a The cells were grown at 37°C to the mid-log phase and concentrated to 2×10^9 /ml. The irradiated phage was plated with 0.15 ml of the concentrated log-phase cells, and incubated at 35°C. NM, Not measurable; ND, not determined.

^b The Prt⁺ Rec⁺ strain EST1528 *lexA*⁺ *ΔrecA*(λ *recA*⁺) was used to determine the survival (S) and was the reference strain for Weigle repair sector (W) = 0.

^c Defined by $W = 1 - \log S_r / \log S_n$, where S_r and S_n are the fractions of irradiated viruses surviving when plated on repairing and nonrepairing cells, respectively, as previously described (17).

^d The mutation frequency per repaired lethal hit, h_r , where $h_r = -W \ln S$.

for the *recA*⁺ allele (16). In the present work, UV-induced mutagenesis was measured for another Prt^c Rec⁻ allele, *recA1206*, which is also devoid of recombinase activity and has a base change at a different site (19). The maximum observed UV-induced mutation frequency in the *recA1206* strain was about as high as that observed for a strong Prt^c Rec⁺ strain, *recA1212* (Fig. 1), particularly when the high spontaneous frequency in the *recA1212* strain was subtracted from the induced frequency. Since there was clearly an absolute increase in the number of mutations for most of the UV fluences applied to the *recA1206* strain, the conclusion that the mutations were UV induced did not depend on the determination of cell survival. We confirmed that UV-induced mutagenesis of *E. coli* does not require recombinase activity.

RecA⁺ protein inhibits activity of RecA (Prt^c Rec⁺) protein in spontaneous mutation. A diploid *recA* strain, EST2214, was constructed in a *lexA* (Def) background by lysogenizing strain EST2076, which contains the *recA*⁺ gene at its normal chromosomal site, with *recA1217* (Prt^c Rec⁺). The two types of RecA protein would be expected to be present in equimolar amounts, with the amount of each being the same as in a *lexA* (Def) strain haploid for *recA*. A haploid *recA1217* strain produced 317 spontaneous *his*⁺ colonies (Table 1). A 10-fold decrease in the spontaneous mutation frequency was found in the diploid. This implies that the RecA⁺ protein inhibits the RecA1217 (Prt^c Rec⁺) protein. The inhibited level of spontaneous mutants, 35 per plate, was still higher than the spontaneous mutant frequency of 5.6 per plate (Table 1).

Weigle repair and mutagenesis of UV-irradiated phage S13 requires RecA protease activity but not recombinase activity. The second role of the RecA protein in Weigle repair and mutagenesis of phage S13 was demonstrated as shown in Table 2. In the *lexA* (Def) *ΔrecA* (Prt⁻ Rec⁻) strain IT1865 there was no detectable repair of the phage relative to the *lexA*⁺ *recA*⁺ (Prt⁺ Rec⁺) strain EST1528. The frequency of phage temperature-sensitive mutants induced in IT1865 was not significantly different from what is observed in *lexA*⁺ *recA*⁺ cells (17). In comparison, the *lexA* (Def) *recA441* (Prt^c Rec⁺) strain EST1640 showed Weigle repair and the high frequency of 14% temperature-sensitive mutants. The specific frequency of 0.071 ± 0.010 mutants per repaired lesion is about what is usually observed for Weigle repair of S13 (unpublished observations) and is in reality a high frequency (see Discussion). We thus see that the RecA protein is needed for a second role in S13 repair and mutagenesis. The *recA441* mutation confers high protease activity at 41°C (6, 15), but the lower activity at 37°C was still adequate for the

second role. We will present additional evidence that relatively little protease activity is needed for the RecA protein to be effective in its second role.

Repair and mutation were normal in the *lexA*⁺ *recA1203* (Prt^c Rec⁻) strain EST1529, proving that the recombinase function was not needed. In this case the background was *lexA*⁺, so the constitutive activity was also needed to cleave the LexA repressor.

To determine semiquantitatively how much protease activity is needed for the second role of RecA, Weigle repair was determined in the series of strains derived from the *lexA* (Def) strain IT1865 by lysogenization with lambda phages bearing *recA* mutations providing different degrees of constitutive protease activity (Table 3). The two very strong (class 1) constitutive protease mutants repaired the phage most effectively. But even the four relatively weak class 4 Prt^c Rec⁺ mutants were very effective.

Thus, relatively little protease activity was needed for the second role. This was illustrated in another way by the *recA441* mutation. There was no measurable repair at 35°C in a *lexA*⁺ background (IT1819), but there was effective repair in a *lexA* (Def) background (EST1640). This was because *recA441* provides insufficient protease activity at 35°C to cleave the LexA repressor and induce the SOS genes (data not shown), its first role, but there was enough activity for its second role, which was apparent in the *lexA* (Def) strain. As expected, at 41°C *recA441* provided enough protease activity to cleave the LexA repressor so that repair was the same in both backgrounds.

The Prt^c Rec⁻ strains that were defective in spontaneous mutagenesis of *E. coli* (Table 1) were nevertheless highly effective in Weigle repair of the UV-irradiated phage, showing that the recombinase function is not essential. The possibility that the recombinase function plays some small role in Weigle repair was not ruled out. Only in strains that were very low in protease activity (Prt[±] Rec[±]) was the repair sector greatly reduced; it was completely absent in the Prt⁻ Rec⁻ strains, which did not even have inducible protease activity.

DISCUSSION

The second, direct role of the RecA protein in spontaneous and UV-induced mutagenesis of *E. coli* and in the Weigle repair and mutagenesis of UV-irradiated phage S13 was explored by using cells deleted for *recA* and lysogenic for λ *recA* mutants representing the phenotypic classes Prt^c Rec⁺, Prt^c Rec⁻, Prt[±], Rec[±], and Prt⁻ Rec⁻; the mutants were all distinct from the wild-type Prt⁺ Rec⁺ strain (15, 16). In most

experiments the cells also contained a defective *lexA* allele to eliminate the need for the first role of the RecA protein, namely facilitating the cleavage of the LexA repressor; in these experiments all effects could be attributed to the second role of RecA.

Weigle repair and mutagenesis of UV-irradiated phage S13 required RecA protease activity but not its recombinase activity. The maximum Weigle repair sector was 0.24 ± 0.02 . This finding that at most only about one in four UV lethal hits could be repaired might give the misleading impression that the repair of UV lesions in S13 DNA by Weigle repair is inefficient. Two separate pieces of evidence suggest that the true efficiency is actually about three times higher than the Weigle repair sector indicates. First, it has been found that only about one in three lethal damages results in pyrimidine dimers (3). Second, studies of photoreactivation of encapsidated as opposed to naked S13 DNA indicate that most of the remaining lethal damages are probably protein-DNA cross-links in the virion, which would prevent infection. The evidence for such nonrepairable damages is that when the intact virus is irradiated, the photoactivable sector is comparable to the Weigle repair sector, being approximately 0.20 ± 0.02 , but when naked DNA is irradiated, the photoreactivable sector increases to approximately 0.55 (17, 18; S. Michalek and I. Tessman, unpublished data). S13 DNA is less repairable if it is irradiated while in the virion, suggesting that many of the lethal hits involve the capsid. Since we are only interested in repairable damages, one should recognize that the phage survival values in the range 3×10^{-8} to 1×10^{-5} (Tables 2 and 3) are in reality equivalent to about 10^{-3} to 10^{-2} for viruses that contain only ordinary DNA hits.

In a *lexA* (Def) background, weak Prt^c mutants gave almost as much Weigle repair as did strong Prt^c mutants, so only a small amount of protease activity per RecA molecule was needed in the direct role of RecA in Weigle repair compared with the amount needed for cleaving LexA repressor. Apparently the presence of a large number of RecA molecules with weak protease activity suffices for nearly maximum Weigle repair.

UV-induced mutation of *E. coli*, like that of phage S13, did not require recombinase activity, but spontaneous mutation of *E. coli* required some aspect of both the protease and recombinase activities. There are two notable features of the dependence of spontaneous mutagenesis on RecA functions.

(i) The direct, second role of RecA in spontaneous mutation required some aspect of protease activity tied to what is needed in its inducing, first role. This was evident from the marked decrease of the spontaneous mutation frequency over the entire range of decreasing protease strength (Table 1). Furthermore, the direct effect of RecA on spontaneous mutation was inhibited by the negative-effector precursor combination C plus G, which also inhibits protease activity toward the LexA protein. It should be emphasized, however, that the direct role may involve only the binding of RecA to some protein, such as UmuDC, without subsequent cleavage of the protein.

(ii) In a *lexA* (Def) background the rate of spontaneous mutation is much lower in $\text{Prt}^c \text{Rec}^-$ strains than in $\text{Prt}^c \text{Rec}^+$ strains, although the $\text{Prt}^c \text{Rec}^-$ strains have greater protease activity than do weak $\text{Prt}^c \text{Rec}^+$ strains (Table 1; 15, 16). Three $\text{Prt}^c \text{Rec}^-$ alleles are known to differ from the wild type by single mutations, each of which confers both constitutive protease activity and recombinase deficiency (19).

The dependence of spontaneous mutation on RecA recombinase activity, as observed in $\text{Prt}^c \text{Rec}^-$ strains, may

TABLE 3. Dependence of Weigle repair of UV-irradiated phage S13 on RecA protease and recombinase activities^a

Strain (temp) ^b	Allele	Phenotype	Constitutive protease strength ^c	Weigle repair sector (W) $\pm 10\%$
EST2578	<i>recA</i> ⁺	$\text{Prt}^+ \text{Rec}^+$	None ^d	0.027
EST1585	<i>recA</i> ⁺	$\text{Prt}^+ \text{Rec}^+$	None ^d	0.074
EST1895	<i>recA1213</i>	$\text{Prt}^c \text{Rec}^+$	Strong	0.23
EST1893	<i>recA1217</i>	$\text{Prt}^c \text{Rec}^+$	Strong	0.24
EST1897	<i>recA1231</i>	$\text{Prt}^c \text{Rec}^+$	Weak	0.22
EST1899	<i>recA1232</i>	$\text{Prt}^c \text{Rec}^+$	Weak	0.22
EST1955	<i>recA1237</i>	$\text{Prt}^c \text{Rec}^+$	Weak	0.24
EST2577	<i>recA1238</i>	$\text{Prt}^c \text{Rec}^+$	Weak	0.20
EST1957	<i>recA1201</i>	$\text{Prt}^c \text{Rec}^-$	Moderate	0.13
EST1901	<i>recA1203</i>	$\text{Prt}^c \text{Rec}^-$	Moderate	0.19
EST1947	<i>recA1204</i>	$\text{Prt}^c \text{Rec}^-$	Moderate	0.17
EST1951	<i>recA1205</i>	$\text{Prt}^c \text{Rec}^-$	Moderate	0.20
EST1949	<i>recA1206</i>	$\text{Prt}^c \text{Rec}^-$	Moderate	0.19
EST1953	<i>recA1207</i>	$\text{Prt}^c \text{Rec}^-$	Moderate	0.21
EST2576	<i>recA1242</i>	$\text{Prt}^\pm \text{Rec}^\pm$	None ^e	0.037
EST1903	<i>recA1243</i>	$\text{Prt}^\pm \text{Rec}^\pm$	None ^e	0.031
EST2579	<i>recA1607</i>	$\text{Prt}^- \text{Rec}^-$	None ^f	<0.01
EST1905	<i>recA1608</i>	$\text{Prt}^- \text{Rec}^-$	None ^f	<0.01
EST1913	<i>recA1609</i>	$\text{Prt}^- \text{Rec}^-$	None ^f	<0.01
EST2580	<i>recA1610</i>	$\text{Prt}^- \text{Rec}^-$	None ^f	<0.01
IT1819 ^g	<i>recA441</i>	$\text{Prt}^c \text{Rec}^+$	None	<0.01
IT1819 (41°C)	<i>recA441</i>	$\text{Prt}^c \text{Rec}^+$	Moderate	0.16
EST1640	<i>recA441</i>	$\text{Prt}^c \text{Rec}^+$	None	0.15
EST1640 (41°C)	<i>recA441</i>	$\text{Prt}^c \text{Rec}^+$	Moderate	0.16

^a The phage was irradiated to a survival of 10^{-7} . All strains were *lexA* (Def) except where otherwise indicated. Strain IT1865 (Table 2) was the reference strain for $W = 0$.

^b All measurements were at 35°C except where otherwise indicated.

^c The qualitative descriptions match the quantitative data in Table 1 and in Tessman and Peterson (15, 16). For *recA441* at 37°C the β -galactosidase specific activity was 12 Miller units.

^d Strongly inducible with mitomycin C.

^e Inducible with mitomycin C to just 15% of the *recA*⁺ inducible value (16).

^f Not inducible with mitomycin C.

^g *lexA*⁺.

help in distinguishing the second, direct role of the RecA protein in mutagenesis from its first, indirect role. The biochemical interactions of RecA in its second role in mutagenesis are not known. This is in contrast to its better understood first role in which in vitro it greatly enhances the proteolytic cleavage of the LexA repressor (10). The strong correlation of the rate of spontaneous mutation with RecA protease activity for the LexA repressor indicates that the second role involves something related to the protease activity. But the additional need for RecA recombinase activity for expression of spontaneous mutation, as observed in $\text{Prt}^c \text{Rec}^-$ strains, indicates a distinct difference between the two roles.

We look for an explanation of this difference in the proteins with which RecA must interact. RecA must complex with the LexA protein in its first role but not in its second, and it likely must complex with the UmuDC protein in its second role but not in its first. A $\text{Prt}^c \text{Rec}^-$ mutation, although conferring the ability to complex constitutively

with LexA, may render RecA unable to complex with UmuDC. The Rec⁺ phenotype includes the ability of RecA to bind to ssDNA, which serves as a positive effector for putting RecA into an active configuration. In nonirradiated *E. coli* the short stretches of ssDNA at the replication forks may activate the Prt^c Rec⁻ protein sufficiently for it to complex with LexA (12, 13) and thereby effect cleavage, but perhaps not sufficiently for it to complex with UmuDC. It is significant that long stretches of ssDNA are more effective in activating the RecA protein (12, 13), which may explain why in UV-induced mutagenesis and Weigle repair the Prt^c Rec⁻ mutants could still perform their second role; the lesions created by UV irradiation would have provided the required long ss regions.

An important implication of this analysis is that the binding of ssDNA to RecA may have at least two consequences in SOS repair. A known consequence is the cleavage of LexA and other repressors; the other could be the complexing of UmuDC with RecA at the site of the block in DNA synthesis to assist synthesis past the block.

Another distinction between the first and second roles of the RecA protein in repair was that in a *lexA* (Def) background, mutants with relatively little constitutive protease activity were able to give practically the maximum amount of Weigle repair. For example, the weak mutant *recA1238* (Prt^c Rec⁺) was about as effective (Table 3) as mutants 17 times stronger in their constitutive induction of the SOS gene *dinD* (15). For UV-irradiated DNA, therefore, the first role of RecA appears to require more activated RecA protein than does the second role. In contrast, for spontaneous mutation, the second role of RecA is more effective the greater the protease strength.

The dependence of spontaneous mutation on RecA recombinase activity also indicates a distinct difference between the second role of RecA in increasing the appearance of spontaneous mutants and its second role in the repair and mutagenesis of UV-irradiated DNA because in the latter case no significant recombinase activity is needed. This is particularly relevant to the idea that in the absence of mutagenic treatment there still may be SOS-mutable cryptic lesions (20), possibly apurinic sites (12), which might account for the enhanced spontaneous mutation in a Prt^c Rec⁺ strain. However, an explanation is needed of why the effect of the putative cryptic lesions should require RecA recombinase activity for expression, whereas the effect of UV lesions does not.

In a diploid strain containing RecA⁺ and RecA (Prt^c Rec⁺) proteins in a *lexA* (Def) background, the wild-type protein inhibited the constitutive protease activity of the mutant protein, although the amount of each protein was expected to be the same as that in the haploid state. There are two ways in which this inhibition may be viewed. (i) The two proteins could compete for ssDNA. RecA⁺ protein may bind to the naturally occurring short stretches of ssDNA, even though such binding would not result in activation. Yet that would deplete the supply of ssDNA that could be used to activate the RecA (Prt^c Rec⁺) protein. (ii) The RecA⁺/RecA (Prt^c Rec⁺) hybrid multimeric form of the protein could have reduced constitutive activity for the presumptive binding to UmuDC, possibly because of decreased binding to ssDNA.

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