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Genetics

Dual role for *Escherichia coli* RecA protein in SOS mutagenesis

(gene regulation/pKM101/DNA repair/UV damage/LexA protein)

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ABSTRACT **Induction of the *Escherichia coli* SOS system increases the ability of the cells to perform DNA repair and mutagenesis. Previous work has shown that this increased mutagenesis is the result of derepression of specific genes through a complex regulatory mechanism controlled by LexA and RecA proteins. One role of RecA protein in this process is to facilitate proteolytic cleavage of LexA protein (the repressor) in response to an inducing signal that reversibly activates RecA protein to perform this function. We show that activated RecA protein plays a second role in SOS mutagenesis, as revealed by analyzing repair of UV-damaged phage λ in host mutants with alterations in the SOS regulatory system. First, phage mutagenesis was not expressed constitutively in a mutant that is derepressed through lack of functional LexA protein; activated RecA protein was still required. Second, phage mutagenesis was constitutively expressed in the presence of *recA* mutations that alter RecA protein so that it is activated in normally growing cells. There was also RecA-dependent constitutive expression of SOS mutagenesis in host mutants that lack functional LexA protein and carry plasmids. We discuss several possible biochemical mechanisms for this second role of activated RecA protein in SOS mutagenesis.**

LexA and RecA proteins play a central role in regulating the *Escherichia coli* SOS system. LexA protein, a repressor of approximately 20 operons, undergoes proteolytic cleavage following DNA damage, derepressing the SOS regulon (1, 2). Cleavage of LexA protein is stimulated by RecA protein that has been reversibly activated by a signaling mechanism, which responds to the inducing treatment (3). Mutants that carry inactive *umuCD* genes are deficient in SOS mutagenesis and are slightly sensitive to UV. In addition, these genes are induced by UV light (4–6), and derepression of *recA* alone is not sufficient for mutagenesis (7), implying that induction of the *umuCD* operon is necessary.

SOS mutagenesis can be measured by infecting cells previously treated with an inducing agent with UV-damaged phage. When the cells reactivate the phage, a high yield of mutants appears (8). Base substitution, frameshift, and double mutants are produced near potential targets for damage. The variety of mutations generated suggests that mutants are produced by more than one molecular mechanism (9). A lower level of mutagenesis that is independent of damage is observed when SOS-induced cells are infected by undamaged phage (10). However, the classes of mutations produced and the genetic requirements of the host cell may be different from those for mutations associated with repair of damage (1, 2, 9–12).

Using a phage mutagenesis assay, we show that host mutants that have no functional LexA protein and, therefore, are expressing *umuCD* constitutively require activated RecA protein for mutagenesis. Hence, RecA protein plays two roles in SOS mutagenesis: (*i*) derepression of the SOS regulon as described earlier (1, 2) and (*ii*) another mutagenesis function similar to one described earlier (1, 13, 14) that is different from derepression.

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MATERIALS AND METHODS

Medium. Tryptone broth was used for routine growth of bacteria and preparation of phage stocks. SM buffer was used for storage of phage stocks (15). M9-Casamino acid agar, LB agar, or MacConkey agar plates (16) were used in various stages of strain construction. When required, additions were made to these plates in the following final concentrations: 100 μg of ampicillin per ml, 20 μg of chloramphenicol per ml, 20 μg of kanamycin per ml, 25 μg of streptomycin per ml, 25 μg of tetracycline per ml, and 0.2% galactose, lactose, maltose, or sorbitol and 50 μg of adenine per ml. Baltimore Biological Laboratory's top agar and agar plates were used for phage plaque and infective center assays (17).

Strains. The genotypes of the *E. coli* K-12 strains, phage λ, and plasmids are listed in Table 1. Strains were propagated as described (16, 20). For bacterial strain construction, detailed in Table 1, we followed standard methods of conjugation, transduction with phage *P1vir* (16), and plasmid transformation (21). For this study, we developed a phage mutagenesis assay using reversion of an amber mutation, which allows rapid scoring of SOS mutagenesis in a large number of host mutants (see Fig. 1).

RESULTS

Host Genetic Requirements for Phage Mutagenesis. We first demonstrated that the phage mutagenesis assay we used to detect SOS mutagenesis was typical of other mutagenesis systems in its requirements (1) for functional RecA and UmuC proteins and a cleavable LexA protein (Table 2; Figs. 1 and 2). The major features were as follows. First, as with other phage mutagenesis systems (9, 10), there was a small (2.4-fold) stimulation of reversion when induced cells proficient in DNA repair were infected with unirradiated phage, compared to infection of unirradiated cells. Second, when both host cell and phage were irradiated, phage reversion was stimulated 50-fold above the level for unirradiated phage and cells (Table 2, cell pair A). Third, mutagenesis required LexA protein cleavage because there was no phage mutagenesis in a host with noncleavable LexA protein (Fig. 1, genotype *recA*$^+$ *lexA3*). Fourth, there was no mutagenesis in a host with the SOS regulon derepressed and the mutation *umuC122*::Tn*5*, demonstrating that the UmuC product is required (Fig. 2). Fifth, there was no mutagenesis in the (*recA-srlR*)306 deletion and *recA1* missense mutants, indicating a requirement for functional RecA protein (Fig. 1). Sixth, infection of UV-treated repair-proficient cells with irradiated phage also resulted in a 5-fold higher phage survival (Fig. 1; refs. 8 and 22). Finally, mutants deficient in mutagenesis did not show an increased ability to reactivate phage (Table 2, cell pairs C–E).

Table 1. List of plasmids, phage, and *E. coli* host mutants

	Relevant markers	Reference or origin
Plasmids		Reference/source
pSK621	*umuCD, aph*$^+$ (Kan^R)	6
pKM101	*mucAB, bla*$^+$ (Amp^R)	18
pBEU436	F'*lac*::Tnl946 (Tn3*BamH*1:: *recA430*)	A. J. Clark
F' *lac, pro*	F'*lacI*^{q1L8}, *proAB*	16
Phage		
λsusL63	*susL63(am)*, cI857	19
Bacteria (*supD43/sup*$^+$)		Parent strain/origin (*supD43/sup*$^+$)
DM2556/DM2558	*recA*$^+$, *lexA*$^+$	DM2550/DM2551[a]
DM2557/DM2559	del(*recA-srlR*)306, *lexA*$^+$	DM2550/DM2551[a]
DM2568/DM2570	*recA*$^+$, *lexA51*	DM2554/DM2555[a]
DM2569/DM2571	del(*recA-srlR*)306, *lexA51*	DM2554/DM2555[a]
DM2572/DM2573	*recA430, lexA51*	DM2554/DM2555[a]
DE175/DE177	*recA*$^+$, *lexA51*, F' *lac, pro*	DM2568/DM2570[b]
DE212/DE214	del(*recA-srlR*)306, *lexA51*, F' *lac,pro*	DM2569/DM2571[b]
DE216/DE218	*recA*$^+$, *lexA*$^+$, F' *lac, pro*	DM2556/DM2558[b]
DE239/DE241	*recA1, lexA*$^+$	DM2556/DM2558[c]
DE270/DE268	*recA430, lexA51*, F' *recA430* (pBEU436)	DM2572/DM2573[d]
DE272/DE274	*recA730, lexA51*	DE190/DE192[a]
DE345/DE347	del(*recA-srlR*)306, *lexA51*, pKM101	DM2569/DM2571[e]
DE369/DE372	*recA*$^+$, *lexA51*, *umuC122*::Tn5	DE353/DE355[f]
DE376/DE378	del(*recA-srlR*)306, *lexA51*, pSK621	DM2569/DM2571[g]
DE405/DE407	*recA*$^+$, *lexA3*	DE190/DE192[h]

Strains were derived from freshly checked stocks of *E. coli* JM1 and DM1187 (13). Note that all strains with *lexA51* also carry *lexA3* (13). Recombinants of JM1 and DM1187 (designated DM1790 and DM3069, respectively) that had lost the weak amber suppressor mutation, *supE44*, were obtained by conjugation crosses with Hfr 3000X74 (from B. Bachmann) and were shown to be Thr$^+$, Leu$^+$, (*lac-pro*)XIII, Gal$^+$, His$^-$, and Str^R (streptomycin resistant). Derivatives of these strains with either *sup*$^+$ or *supD43* [those from DM1790 were denoted DM2550 and DM2551, and those from DM3069 were denoted DM2554 and DM2555], respectively, were from a conjugation cross with Hfr JG75 (from J. Gross), selecting for His$^+$, Str^R recombinants and screening for the amber suppressor *supD43*. DE190 and DE192 are as DM2568 and DM2570, respectively, but are *srl*$^+$. DE353 and DE355 are as DE190 and DE192, respectively, but *purB58* and *fadR613*::Tn*10* were cotransduced from RS3032 (from B. Bachmann).

[a] *recA* alleles were transduced by phage P1 with *srl*::Tn*10*.
[b] F' *lac-pro* strain was mated with DE44.
[c] *recA1* strain was mated from KL16-99 (from K. B. Low).
[d] F' *recA430* was mated from BEU328 (from A. J. Clark).
[e] Plasmid pKM101 was from TA92 (from B. Ames).
[f] *umuC122*::Tn5 was moved by phage P1 transduction from GW2100 (from D. Lackey and S. Linn), selecting *purB*$^+$, Kan^r transductants and screening them for loss of the closely-linked Tn*10* insertion in *fadR*.
[g] pSK621 was introduced by transformation, selecting Kan^r.
[h] *lexA3* was cotransduced by phage P1 with *malB*::Tn*9*.

Mutagenesis in Derepressed Host Mutant Requires Activated RecA Protein. As demonstrated above, mutagenesis of UV-damaged phage was not observed in the absence of functional RecA protein or presence of noncleavable LexA protein. This result is most simply explained by the requirement of increased transcription from the SOS regulon for mutagenesis. However, unirradiated cells lacking LexA protein (*recA*$^+$ *lexA51*) showed no greater capacity for phage mutagenesis than did unirradiated *recA*$^+$ *lexA*$^+$ cells (Fig. 2; Table 2, cell pairs A and B), but after irradiation they exhibited as much mutagenesis.

Table 2. SOS repair and mutagenesis of phage in repair-deficient hosts

Cell pair	Relevant genotype *recA*	Relevant genotype *lexA*	UV dose to cell, J/m^2	Sus$^+$ phage per 10^7 progeny: Without UV	Sus$^+$ phage per 10^7 progeny: With UV	Phage survival, S/So
A	+	+	0	5.8	34	0.045
			10	5.2	270	0.060
			18	14	280	0.28
B	+	51	0	6.4	11	0.056
			10	5.6	310	0.024
			18	11	300	0.45
C	306	51	0	2.8	11	0.055
			10	5.0	8	0.045
D	430	51	0	5	17	0.024
			10	7	40	0.027
E	430	51	0	3	30	0.020
	(+F'*recA430*)		10	4	25	0.030
F	730	51	0	49	450	0.37
			10	76	510	0.44

Mutant pair A was DM2556/DM2558, B was DM2568/DM2570, C was DM2569/DM2571, D was DM2572/DM2573, E was DE270/DE268, and F was DE272/DE274. The experimental conditions are described in Fig. 1. The first member of each strain pair in column 1 carries *supD43*, and the second is *sup*$^+$. With or without UV indicates phage were or were not irradiated, respectively. Note that the Sus$^+$ revertants are those phage mutants that have acquired the ability to grow on the host strain that lacks the amber suppressor mutation *supD43*. S/So is the fraction of phage surviving.

We considered the unlikely possibility that *recA*$^+$ *lexA51* cells must be given an inducing treatment because the mutant LexA protein has residual repressor activity that must be destroyed for mutagenesis. However, other mutants with the *lexA71*::Tn5 insertion (23) or with *lexA* deletions (unpublished observations) showed a similar requirement (data not shown). Finally, the requirement for UV treatment of a *recA*$^+$ *lexA51* host is not confined to the reversion of *susL63*, since a similar requirement was previously observed for the induction of forward mutations in the phage λ cI gene (13).

Mutagenesis in *recA-lexA51* Mutants. To analyze further the role of RecA protein in SOS mutagenesis and to compare this role to the other functions of RecA protein, we analyzed mutagenesis in several *recA* mutants. Induction of SOS mutagenesis did not occur in a *lexA51* Δ(*recA-srl*) host (Fig. 2; Table 2, cell pair C), indicating that RecA protein has a mutagenesis function that must be activated by irradiation of the derepressed host.

A *recA430 lexA*$^+$ mutant has been shown to be deficient in repair and mutagenesis of phage λ (24). According to the above analysis, the *recA430* mutant may be deficient in repair and mutagenesis of phage either because it is unable to promote enough cleavage of LexA protein *in vivo* to derepress the SOS regulon or because the mutant protein is unable to support the RecA mutagenesis function. To distinguish these possibilities, we analyzed a double host mutant lacking LexA protein and producing the altered RecA430 protein (*recA430 lexA51*). The level of phage mutagenesis was drastically reduced (Table 2; Fig. 1). To double the number of copies of *recA430*, we introduced a second copy of *recA430* on an F factor, but there was still no detectable mutagenesis (Table 2). A similar deficiency in mutagenesis in

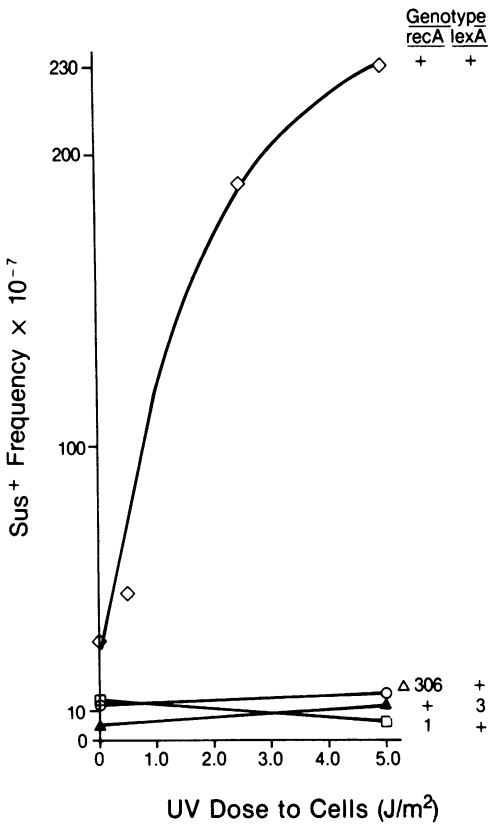

FIG. 1. Genetic requirements for reversion of UV-irradiated phage λ*susL63* by SOS mutagenesis. Phage stocks were diluted in SM diluent (15), and aliquots were irradiated with a UV fluence of 100 J/m², giving a survival of 5% when assayed on *E. coli* strain DM2556. Pairs of host strains of a particular genotype with respect to DNA repair, one carrying the amber suppressor *supD43* and the other *sup*⁺, were grown to midlogarithmic phase in tryptone broth supplemented with maltose and 10 mM $MgSO_4$, centrifuged, resuspended to 1/10th of their original concentration in 10 mM $MgSO_4$ at 4°C, irradiated with agitation, centrifuged, resuspended to 2-fold their original concentration in 10 mM $MgSO_4$, and infected with phage λ*susL63* at a multiplicity of infection of <0.5 phage particles per cell. After adsorption, additional unirradiated cells of the same genotype were sometimes added when survival of the host strain was too low to support plaque development, 2.5 ml of top agar was added, and the mixture was poured on plates. After overnight incubation at 37°C, total progeny and Sus⁺ revertants were scored. All manipulations were performed in the dark to avoid photoreactivation. All data points are the average of at least two and as many as four independent experiments. Full strain genotypes are given ◇, D2556/DM2558; ○, DM2557/DM2559; ▲, DE405/DE407; □, DE239/DE241.

a *recA430 lexA51* strain for induction of cellular nonsense suppressor mutations was reported by Blanco *et al.* (14). The additional observation that there was no phage (data not shown) or cellular (2) mutagenesis in a *recA430 lexA71*::Tn*5* mutant rules out the possibility that the mutagenesis deficiency in strains with *recA430* is due to a residual requirement for activated RecA protein for LexA protein cleavage. The observation described above that mutagenesis is also blocked by a *recA* deletion (Fig. 2; Table 2, cell pair C) indicates that RecA protein plays a direct stimulatory role in mutagenesis. This conclusion in turn suggests that RecA430 protein is not blocking mutagenesis by poisoning a reaction but that the mutant protein is deficient in performing the RecA mutagenesis function described above.

To test whether a high level of the UmuC and UmuD products or the functionally homologous MucA and MucB gene products (25) would restore mutagenesis in a RecA-deleted host, we introduced pSK621, a multicopy plasmid carrying the *umuCD* operon (7), or pKM101 carrying the *mucAB* operon (18) into mutant hosts that carried defective *lexA* and *recA* functions. There was no detectable mutagenesis in the absence of functional RecA protein (Fig. 3). We conclude that the requirement for activated RecA protein for SOS mutagenesis cannot be bypassed by introducing a plasmid that should increase the level of the MucAB or UmuCD products.

Constitutive Expression of the RecA Mutagenesis Function. The *recA730* mutant expresses the SOS system constitutively and promotes repressor cleavage in the absence of an inducing treatment (26, 27), causing derepression of the SOS regulon. We wished to test whether the newly identified mutagenesis function of RecA protein is also expressed constitutively in the *recA730* mutant. A dramatic level of phage mutagenesis was observed in untreated *recA730* cells that was almost twice the level induced in normal cells, and there was little additional increase with irradiation of the host cells (Table 2, cell pairs B and F; Fig. 2). Since there was much less mutagenesis of undamaged phage, most mutagenesis by RecA730 protein is a response to the presence of damage. We conclude that the RecA730 protein expresses the mutagenesis function constitutively and to a greater extent than is achievable by inducing normal cells.

We further discovered that the presence of plasmids promotes induction of mutagenesis in the absence of DNA damage. The presence of the sex factor F in *lexA51*(Def⁻) cells stimulated mutagenesis 10-fold in the absence of any inducing treatment, and a small additional increase was observed when the host cell was UV-irradiated (Fig. 3). A similar and slightly larger increase of 20- to 40-fold in mutagenesis without induction was observed in the same *lexA51* mutant carrying pBR322 or several of its derivatives (data not shown). No effect of factor F or pBR322 on mutagenesis in *lexA*⁺ strains was observed, although mutagenesis remained normally inducible by UV (Fig. 3 and data not shown). There was also no stimulation of mutagenesis by factor F in a *recA*-deleted, derepressed host (Fig. 3), indicating that the plasmid only stimulates the mutagenesis function of RecA protein rather than substituting for it. We conclude that these plasmids can lead to partial constitutive expression of the RecA mutagenesis function but that they are not capable of stimulating both LexA protein cleavage and mutagenesis.

DISCUSSION

We have defined the genetic requirements for a new mutagenesis function for activated RecA protein in the SOS response. This function is not constitutively expressed in *E. coli* mutants with a derepressed SOS regulon; these mutants must be treated with an inducing agent such as UV to observe its expression. Our results clearly show that the RecA protein mutagenesis function can be *trans*-activated (i.e., by plasmids as in Fig. 3 or by a damaged host replicon), indicating the involvement of a diffusible product. Evidence for a required activation of RecA protein for cellular mutagenesis was also described earlier by Blanco *et al.* (14) and for phage mutagenesis by Mount (13).

UV-irradiation can induce both derepression and mutagenesis in RecA⁺ LexA⁺ cells. In contrast, we observed that plasmids only stimulate mutagenesis in an already derepressed host. We attribute this result to a requirement for a stronger or different inducing signal for repressor cleavage than for mutagenesis and to the production of a weaker or different signal by the plasmid. We suggest further that the full signal generated by UV irradiation might be a population of different species of molecules and that some plasmids could be generating or mimicking a subset of this population. Due to the striking increase in mutagenesis promoted by

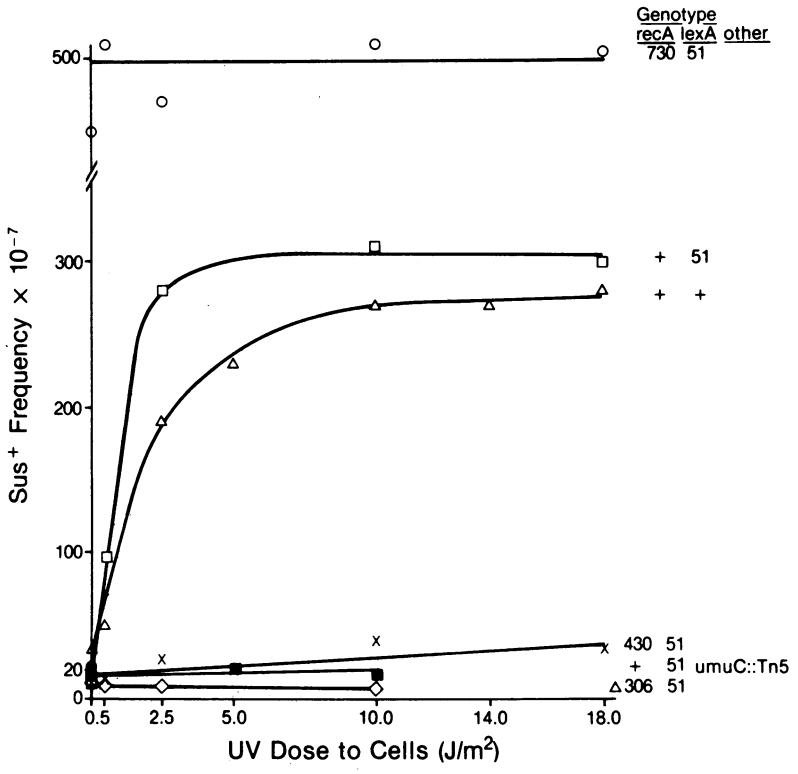

FIG. 2. Influence of *recA* upon SOS phage mutagenesis. The experimental conditions were as described in Fig. 1, and full strain genotypes are given in Table 1. ○, DE272/DE274; □, DM2568/DM2570; △, DM2556/DM2558; ×, DM2572/DM2573; ■, DE369/DE372; ◇, DM2569/DM2571.

plasmids with no accompanying increase in repressor cleavage (unpublished results), we propose that these RecA-activated functions are separable. Other plasmids that carry specific alterations in their origins of replication can fully induce the SOS system (28), presumably because they produce all the necessary signals.

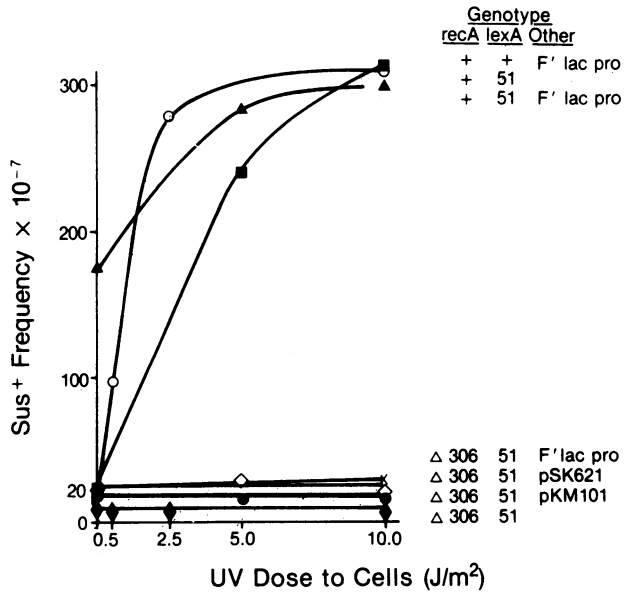

FIG. 3. Influence of plasmids and their products on RecA mutagenesis function. Experimental conditions were as in Fig. 1, and full strain genotypes are given in Table 1. For strains harboring pKM101 or pSK621, antibiotics were used in the growth medium before irradiation to avoid plasmid segregation and loss. ■, DE216/DE218; ○, DM2568/DM2570; ▲, DE175/DE177; ×, DE212/DE214; ◇, DE376/DE378; ●, DE345/DE347; ◆,DM2569/DM2571.

What is the biochemical nature of the additional cellular function that must be induced for mutagenesis and what role does activated RecA play? Considering the known biochemical properties of RecA protein, it could act in one of several possible ways to modify DNA replication and make the process more tolerant of DNA damage and prone to error. Activated RecA protein could: (*i*) promote cleavage of additional repressors, inducing more operons like *umuCD* whose products are mutagenic; (*ii*) activate one or more mutagenic products by promoting their cleavage, decreasing fidelity of DNA replication; (*iii*) be involved in protein–protein interactions with proteins that replicate DNA in a manner that decreases replication fidelity; or, (*iv*) perform types of DNA strand exchanges (29, 30) that aid replication of damaged DNA. We shall discuss these possibilities below.

A function for activated RecA protein in mutagenesis that is related to its ability to facilitate cleavage of repressors is implied by the defect in RecA430 protein in both phage λ mutagenesis and induction (24) and in λ *c*I protein cleavage (31). However, cleavage of LexA protein *in vivo* in *recA430* mutants occurs at 40% of the normal rate (unpublished observations), and other phages are partially or fully inducible in this mutant (32). Such selectivity could be due to an inability of the RecA430 protein to promote cleavage of some repressors. Witkin and Kogoma have presented evidence that SOS mutagenesis does not require inactivation of other repressors (33). Such a mechanism also could not be easily reconciled with our observation, described above, that the RecA functions for LexA proteolysis and SOS mutagenesis are separable.

Two of the above mechanisms propose that RecA protein modifies the activity of proteins involved in DNA replication. A large proportion of the mutations induced in the phage mutagenesis system we used are base substitutions (9) that could arise through decreased replication fidelity. Specific proteins in the polymerase III holoenzyme complex that replicates the *E. coli* chromosome play a major role in

regulating fidelity (34, 35). Addition of RecA protein to this complex in an *in vitro* DNA synthesis system decreases fidelity slightly (36). A modified form of DNA polymerase I called "PolI*," which replicates DNA with lower fidelity *in vitro*, has also been found in SOS-induced cells (37). Since this activity is found in $RecA^-$ and $UmuC^-$ cells that have been treated with an inducing agent (D. Lackey and S. Linn, personal communication), the biological role of PolI* is not yet clear. Echols (38) has suggested that activated RecA protein binds to damaged sites in DNA, enhancing replication past the sites with decreased fidelity.

Mutagenesis by a recombinational mechanism is made plausible by the variety of molecular interactions between DNA strands promoted by RecA protein (29, 30), leading to the supposition that there might be reactions involving damaged DNA that are mutagenic. The properties of RecA430 protein also could be explained by a deficiency in performing such mutagenic exchanges while retaining normal ability to promote strand exchanges in undamaged DNA. Miura and Tomizawa (39) showed (*i*) that mutagenesis of UV-damaged phage λ is not higher in phage that have undergone genetic recombination in the region scored for mutagenesis and (*ii*) that mutations in the host *recBC* and phage *red* functions (40) did not reduce mutagenesis. Although these results appear to argue against a recombinational model for mutagenesis, our results suggest that activated RecA protein determines a mutagenic event that may not yield a recombinant phage.

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