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Genetic Separation of *Escherichia coli recA* Functions for SOS Mutagenesis and Repressor Cleavage

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Evidence is presented that *recA* functions which promote the SOS functions of mutagenesis, LexA protein proteolysis, and λ cI repressor proteolysis are each genetically separable from the others. This separation was observed in recombination-proficient *recA* mutants and *rec*$^+$ (F′ *recA56*) heterodiploids. *recA430*, *recA433*, and *recA435* mutants and *recA*$^+$(F′ *recA56*) heterodiploids were inducible for only one or two of the three functions and defective for mutagenesis. *recA80* and *recA432* mutants were constitutively activated for two of the three functions in that these mutants did not have to be induced to express the functions. We propose that binding of RecA protein to damaged DNA and subsequent interaction with small inducer molecules gives rise to conformational changes in RecA protein. These changes promote surface-surface interactions with other target proteins, such as cI and LexA proteins. By this model, the *recA* mutants are likely to have incorrect amino acids substituted as sites in the RecA protein structure which affect surface regions required for protein-protein interactions. The constitutively activated mutants could likewise insert altered amino acids at sites in RecA which are involved in the activation of RecA protein by binding small molecules or polynucleotides which metabolically regulate RecA protein.

Following DNA damage or inhibition of DNA synthesis, *Escherichia coli* shows dramatic physiological changes known as the SOS response. Many of the changes are due to coordinate derepression of approximately 20 unlinked operons of widely divergent functions called the SOS regulon. Derepression causes increased DNA excision repair, recombinational repair and SOS mutagenesis, reinitiation of DNA replication, lytic induction of phage λ, and inhibition of cell division (12, 15, 35, 38). The products of two SOS genes, *lexA* and *recA*, regulate the SOS response. In uninduced cells, transcription of SOS genes is repressed by LexA protein. After DNA damage, LexA protein is inactivated by proteolytic cleavage (15). This cleavage is stimulated by RecA protein being reversibly activated to a new conformation (RecA*). This activation step is thought to involve binding of RecA protein to damaged chromosomal sites, followed by interaction with small inducer molecules and proteins such as repressors.

We are interested in genetic analysis of the regulatory and mutagenic activities of RecA protein. *recA* function has been studied previously through analysis of *recA* mutants and *recA*$^+$/*recA* heterozygous strains (21, 24, 37, 39). *recA* mutants have been isolated which constitutively express *recA* functions or which are defective in specific *recA* functions, such as mutagenesis. Other genetic studies have shown that certain *recA* mutations which abolish homologous genetic recombination are dominant over *recA*$^+$ for this property, suggesting that RecA protein acts as a multimer, now verified by both biochemical and structural analyses (13, 21, 36).

The process of SOS mutagenesis requires functional UmuDC and RecA proteins, derepression of specific SOS genes, and damage to the mutagenic target (6, 15, 34). RecA protein plays multiple roles in this process, including derepression of *recA* and *umuDC* (1, 6, 7; A. T. Thliveris, D. G. Ennis, L. K. Lewis, and D. W. Mount, *in* M. Riley and K. Drlica, ed., *The Bacterial Chromosome: Structure and Functional Organization*, in press), proteolysis of UmuD protein, and other as yet unidentified roles (2, 23, 28). Recent work has shown that production of the COOH proteolytic fragment of UmuD protein is necessary for mutagenesis (23). The role of this fragment in SOS mutagenesis has not yet been identified. Echols and colleagues observed binding of RecA protein to damaged single-stranded and double-stranded DNA (16). They proposed that RecA protein binding relaxes the fidelity of a DNA polymerase and permits DNA synthesis to proceed past the damage (5, 16). This process is postulated to result in misincorporation of erroneous nucleotides opposite lesions in the template strand (5, 16).

We have extended the above types of genetic analysis to a large number of *recA* mutants to determine which activities of RecA protein are biochemically related. We show that the RecA mutagenesis functions are genetically separable from two other activities, LexA proteolysis and λ cI proteolysis. Our analysis also showed that the LexA and cI proteolysis activities are also separable. Based on these results, we propose that the proteolysis of LexA and λ cI proteins and SOS mutagenesis appears to be mediated by separable RecA functions.

MATERIALS AND METHODS

Media and strains. The genotypes of the *E. coli* K-12 strains and phage λ strains are listed in Table 1. Strains were propagated for experimental purposes and for the construction of strains by routine conditions that were described previously (6, 19). For various bacterial strain construction steps, M9-casamino acid agar, LB agar, LC agar, or MacConkey agar plates were used. When required, additions were made to these plates with the following final concen-

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TABLE 1. List of phages and *E. coli* K-12 host strains

Phage, plasmid, or strain	Relevant markers	Allele no.			Reference or origin
		recA	*lexA*	*sulA*	
Phages					
λ	λ$^+$				This laboratory
	λ *c*I60				This laboratory
	λ *c*I *ind*$^-$ *1*				This laboratory
	λ *c*I *ind*^{s} *1*				This laboratory
DE57	λ *L63 c*I857				6
DE83 (MMS1006)	λ *spi6 imm*^{434}				F. Stahl
DE177	λ *c*I *ind*^{r}*543*				GY4768[a]
Plasmid pBEU33	F′ *lac*::Tn*1943* (Tn*3Bam*HI::*recA56*)				JC10287
E. coli					
DE1337	*thr-1 leuB6 thi-1 lacY1 supE44 tonA21 malB*::Tn*9*				C600[b]
GY4768	C600(λ *c*I *ind*^{r}*543*)				R. Devoret (4)
JC10287	Δ(*recA-srlR*)*304*::Tn*10*(pBEU33)				A. J. Clark
Mutagenesis strain pairs (*supD43*/*sup*$^+$)					
DM2572/CM2573		*430*	*51*	*211*	6
DE183/DE185		*+/56*	*51*	*211*	DM2568/DM2570[c]
DE190/DE192		*+*	*51*	*211*	6
DE272/DE274		*730*	*51*	*211*	6
DE277/DE259		*56*	*51*	*211*	DE190/DE192[d]
DE441/DE443		*730/56*	*51*	*211*	DE272/DE274[c]
DE732/DE736		*80*	*51*	*211*	DE190/DE192[d]
DE858/DE860		*432*	*51*	*211*	DM2568/DM2570[e]
DE862/DE864		*435*	*51*	*211*	DM2568/DM2570[e]
DE866/DE868		*433*	*51*	*211*	DM2568/DM2570[e]
DE1185/DE1187		*432*	*+*	*211*	DE858/DE860[f,g]
DE1294/DE1296		*432*	*+*	*+*	DE1185/DE1187[h,i]
λ plaque morphology hosts					
DE278		*441*	*51*	*211*	DM2554[e]
DE464		*730*	*51*	*211*	DE274[j]
DE492		*730*	*3*	*211*	DE464[k]
DE902		*801*	*51*	*211*	DE874[e]
DE1277		*730*	*41*	*211*	DE492[l]
λ$^+$ lysogens					
DE621		*+*			DE190[m]
DE1265		*433*			DE8661[m]
DE1298		*305*			DE1265[d]
DE1368		*430*			DM2573[m]
sulA fusion strains					
NO56		*+*			26
NO62		*730*			NO56[d]
DE939		*433*			DE880[e]
DE894		*432*			DE880[e]
DE975		*430*			NO56[e]

[a] Isolates of the *c*I *ind*^{r} phage were obtained following superinfection of GY4768 with λ DE83; Spi$^+$ phage were selected on a *recA* host, and isolates with λ immunity were characterized further.
[b] *malB*::Tn*9* was from DE405 (6) by P1 transduction.
[c] F′ *recA56* was transferred by conjugation from JC10287.
[d] *recA* allele was cotransduced with *srl*::Tn*10*.
[e] *recA* allele was cotransduced with *srl*$^+$.
[f] *lexA3* was cotransduced with *malB*::Tn*9* from DE405.
[g] *lexA*$^+$ was cotransduced with *mal*$^+$.
[h] *pyrD* marker was cotransduced with *sulA*::Tn*5* from DE1243.
[i] *sulA*$^+$ was cotransduced with *pyrD*$^+$.
[j] Spontaneous precise excision of Tn*10*.
[k] *lexA3* and *malB45* were cotransduced with *zja-505*::Tn*10* (26).
[l] *lexA*$^+$ and *zja*$^+$ were cotransduced with the *mal*$^+$ marker.
[m] Lysogenized by λ$^+$.

trations: 20 μg of chloramphenicol per ml, 20 or 80 μg of kanamycin per ml, 25 μg of tetracycline per ml, 0.2% maltose, 0.2% sorbitol, 50 μg of adenine per ml, and 50 μg of uracil per ml.

Most bacterial strains were built from others described previously (6, 26) by standard methods of P1 transduction. Bacterial strain constructions are detailed in Table 1. *sulA* fusion strains were derived from GC4572 (obtained from R. D'Ari); except for various recA markers, they all carried HfrH Δ(*lac-argF*)*U169 relA1 thi-1 cps-3 malF55*::Tn*5* *sulA*::Mu d(*lac* Ap)XCam (Mu^+). DE880 was the same as NO56 except it carried *srlC300*::Tn*10*. In addition to the relevant markers in Table 1, all other strains carried Δ(*lac-gpt*)*5 rpsL31 thi-1 mtl-1 ilv*(Ts) (6). The first member of each mutagenesis strain pair carried *supD43* and the second carried sup^+. All strains used for λ clear-plaque morphology tests were genetically the same as the sup^+ hosts listed in the mutagenesis strain pairs. DE874 is essentially the same as DE192 except that it carries Δ(*recA-srlR306*)::Tn*10* and Δ(*uvrB-chlA*); *recA801* was then transduced by phage P1 into DE874 from MV1200 (31) to generate DE902. *recA801* was crossed from this strain into a *recA* recipient to confirm that the selected Rec^+ Srl^+ recombinant carried the mutation. The *recA801* marker was then backcrossed by P1 transduction into the appropriate *recF* mutant to confirm the DE902 construction. DE874 was used because of its *uvrB* deletion as a precaution against the potential lethality that had been noted previously when this *recA* allele was introduced into some uvr^+ hosts (31). Later studies indicated that in our genetic background, a uvr^+ *recA801* double mutation is a viable combination (Ennis and Mount, data not shown). *recA80* was transduced from NO60; *recA432*, *recA435*, and *recA433* were transduced from TK504, TK505, and TK508, respectively (10, 11); Δ(*recA-srl*)*305* and *srlR301*::Tn*10* were transduced from JC10288 (A. J. Clark); *recA430* was transduced from DM2572 (6); Δ(*recA-srlR*)*306*::Tn*10* was transduced from DM2569; *recA730* was transduced from DE272 (6); and *recA56* was transduced from DE64 (C600 *recA56* *srlC300*::Tn*10*). The *recA80* allele was isolated following standard methods of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (19); its isolation and characterization will be described elsewhere. The *sulA211* marker was formerly designated *sfiA11*, the Δ(*lac-gpt*)*5* deletion was formerly called Δ(*lac-pro*)*X111*, *recA801* was *srf-801*, and *lexA51* was *spr-51*, and *recA432*, *recA433*, and *recA435* were first designated *umuB52*, *umuB64*, and *umuB108*, respectively (11), and then later *lexB32*, *lexB33*, and *lexB35*, respectively (10).

Experimental conditions. SOS mutagenesis was measured by using a λ phage reversion assay as described previously (6) and noted in the Fig. 1 legend. Conditions for the SOS induction of phage λ from lysogens are described in the footnotes to Table 2. A clear-plaque assay was used to indicate constitutive λ cI cleavage and has also been extensively described elsewhere (9, 22). We used a variation of this assay as noted in the footnotes to Table 3 and Results. β-Galactosidase activity was measured as described by Miller (19) with modifications (26).

RESULTS

Strategy of experiment. The objective of this work was to use genetic methods to discover how the separate activities of RecA protein are related and to use this information as a basis for modeling the behavior of RecA protein. One set of *recA* mutations abolished all RecA protein activities, including induction of SOS functions, because the protein was missing or defective. Another set caused a so-called split mutant phenotype, in which expression of a subset of *recA* functions was abnormal. The properties of this type of mutant may reflect a general decrease in activity of RecA protein or of an active protein that performs some *recA* functions but not others. Analysis of the latter type of mutant has previously provided a method for genetic dissection of RecA protein activities.

To analyze *recA* functions by this method, the properties of a group of 65 *recA* mutants were compared. The mutations included 31 alleles constitutively activated for cleavage of LexA protein, 19 putative null alleles (recombination deficient or Rec^-), 6 alleles hypersensitive to UV light but recombination proficient (formerly designated *lexB* alleles), 4 conditional alleles (amber or temperature sensitive), 3 alleles that suppress a $RecF^-$ phenotype, and 2 alleles with miscellaneous phenotypes (*recA142* and *recA718*). From this collection of mutants, a subset of *recA* alleles were chosen for further study based on unusual properties found in preliminary experiments. This subset included *recA80* and *recA730* from the constitutively activated class of alleles, *recA56* from the null class, *recA430*, *recA432*, *recA433*, and *recA435* from the *lexB* class, and *recA801* from the *recF*-suppressing class. In addition, we also examined the properties of *recA*(F' $recA^+$) heterozygotes, each carrying a representative mutant allele from the above set, and determined that of this set, $recA^+$(F' *recA56*) and *recA730*(F' *recA56*) merodiploid strains were unique in showing genetic separation of *recA* functions.

Genetic separation of SOS mutagenesis from LexA protein cleavage. As indicated above, RecA protein has at least two distinct roles in SOS mutagenesis, cleavage of LexA protein leading to induction of the SOS regulon, and one or more additional roles (1, 6, 15). *recA* mutants might be deficient in mutagenesis due to a regulatory defect involving LexA cleavage or to a defect in some other mutagenesis function. To distinguish between these two possibilities, several *recA* mutants previously found to be defective in SOS mutagenesis were analyzed for SOS mutagenesis and LexA cleavage.

LexA protein cleavage was scored by measuring expression of β-galactosidase from a *sulA-lacZ* fusion [*sulA*::Mu d (Ap *lac*)XCam] in the presence or absence of two SOS-inducing agents, UV light and mitomycin (MC). We chose this particular fusion because its unusually large induction ratio (as high as 400-fold) makes possible detection of subtle differences in *sulA* expression (26).

SOS mutagenesis was scored by a λ *L*am63 → λ L^+ Sus^+ reversion assay described previously in host strains which do not produce functional LexA protein (6). In this assay, the phage may be damaged to induce mutagenic lesions independently of treatment to induce the SOS response. Noninduced cultures of constitutive host mutants will give partial or full mutagenesis of UV-irradiated phage. Thus, this procedure makes possible the unambiguous detection of mutants which express SOS mutagenesis constitutively (6). Mutagenesis was measured in cells that had their LexA gene mutationally inactivated by the *lexA51* null allele in order to bypass any defect in LexA protein cleavage in any particular *recA* mutant. *lexA51* derepresses all of the SOS genes, e.g., increases the level of RecA protein 3- to 20-fold. Activities of mutant and wild-type RecA proteins were compared in the same *lexA51* background.

As observed previously, $recA^+$ cells required UV or MC inducing treatments for the expression of SOS mutagenesis and LexA cleavage, and both of these activities were expressed constitutively in *recA730* cells (Fig. 1) (6, 15). In

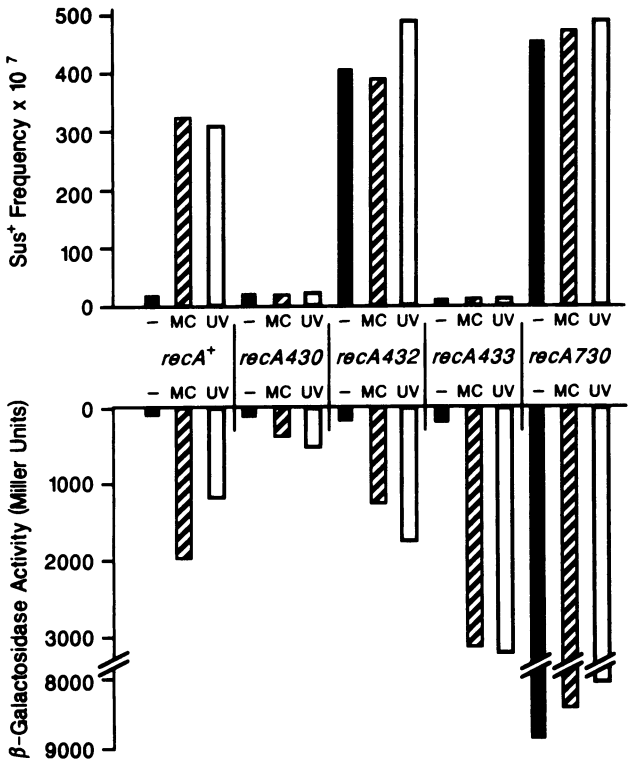

FIG. 1. Influence of *recA* on phage mutagenesis and LexA repressor cleavage. The top panel represents measurements of the SOS mutagenesis assay using λ *sus* *L*63 (λ DE57) as described previously. λ (*sus* L^+) revertants are expressed as number of Sus^+ phage per 10^7 surviving phage when plaque assayed on a given *recA* cell pair under the indicated conditions. The frequency of revertants was the ratio of the plaque count on a sup^+ strain over the count on the *supD43*-containing host. The first member of each strain pair carried *supD43*, and the second carried sup^+. All cell pairs carry the *lexA51* null allele. Strain genotypes are given in Table 1: $recA^+$, DE190/DE192; *recA430*, DM2572/DM2573; *recA432*, DE858/DE860; *recA433*, DE866/DE868; *recA730*, DE272/DE274. All data points are the average of two to four experiments. MC signifies that the cells were exposed to 0.5 μg of MC per ml for 40 min before being used for plaque assay of damaged λ DE57 (7). UV indicates similar treatment of cells with 18 J of UV light per m^2. A minus sign indicates that untreated cells were used. The bottom panel represents β-galactosidase activity expressed from the LexA-regulated *sulA*::Mu d(Ap *lac*)XCam fusion. Procedures were as described previously (8, 18) except for the doses. Strain genotypes are in Table 1; $recA^+$, NO56; *recA430*, DE975; *recA432*, DE894; *recA433*, DE939; *recA730*, NO62. All values are expressed as the average of four different β-galactosidase measurements.

untreated *recA730* cells, twofold-greater mutagenesis and sixfold-greater *sulA-lacZ* expression were observed than in induced $recA^+$ cells. Also as reported previously, strains carrying the *recA430* allele were defective in promoting SOS mutagenesis (1, 6). However, as judged by expression of the *sulA-lacZ* fusion, LexA proteolysis could be induced to moderate levels in *recA430* mutants (Fig. 1). Mutagenesis was similarly deficient in *recA433* (Fig. 1) and *recA435* (Fig. 2) mutants, whereas the *sulA* gene was induced to levels nearly twice those of induced $recA^+$ cells in the latter two strains (Fig. 1 and data not shown). We conclude that *recA430*, *recA433*, and *recA435* define a class of mutants that are defective in mutagenesis but proficient in LexA cleavage.

In contrast to the above class of mutants, the *recA432* mutant expressed maximal levels of mutagenesis of UV-damaged phage in the absence of induction comparable to that of *recA730*, and there was little further increase following an inducing treatment (Fig. 1). Although *recA432* cells expressed mutagenesis in the absence of an inducing treatment, they did not apparently promote cleavage of LexA protein under these conditions. However, following induction, these cells did promote LexA cleavage (Fig. 1). Thus, the activities of RecA432 protein in uninduced cells are functionally the opposite of RecA433 activities in induced cells.

Genetic separation of SOS mutagenesis from cI protein cleavage. *recA* mutants were screened for the constitutive expression of λ cI proteolysis by a series of rapid assays on the set of *recA* mutants. First, the plaque type of λ^+ was determined—a clear plaque indicating strong constitutive expression; second, stable lysogens of λ^+ were produced, and the release of free λ phage was measured following induction from those host mutants which could be lysogenized. To eliminate any indirect effect of expression of the SOS regulon on phage induction, host mutants lacking functional LexA protein were used in these assays.

Clear-phage phenotype has been shown to be due to constitutive cleavage of cI protein as a consequence of increased activity and elevated intracellular concentrations of RecA protein (9, 22; Ennis and Mount, unpublished observations). To improve the sensitivity range of the clear-plaque assay, we used three mutant phage strains with different mutant *c*I alleles whose products vary in their susceptibility to RecA-mediated proteolysis (4); in addition to λ^+, these alleles include a hypocleavable mutant (λ *c*I ind^r*543*), a hypercleavable mutant (λ *c*I ind^s*1*), and a non-cleavable mutant (λ *c*I ind^-*1*) (4, 22). These mutants were also used to determine whether a given mutant could be activated further by the addition of 0.5 μg of MC per ml to the top agar as an inducing treatment. As expected, $recA^+$ lysogens efficiently induced λ following induction with MC, and no induction of phage was observed in lysogens of Δ*recA306* or *recA430* mutants (Table 2). This failure is due to absence of RecA protein in the Δ*recA306* mutant and to loss of the cI proteolysis activity by RecA430 protein, respectively (15, 20, 27).

With this plaque assay, the constitutive *recA730* mutant produced clear plaques for all phage strains except *c*I ind^-*1*, and the $recA^+$ strain produced turbid plaques of all phage strains. The other *recA* mutants could be ranked in the following order with respect to their constitutive cI cleavage activities; nonactivated ($recA^+$, *recA430*, *recA433*, *recA435*), slightly activated (*recA80*, *recA718*, *recA801*), moderately activated (*recA441*), and highly activated (*recA432*, *recA730*) (Table 2 and data not shown). *recA433* and *recA435* lysogens were found to induce λ efficiently and slightly better than the $recA^+$ parental strain. *recA433* and *recA435* strains thus stimulated LexA and cI protein cleavage but did not stimulate SOS mutagenesis (Fig. 1 and 2, and data not shown). The *recA80* mutant promoted only weak cleavage of λ cI repressor in the absence of induction (Table 2). *recA80* strains, however, expressed SOS mutagenesis at levels comparable to those in SOS-induced $recA^+$ strains (Fig. 2). This pattern of RecA protein activities in *recA80* cells is the functional opposite of the RecA433 protein (and RecA435 protein) pattern described earlier.

Genetic separation of LexA and cI protein cleavages. The abilities of various RecA proteins to promote cleavage of LexA and cI proteins is shown in Table 2. In particular, we note that RecA432 protein was inducible for LexA cleavage

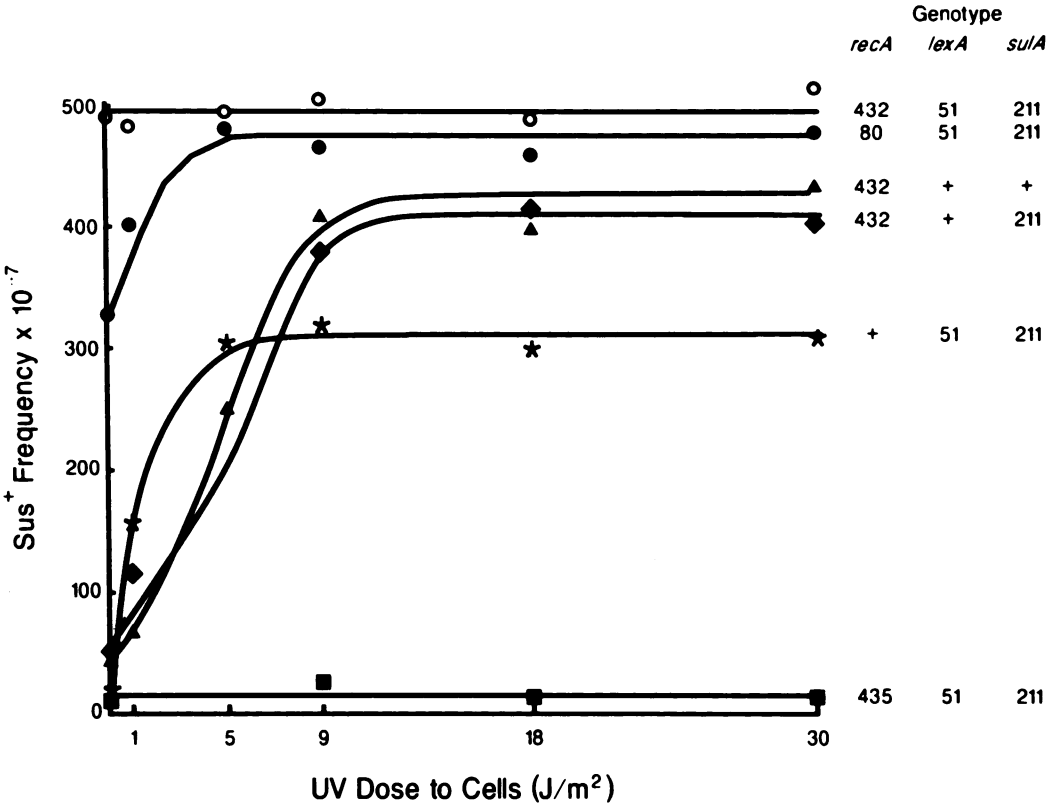

FIG. 2. SOS mutagenesis of UV-damaged phage λ in *recA* mutant hosts. The experimental conditions were the same as in Fig. 1, except that the UV dose was varied and no MC was used. Strain genotypes are shown in Table 1, and relevant genetic markers are shown in the figure. Symbols: ○, DE858/DE860; ●, DE732/DE736; ▲, DE1294/DE1296; ◆, DE1185/DE1187; ★, DE190/DE192; ■, DE862/DE864.

and constitutive for cI cleavage and that RecA80 had the opposite pattern of activities. As shown in Fig. 1 and Table 2, cells that produced RecA430 protein were defective for λ cI proteolysis but partially proficient for LexA proteolysis. Therefore, RecA430 protein from induced cells exhibits a pattern of properties opposite that of RecA432 protein from uninduced cells. *recA80* and *recA730* cells both showed high levels of expression of the *sulA-lacZ* fusion (Fig. 1 and Table 2), indicating that LexA cleavage is expressed constitutively. However, unlike in *recA730* strains, only a low level of cI cleavage was detected in *recA80* strains (Table 2). We conclude that noninduced *recA80* strains also express a split phenotype (high-level LexA cleavage but low-level cI cleavage) that contrasts with the phenotype of noninduced *recA432* strains. This property of *recA80* might also indicate genetic separation of LexA and cI protein cleavages. Alternatively, since LexA protein cleavage is more easily facilitated by RecA protein than is cI protein cleavage (14), the properties of the *recA80* mutant could also be explained by a reduced activity of RecA80 protein towards both LexA and cI proteins. However, by being constitutive for cI protein cleavage and inducible for LexA protein cleavage, RecA432 protein displays a clear preference for promoting cleavage of cI protein, the less easily cleaved of the two proteins. From this preference, we may conclude that these two *recA* functions are genetically separable.

Genetic separation of *recA* functions in *recA*$^+$(F′ *recA56*) heterodiploids. An alternative method of separating *recA* activities in mutants is to analyze the properties of partially diploid strains which carry both mutant and functional copies of *recA*. The merodiploid strains produce both defective and functional RecA proteins in the same cell, which presumably leads to the formation of mixed multimers (21, 24). We wished to determine whether such a condition can lead to selective poisoning of some *recA* functions by the mutant protein. The properties of merodiploid strains might therefore indicate which functions of RecA protein are separable and whether these functions are attributable to the multimeric structure of RecA protein (21).

A series of such merodiploid strains carrying various mutant *recA* alleles were prepared. In these strains, the presence of the mutant *recA* allele on a low-copy-number F plasmid avoided a bias in gene dosage (24, 28). Whereas many of these alleles did not show a significant degree of dominance over a functional *recA* gene, *recA56* exhibited a moderate degree of dominance over *recA*$^+$ for induction of λ prophage (Table 2) and a high degree of dominance for SOS mutagenesis (Fig. 3). Only a marginal degree of dominance was shown for LexA protein cleavage (Table 2). The results were identical when the mutations were in the opposite genetic orientation [*recA56*(F′ *recA*$^+$)] (data not shown). *recA56* was also dominant over the constitutive mutation *recA730* (Fig. 2), indicating that RecA56 protein can interfere with the function of constitutively activated RecA protein. Our data revealed that homologous recombination was only slightly inhibited in *recA*$^+$/*recA56* diploid strains (data not shown). In combination with the above mutagenesis data, these observations suggest that the *recA* mutagenesis function is a property of RecA protein multimers. These

TABLE 2. Summary of in vivo *recA*-mediated functions

recA allele	Selected phenotype[b]	Inducing treatment[c]	*recA*-mediated function[a]		
			SOS mutagenesis[d]	LexA cleavage[d]	λ cI cleavage[e]
+	None	No	−	−	−
		Yes	++	++	++
306	Rec⁻[f]	No	−	−	−
		Yes	−	−	−
441	SOS constitutive[g]	No	++	+	++
		Yes	+++	+++	+++
730	SOS constitutive[h]	No	+++	+++	+++
		Yes	+++	+++	+++
430	No phage induction[i]	No	−	−	−
		Yes	−	+	−
433	No SOS mutagenesis[j]	No	−	−	−
		Yes	−	+++	++
432	No SOS mutagenesis[j]	No	+++	−	+++
		Yes	+++	++	+++
80	SOS constitutive[k]	No	++	++++	+/−
		Yes	+++	++++	+++
+/56	—[l]	No	−	−	−
		Yes	−	++	+

[a] SOS mutagenesis and λ *c*I cleavage were examined in cells which lacked functional LexA protein to obviate any regulatory effect of reduced LexA cleavage on expression of the *recA* function. The − and ++++ scores indicate no detectable and highest expression scored, respectively. The +, ++, and +++ scores indicate gradually increasing expression of the function, as detectable in each of the individual tests.

[b] The phenotype that was originally used to isolate each mutant.

[c] Use (yes) or nonuse (no) of the SOS-inducing agents MC and UV light as described in the legend to Fig. 1 and Materials and Methods.

[d] SOS mutagenesis and LexA protein cleavage were measured as described in the legend to Fig. 1 and Materials and Methods.

[e] Extent of constitutive expression of *c*I cleavage was assessed by plaque assay of λ *c*I alleles with different susceptibilities to *recA*-mediated cleavage (see text) or by plaque assay of phage particles induced by treatment of cultures with 0.5 μg of MC per ml.

[f] A Rec⁻ UV-sensitive mutant with a deletion extending from the neighboring *srl* operon through *recA*.

[g] A mutant showing induction of phage and SOS functions by growth at 42°C, especially when grown on minimal medium in the presence of adenine.

[h] A derivative of *recA441* which is constitutively activated at all temperatures.

[i] A mutant not showing phage induction and also found to be Rec⁺ and UV sensitive.

[j] A mutant not showing SOS mutagenesis and also found to be Rec⁺ and UV sensitive.

[k] A mutant showing constitutive LexA proteolysis.

[l] *recA56* was isolated as a recombination-deficient mutant. The *rec*⁺(F′ *recA56*) heterodiploid used here was recombination proficient.

data also provide further evidence that the mutagenesis and LexA cleavage functions are separable.

DISCUSSION

We have presented evidence that the functions of RecA protein which promote SOS mutagenesis, LexA proteolysis, and λ cI repressor proteolysis are separable by mutation. This separation of *recA* function was observed in three classes of *recA* mutants and also in *rec*⁺(F′ *recA56*) heterodiploids. The first class of mutants, represented by *recA430*, *recA433*, and *recA435*, was inducible for only one or two of the functions following an inducing treatment. *recA*⁺(F′ *recA56*) heterodiploids were like this first class in being inducible for LexA protein cleavage but not for mutagenesis. The second class, typified by *recA730*, was constitutively activated for all three functions. The third class, represented by *recA480* and *recA432*, was differentially activated by being constitutively activated for two of

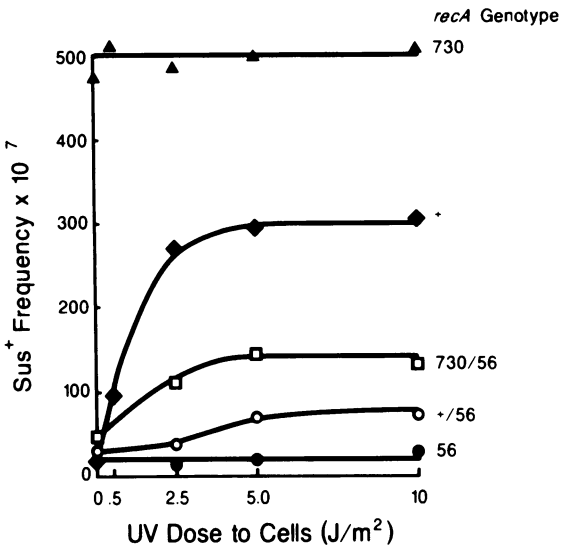

FIG. 3. Dominance of *recA56* on SOS mutagenesis of UV-damaged phage λ. The experimental conditions are described in the legend to Fig. 1, except that the UV dose was varied and no MC was used. Strain genotypes are shown in Table 1, and relevant genetic markers are shown in the figure. All cell pairs carry the *lexA51* null allele. Symbols: ▲, DE272/DE274; ◆, DE190/DE192; □, DE441/DE443; ○, DE183/DE185; ●, DE277/DE259.

the three functions studied and inducible for the third function. *recA* mutants used in these experiments were proficient for homologous recombination, indicating that the ability for the interactions between DNA strands and RecA protein that are required for recombination is retained in these mutant RecA proteins. Tessman and Peterson (30) have isolated recombination-defective, mutagenesis-proficient *recA* mutants, providing further evidence for separation of the recombination and mutagenesis functions of RecA protein.

The *recA432* mutant was found to be constitutive for SOS mutagenesis and cI protein cleavage but inducible for LexA protein cleavage (Fig. 1 and 2 and Table 2). This mutant was previously found to be defective in cellular mutagenesis (11). This difference in mutagenesis results might be attributable to the different *E. coli* genetic backgrounds used or to the different methods used to measure mutation frequency. After exposure to UV light, the original strain of Kato and Shinoura (11) showed an unusually high degree of filamentation in our hands (data not shown). The genetic background used by us differed from that of the original Kato and Shinoura strain (TK504) in carrying null alleles in the *lexA* and *sulA* genes. When we restored functional *lexA* and *sulA* genes to our *recA432* strains, we found that they also showed extensive filamentation (preliminary observations). We also found that cellular mutagenesis could be restored to TK504 when filamentation was prevented by introduction of a null *sulA* mutation. This result suggested that the filamentation observed in some fashion interfered with the detection of mutant colonies. Lethal filamentation proved not to interfere with the detection of mutants in the phage assay (compare *recA432 lexA*⁺ *sulA211* and *recA432 lexA*⁺ *sulA*⁺ strains in Fig. 2).

Another mutant, *recA718*, appeared to be activated for mutagenesis but inducible for other properties (38). Differential activation could also account for the properties of the *recA801*, *recA802*, and *recA803* mutants (Thliveris et al., in

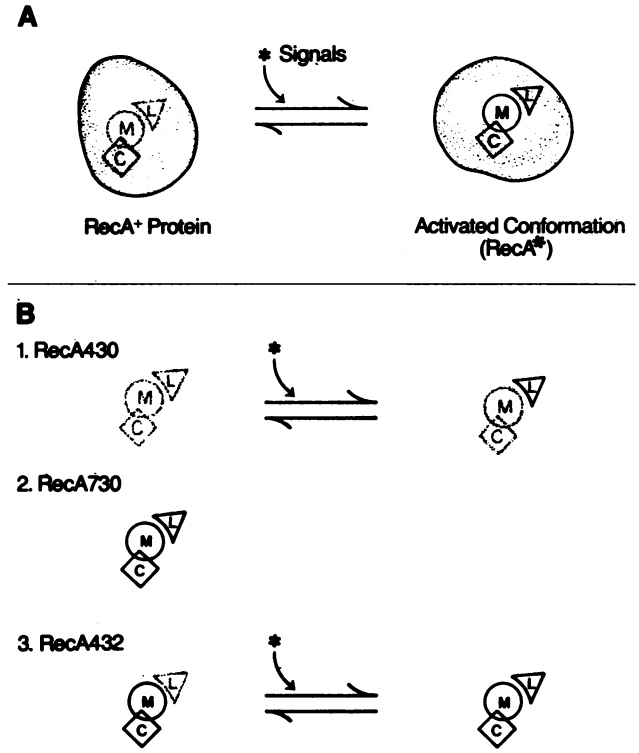

FIG. 4. Model showing activation of RecA protein functions. The spherical object indicates a RecA protein monomer and its transition to an activated conformation as a result of interaction with polynucleotide and small, as yet unidentified, molecules acting as inducing signals. The shapes on the surface of the molecule represent sites of interaction between target proteins and the RecA monomer. L, C, and M indicate sites of interaction with LexA, cI, and mutagenesis proteins, respectively. The shapes outlined with stippled and solid lines represent inactive and active surfaces, respectively. (A) Activation of RecA$^+$ protein for all three functions. (B) Three classes of mutant proteins. RecA430 is only inducible for LexA cleavage; RecA730 is constitutive for all three functions; RecA432 is constitutive for cI cleavage and mutagenesis functions and can be activated for LexA protein cleavage following an inducing treatment.

press). These mutants showed some constitutive cI cleavage but are normally inducible for LexA cleavage (31; Thliveris et al., in press; Ennis and Mount, data not shown). In addition, we showed previously that the presence of a high-copy-number plasmid can also activated mutagenesis but not LexA cleavage in induced *recA*$^+$ cells (6).

To account for the above observations, we propose the model for activation of RecA protein shown in Fig. 4. Activation normally occurs when RecA protein combines with inducing signals derived from nucleic acids or damaged sites on DNA. Activation gives rise to conformational changes in RecA protein which promote surface-surface interactions with other target proteins, such as the cI and LexA proteins. We propose that RecA protein can be altered by mutation to a form that is unable to interact with a subset of target proteins or that is conformationally active in the absence of the normal endogenous inducing signal. This model is supported by the existence of two classes of mutants. The first class, B1 in Fig. 4, is defective for a subset of functions normally observed following activation. In agreement with this expectation, RecA430 protein is known to be defective in cleavage of cI and UmuD proteins (2, 27, 28) (Table 2) but supports partial cleavage of LexA protein (Fig. 1). Class B3 (Fig. 4) is constitutively activated for a subset of RecA functions, SOS mutagenesis, and cI cleavage, but requires an inducing treatment to activate the LexA cleavage function. The above results with RecA mutants (class B3) also imply that a particular type of damage may induce the synthesis of a specific class of signal molecules. As a result, a given type of damage could induce a certain class of RecA functions through metabolic regulation of RecA protein by these molecules. This kind of differential activation of RecA protein was proposed previously to account for induction of lambdoid phages (18) but not LexA protein cleavage (3) by amino acid starvation.

The constitutive class of mutants might arise by mutation at the binding site of an inducer molecules, so that a different molecule can act as an inducer or so that binding of inducer is not necessary to bring the conformational change. Genetic studies with cyclic AMP (cAMP) receptor protein (CRP) have led to the isolation of such types of mutants. Mutant forms of CRP have been isolated which are activated by cGMP as well as cAMP (35). Other mutant CRP proteins have an altered conformation which results in activation without binding of cAMP or any other allosteric molecule (8, 35).

RecA protein has several roles in SOS mutagenesis which are separable in *recA* mutants. First, the protein is required for derepressing genes in the SOS regulon by cleavage of LexA protein. Second, RecA protein is used for posttranslational modification of one of the SOS-induced proteins (UmuD protein). Defects in either of these roles can be overcome by mutation; first, derepression of the SOS regulon by a *lexA*(Def$^-$) mutation; and second, synthesis of the COOH fragment of UmuD protein which is active in mutagenesis (UmuD*) by the appropriate plasmid (23). These types of genetic suppression provide a method for assessing the specific mutagenesis defect in *recA* mutants. For instance, UmuD* can overcome the defect in UV-induced cellular mutagenesis in *recA430* mutants, indicating that RecA430 mutant protein is defective in promoting UmuD protein cleavage (23). Using the phage mutagenesis assay described above, we have shown in preliminary experiments that mutagenesis is also restored to *recA433 lexA*(Def$^-$) and *recA435 lexA*(Def$^-$) hosts when UmuD* is expressed by the appropriate plasmid (Ennis and Mount, unpublished observations). RecA433 and RecA435 proteins thus may also be defective in promoting UmuD protein cleavage. However, in contrast to RecA430 protein, RecA433 and RecA435 proteins are fully capable of promoting cleavage of LexA and cI proteins. Accordingly, RecA433 and RecA435 proteins may be uniquely defective in their UmuD protein cleavage function.

Nohmi et al. (23) showed further that *recA*-deleted *lexA*(Def$^-$) cells remained nonmutable even in the presence of UmuD* protein. This lack of mutagenesis indicates that RecA protein plays a third role in mutagenesis. The *recA1730* mutant appears to be defective in this third role, since UmuD* protein was not able to suppress the mutagenesis defect in *lexA*(Def$^-$) derivatives of this mutant (Battista and Walker and Dutreix and Devoret, personal communications). Nohmi et al. (23) have suggested that detection of cellular mutagenesis in mutants hypersensitive to DNA damage (such as *recA*-deleted strains) is difficult in part because doses of UV light sufficient to induce a detectable number of mutations in these strains are likely to be lethal. This potential difficulty is reduced by use of the phage mutagenesis assay, which allows use of smaller doses of UV

light to *E. coli* necessary only to induce mutagenesis functions. With the phage mutagenesis assay, the level of host viability at a given dose of UV light (e.g., 20 J/m^2) was not found to be a significant factor in the detection of phage mutants in host mutants hypersensitive to DNA damage (D. G. Ennis, Ph.D. thesis, University of Arizona, Tucson, 1988). Accordingly, we have been able to measure mutagenesis in *recA*-defective hosts with greater confidence.

The extent of phage mutagenesis was measured in *recA56 lexA51* cells that express the UmuD* fragment. In preliminary experiments, UmuD* was unable to restore mutagenesis under these conditions (Ennis and Mount, unpublished observations). These results are also consistent with a third role for RecA protein in SOS mutagenesis and suggest further that RecA56 mutant protein is defective in this mutagenesis function. Additional genetic experiments with UmuD* protein should be informative in determining the nature of the mutagenesis defect in other *recA* mutants and may offer new insight into the third role of RecA protein in mutagenesis.

The dominance of *recA56* in *recA*$^+$(F′ *recA56*) heterodiploids for SOS mutagenesis indicates that RecA56 protein can form mixed protein multimers with RecA$^+$ protein and poison the mutagenesis function of RecA protein. Consistent with this interpretation, RecA56 protein has been found to inhibit wild-type RecA$^+$ protein activities by forming RecA$^+$/RecA56 mixed filaments with RecA$^+$ protein on single-stranded DNA (Kowalczykowski and Lauden, personal communication). These observations lead to the conclusion that the mutagenesis function of RecA protein is due to the action of RecA multimers. Multimers may promote cleavage of UmuD protein or act in an as yet unidentified role. This role might involve interactions with components of DNA polymerase III holoenzyme to permit replication forks to proceed past the damaged sites, as proposed previously (5, 16).

All of the mutagenesis-defective (Mut$^-$) *recA* mutants described above remained sensitive to UV light even when the cells lacked functional LexA repressor and expressed the SOS regulon constitutively; in contrast, all Mut$^+$ alleles were resistant to UV light. Accumulation of UV photoproducts in DNA inhibits DNA replication, but eventually DNA replication recovers. This recovery (called induced replisome reactivation [IRR]) is SOS inducible and a function of RecA protein (12, 38). Inability to perform IRR increases the sensitivity of *E. coli* to DNA damage. The strong correlation between the defects in mutagenesis and DNA repair observed in the *recA* mutants which we examined suggests that these two functions of RecA protein are related. In the case of the *recA430* mutant, this UV-sensitive mutant is defective in both IRR function and mutagenesis (38). As noted above, although *recA*$^+$/*recA56* heterodiploid strains were mutagenesis deficient, they were fully resistant to UV light, demonstrating another separation of these two functions. Other work has also suggested that these processes are genetically separable (Ennis and Mount, data not shown; E. Witkin and R. Devoret, personal communications).

Mutations in *E. coli* genes such as *recF* whose products appear to participate in the activation of RecA protein confer a UV-sensitive phenotype (17). One possible explanation for this phenotype is that *recF* mutations prevent activation of RecA protein IRR function. The *recA441*, *recA730*, and *recA801* alleles do not require *recF* for functional activation (31, 33; Ennis and Mount, unpublished observations). These mutant RecA proteins might therefore express their IRR functions constitutively.

Recently, an impressive and consistent body of evidence demonstrating that one role of RecA* protein in SOS mutagenesis is to promote the cleavage of UmuD protein has been published (2, 23, 28). A proteolytic fragment of UmuD protein is the active form for mutagenesis. Thus, the role for RecA* in SOS mutagenesis is regulated by two protein cleavage reactions; first, LexA cleavage, which leads to the derepression of the SOS regulon, and second, UmuD cleavage. Experiments with purified proteins have shown that proteolysis of the LexA, λ cI, and UmuD proteins is catalyzed by a common proteolytic mechanism called autodigestion (2, 14). In each case, RecA protein is thought to stimulate the proteolytic event in vivo by interacting with the target protein in a positive allosteric manner that promotes autodigestion. From these genetic and biochemical experiments, we propose that the allosteric effector surfaces of RecA protein are nonidentical and can be independently altered by mutation (Fig. 4). However, these studies do not preclude the possibility that the effector surfaces are physically overlapping. The RecA-mediated proteolysis of λ cI protein in vitro was shown to be competitively inhibited by the presence of a noncleavable LexA protein (29). This result suggested that the RecA protein surfaces which interact with these two target proteins may be close or overlapping.

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