JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 7373–7382
0021-9193/93/227373-10$02.00/0

Vol. 175, No. 22

Novel Mechanism for UV Sensitivity and Apparent UV Nonmutability of *recA432* Mutants: Persistent LexA Cleavage following SOS Induction†

DON G. ENNIS,[1,2]‡* JOHN W. LITTLE,[1,3] AND DAVID W. MOUNT[1]

Department of Molecular and Cellular Biology[1] and Department of Biochemistry,[3] University of Arizona, Tucson, Arizona 85721, and Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland 20892[2]

Received 7 June 1993/Accepted 16 September 1993

The *recA432* mutant allele was isolated (T. Kato and Y. Shinoura, Mol. Gen. Genet. 156:121–131, 1977) by virtue of its defect in cellular mutagenesis (Mut$^-$) and its hypersensitivity to damage by UV irradiation (UV^s), which were phenotypes expected for a *recA* mutant. However, we found that in a different genetic background (*lexA51 sulA211 uvrB*$^+$), *recA432* mutants expressed certain mutant phenotypes but not the Mut$^-$ and UV^s phenotypes (D. G. Ennis, N. Ossanna, and D. W. Mount, J. Bacteriol. 171:2533–2541, 1989). We present several lines of evidence that these differences resulted from the *sulA* genotype of the cell and that the apparent UV^s and Mut$^-$ phenotypes of the *sulA*$^+$ derivatives resulted from lethal filamentation of induced cells because of persistent derepression of *sulA*. First, transduction of *sulA*(Def) mutations into the *recA432* strains restored cellular mutagenesis and resistance to UV. Second, *recA432 sulA*$^+$ strains underwent filamentous death following SOS-inducing treatments. Third, cleavage of LexA repressor in a *recA432* strain continued at a rapid rate long after UV induction, at a time when cleavage of the repressor in the *recA*$^+$ parental strain had substantially declined. Fourth, we confirmed that a single mutation (*recA432*) conferring both the UV^s and Mut$^-$ phenotypes mapped to the *recA* gene. These findings indicate that the RecA432 mutant protein is defective in making the transition back to the deactivated state following SOS induction; thus, the SOS-induced state of *recA432* mutants is prolonged and can account for an excess of SulA protein, leading to filamentation. These results are discussed in the context of molecular models for RecA activation for LexA and UmuD cleavage and their roles in the control of mutagenesis and cell division in the SOS response.

Irradiation of cells with UV light or treatment with chemical agents that introduce bulky DNA adducts induces the SOS response. This response is controlled by two major regulatory proteins, LexA repressor and RecA, which is activated by DNA damage to a form (designated RecA*) which promotes the cleavage of the LexA repressor, resulting in increased transcription of about 20 genes (the SOS regulon). Activation of RecA protein presumably occurs by its binding to small diffusible signal molecules. Although the molecular nature of these signals is still unknown, they are believed to be derived from nucleic acids and are perhaps the by-products of DNA damage. Derepression of the widely divergent genes from the SOS regulon increases DNA excision repair, SOS mutagenesis, reinitiation of stalled DNA replication, and inhibition of cell division; if the cell contains a lambdoid prophage, cleavage of the phage repressor leads to prophage induction (for reviews, see references 32, 45, 47, 57, and 60).

RecA protein is required to promote multiple roles in SOS mutagenesis (3, 14), including derepression of *recA* and *umuDC* (17, 32, 55), proteolysis of UmuD protein to its active form (UmuD′) (4, 42, 51, 61), and another as yet unidentified role (9, 15, 16, 53). *recA* mutants which are altered in one or more of these mutagenesis functions have been identified; these include the split-phenotype mutants, which are unable to promote some RecA functions but have retained other functions (8, 9, 14, 16, 41). For example, several mutants (e.g., *recA430*, *recA433*, and *recA435*) are essentially nonmutable because they are unable to promote UmuD cleavage; mutagenesis can be restored to these mutants either if UmuD′ is supplied or if the functional *mucAB* analog is present (15, 16, 20, 42). Conversely, *recA* mutants have been identified which promote SOS functions such as UmuD cleavage without the normal requirement for inducing treatments (14, 15, 29, 51, 54, 61).

We have shown that the *recA432* mutant allele expresses phage mutagenesis and lambda CI cleavage constitutively but requires induction for LexA cleavage and derepression of LexA-regulated genes such as *sulA* (16). The observation that our *recA432* strains were proficient in SOS mutagenesis (Mut$^+$) was surprising, because this *recA* allele was originally identified as a mutant defective in cellular mutagenesis (Mut$^-$ [24]). Also in conflict with the original and subsequent characterizations of this mutant (19, 24), our *recA432* mutant strains were not hypersensitive to UV and other DNA-damaging treatments; instead they were almost as resistant to damage as their *recA*$^+$ parental strains. The differences in these results were attributed to one or more possibilities, such as genetic differences in strain backgrounds, the different mutagenesis assays used, or genetic changes of the original *recA432* mutant strain (TK504) that may have accumulated during more than a decade of storage (16).

In this report, we trace these apparent discrepancies to the different *sulA* genotypes of the strains used. We found that the observed Mut$^-$ and UV^s phenotypes of *recA432* were suppressed in strains which carried null mutations of *sulA* and that

* Corresponding author.

† This article is dedicated to the memory of our friend and colleague Hatch Echols.

‡ Present address: Laboratory of Molecular Genetics, Building 6B, Room 2B-231, National Institute of Child Health and Human Development, Bethesda, MD 20892.

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

Strains	Relevant genotype	Source or reference
Bacterial		
AB1157	*thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1*	Mount collection
AB1885[a]	*uvrB5*	Mount collection
AB2463[a]	*recA13*	Mount collection
KL399	*ara-14 leuB6 lacZ36 proC32 hisF860 recA200*(Ts) *thyA54 rpsE2115 rpsL109 metE70 malA38 xyl-5 mtl-1 thi-1 deo-85*	K. B. Low
JC2924	*recA56*	A. J. Clark
JC3890[a]	Δ(*gal-uvrB*)*301*	24
JC10244[a]	*srlC300*::Tn*10 alaS3*(Ts)	7
JC10288[a]	*srlR301*::Tn*10* Δ(*srl-recA*)*305*	7
JC10289[a]	Δ(*srlR-recA*)*306*::Tn*10*	7
JC10290[a]	Δ(*srlR-recA*)*302*	7
JC11597[a]	JC10289/pJC745 (pBEU28 *Bam*HI::*recA99*)	6
JC13062[a]	*recA430*	33
TK504[a]	Δ(*gal-uvrB*)*301 recA432*	24
TK505[a]	Δ(*gal-uvrB*)*301 recA435*	24
TK508[a]	Δ(*gal-uvrB*)*301 recA433*	24
DM455	*recA99*(Am) *galK2 galT22 rpsL179 lac3350*	Mount collection
DM1760	*recA13 srlC300*::Tn*10*	Mount collection
DM2569[b]	Δ(*srlR-recA*)*306*::Tn*10 lexA51 sul211 supD43*	14
DM2572[b]	*recA430 srlC300*::Tn*10 lexA3 sulA211 supD43*	14
NO56	*HfrH* Δ(*lac-argF*)*U169 relA1 thi-1 cps-3 malF59*::Tn*5 sulA1*::Mu *d*(Ap *lac*)*1* X(Cam)	16
DE190[b]	*recA*$^+$ *lexA51 sulA211 supD43*	14
DE405[b]	*recA*$^+$ *lexA3 sulA211 supD43*	14
DE407[b]	*recA*$^+$ *lexA3 sulA211*	14
DE463[b]	*recA730 lexA51 sulA211 supD43*	16
DE542[b]	*recA200*(Ts) *srlC300*::Tn*10 lexA51 sulA211 supD43*	DE190[c]
DE548[b]	*recA431 lexA51 sulA211 supD43*	DM2568[d]
DE858[b]	*recA432 lexA51 sulA211 supD43*	16
DE862[b]	*recA435 lexA51 sulA211 supD43*	16
DE866[b]	*recA433 lexA51 sulA211 supD43*	16
DE880[c]	*malF*$^+$ *srlC300*::Tn*10*	16
DE894[c]	*malF*$^+$ *recA432*	16
DE943	Δ(*srlR-recA*)*306*::Tn*10 galK2 galT22 rpsL179 lac3550* (P2)	594(P2)[c]
DE951	Δ(*srlR-recA*)*306*::Tn*10 pro mel thi supF* (P2) mutant	QD5003 (P2)[c]
DE991[a]	*recA432* Δ(*gal-uvrB*)*301 sulA100*::Tn*5 pyrD* mutant	TK504[f]
DE998[a]	*recA433* Δ(*gal-uvrB*)*301 sulA100*::Tn*5 pyrD* mutant	TK508[f]
DE1046[a]	Δ(*gal-uvrB*)*301* Δ(*srlR-recA*)*306*::Tn*10*	JC3890[c]
DE1048[a]	*recA430 srlC300*::Tn*10*	JC3890[c]
DE1052[a]	Δ(*gal-uvrB*)*301 sulA100*::Tn*5 pyrD* mutant	JC3890[f]
DE1078[a]	Δ(*gal-uvrB*)*301* Δ(*srlR-recA*)*306*::Tn*10*/F′ *lac*,::Tn*3-recA*$^+$	TK504[c,g]
DE1133[c]	*recA56 srlC300*::Tn*10*	NO56[c]
DE1185[b]	*recA432 lexA*$^+$ *sulA211 supD43*	16
DE1242[a]	*recA432 srlR301*::Tn*10 sulA100*::Tn*5 pyrD* mutant	DE991[f]
DE1294[b]	*recA432 lexA*$^+$ *sulA*$^+$ *supD43*	16
DE1489[a]	*recA432* Δ(*gal-uvrB*)*301*	DE1046[d]
DE1500[b]	*recA*$^+$ *lexA*$^+$ *sulA211 supD43*	61
BEU317[a]	Δ(*srl-recA*)*304*/pBEU34 (F′*lac*$^+$, Tn*3-recA*$^+$)	A. J. Clark
Lambda		
λDE57	*L63*(Am) *c*I857	14
λNM1151	Δ(RI_1-RI_2) *imm*^{21} *c*I(Ts) *nin5*	N. Murray
λDE212	Δ(RI_1-RI_2) *gam-kil*::Tn*3-recA99 imm*^{21} *c*I(Ts) *nin5*	λNM1151[h]
λ11	v_2 v_3	46
λDE170	*c*II2002 *susR221*(Am)	31

[a] These strains descended from strain AB1157 and are known to share most of its genetic markers; any known changes from AB1157 or from the indicated parental strain are described in the genotype column.

[b] These strains were derived from either DM2568 or DM2570; both were *thi-1 lexA51 sulA211 srlC300*::Tn*10* Δ(*lac-gpt*)*5 ilv*(Ts) *mtl-1 rpsL31* (*supD43* or *sup*$^+$, respectively). Known changes from these strains or from the indicated parental strain are listed.

[c] *recA* allele was cotransduced with *srl*::Tn*10*.

[d] *recA* allele was cotransduced with *srl*$^+$.

[e] These strains were derived from NO56; changes are listed.

[f] *pyrD* mutant and *sulA100*::Tn*5* were obtained by selecting for Kan^r transductants that required uracil on minimal plates.

[g] F′ *recA*$^+$ was transferred by conjugation from strain BEU317, obtained *bio* mutant Amp^r Rec$^+$ transconjugants.

[h] To obtain phage that carry the Tn*3-recA99* transposon, this phage was propagated on host JC11597. By plating this lysate on strain DE951, we selected for transposon insertion events that yielded λSpi$^-$::*recA99* phage (see mapping section of Materials and Methods); the insertions yielding turbid plaques would be expected to be distal to *c*III (and polar on *gam* and *red*) and proximal insertions should have clear phenotypes. Although most of the isolates had clear plaque phenotypes; some isolates formed turbid plaques, efficiently lysogenized host DM2569, and as a prophage, conferred Rec$^+$ and Amp^r phenotypes. These phenotypes would indicate that the insertion was distal to *c*III (λ*gam-kil* interval); deletion mapping and preliminary restriction mapping of the insertion point for this isolate was consistent with this assignment (11). As expected, this phage carrying *recA99*(Am) was unable to form plaques on a similar strain which did not carry an amber suppressor (DE943).

recA432 sulA$^+$ strains lethally filamented following SOS induction. We have thus concluded that the apparent Mut$^-$ and UV^s phenotypes were the indirect consequences of lethal filamentation. The *sulA* gene is negatively regulated by LexA and encodes a product which, upon derepression, blocks septation (18, 22, 32); because an excess of SulA has been shown to result in lethal filamentation, we presume that the induced *recA432* mutants were also overproducing SulA protein. In this work, we have examined this possibility further by comparing the cleavage patterns of LexA in induced *recA*$^+$ and *recA432* strains; we found that unlike the *recA*$^+$ parental strain, these mutant cells remain fully induced long after induction. We propose that RecA432 protein is in some manner defective in making the transition to a deactivated state, causing elevated levels of SulA. This is the first report known to us that the apparent Mut$^-$ and UV^s phenotypes of a *recA* mutant resulted from complications associated with the overproduction of an SOS gene product (SulA) rather than a loss of some RecA activity.

TABLE 2. UV survival and His$^+$ reversion in *recA* mutant cells[a]

Strain	*recA* genotype	UV dose required for 10% survival (J/m^2)	His$^+$ frequency per 10^6 surviving cells at 1.0 J of UV irradiation/m^2
A (JC3890)	Wild type	3.8	35
B (TK504)	*432*	0.25	3.5
C (TK508)	*433*	0.25	2.9
D (TK505)	*435*	0.25	1.2
E (DE1048)	*430*	0.90	~1[b]
F (DE1253)	*432*	0.29	1.9
G (DE1078)	Δ/wild type	3.9	29
H (DE1489)	*432*	0.30	1.4

[a] Experimental conditions were described in Materials and Methods. Strain G (DE1078) carries all markers of TK504, except that the *srlR-recA* interval was deleted and subsequently a *recA*$^+$ copy was introduced with an F′ construct (pBEU34 from strain BEU317). Full genotypes of the strains presented above are given in Table 1. All His$^+$ reversion values are the averages of three separate experiments except where noted otherwise.

[b] His$^+$ reversion value was the result of only one experiment.

MATERIALS AND METHODS

Bacterial and phage strains. Strains with genotypes and sources are listed in Table 1; strains were propagated for experimental purposes and for construction of strains under routine conditions that were described previously (14, 40, 56). Most strains were constructed from others described previously (16, 17, 24, 33) by standard P1 transduction.

Media. For various bacterial and phage strain construction steps, the following media were utilized: Luria broth, 3× lambda broth, 1× A liquid medium, SM diluent, M9-Casamino Acid agar, SEM agar, Luria-Bertani agar, and LC agar. BBL-lambda agar or MacConkey agar plates were used (1, 16, 23, 40). For measurements of LexA cleavage, M9 minimal medium containing 0.4% glucose and supplemented with seven amino acids was as described previously (30).

Determination of cellular and phage mutagenesis. UV-induced cellular mutagenesis of the *hisG4* nonsense mutation was conducted essentially as described previously (23, 24), except that in some experiments in which *pyrD* mutants were used, SEM plates were supplemented with uracil. Control experiments with strains JC3890, TK504, TK505, and TK508 (Table 1) indicated that the addition of uracil to SEM plates had no detectable influence on *his* reversion frequencies. In general, the UV-induced His$^+$ frequencies we obtained from strains received from T. Kato were about fivefold higher (Table 2) than those published under identical conditions that were described by Kato and Shinoura (24). However, despite the differences in experimental values, we also obtained 10-fold differences in reversion frequencies when comparing a mutagenesis-proficient strain (JC3890) with several defective strains (TK504, TK505, TK508, and DE1048). Rifampin-resistant (Rif^r) mutants were assayed by the technique of Sedgwick and Goodwin (50). The UV-induced Rif^r frequencies that we obtained compared satisfactorily with the values published previously for similar strains (5, 9).

SOS mutagenesis of phage lambda was measured with two different reversion assays: the λ*L63*(Am)→λL$^+$ we described previously (14) and the λ*R221*(Am)→λR$^+$ reversion assay described by Maenhaut and Caillet-Fauquet (35). The lambda forward assay ($\lambda v_2\ v_3 \rightarrow \lambda vir$) described by Quillardet and Devoret (46) was also employed. Slight differences in the experimental procedures for λ*L63* reversion mutagenesis were required for some experiments with *supE44* strains, bacterial hosts that were derived from JC3890 or AB1157. These *supE44* hosts were used as infective centers and plated on lawns of *supD43* and *sup*$^+$ nonmutable hosts (DE405 and DE407, respectively) to determine Sus$^+$ frequencies. Experimental values obtained with this method were usually slightly higher than those obtained with our standard method (14) but were generally in agreement with our previous results. As before, precautions were taken to avoid photoreactivation (14).

UV survival determination. Conditions for UV irradiation of cell suspensions were essentially as described previously (16, 44). Rapid, qualitative plate screens were also employed in the mapping studies to indicate UV^s snd UV^r phenotypes (12). Colonies were streaked across BBL plates with sterile toothpicks and irradiated at three different UV doses by moving an index card at different time intervals under a UV lamp. The differences in UV^s that were demonstrated with quantitative measurements in Fig. 1 and elsewhere (24, 41) could be qualitatively reproduced with the plate screen. For *uvr*$^+$ strains, cross-streaked colonies were irradiated at about 0, 18, 72, and 180 J of UV irradiation per m^2 and for *uvrB* mutant strains, they were irradiated at about 0, 2, 4, and 8 J/m^2. The best distinctions were obtained after approximately 16 h of incubation at 32°C.

β-Galactosidase assays. β-Galactosidase measurements were conducted at the Du Pont Experimental Station (Wilmington, Del.) with a semiautomatic system developed by Rolf Menzel (38). This robot integrates the use of automatic pipetting devices, microtiter readers, and microcomputers, which allowed us to perform 88 individual β-galactosidase assays which were monitored simultaneously. Bacterial cultures were propagated with lambda tryptone broth (40) at 37°C in wells of a microtiter plate. *sulA*::*lacZ* fusion strains were exposed to one of seven different concentrations of mitomycin (MC) (0.005 to 0.5 μg/ml; see Fig. 4B); after 2 h of exposure at 37°C, β-galactosidase activities were measured and computed. Previous *sulA* fusion experiments with 0.5 μg of MC have shown that 2 h with MC yielded approximately half-maximal β-galactosidase levels and that 4 h were required for maximal expression (see Results and Fig. 4) (11, 16, 44). In addition, during the same experiment we also measured the early kinetics (0 to 120 min) of *sulA*::*lacZ* induction at 0.5 μg of MC per ml (see Fig. 4A). Each of the 40 experimental conditions was run in duplicate and compared with β-galactosidase levels obtained in unexposed control cultures. Reaction rates and specific activities were calculated electronically from 12,320 separate spectrophotometric readings by a spreadsheet pro-

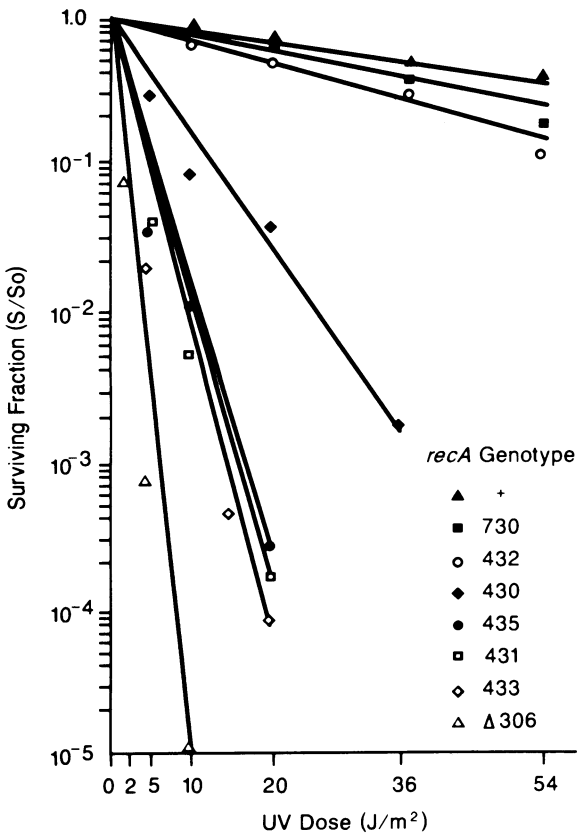

FIG. 1. Influence of *recA* on sensitivity to UV damage. UV survival was measured as described in the Materials and Methods section. Full genotypes are given in Table 1. ▲, DE190; ■, DE463; ○, DE858; ◆, DM2572; ●, DE862; □, DE548; ◇, DE866; △, DM2569.

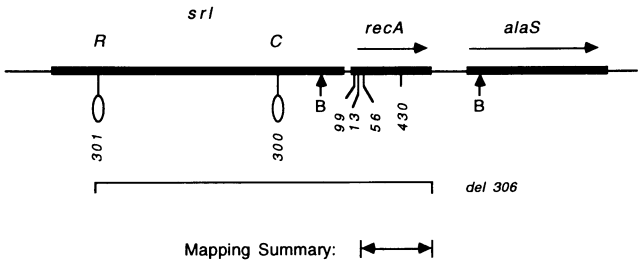

FIG. 2. Genetic map for the *srl-recA-alaS* interval of *E. coli*. The *srl*, *recA*, and *alaS* structural genes are indicated by the heavy lines. The horizontal arrows below *recA* and *alaS* indicate the direction of transcription of each gene. The elliptical symbols labeled *301* and *300* indicate the positions of the *srlR301*::Tn*10* and *srlC300*::Tn*10* insertion mutations, respectively. The allele numbers beneath the *recA* gene indicate the approximate position of each of the mutations; *recA99*, *recA13*, *recA56*, and *recA430* alleles encode amino acid substitutions at residues 7, 51, 60, and 204, respectively. The bracketed area designated *del 306* represents the 6.8-kb deleted interval of Δ(*srlR-recA*)*306*::Tn*10*; this deletion extends from the right border of the Tn*10* in *srlR* to sequences just distal to the *recA* gene. The *Bam*HI restriction sites in the *srlC* and *alaS* genes are indicated by B; these sites are separated by 3.3 kb. The positions of the different markers and the sizes of genes have been drawn approximately to scale (7, 9, 21, 25, 34, 48, 59). With a combination of four different strategies, the UV^s mutation of TK504 was mapped to an interval that would correspond to about 1 kb (see Materials and Methods and Results). This interval is indicated by double arrows, labeled Mapping summary. The boundaries of this interval are the *recA13* mutational change (codon 51) to the rightward border of the (*srlR-recA*)*306* deletion.

gram (Lotus 1-2-3, version 2.01). The values from the robot (at 0.5 μg of MC per ml [see Fig. 4B]) for the *recA*$^+$ and *recA432* strains were lower than we obtained previously with the same fusion strains at the same dose by conventional methods (16). These differences are likely the consequence of shorter exposure time to MC (2 rather than 4 h), although the different culture medium and differences in aeration may also have contributed to the lower values.

Genetic mapping. A combination of strategies was used to map the UV^s and Mut$^-$ mutation(s) of strain TK504. The first approach mapped the UV^s marker with respect to the (*srlR-recA*)*306* deletion (Fig. 2) essentially as conducted by Volkert and Hartke (56). Using this approach, we selected for the *srl*$^+$ transductants of the deletion strain DE1046 and then UV^s and UV^r phenotypes were screened. As expected, 100% cotransduction frequencies were observed for *srl*$^+$ and each of the *recA* markers examined (*recA*$^+$, *recA200*, *recA430*, *recA433*, and *recA435*, with donor strains AB1157, KL399, JC13062, TK508, and TK505, respectively). All of the 395 Srl$^+$ transductants from strain TK504 were found to confer UV^s phenotypes that were equivalent to that of TK504, indicating that the marker is either within the deleted interval or is just outside its boundaries. As described previously for analogous deletion mapping experiments (56), the maximum distance of the TK504 UV^s marker from the ends of the deletion was estimated with the formula of Wu (62); this distance was calculated to be <0.00169 min. With the average size of 47 kb/min from Kohara et al. (26), this genetic distance could be converted to an approximate value of <80 bp.

By the three-factor mapping strategy of Csonka and Clark (7), the UV^s marker form TK504 was mapped between the *srlC300*::Tn*10* and *alaS3*(Ts$^-$) markers of strain JC10244 (11). Control crosses with other *recA* mutant alleles (*recA13*, *recA435*, *recA430*, *recA433*, and *recA435*) were conducted and also mapped between the *srl* and *alaS* markers (data not shown). In addition, we found that the UV^s marker could be crossed onto a lambda phage carrying a 3.3-kb *Bam*HI fragment that includes the *recA* structural gene (11); recombinant phage were selected for by propagating λDE212 on TK504 and then were plated on host DE943. With these data taken together, the UV^s marker of TK504 was positioned within an interval of about 2 kb (e.g., between the leftward *Bam*HI site and the rightward border of *del306* [Fig. 2]).

To place the UV^s marker of TK504 with respect to defined *recA* point mutants (Fig. 2), transductional crosses between UV^s parents were conducted in which only the donor carried the *srlC*::Tn*10* selectable marker. Tet^r transductants were selected and screened for their UV^r and UV^s phenotypes by cross-streaking (described above). Detection of UV^r transductants from a cross was interpreted to indicate that a recombinational event had occurred between the two UV^s mutational changes, presumably regenerating a wild-type interval. This expectation assumes that recombinants with both mutational changes would be UV^s and that gene conversion events would be rare. Because both of the UV^s markers in these crosses are within a small interval, we would expect UV^r recombinants to be infrequent, even if the mutation in the donor were distal to the mutation in the recipient. The appearance of UV^r recombinants from transductional crosses conducted in the opposite orientation (e.g., the donor mutation was proximal) would be exceptionally infrequent, because it would require a recombinational event in each of the four marked intervals.

As a test of these expectations, intragenic control crosses were conducted between proximal mutations (*recA99*, *recA13*, and *recA56*, with mutational changes at codons 7, 51, and 60, respectively) and the more distal *recA430* mutant (change at codon 204 [Fig. 2]). In crosses with a *recA430* mutant as the transductional donor (DM2572), rare UV^r recombinants were obtained with the *recA99* (DM455) and *recA13* (AB2463) strains (6 of 318 and 2 of 270, respectively). Again, consistent with our expectations, UV^r transductants were not detected in a control cross with the opposite orientation (0 of 350) in which the proximal *recA13* was the donor and the distal *recA430* mutant was the recipient (DM1760 and JC13062, respectively). Despite repeated efforts, we found that it was difficult to obtain Tet^r transductants from crosses involving *recA56* and *recA430* in either orientation and thus were unable to map these mutants. Experiments with standard transductional crosses or an inversion phage assay (13, 40) in either *recA56*/F′ *recA430* or *recA430*/F′ *recA56* heterodiploid strains, indicated that *recA56* was dominant for homologous recombination (11, 12), thus explaining our difficulty in obtaining transductants.

To map the position of the UV^s marker of TK504 with respect to the *recA* mutants, equivalent crosses were also conducted with strain TK504 and *srl*::Tn*10* derivatives. Rare UV^r transductants were again obtained when *recA99* or *recA13* (4 of 300 and 3 of 320, respectively) mutants were used as the recipients. In a transductional cross in which *recA13* mutant was used as the donor and strain TK504 was the recipient, no UV^r recombinants were detected (0 of 380). These results suggest that, like *recA430*, the UV^s marker of TK504 is distal to the *recA99* and *recA13* mutations, which in turn places this marker in an interval of approximately 1 kb, and that the UV^s marker of TK504 probably resides within the *recA* structural gene (Fig. 2).

Analysis of in vivo LexA cleavage. The rate of in vivo LexA cleavage was determined as previously described (30), with procedure B, except that a mixture of [^{35}S]methionine and [^{35}S]cysteine was used to label irradiated cells. This should make no difference to the outcome of the experiment, because LexA contains no Cys residues. This method is essentially a pulse to exhaustion; almost all of the label to be incorporated into protein is incorporated within 1 min. Samples were taken at the indicated intervals, frozen, and lysed; LexA and cleavage products were recovered by immunoprecipitation and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.

RESULTS

Genetic characterization of the UV^s and Mut^- phenotypes in strain TK504. Kato and Shinoura (24) reported that the *recA432* strain TK504 displayed two mutant phenotypes: a deficiency in cellular mutagenesis (Mut^-) and hypersensitivity to UV damage (UV^s). In addition, they described (24) two other *recA* mutants (*recA433* and *recA435*) that also conferred comparable Mut^- and UV^s phenotypes. When *recA432* was transduced from TK504 into our bacterial strains, however, the *recA432* derivatives were nearly as resistant as the isogenic $recA^+$ control (Fig. 1) and were highly proficient in promoting SOS mutagenesis of phage and cellular markers (16) (see below). In contrast, when the *recA433* and *recA435* alleles were introduced into the same background, the resulting strains retained the Mut^- and UV^s phenotypes (see Fig. 1 in reference 16).

We tested whether our isolates of TK504 and related strains gave the expected behavior (Table 2). By comparison with the $recA^+$ strain (strain A), all three mutant strains (strains B to D) were highly sensitive to UV irradiation and were Mut^- by the same tests used by Kato and Shinoura (24). These strains were also Mut^- as judged by an assay measuring forward mutagenesis to Rif^r (11). Identical behavior was shown by an isogenic strain carrying *recA430*, a well-characterized Mut^- and UV^s mutant allele (strain E [3, 14, 41, 42]). We conclude that the differences between our strains and those from Kato (24) did not result from changes in the latter strains.

Because TK504 was isolated from an ethyl methanesulfonate (EMS) mutagenesis treatment, it was possible that it also contained an additional mutation that contributed to the Mut^- and UV^s phenotypes of this strain. To test whether the mutations responsible for these phenotypes were linked to *recA*, we transduced the *srlR-recA* interval from TK504 into an isogenic strain which carried the (*srlR-recA*)*306* deletion (DE1046), a strain which had not received this EMS mutagenic treatment. All of the 395 Srl^+ transductants screened were UV^s; of these, 48 of 48 tested were found to display apparent Mut^- phenotypes as judged by *his* reversion (see Materials and Methods). The results obtained from one of these transductants are shown in Table 2 (strain F). These deletion-mapping experiments indicated that this UV^s mutation lies within the boundaries of the deletion or at least very close to these boundaries (<80 bp [see Materials and Methods]). We also showed that the Mut^+ and UV^r phenotypes could be restored to the original strain by first deleting the *srlR-recA* interval from TK504 and then introducing a $recA^+$ copy that was carried on an F′ (strain G) or by transduction (11, 12). The mutation conferring UV^s was further mapped within a distal portion of the *recA* structural gene (Fig. 2; also Materials and Methods). Together, these results indicate that the mutation(s) from TK504 conferring the Mut^- and UV^s phenotypes lies within the *recA* structural gene; in addition, we could conclude that TK504 did not contain any other unlinked UV^s markers.

To test whether the mutations responsible for these UV^s and Mut^- phenotypes were present in one of our *recA432* mutant strains (DE858) which did not show these phenotypes (16) (Fig. 1), we backcrossed the *srl-recA* interval into the same *recA* deletion strain that was used for the deletion-mapping experiments described above (DE1046). All of the transductants examined from this cross (42 of 42) conferred the same Mut^- and UV^s phenotypes, and experimental results obtained from one representative transductant are shown in Table 2 (strain H).

From these mapping studies, we could conclude the following. First, the strains obtained from T. Kato had retained their expected phenotypes. Second, the mutation(s) responsible for the Mut^- and UV^s phenotypes of strain TK504 mapped to a distal position of the *recA* gene. Finally, the expression of Mut^- and UV^s phenotypes was strain dependent. The observation that these mutant phenotypes were not expressed in some of our *recA432* strains suggested that these strains carried a suppressor for these phenotypes.

***sulA* mutations suppress the UV hypersensitivity and cellular mutagenesis defect for *recA432* mutants.** Although the strains we have used for phage mutagenesis studies (e.g., DE858 and DE860 [16]) and those obtained from T. Kato had similar origins, several genetic differences were known to exist (Table 1). In addition to differences in some of the auxotrophic markers, genetic differences at three different SOS genes were present (*uvrB*, *lexA*, and *sulA*). The Kato strains carried a deletion of the *gal-uvrB* region [Δ(*gal-uvrB*)*301*], while our strains were wild type for this interval. In addition, our strains carried null mutations in the *lexA* and *sulA* genes (*lexA51* and *sulA211*), whereas the Kato strains were $lexA^+$ and $sulA^+$.

Differences at these loci were examined to determine

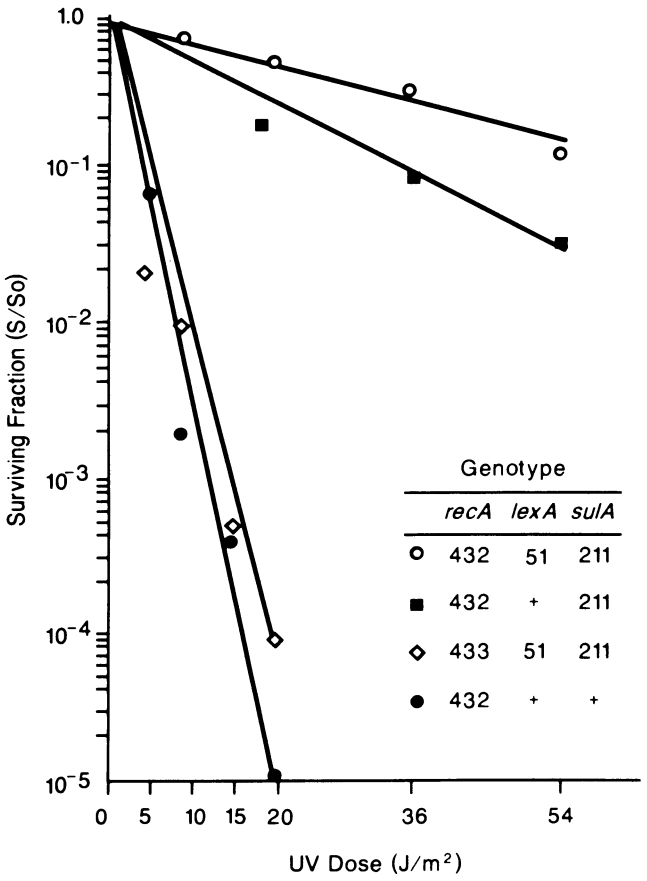

FIG. 3. *sulA* mutations suppress the UV^s phenotype of *recA432* mutant strains. UV survival was measured as indicated in Materials and Methods. Full strain genotypes are given in Table 1, and relevant SOS genotypes are given in the lower right corner of the figure. ○, DE858; ■, DE1185; ◇, DE866; ●, DE1294.

whether they might be responsible for the apparent suppression in our strains. Replacement of the *gal-uvrB* deletion of strain TK504 by genetic material that included either the *uvrB5* frameshift mutation (2) or replacement by material with a *uvrB*$^+$ copy did not reverse the UV^s phenotype of this *recA432* mutant compared with those of the isogenic *recA*$^+$ control strains (11 [see below]) and indicated that the *(gal-uvrB)301* deletion was not responsible for the contrasting phenotypes.

To determine whether the suppression was due to mutations in *lexA* or *sulA*, we restored *lexA*$^+$ and then *sulA*$^+$ copies to our strain DE858 (*recA432 lexA51 sulA211*). [Note that a *lexA* (Def) *sulA*$^+$ strain is inviable because of lethal filamentation.] A *lexA*$^+$ derivative was found to be only slightly more sensitive to UV damage than strain DE858 (Fig. 3), suggesting that the *lexA51*(Def$^-$) mutation was not a major contributor to the suppression. Restoration of both *lexA*$^+$ and *sulA*$^+$ copies, however, dramatically decreased the resistance of strain DE858 to UV damage (at 20 J/m^2, about a 50,000-fold decrease in survival [Fig. 3]), indicating that the *sulA211* allele was principally responsible for the suppression of the UV^s phenotype. In fact, the *lexA*$^+$ *sulA*$^+$ derivative of DE858 became as sensitive as the hypersensitive *recA433* mutant strain (Fig. 3). We therefore can conclude that the *sulA211* marker suppressed the UV^s phenotype in strain DE858.

Two lines of evidence confirmed this conclusion and showed that *sulA* mutations also suppress the Mut$^-$ phenotype. First, other null alleles of *sulA* [e.g., *sulA100*::Tn*5* and *sulA1*::Mu *d*(Ap *lac*)1] were also able to suppress the UV^s phenotype of *recA432* mutants, indicating that the suppression resulted from a loss of *sulA* function (11 [see below]). In addition, spontaneous UV^r mutants of the *recA432 sulA*$^+$ strains were identified, approximately half of which (6 of 11) were found to map to *sulA* (11). High levels of Rif^r cellular mutagenesis were measured in strain DE858 (1.4 Rif^r mutants per 10^5 survivors [see Materials and Methods]) following exposure to 10 J of UV irradiation per m^2. The irradiated *lexA*$^+$ derivative was also proficient at Rif^r mutagenesis (3.8×10^{-6}). However, UV-induced Rif^r mutants were not detected in the *lexA*$^+$ *sulA*$^+$ derivative ($<3 \times 10^{-7}$), showing that introduction of a *sulA*$^+$ copy into this strain also conferred a loss of induced cellular mutagenesis (see below).

Second, we introduced a *sulA100*::Tn*5* mutation into the original *recA432* strain, TK504, as well as into the *recA*$^+$ and *recA433* companion strains (JC3890 and TK508, respectively). The presence of this *sulA*::Tn*5* mutation in strain TK504 resulted in a substantial increase in survival (at 1 J/m^2, about a 2,000-fold increase) and restored UV-induced *his* reversion mutagenesis (compare strains C and D in Table 3) and Rif^r forward mutagenesis (11). The *sulA* insertion mutation had no significant effect on UV-induced *his* mutagenesis or on survival for the *recA*$^+$ and *recA433* control strains (Table 3, strains B and F).

TABLE 3. A mutation in *sulA* suppresses the UV^s and Mut$^-$ phenotypes for strain TK504[a]

Strain	Genotype		UV dose required for 10% survival (J/m^2)	His$^+$ frequency/10^6 surviving cells at 1.0 J of UV irradiation/m^2	Presence of filamentous cells:	
	recA	*sulA*			Without UV	With UV[b]
A (JC3890)[c]	Wild type	Wild type	3.8	35	–	+/–
B (DE1052)	Wild type	*100*	4.1	~50[d]	–	–
C (TK504)[c]	*432*	Wild type	0.25	3.5	+/–	+++
D (DE991)	*432*	*100*	1.9	19	–	–
E (TK508)[c]	*433*	Wild type	0.25	2.9	–	+
F (DE998)	*433*	*100*	0.30	~2[d]	–	–

[a] Experimental conditions for induced cellular mutagenesis and cell survival determinations were as described in Materials and Methods. UV-irradiated and unirradiated cultures were examined by light microscopy for the presence of filamentous cells; irradiated cells were microscopically examined after 30 min and 1, 2, and 4 h of incubation in Luria-Bertani broth at 37°C. Full genotypes of all strains are listed in Table 1. Except where noted otherwise, all His$^+$ reversion values were the averages of three separate experiments.

[b] Cells were irradiated at 1.0 J/m^2.

[c] The survival and His$^+$ frequencies for these strains were also cited in Table 2.

[d] These His reversion values are the results of a single experiment.

Like other LexA-regulated genes, *sulA* is derepressed by SOS induction (22). Elevated SulA levels arrest cell division by blocking septum formation; under normal inducing conditions, this is thought to allow time for repair of damage prior to the resumption of cell division (18, 22). Overexpression of SulA is lethal, blocking septation and resulting in the formation of long filamentous cells. Filamentation was observed in the irradiated cultures of strain TK504 (Table 3, strain C) but not in the *sulA* mutant derivative (strain D) or any of the other related strains examined (strains A, B, E, and F); although some filamentation was also detected for the *recA433 sulA*$^+$ mutant, the *sulA* genotype does not appear to affect sensitivity to DNA damage or to alter the Mut$^-$ phenotype (strains E and F). These observations strongly suggested that SOS induction results in an excess of SulA, which in turn leads to lethal filamentation of the *recA432 sulA*$^+$ strains.

***sulA* expression and LexA proteolysis in *recA432* mutants.** An excess of SulA protein in *recA432* mutant cells might be achieved if abnormally high levels of LexA repressor cleavage occurred with induction. Some *recA* mutants were previously shown to be hyperactive for LexA cleavage, resulting in abnormally high expression of the *sulA* gene (11, 16, 18, 22). However, excess SulA in *recA432* mutants does not appear to be achieved by this mechanism; indeed, the induced *sulA* expression was slightly lower in the *recA432* mutant than in the induced *recA*$^+$ control (16). Alternatively, an excess of SulA could be achieved if the induced state of the mutant were abnormally long, allowing greater time for the *sulA* gene products to accumulate.

A prolonged induced state might occur if the mutant RecA432 protein were hyperactivated (or a "hair-trigger"), that is, activated by abnormally low concentrations of the inducing signals. We had two expectations for this postulated hair-trigger mutant. First, the mutant would exhibit an abnormally rapid transition to an activated state, and second, the mutant might also be more readily activated by weak SOS-inducing treatments. To test the first expectation, we examined the early kinetics of *sulA* derepression following exposure to 0.5 μg of MC per ml, and to test the second expectation, we measured the effects of very low to moderate MC concentrations (0.005 to 0.5 μg/ml). Both the kinetic and dose-response experiments were conducted simultaneously by using the semiautomatic robot developed by Menzel (38) (see Materials and Methods). The time intervals for MC exposure in the kinetic experiments (0 to 2 h) and the dose-response experiments (2 h) were chosen because previous studies had indicated that these conditions could detect subtle differences in *sulA-lacZ* expression levels (11, 44) (see Materials and Methods). As indicated in Fig. 4A, the induction kinetics for a *sulA-lacZ* fusion were actually slower in the *recA432* mutant than in the *recA*$^+$ parental control. We also discovered that the *recA432* fusion strain did not respond to lower MC doses than the *recA*$^+$ control (Fig. 4B). These results indicated that the *recA432* mutants did not exhibit the proposed hair-trigger response to damage.

Alternatively, a prolonged induced state could be achieved if the activated *recA432* cells were slow to make the transition back to a repressed state. This hypothesis would predict that the time which RecA432 remained activated for LexA cleavage would be abnormally long. By using the pulse-chase methods of Little (30), the stability of LexA repressor in *recA*$^+$ and *recA432* strains was compared at different time intervals following UV irradiation. At 30 min after irradiation, the LexA half-lives for both strains were found to be essentially the same (about 30 s [Fig. 5]). As the *recA*$^+$ cells began to recover from damage, the LexA half-life increased from 30 s to about 750 s

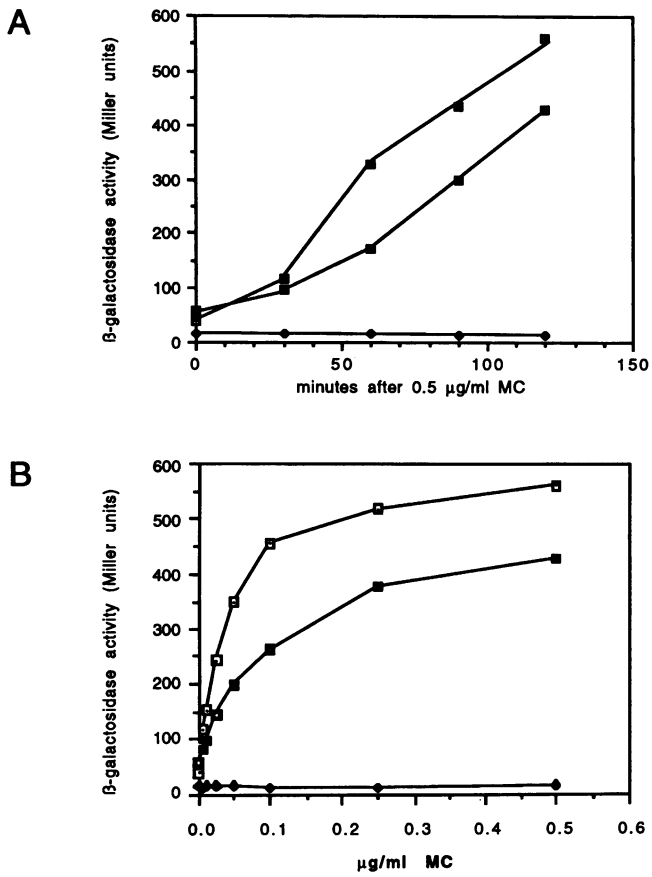

FIG. 4. Influence of *recA* on the MC-induced expression of a *sulA-lacZ* fusion. (A) Kinetics of *sulA* expression. β-Galactosidase levels were measured following different times of exposure to 0.5 μg of MC per ml. (B) MC dose-response curve of *sulA* expression. The fusion strains were exposed to 0, 0.005, 0.01, 0.025, 0.05, 0.10, 0.25, or 0.50 μg of MC per ml for 2 h. β-Galactosidase levels are expressed in Miller units (40) and were measured with the semiautomatic devices of Menzel (38 [see Materials and Methods]). The *recA*$^+$ strain was DE880 (□), the *recA432* strain was DE894 (■), and the *recA56* strain was DE1133 (◆). Full genotypes are given in Table 1.

at the 2-h time point, as seen previously (30). In contrast, the LexA half-lives for the *recA432* mutant cells remained constant at all time points (about 30 s); thus, at 2 h after irradiation, the LexA half-life for the *recA*$^+$ strain was 25-fold greater than that for the *recA432* strain. These results therefore demonstrate that high levels of LexA repressor cleavage continue in the *recA432* mutant long after a substantial decline was observed in the *recA*$^+$ control strain.

We conclude that *recA432* mutants remain in the induced state for an abnormally long period of time, which presumably results in an excess of SulA and lethal filamentation. A mutant that can be activated for LexA cleavage by damage but remains locked in this state has not been described previously.

DISCUSSION

Several lines of evidence indicate that the apparent Mut$^-$ and UV^s phenotypes conferred upon wild-type strains by the *recA432* mutant allele are an indirect result of altered RecA regulatory functions. First, these phenotypes were suppressed by mutations in the *sulA* gene. This finding, together with the

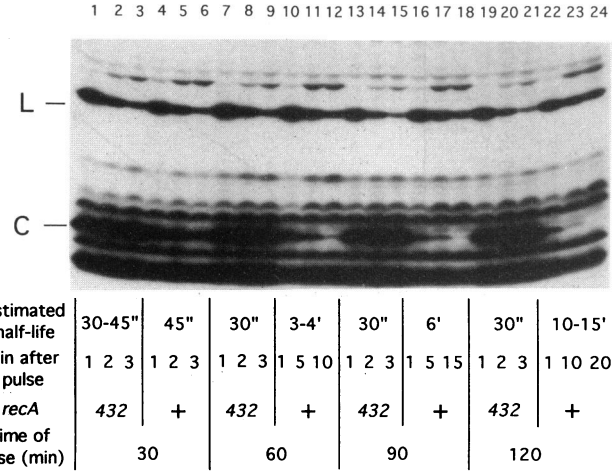

FIG. 5. LexA proteolysis is prolonged in *recA432* mutants. The rate of LexA cleavage in irradiated *recA*$^+$ and *recA432* cells is shown. Strains DE1185 (*recA432*) and DE1500 (*recA*$^+$) were grown at 37°C to a density of about 2×10^8 cells per ml, and irradiated with UV light (10 J/m^2); aliquots were shaken in red flasks at 37°C. At the indicated times after irradiation, aliquots were pulse-labeled, and samples were taken at the indicated times after labeling began. Estimated half-lives were derived by visual inspection as the time required to reduce the intensity of intact LexA by half. Only the bottom portion of the autoradiogram is shown. L, intact LexA; C, C-terminal fragment; +, wild-type.

observation that a *recA432 sulA*$^+$ strain forms long filaments after SOS-inducing treatments, implies that the UV^S is likely to result from filamentous death, a condition that results from high levels of SulA protein. Second, unlike the mutagenesis of cellular markers, the mutagenesis of sequences in phage λ was not impaired in a *recA432 sulA*$^+$ strain, implying that the mutant strain had the capacity to promote mutagenesis (11, 16), but mutant cells were not recovered because they also filament and die. In support of this view was the observation that *sulA*(Def) mutants not only suppressed the filamentation but also suppressed the apparent Mut$^-$ phenotype of the *recA432* mutant strains (Table 3). Finally, direct measurement of LexA stability revealed that activation of RecA432 protein persists for at least 2 h after UV treatment, in contrast with the rapid return of a *recA*$^+$ strain to the SOS-repressed state (Fig. 5). A possible explanation for the persistent induction would be the failure of the mutant to efficiently repair damage which would leave inducing lesions in the DNA, as was observed for excision-defective mutants (22, 47, 49); however, the lack of sensitivity in the *sulA*(Def) derivatives argues against this. We presume that this prolonged LexA cleavage by RecA432 leads to persistent high levels of SulA protein. We conclude that the primary defect in the RecA432 mutant protein has to do with an abnormal response to inducing signals and that the original Mut$^-$ phenotype by which it was identified is an indirect and distant consequence of the aberrant operation of the SOS regulatory network and the proteins it controls.

Several other findings also indicate that RecA432 differs from wild-type RecA in its interaction with effector molecules. Primary among these is that *recA432 lexA*(Def) strains support cleavage of two proteins, UmuD and λ CI repressor, without inducing treatments (11, 12, 15, 16). In this respect, it resembles other constitutively activated alleles (*recA**), such as *recA730* and *recA1202*, that are activated for cleavage of these target proteins even without the normal requirements for inducing treatments. These RecA* mutant proteins are thought to be constitutively activated because they bind to normal cellular components (endogenous signals), presumably nucleic acids (28, 32, 37, 49). Consistent with these interpretations, biochemical data show that RecA1202 protein can be activated in vitro by binding to tRNA or rRNA (58), which do not induce cleavage with RecA$^+$ protein in vitro (32, 37). However, *recA432* strains differ from these alleles in that they do not display constitutive LexA cleavage (as required from the manner in which they were isolated), and hence *recA432 lexA*$^+$ strains contain low levels of RecA432 protein and normal levels of LexA. The lack of constitutive LexA cleavage in a mutant that expressed UmuD and CI cleavage constitutively was especially surprising because LexA proteolysis is more efficient for RecA$^+$ protein than the cleavage of UmuD or CI (4, 20, 32).

How can we rationalize our findings for *recA432* mutants at a mechanistic level? We have considered the possibility that the RecA432 mutant protein may be differentially activated (16). We proposed that this protein is activated to cleave some target proteins (e.g., UmuD and CI) by endogenous signals but required signals produced by damage for activation of the LexA cleavage functions. One difficulty with this model, however, is that it predicts the existence of different forms of activated RecA that interact with different substrates; there is little biochemical evidence to support this prediction (27). In addition, it is difficult to make a direct comparison of the requirements for cleavage of the various substrates from the previous data; whereas the evidence for constitutive CI and UmuD cleavage was obtained in a *lexA*(Def) strain, which contains high levels of RecA432 protein, that for LexA cleavage was obtained in a *lexA*$^+$ strain, which initially contains low levels of the mutant RecA because of repression by LexA.

Our present results lead us to suggest a different model to explain the behavior of RecA432 protein. We propose that RecA432 protein can be activated constitutively by effectors normally present in the cell, but only when the protein is present at high levels as in a *lexA*(Def) mutant (15, 16). Accordingly, when the mutant protein is present at low levels in an uninduced *recA432 lexA*$^+$ strain, it was not activated; it can still be activated by the same kind of novel effectors produced following damage that activates wild-type RecA (Fig. 4). However, it differs from wild-type RecA in that, once the SOS system has been turned on, RecA432 builds up to high levels and no longer needs the damage-induced signal molecules to perpetuate the activated state; its activation by endogenous molecules suffices to lead to chronic activation and therefore to a persistence of the induced state of the SOS system.

This hypothesis predicts that constitutive cleavage of certain target proteins (e.g., UmuD) would no longer occur when RecA432 is present at low repressed levels. To test these expectations, cleavage in *recA432* strains that also carried a noncleavable repressor [LexA3(Ind$^-$)] was studied. Because of the repressing action of LexA3, RecA protein remains fixed at a low basal level even following induction. Very little cleavage of plasmid-encoded UmuD was observed in these *recA432 lexA3*(Ind$^-$) cells without induction, substantially less than was observed in the *recA730 lexA3*(Ind$^-$) control strains (15, 61). Impaired proteolysis of a cleavable LexA substrate was also observed in *recA432* strains which carried a dominant LexA3(Ind) repressor (31). These results are therefore consistent with the expectations of the model described above that elevated levels of RecA432 protein are required for forming efficient constitutively activated complexes. Under the appropriate in vitro conditions, RecA cooperatively binds to single-

stranded DNA to form filamented complexes; these complexes have been implicated as the form which is activated for cleavage (27, 52). Like the RecA1730 mutant protein (8), perhaps RecA432 is also impaired in the assembly of nucleoprotein complexes, requiring elevated RecA432 levels to form activated complexes.

These properties of *recA432* mutants resemble those described for *recA718* (36), which also expressed some SOS functions constitutively when the protein was expressed at high levels (e.g., mutagenesis). However, phenotypic differences have been noted for these mutants; for example, the level of constitutive mutagenesis and CI cleavage was less for the *recA718 lexA*(Def) strains than for *recA432 lexA*(Def) mutants (11, 12, 15, 16, 53). In addition *recA718 sulA*$^+$ strains do not exhibit an extreme UV^s phenotype nor do they confer a Mut$^-$ phenotype (11, 15), implying that once induced, the activated state is not perpetuated to the same extent as observed in *recA432* mutants.

Persistent activation in *recA432* mutants is formally similar to two other examples of bistable switches in prokaryotic regulatory circuits. In all three cases, cells of the same genotype display different phenotypes, depending on their history. First, in the *lac* operon, a low level of inducer suffices to keep the operon induced if it has been previously turned on by a high level of inducer, because the Lac permease is expressed and imports the inducer efficiently, perpetuating the induced state; by contrast, cells that had not been induced do not contain a sufficiently high level of inducer to turn on the operon, and it remains in the repressed state (43). Second, in phage λ, the lysis-lysogeny decision can be perpetuated in a host carrying a *c*I857(Ts) prophage that is also an *N O* (or *N P*) mutant. At low temperatures, the lysogen is immune, because CI represses Cro; at high temperatures, it is an anti-immune state, because Cro represses CI (10, 39). In the present case, the LexA and RecA regulatory proteins contend for supremacy; LexA is a negative regulator and RecA is a positive regulator, and the mutant form of RecA allows an alternative state of the system to become established and maintained.

ACKNOWLEDGMENTS

This research was supported in part by Public Health Service grants GM24496 to D.W.M. and GM24178 to J.W.L. and support to D.G.E. from the Scolnik-Quisenberry fund of the Plant Molecular Biology Group (Du Pont Experimental Station, Wilmington, Del.).

We are grateful to T. Kato, H. Shinagawa, F. Stahl, A. J. Clark, K. B. Low, G. Smith, N. Murray, and B. Bachmann for providing strains. We are also grateful to R. Menzel (formerly at the Du Pont Experimental Station) for making available the robot for measuring β-galactosidase activities and A. Segall and R. Menzel for technical assistance in its operation. We thank T. Kato, H. Shinagawa, E. Witkin, H. Echols, M. Brito, F. Chumley, A. Ganesan, and K. Peterson for helpful comments and for offering a diverse collection of hypotheses to account for the unusual properties of *recA432* mutants and R. Woodgate, P. Nixon, and R. Crouch for critical reading of the manuscript. We thank D. Bryan, D. Poulson, M. Feldmann, B. Valent, and F. Chumley for encouragement and advice.

REFERENCES

1. **Arber, W., L. Enquist, B. Hohn, N. Murray, and K. Murray.** 1983. Experimental methods for use with lambda, p. 433–466. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. **Bakendorf, C., H. Spanik, A. P. Barbeiro, and P. Van de Putte.** 1986. Structure of the *uvrB* gene of *Escherichia coli*. Homology with other DNA repair enzymes and characterization of the *uvrB5* mutation. Nucleic Acids Res. **14:**2877–2890.
3. **Blanco, M., G. Herrera, P. Collado, J. E. Rebollo, and J. M. Botella.** 1982. Influence of RecA on induced mutagenesis. Biochimie **64:**633–636.
4. **Burckhardt, S. E., R. Woodgate, R. H. Scheuermann, and H. Echols.** 1988. UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification and cleavage by RecA. Proc. Natl. Acad. Sci. USA **85:**1811–1815.
5. **Caillet-Fauquet, P., and G. Maenhaut-Michel.** 1988. Nature of SOS mutator activity: genetic characterization of untargeted mutagenesis in *Escherichia coli*. Mol. Gen. Genet. **213:**491–498.
6. **Clark, A. J.** 1982. *recA* operator mutations and their usefulness. Biochimie **64:**669–675.
7. **Csonka, L. N., and A. J. Clark.** 1979. Deletions generated by the transposon Tn*10* in the *srl recA* region of the *Escherichia coli* chromosome. Genetics **93:**321–343.
8. **Dutreix, M., B. Burnett, A. Bailone, C. M. Radding, and R. Devoret.** 1992. A partially deficient mutant, *recA1730*, that fails to form normal nucleoprotein filaments. Mol. Gen. Genet. **232:**489–497.
9. **Dutreix, M., P. L. Moreau, A. Bailone, F. Galibert, J. R. Battista, G. C. Walker, and R. Devoret.** 1989. New *recA* mutations that dissociate the various RecA protein activities in *Escherichia coli* provide evidence for an additional role for RecA protein in UV mutagenesis. J. Bacteriol. **171:**2415–2423.
10. **Eisen, H., P. Brachet, L. Pereira da Silva, and F. Jacob.** 1970. Regulation of repressor expression in λ. Proc. Natl. Acad. Sci. USA **66:**855–862.
11. **Ennis, D. G.** Unpublished data.
12. **Ennis, D. G.** 1988. Ph.D. thesis. University of Arizona, Tucson.
13. **Ennis, D. G., S. K. Amundsen, and G. R. Smith.** 1987. Genetic functions promoting homologous recombination in *Escherichia coli*: a study of inversions in phage λ. Genetics **115:**11–24.
14. **Ennis, D. G., B. Fisher, S. Edmiston, and D. W. Mount.** 1985. Dual role for *Escherichia coli* RecA protein in SOS mutagenesis. Proc. Natl. Acad. Sci. USA **82:**3325–3329.
15. **Ennis, D. G., A. S. Levine, and R. Woodgate.** Submitted for publication.
16. **Ennis, D. G., N. Ossanna, and D. W. Mount.** 1989. Genetic separation of *Escherichia coli recA* functions for SOS mutagenesis and repressor cleavage. J. Bacteriol. **171:**2533–2541.
17. **Ennis, D. G., K. Peterson, and D. W. Mount.** 1988. Increased expression of the *E. coli umuDC* operon restores SOS mutagenesis in *lexA41* cells. Mol. Gen. Genet. **213:**541–544.
18. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit mutator properties of *tif*. Mol. Gen. Genet. **140:**390–432.
19. **Glickman, B. W., N. Guijf, and P. Morand.** 1977. The genetic characterization of *lexB32*, *lexB33* and *lexB35* mutations of *Escherichia coli*: location and complementation pattern for UV resistance. Mol. Gen. Genet. **157:**83–89.
20. **Hauser, J., A. S. Levine, D. G. Ennis, K. M. Chumakov, and R. Woodgate.** 1992. The enhanced mutagenic potential of MucAB proteins correlates with the highly efficient processing of the MucA protein. J. Bacteriol. **174:**6844–6851.
21. **Horii, T., T. Ogawa, and H. Ogawa.** 1980. Organization of the *recA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77:**313–317.
22. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290:**797–799.
23. **Kato, T., R. H. Rothman, and A. J. Clark.** 1977. Analysis of the recombination and repair in mutagenesis of *Escherichia coli* by UV irradiation. Genetics **156:**1–18.
24. **Kato, T., and Y. Shinoura.** 1977. Isolation and characterization of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156:**121–131.
25. **Kawashima, H., T. Horii, T. Ogawa, and H. Ogawa.** 1984. Functional domains of *Escherichia coli* RecA protein deduced from the mutational sites in the gene. Mol. Gen. Genet. **193:**288–292.
26. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for

rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
27. **Kowalczykowski, S. C.** 1991. Biochemical and biological function of *Escherichia coli* RecA protein: behavior of mutant RecA proteins. Biochimie **73:**289–304.
28. **Lavery, P. E., and S. C. Kowalczykowski.** 1992. Biochemical basis for the constitutive cleavage activity of recA730 protein. J. Biol. Chem. **267:**20648–20658.
29. **Lieberman, H. B., and E. M. Witkin.** 1983. DNA degradation, UV sensitivity and SOS-mediated mutagenesis in strains of *Escherichia coli* deficient in single-stranded DNA binding protein: effects of mutations and treatments that alter levels of exonuclease V or RecA protein. Mol. Gen. Genet. **190:**92–100.
30. **Little, J. W.** 1983. The SOS regulatory system: control of its state by level of RecA protease. J. Mol. Biol. **167:**791–808.
31. **Little, J. W.** Unpublished data.
32. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29:**11–22.
33. **Lovett, S. T., and A. J. Clark.** 1983. Genetic analysis of regulation of the RecF pathway of recombination in *Escherichia coli* K-12. J. Bacteriol. **153:**1471–1478.
34. **Madiraju, M. V., A. Templin, and A. J. Clark.** 1988. Properties of a mutant *recA*-encoded protein reveal a possible role for *Escherichia coli recF*-encoded protein in genetic recombination. Proc. Natl. Acad. Sci. USA **85:**6592–6596.
35. **Maenhaut, G., and P. Caillet-Fauquet.** 1984. Effect of *umuC* mutations on targeted and untargeted ultraviolet mutagenesis. J. Mol. Biol. **177:**181–187.
36. **McCall, J. O., E. M. Witkin, T. Kogoma, and V. Roegner-Maniscalco.** 1987. Constitutive expression of the SOS response in *recA718* mutants of *Escherichia coli* requires amplification of RecA718 protein. J. Bacteriol. **169:**728–734.
37. **McEntee, K., and G. M. Weinstock.** 1981. *tif-1* mutation alters polynucleotide recognition by the *recA* protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78:**6061–6065.
38. **Menzel, R.** 1989. A microtiter plate-based system for the semiautomated growth and assay of bacterial cells for β-galactosidase activity. Anal. Biochem. **181:**40–50.
39. **Mieschendahl, M., H.-W. Grießer and B. Muller-Hill.** 1981. λ immunity phase shift in λN^{-}-*lacZ*$^{+}$ fusion. Mol. Gen. Genet. **181:**202–204.
40. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Morand, P., M. Blanco, and R. Devoret.** 1977. Characterization of *lexB* mutations in *Escherichia coli* K-12. J. Bacteriol. **131:**572–582.
42. **Nohmi, T., J. R. Battista, L. A. Dodson, and G. C. Walker.** 1988. RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationships between transcriptional derepression and posttranslational activation. Proc. Natl. Acad. Sci. USA **85:**1816–1820.
43. **Novick, A., and M. Weiner.** 1957. Enzyme induction as an all-or-none phenomenon. Proc. Natl. Acad. Sci. USA **43:**553–570.
44. **Peterson, K., and D. W. Mount.** 1987. Differential repression of SOS genes by unstable *lexA41* (*tsl-1*) protein causes a "split-phenotype" in *Escherichia coli* K-12. J. Mol. Biol. **193:**2533–2541.
45. **Peterson, K. R., N. Ossanna, A. T. Thliveris, D. G. Ennis, and D. W. Mount.** 1988. Derepression of specific genes promotes DNA repair and mutagenesis in *Escherichia coli*. J. Bacteriol. **170:**1–4.
46. **Quillardet, P., and R. Devoret.** 1982. Damaged-site independent mutagenesis of phage λ produced by induced error-prone repair. Biochimie **64:**789–796.
47. **Sancar, A., and G. B. Sancar.** 1988. DNA repair enzymes. Annu. Rev. Biochem. **57:**29–67.
48. **Sancar, A., C. Stachelek, W. Konigsberg, and W. D. Rupp.** 1980. Sequences of the *recA* gene and protein. Proc. Natl. Acad. Sci. USA **77:**2611–2615.
49. **Sassanfar, M., and J. W. Roberts.** 1990. Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. J. Mol. Biol. **212:**79–96.
50. **Sedgwick, S. G., and P. A. Goodwin.** 1985. Differences in mutagenic and recombinational DNA repair in enterobacteria. Proc. Natl. Acad. Sci. USA **82:**4172–4176.
51. **Shinagawa, H., H. Iwasaki, T. Kato, and A. Nakata.** 1988. RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis. Proc. Natl. Acad. Sci. USA **85:**1806–1810.
52. **Story, R. M., I. T. Weber, and T. A. Steitz.** 1992. The structure of the *E. coli recA* protein monomer and polymer. Nature (London) **355:**318–325.
53. **Sweasy, J. B., E. M. Witkin, N. Sinha, and V. Roegner-Maniscalco.** 1998. RecA protein of *Escherichia coli* has a third essential role in SOS mutator activity. J. Bacteriol. **172:**3030–3036.
54. **Tessman, T. S., and P. Peterson.** 1985. Plaque color method isolation of novel *recA* mutants of *Escherichia coli* K-12: new classes of protease-constitutive *recA* mutants. J. Bacteriol. **163:**677–687.
55. **Thliveris, A. T., D. G. Ennis, L. K. Lewis, and D. W. Mount.** 1990. SOS functions, p. 381–387. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
56. **Volkert, M. R., and M. A. Hartke.** 1984. Suppression of *Escherichia coli recF* mutations by *recA*-linked *srfA* mutations. J. Bacteriol. **157:**498–506.
57. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:**60–93.
58. **Wang, W. B., E. S. Tessman, and I. Tessman.** 1988. Activation of protease-constitutive RecA proteins of *Escherichia coli* by rRNA and tRNA. J. Bacteriol. **170:**4823–4827.
59. **Willis, D. K., B. E. Uhlin, K. S. Amini, and A. J. Clark.** 1981. Physical mapping of the *recA srl* region of *Escherichia coli*: analysis of Tn*10* generated insertions and deletions. Mol. Gen. Genet. **183:**497–504.
60. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40:**869–907.
61. **Woodgate, R., and D. G. Ennis.** 1991. Levels of chromosomally encoded Umu proteins and requirements for *in vivo* UmuD cleavage. Mol. Gen. Genet. **229:**10–16.
62. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54:**405–410.