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Constitutive Expression of the SOS Response in *recA718* Mutants of *Escherichia coli* Requires Amplification of RecA718 Protein

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In *recA718 lexA*$^+$ strains of *Escherichia coli*, induction of the SOS response requires DNA damage. This implies that RecA718 protein, like RecA$^+$ protein, must be converted, by a process initiated by the damage, to an activated form (RecA*) to promote cleavage of LexA, the cellular repressor of SOS genes. However, when LexA repressor activity was abolished by a *lexA*-defective mutation [*lexA*(Def)], strains carrying the *recA718* gene (but not recA$^+$) showed strong SOS mutator activity and were able to undergo stable DNA replication in the absence of DNA damage (two SOS functions known to require RecA* activity even when cleavage of LexA is not necessary). λ lysogens of *recA718 lexA*(Def) strains exhibited mass induction of prophage, indicative of constitutive ability to cleave λ repressor. When the cloned *recA718* allele was present in a *lexA*$^+$ strain on a plasmid, SOS mutator activity and β-galactosidase synthesis under LexA control were expressed in proportion to the plasmid copy number. We conclude that RecA718 is capable of becoming activated without DNA damage for cleavage of LexA and λ repressor, but only if it is amplified above its base-line level in *lexA*$^+$ strains. At amplified levels, RecA718 was also constitutively activated for its roles in SOS mutagenesis and stable DNA replication. The nucleotide sequence of *recA718* reveals two base substitutions relative to the *recA*$^+$ sequence. We propose that the first allows the protein to become activated constitutively, whereas the second partially suppresses this capability.

The RecA protein of *Escherichia coli* is activated by DNA damage or by certain *recA* mutations to an activated form (RecA*) that derepresses the SOS response by effecting proteolytic cleavage of LexA protein, the cellular repressor of DNA-damage-inducible genes (for reviews, see references 14, 27, and 30). In addition to its antirepressor function, RecA* participates in essential but unidentified ways in the expression of at least three individual components of the SOS response: SOS mutagenesis (1, 6, 31), stable DNA replication (31), and association of RecA with the membrane fraction of cell extracts (7). Evidence from in vitro studies suggests that RecA is activated to perform these functions by its interaction with single-stranded DNA and an adenine nucleotide to form a ternary complex (3). RecA$^+$ protein requires DNA damage to become activated, presumably because the single-stranded DNA (and possibly also the appropriate adenine nucleotide) required to activate wild-type RecA is not available at sufficient levels in undamaged cells.

Mutations in the *recA* gene have been described which permit the mutant RecA protein to become SOS activated without the need for DNA damage and consequently cause either heat-inducible (*recA441*) or constitutive (*recA730* and *recA1211*) expression of the SOS response (2, 28, 32). For RecA441, it has been shown that the affinity of the protein in vitro for single-stranded oligonucleotides and for certain adenine nucleotides is greatly increased compared with that of RecA$^+$, which suggests that the levels of cofactors that are present in the undamaged cell may be sufficient for activation of RecA441 (16, 22).

In a previous report, the isolation and some of the properties of two new *recA* mutants, strains SC30 (*recA730*) and SC18 (*recA718*), were described (32). Both mutants originated as stable components of a single mixed colony which also contained cells carrying the unaltered parental *recA441* allele. Because all three stable genotypes were present as substantial fractions of the mixed colony, we assume that *recA730* and *recA718* originated, either independently or sequentially, during the early divisions of the *recA441* cell from which the colony developed. The *recA730* allele causes constitutive, rather than heat-inducible, expression of the SOS response, whereas the *recA718* mutant strain SC18, like the wild type, requires DNA-damaging treatment to induce SOS expression (32).

In this report, we present evidence that the RecA718 protein actually retains the capacity to become constitutively activated for SOS expression, a capacity that is potentiated by amplification of the protein above its base-line level. We also describe the nucleotide sequence of *recA718* and discuss its possible relation to the properties of the mutant protein and to the origin of the mutant allele.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains and plasmids used are listed in Table 1.

Culture media. Liquid media were nutrient broth (NB) (Difco Laboratories) with 0.5% NaCl, LB broth (19), and LB broth supplemented with 5 mM $CaCl_2$ and 10 mM of $MgSO_4$ (LC broth). MCHT was minimal medium E (29) supplemented with Casamino Acids (0.4%) (Difco) and tryptophan (20 μg/ml). ET was minimal medium E supplemented only with tryptophan (20 μg/ml). Nutrient agar and LB agar were NB and LB, respectively, each solidified with 1.5% Difco agar. Soft LC agar was LC broth solidified with 0.7% Difco

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TABLE 1. Bacterial strains and plasmids

E. coli strain or plasmid	Relevant genotype[a] or description	Source or reference
Strains		
SC18	*recA718 lexA*$^+$	32
SC18-SP	*recA718 lexA71*::Tn*5*	This study, from SC18
SC18-RP1	*recA*$^+$ *lexA*$^+$	*alaS*$^+$ revertant of SC18-RP (31)
SC18-RP-SP	*recA*$^+$ *lexA71*::Tn*5*	This study, from SC18-RP1
SC30	*recA730 lexA*$^+$	32
SC30-SP	*recA730 lexA71*::Tn*5*	31
SC30-TF	*recA441 lexA*$^+$	31
SC30-TF-SP	*recA441 lexA71*::Tn*5*	31
SC30-RP1	*recA*$^+$ *lexA*$^+$	This study, from SC30
SC18-MP(λ)	*recA718 lexA*$^+$ *lamB*$^+$ (λ)	32
SC18-MP-SP(λ)	*recA718 lexA71*::Tn*5* *lamB*$^+$ (λ)	This study, from SC18-MP(λ)
SC30-MP(λ)	*recA730 lexA*$^+$ *lamB*$^+$ (λ)	32
$WP2_s$-MP(λ)	*recA*$^+$ *lexA*$^+$ *lamB*$^+$ (λ)	This laboratory, from WP2
OM10	Δ(*srl-recA*)*306*::Tn*10 lexA*$^+$	This study (Tet^r transductant of $WP2_s$)
GC2181	*recA*$^+$ *lexA*$^+$ *lac* Δ*U169 sfiA*::Mu *c*ts d(Ap *lac*) (Mu *c*$^+$)	4
K250	Like GC2181, except *recA718*	This study, from GC2181
Plasmids		
pBEU33	F′ *lac*$^+$, carries Tn*1943*; Tn*1943* is a derivative of Tn*3* carrying a chromosomal *Bam*HI fragment containing *recA56*(Def) in its single nonessential *Bam*HI site (Ap^r)	B. E. Uhlin and A. J. Clark, personal communication
pOM4	Like pBEU33, except carries Tn*3718* instead of Tn*1943*; Tn*3718* is Tn*1943* with *recA718* instead of *recA56*(Def)	This study (see Materials and Methods)
pBEU28	A plasmid exhibiting runaway replication at elevated temperatures (Km^r)	25
pOM5	pBEU28 carrying Tn*3718* (*recA718* inserted in Tn*3*) (Ap^r Km^r)	This study (see Materials and Methods)

[a] All SC strains are B/K-12 hybrids with the restriction/modification of strain B and carrying *lon-11*, *sulA1*, *trpE65*, and *uvrA155* alleles derived from their B/r parent, strain $WP2_s$. GC2181 and K250 are K-12 strains, also *thi-1 relA1*.

agar. MCHt was MCHT solidified with 1.5% Difco agar, except that the tryptophan supplement was 0.5 μg/ml.

UV irradiation and selection of Trp$^+$ mutants. Cultures were grown in NB to log-phase titers of 2×10^8 to 4×10^8 cells per ml and centrifuged, and the pellets suspended in the same volume of saline solution (0.9% NaCl). Trp$^+$ mutants were selected by plating samples (0.1 ml) of the undiluted saline suspensions on MCHt agar. Dilutions of the saline suspensions were plated on the same medium to determine viable-cell titers. Plates used to determine the initial titer and the frequency of spontaneous Trp$^+$ mutants were incubated without exposure to UV irradiation. The bacteria on plates used to determine survival levels and induced mutation frequencies were irradiated with UV light immediately after plating, while on the surface of the agar. (This procedure yields more reproducible results than irradiation in liquid suspension at the extremely low UV dose range [<1 J/m^2] used here). Counts of colonies of Trp$^-$ survivors and Trp$^+$ mutants were made after 2 and 3 days of incubation, respectively, at 30°C. The incubation times were reduced by 1 day when incubation was at 42°C. The source of UV irradiation and other details were described previously (29).

Stable DNA replication. The ability to synthesize DNA in the presence of chloramphenicol (CAP) (25 μg/ml) was assessed as described previously (13).

Spontaneous induction of λ prophage. Cultures of lysogenic strains were initially grown overnight in NB (a medium that inhibits the SOS-constitutive phenotype and therefore permits normal growth of λ lysogens of SOS-constitutive strains such as SC30). Low levels of inoculum (10^2 to 10^3 cells) from these NB cultures were used to start another round of overnight cultures in 10 ml of MCHT at 30 or 42°C. These cultures were centrifuged, and the titer of λ phage particles in the supernatant of each culture was assayed in soft LC agar. About 10^6 cells of the indicator strain SC30-MP grown in LC broth to log-phase titers of 1×10^8 to 2×10^8 were mixed with 2 ml of soft agar just before 0.1-ml portions of appropriate dilutions of the supernatants were added. The soft agar was then poured and spread evenly on the surface of LB agar. Plaques were counted after overnight incubation at 37°C.

Assay of β-galactosidase activity. The method of Miller (19) was used to assay β-galactosidase activity.

Antibiotics. When strains owing their resistance to antibiotics to transposons or plasmids or both were used, the appropriate antibiotics were routinely added to the overnight culture medium. Kanamycin, tetracycline, or ampicillin (all from Sigma Chemical Co.) or any combination thereof were added to final concentrations of 25, 15, and 50 μg/ml, respectively.

Cloning of *recA718*. The *recA718* allele was cloned by an unpublished procedure (B. E. Uhlin and A. J. Clark, personal communication) which uses two specially constructed plasmids, pBEU28 (25) and pBEU33. Plasmid pBEU33 carries F′ *lac*$^+$::Tn*1943*. Tn*1943* is a derivative of Tn*3* containing a *recA56*-defective mutation [*recA56*(Def)]. The *recA718* allele was moved from the chromosome onto pBEU33 by recombination in vivo. Recombinant plasmids were selected for by conjugation into a *recA*(Def) *polA12*(Ts) recipient, and transconjugants were scored for survival at

TABLE 2. Spontaneous mutability (Trp$^-$ to Trp$^+$) in various *E. coli* strains

Strain	Chromosomal genotype		No. of Trp$^+$ colonies/plate[a]			
	recA	*lexA*	30°C		42°C	
			−Ade	+Ade	−Ade	+Ade
SC18	*718*	+	19, 18	11, 17	12, 9	13, 10
SC18-SP	*718*	Def	354, 327	349, 388	456, 468	707, 605
SC18-RP1	+	+	14, 18	13, 9	11, 11	14, 16
SC18-RP-SP	+	Def	32, 45	28, 37	24, 21	13, 21
SC30	*730*	+	166, 202	380, 395	607, 712	939, 1,119
SC30-SP	*730*	Def	545, 511	797, 913	667, 735	1,101, 1,143
SC30-TF	*441*	+	32, 25	44, 39	78, 90	497, 586
SC30-TF-SP	*441*	Def	57, 63	127, 131	306, 224	501, 483
OM10(pOM5)[b]	Δ*306*	+	29, 35	41, 37	108, 103	253, 260
SC18-SP(pOM5)	*718*	Def	333, 339	338, 348	379, 405	764, 772

[a] Data are representative of six similar experiments. Counts from duplicate plates are given. ±Ade, MCHt medium with or without adenine (100 μg/ml).
[b] pOM5 carries *recA718* inserted into Tn*3* (Table 1); we estimated the copy number of pOM5 per cell in SC18(pOM5) and SC18-SP (pOM5) relative to the copy number of pBR322 under the same conditions, which was assumed to be 30 copies per cell. On this basis, the copy number of pOM5 is 4 at 30°C, 10 at 37°C, and 14 at 42°C in the *lexA*$^+$ strain SC18; it is 2.5 at 30°C, 3.6 at 37°C, and 6.7 at 42°C in the *lexA*(Def) strain SC18-SP. The copy number was determined as previously described (N. L. Subia and T. Kogoma, J. Mol. Biol., in press).

43°C (RecA$^-$ PolA$^-$ is a lethal phenotype [8]). The transposon, containing *recA718*, was then transposed onto pBEU28, a plasmid with runaway replication at high temperatures. The resulting plasmid, pOM5, did not express runaway replication at 42°C, although its copy number per cell increased from 2 to 5 at 30°C to 10 to 20 at 42°C.

DNA sequencing. The sequence of *recA718* was determined by the dideoxy method of Sanger et al. (24). Gene fragments were subcloned into the bacteriophage M13 sequencing vectors mp8, mp9, and mp18 (17, 18, 20). Vectors mp8 and mp9 were purchased from Pharmacia PL; mp18 was a gift from Ron Morris. The sequencing reactions were labeled with [α-^{35}S]dATP (400 Ci/mmol; Amersham Corp.). T4 ligase and restriction enzymes, *Eco*RI, *Bam*HI, and *Pst*I were purchased from Bethesda Research Laboratories, Inc. Deoxynucleoside triphosphates were purchased from Sigma. Klenow fragment and dideoxynucleoside triphosphates were purchased from Pharmacia PL. Universal sequencing primer was purchased from New England BioLabs, Inc. A second primer was made on an Applied Biosystems MS380A DNA synthesizer. The primer sequence is 3′-T-GGACACGCAAATAG-5′, corresponding to base pairs 316 to 330 of the *recA* gene. Because the wild-type *recA* sequence was available for comparison (23), only one strand was sequenced, except in three regions where the confirming complementary sequence was deemed necessary.

Two-dimensional polyacrylamide gel electrophoresis. The method of O'Farrell (21) was used.

RESULTS

Spontaneous mutability of a *recA718 lexA*(Def) strain. In our previous report (32), we characterized some of the phenotypic effects of *recA718* in the *lexA*$^+$ background of strain SC18. SC18 is not SOS inducible by heat or adenine but is inducible by DNA damage. In this respect and in its normal spontaneous mutability, it appears to be a RecA$^+$ revertant of its *recA441* parent. However, SC18 differs from its *recA*$^+$ transductant in its considerably greater sensitivity to UV irradiation at 30°C and its hypermutability at low UV doses. The spontaneous mutability of the *recA718* strain SC18 and its *lexA*(Def) transductant is compared with that of some closely related strains in Table 2. Although the mutability of SC18 was similar to that of the *recA*$^+$ *lexA*$^+$ strain, derepression of the LexA regulon by a *lexA*(Def) allele in strain SC18-SP resulted in strong mutator activity. This response contrasts markedly with the responses of the other *recA* alleles shown. In the *recA*$^+$ strains, spontaneous mutability increased only slightly when *lexA*(Def) replaced *lexA*$^+$. In *recA441* strains, strong mutator activity was expressed at 42°C with added adenine in the presence of either the *lexA*$^+$ or the *lexA*(Def) allele. The *recA730* strains showed considerable mutator activity even at 30°C without adenine in both *lexA*$^+$ and *lexA*(Def) derivatives, and this activity reached about the same maximal level in the presence of either *lexA* allele at 42°C with adenine in the medium. Thus, in its spontaneous mutability, *recA718* acted like

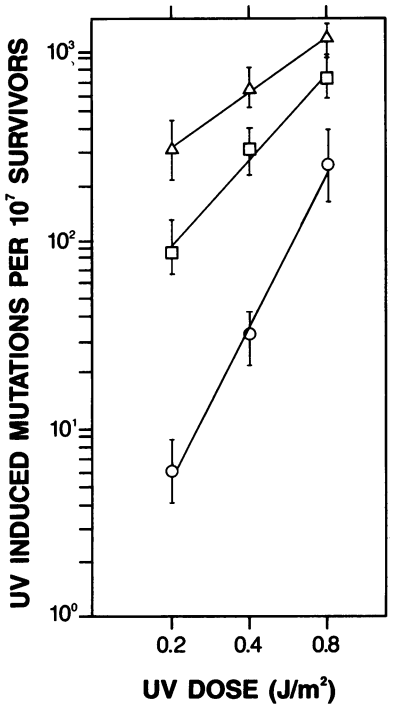

FIG. 1. UV-induced mutations (Trp$^-$ to Trp$^+$) at low UV doses in strain SC18 and derivatives. Symbols: □, SC18 (*recA718 lexA*$^+$); ○, SC18-RP1 (*recA*$^+$ *lexA*$^+$); △, SC18-SP (*recA718 lexA71*::Tn*5*). At the doses shown, survival levels did not fall below 50% for any of the strains.

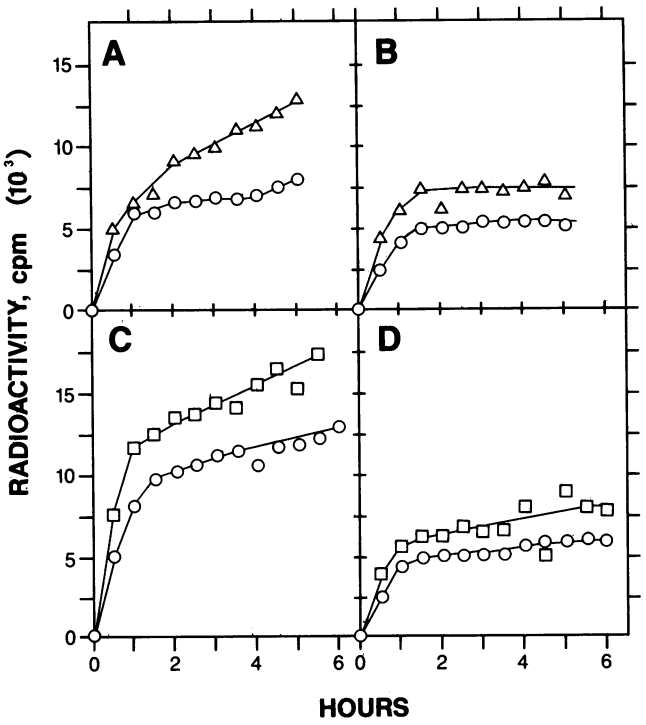

FIG. 2. DNA replication in the presence of CAP in strain SC18 and derivatives. Strains SC18-SP (*recA718 lexA71*::Tn*5*) (A) and SC18 (*recA718 lexA*$^+$) (B) were incubated in the presence of CAP and [^{3}H]thymidine (10 μCi/ml; specific activity, 1 μCi/μg) at 30°C (○) and 42°C (△); strains SC18-SP(pOM5) (C) and SC18(pOM5) (D) were incubated in the presence of CAP and [^{3}H]thymidine at 30°C (○) and 37°C (□). Conditions were as described previously (30) except that adenine was not added to the medium at any temperature. pOM5 carries *recA718* (Table 1).

recA$^+$ when *lexA*$^+$ was present but more like *recA441* and especially (in its pattern of response to temperature) like *recA730* in the presence of *lexA*(Def). The maximum mutability promoted by *rec718* was somewhat lower than the maximum level seen in the *recA730* strains but higher than the maximum level promoted by *recA441*.

UV mutability of *recA718* strains. The UV mutability of various strains at low radiation doses is shown in Fig. 1. The hypermutability promoted in this dose range by *recA718*, which was previously reported (32), was enhanced when *lexA*(Def) was also present.

Stable DNA replication in *recA718* strains. Constitutive expression of another RecA*-dependent function, stable DNA replication (the ability to undergo repeated rounds of DNA replication in the presence of CAP) (13), requires not only derepression of the LexA regulon but also the presence of activated RecA (31). The *lexA*(Def) strain SC18-SP (but not the *lexA*$^+$ strain SC18) expressed stable DNA replication without DNA damage at 42°C (Fig. 2A and B).

TABLE 3. Spontaneous production of λ bacteriophage in various lysogenic *E. coli* strains

Strain	Genotype		No. of free phage particles/ml in supernatants of MCHT cultures[a]	
	recA	*lexA*	Expt 1	Expt 2
WP2$_s$-MP(λ)	+	+	1.6×10^5	5.0×10^5
SC18-MP(λ)	*718*	+	2.5×10^4	3.6×10^4
SC18-SP-MP(λ)	*718*	Def	4.3×10^9	5.7×10^9
SC30-MP(λ)	*730*	+	1.9×10^9	1.8×10^9

[a] Cultures were incubated overnight at 30°C; see Materials and Methods for details.

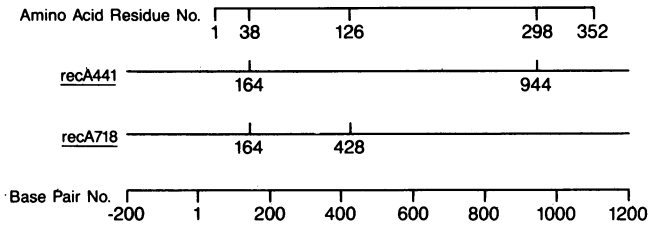

FIG. 3. Location of base substitutions distinguishing *recA718* from *recA*$^+$ and *recA441*. The change at nucleotide 164 in *recA441* (9, 11) is also present in *recA718*, causing the change from Glu-38 to Lys-38 in both mutant proteins. The second change in *recA718*, at nucleotide 428, changes Leu-126 to Val-126. The second change in *recA441* changes Ile-298 to Val-298 (11); the corresponding base change, indicated at base pair 944, has not been confirmed by DNA sequencing and may be one base removed from this site.

Induction of λ prophage in *recA718* strains. Spontaneous λ prophage induction in various lysogens is shown in Table 3. The high free-phage titer found in overnight MCHT cultures of the SOS-constitutive *recA730* strain reflected the constitutive ability of the RecA730 protein to promote the cleavage of λ repressor in this medium, whereas the free-phage titer of the *recA*$^+$ strain was orders of magnitude lower, as previously shown (32). The free-phage titers in the supernatants of overnight cultures of the *lexA*$^+$ *recA718* strain were in the same range as those in the *recA*$^+$ supernatants, whereas the supernatants of the *lexA*(Def) *recA718* lysogen contained about as much free phage as those of the SOS-constitutive *recA730* strain. In its ability to effect cleavage of λ repressor, as well as in SOS mutator activity, RecA718 was similar to RecA$^+$ in *lexA*$^+$ strains but was more like RecA730 when *lexA*(Def) was present.

Sequence changes in *recA718*. We cloned and sequenced the *recA718* allele, as described in Materials and Methods. The deviations from the wild-type sequence (23) in *recA718* and *recA441* are shown in Fig. 3. The same base substitution (a G · C to A · T transition) that is present in *recA441* at nucleotide 164 (10, 12) is also present in *recA718*, causing a change from Glu-38 to Lys-38 in both mutant proteins. In addition, *recA718* contains a second base substitution (a C · G to G · C transversion) at nucleotide 428 that changes Leu-126 to Val-126. The second mutation in *recA441*, deduced from the amino acid change from Ile-298 to Val-298 in the RecA441 protein (12), is not present in *recA718*.

Migration in RecA718 during two-dimensional gel electrophoresis. The migration of RecA718, RecA$^+$, and RecA441 during two-dimensional gel electrophoresis is shown in Fig. 4. It is known (9, 15) that RecA441 migrates to a position more basic than does RecA$^+$; Fig. 4 confirms this conclusion and shows that RecA718 comigrated with RecA441, as would be predicted from the nature of amino acid substitution (Glu-38 to Lys-38) shared by the two mutant proteins.

Expression of *recA718* cloned on pOM5. The UV sensitivity of the *recA*-deleted strain OM10 carrying *recA718* cloned on pOM5 (as described in Materials and Methods) and its hypermutability at low UV doses were essentially the same as those of strain SC18 (results not shown). The spontaneous mutability of the *lexA*$^+$ strain OM10 carrying the cloned

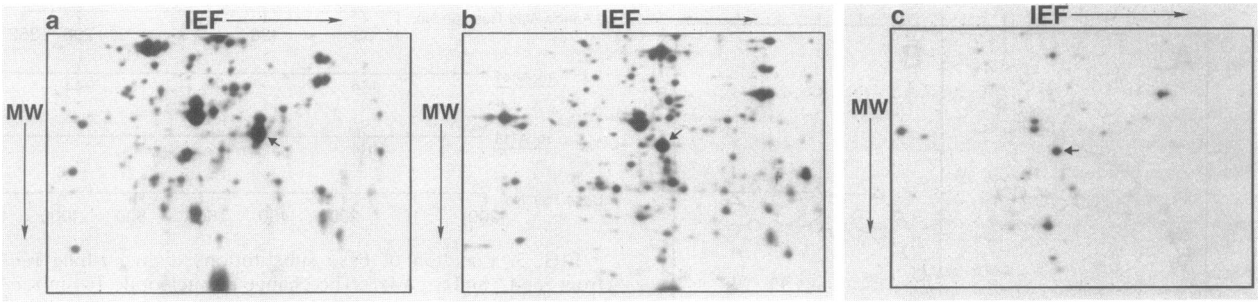

FIG. 4. Portions of autoradiograms of two-dimensional polyacrylamide gels containing proteins from whole-cell extracts of strains SC30-RP1 (*recA*$^+$) (a), SC30-TF (*recA441*) (b), and SC18 (*recA718*) (c). Isoelectric focusing (IEF) ran from left to right (left = basic; right = acidic); molecular weight (MW) ran from top to bottom. RecA protein synthesis was induced by nalidixic acid. The identity of the spots indicated by arrows as RecA was confirmed by their absence or marked reduction in size on gels made from extracts of uninduced cultures or from uninducible [*lexA*(Ind$^-$)] derivatives of these strains (results not shown).

recA718 allele on plasmid pOM5 was only slightly higher than that of strain SC18 at 30°C but increased 10-fold at 42°C (a temperature that increases the copy number of the plasmid per cell) and was enhanced at both temperatures by adenine (Table 2). No increase in spontaneous mutability was observed when the *recA*-deleted strain was transformed with pBEU28, the plasmid from which pOM5 was derived (data not shown). These results imply that the amplification of the level of the RecA718 provided when the plasmid copy number is about 14 per cell is sufficient to permit considerable constitutive cleavage of LexA, since derepression of *umuDC* is a requirement for bacterial SOS mutator activity. However, the maximum level of mutability expressed at this amplification of RecA718 was less than 40% of the maximum level expressed in the *lexA*(Def) *recA718* strain SC18-SP, in which the amplification of RecA718 is presumably much greater. Introducing the plasmid carrying *recA718* into the *lexA*(Def) *recA718* strain SC18-SP did not cause any further increase in the mutator activity (Table 2). This suggests that the limiting factor in the level of mutator activity in these *lexA*(Def) strains is probably not the amount of RecA718 available.

Ability of RecA718 to cleave LexA without DNA damage. The results described above imply that RecA718 at its base-line level in strain SC18 behaves like RecA$^+$ in its inability to cleave LexA protein or λ repressor without DNA damage but that, unlike RecA$^+$, RecA718 is well able to perform its essential roles in expression of SOS mutator activity and stable DNA replication and to cleave λ repressor, all without DNA damage, when the LexA regulon is derepressed by a *lexA*(Def) allele. We compared the ability of RecA718 to cleave LexA with that of RecA$^+$ more directly by measuring the constitutive level of β-galactosidase synthesized under LexA control in a pair of *lexA*$^+$ strains carrying a Mu d(Ap^r *lacZ*) insertion in the SOS-inducible *sfiA* gene (Table 4). We found no significant difference in the level of β-galactosidase synthesized in exponentially growing cultures of the *recA*$^+$ strain and its *recA718* transductant. The effect on constitutive β-galactosidase synthesis of amplifying RecA718 by introducing plasmid pOM5 into strain K250 is also shown in Table 4. The plasmid increased the constitutive level of β-galactosidase synthesis about 10-fold, approximately proportional to the copy number of the plasmid at 37°C. This confirms that RecA718 is able to effect some cleavage of LexA without DNA damage when it is amplified above its base-line level.

Effect of pOM5 on expression of stable DNA replication. The addition of pOM5 to *lexA*$^+$ strains caused constitutive expression of both SOS mutator activity (Table 2) and β-galactosidase synthesis (Table 4). The presence of the plasmid carrying *recA718* stimulated expression of stable DNA replication only slightly in the *lexA*$^+$ strain SC18 (cf. 2B and D) and in SC18-SP at 30°C (cf. 2A and C). At the higher temperatures, the *lexA*(Def) strain SC18-SP synthesized DNA continuously in CAP whether or not pOM5 was present. Thus, RecA718 can apparently promote stable DNA replication without DNA damage only at a relatively high level of amplification.

TABLE 4. Constitutive and UV-induced levels of β-galactosidase synthesized under LexA control at 37°C in various *lexA*$^+$ strains

Strain	*recA* genotype	Amt of β-galactosidase (U/mg of protein) with:		
		No UV irradiation		UV irradiation (20 J/m^2)
		Expt 1	Expt 2	
GC2181	+	107	113	5,385
K250	*718*	109	140	5,017
K250(pOM5)[a]	*718*	1,396	1,246	5,976

[a] pOM5 carries *recA718* inserted into Tn*3* (Table 1); the copy number of pOM5 at 37°C in the *lexA*$^+$ strain SC18(pOM5) was estimated to be 10 per cell (Table 2, footnote *b*). Cultures were grown in ET medium supplemented with thiamine (1 μg/ml); cells were exposed to UV irradiation while in the growth medium and were incubated at 37°C for 90 min after irradiation before β-galactosidase levels were determined. Transformation of strain K250 with pBEU28 did not significantly increase the level of β-galactosidase synthesized.

DISCUSSION

In undamaged populations of *recA718 lexA*$^+$ strains such as SC18, as in *recA*$^+$ *lexA*$^+$ strains, spontaneous mutability, β-galactosidase synthesis from a *lacZ* gene controlled by LexA, and spontaneous induction of λ prophage were all low, indicating that RecA718 protein, like RecA$^+$, is not constitutively activated at its base-line level for cleavage of either LexA or λ repressor. This conclusion is reinforced by the inability of RecA718 to derepress its own synthesis in untreated cultures of strain SC18, although this protein is copiously amplified after UV irradiation (32). RecA718, like RecA$^+$, requires a signal generated by DNA damage to become activated to effect proteolytic cleavage of LexA or λ repressor. In this regard, RecA718 differs from RecA441, which is activated by heat plus adenine, and from RecA730, which is constitutively activated to cleave both repressors.

RecA718 did not require DNA damage to become activated for cleavage of λ repressor (Table 3) or for its essential roles in SOS mutator activity (Table 2) and stable DNA

replication (Fig. 2) when *lexA*$^+$ was replaced by *lexA*(Def). These observations tell us that the base-line level of RecA718 is inactive in cleavage of LexA but that highly amplified levels of this RecA protein are active in the other RecA* functions examined. In themselves, they do not reveal whether highly amplified levels of RecA718 are also proficient in cleavage of LexA. The ability of RecA718 to effect cleavage of LexA substantially (albeit not maximally) at relatively modest levels of amplification was demonstrated by the effects of introducing pOM5, carrying cloned *recA718*, into *lexA*$^+$ strains. The presence of the plasmid resulted in SOS mutator activity (Table 2) and in elevated synthesis of β-galactosidase (Table 4), both indicators of significant cleavage of LexA at the level of amplification of REC718 promoted by the plasmid at the higher temperatures. We conclude that RecA718 has the capacity to cause cleavage of both LexA and λ repressor constitutively, a capacity that is potentiated only when the mutant RecA protein is amplified above its base-line level.

Wang and Tessman (28) sequenced several SOS-constitutive *recA* mutants with phenotypes similar to the *recA730* strain SC30 and found two with sequences that differ from that of *recA*$^+$ by a single nucleotide substitution, causing a change from Glu-38 to Lys-38. Knight et al. (12) showed that RecA441 contains the same substitution at the same site plus a second substitution at amino acid 298. Wang and Tessman concluded that the substitution at amino acid 38 causes constitutive protease activity, whereas the change in RecA441 at amino acid 298 suppresses this activity at low temperatures, resulting in the thermal inducibility of *recA441* mutants. Because *recA718* also contains the Glu-38 to Lys-38 change, we suggest that the ability of this protein to become constitutively activated when amplified is due to this substitution and that the second change (Leu-126 to Val-126) partially suppresses this capability, in a relatively temperature-independent way, so that is is effectively manifested only when the base-line level of the protein is amplified. The ability of RecA441 to become activated without DNA damage has been ascribed to its increased affinity for shorter regions of single-stranded DNA and certain adenine nucleotides (16, 22). If this property is due to the amino acid substitution at position 38, the phenotype conferred by *recA718* can be explained by assuming that the second substitution in RecA718 at position 126 reduces, but does not abolish, the increased affinity for one or both cofactors of activation available in the undamaged cell. Thus, increasing the concentration of RecA718 could partially compensate for its reduced affinity for cofactor(s) of activation.

The established nucleotide sequences of *recA441* and *recA718* and the knowledge that the change from Glu-38 to Lys-38 causes an SOS-constitutive phenotype like that of the *recA730* strain SC30 suggests an explanation for the unusual mode of origin of the mutant alleles *recA730* and *recA718*. It is highly probable that both *recA730* and *recA718* arose from the parental allele *recA441*, because both new alleles share with *recA441* the capacity to become activated without DNA damage, as well as the susceptibility to stimulation by adenine in promoting SOS mutagenesis. Furthermore, *recA718* also shares with *recA441* the base substitution causing the change from Glu-38 to Lys-38, which probably accounts for their comigration during two-dimensional gel electrophoresis at a position more basic than that of the wild-type protein (Fig. 4). The sequence of *recA730* is not known, but if it also arose from *recA441*, as seems likely, it may have arisen by loss of the second base substitution in *recA441*, leaving the Glu-38 to Lys-38 substitution as the only difference from the wild-type protein, a substitution known to result in constitutive SOS activity (28). This possibility is consistent with our unpublished observation, confirmed by Garvey et al. (7), that RecA730 protein also comigrates during electrophoresis in two-dimensional gels with RecA441 and RecA718. Perhaps the simplest way to explain the origin of the two new alleles is that they arose sequentially from a *recA441* cell which first gave rise to *recA730* by loss of the second substitution in *recA441* in a very early DNA replication, an event rapidly followed by the occurrence of a second mutation in a *recA730* daughter molecule, which caused the change of Leu-126 to Val-126.

The need for RecA* activity not only for repressor cleavage but also for expression of several individual SOS phenotypes raises questions about the nature of the activated state. In *recA*$^+$ strains and most known *recA* mutants, activation of RecA to perform the various RecA*-dependent functions appears to be all or none based on the available evidence. For example, RecA441 effects cleavage of LexA poorly at 30°C without adenine and also, in *lexA*(Def) strains, promotes relatively weak SOS mutator activity and stable DNA replication under these conditions, compared with its performance at 42°C with adenine (31), the conditions which are also optimal for its LexA and λ repressor clavage activity. In *recA730* strains, the ability to perform all RecA*-dependent functions so far examined is strong at 30°C and optimal at 42°C with adenine. These observations and others are consistent with the possibility that the requirements for RecA* activity in all of its known functions are the same and that mutations affecting the efficiency or temperature response of any one RecA*-dependent function are likely to affect them all. A possible exception is *recA430*, which seems to exert differential effects on individual RecA*-dependent activities of the protein: it prevents UV induction of λ prophage but not φ80 prophage, indicating that UV irradiation activates RecA430 for φ80 repressor cleavage but not for cleavage of λ repressor (5).

RecA430 also effects cleavage of LexA efficiently in vitro (J. W. Roberts, personal communication) and at a substantial fraction of the normal rate in vivo (4; J. W. Little, personal communication), yet completely suppresses UV mutability even in *lexA*(Def) strains (1; E.M.N. unpublished observation). The UV nonmutability of *recA430* strains could reflect a deficiency in the ability of RecA430 to perform the direct role of RecA protein in SOS mutagenesis. On the other hand, because UV mutagenesis requires DNA replication, the UV nonmutability could be a secondary consequence of the inability of RecA430 to promote recovery from UV-induced inhibition of DNA synthesis (11). The recovery process requires both RecA and induction of an unidentified SOS function, and RecA430 could be defective in either or both of these requirements. In any case, the properties of *recA430* strains suggest that diverse RecA*-dependent functions can be uncoupled by mutation. Whereas all RecA*-dependent activities may require that RecA be able to interact with single-stranded DNA and an adenine nucleotide, some mutations in the recA gene that still allow the active ternary complex to form may alter its substrate specificity, possibly in a variety of different patterns, thereby causing "split phenotypes" as observed in *recA430* strains.

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