Involvement of the activated form of RecA protein in SOS mutagenesis and stable DNA replication in *Escherichia coli*

(*lexA* gene/tease/ultraviolet mutagenesis/*din* genes)

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ABSTRACT DNA damage activates RecA protein of *E. coli* to a form (RecA*) that promotes proteolytic cleavage of LexA protein, the repressor of at least 17 DNA damage-inducible genes, resulting in expression of the SOS response. In addition to this known role, RecA* performs another function necessary for expression of SOS mutagenesis [Blanco, M., Herrera, G., Collado, P., Rebollo, J. & Botella, L. M. (1982) *Biochimie* 64, 633–636]. The additional role of RecA* could be (i) cleavage of another repressor, (ii) proteolytic processing of one or more proteins, or (iii) mechanistic interaction with DNA or with one or more other proteins. We describe experiments designed to test the first possibility. Our results suggest that neither SOS mutator activity nor ultraviolet mutagenesis requires induction by RecA* of any gene(s) outside the LexA regulon and that the additional role of RecA* is not cleavage of another repressor. We show that stable DNA replication, another DNA damage-inducible function [Kogoma, T., Torrey, T. A. & Connaughton, M. J. (1979) *Mol. Gen. Genet.* 176, 1–9], shares with SOS mutagenesis the requirement for RecA* activity, even in a strain constitutively expressing all LexA-controlled genes. In this strain, conditions that activate RecA initiate expression of stable DNA replication in the presence of chloramphenicol, without an intervening period of protein synthesis. We conclude that the additional function of RecA* in stable DNA replication is not another antirepressor activity.

Mutations in the *recA*, *lexA*, and *umuDC* genes of *Escherichia coli* eliminate mutability by UV and by other mutagens that induce the SOS response (for reviews, see refs. 1–3). Current understanding of SOS regulation readily accounts for these effects. DNA damage caused by the inducing agents generates an induction signal, probably single-stranded DNA plus an adenine nucleotide, that activates the latent ability of the RecA protein to promote proteolysis. Activated RecA (RecA*) then causes cleavage of LexA, the common repressor of at least 17 genes scattered about the chromosome, thereby inducing the pleiotropic SOS response, including the mutagenic activity promoted by the *umuDC* gene products (3). The induced mutagenic activity elevates spontaneous mutation rates (SOS mutator activity, untargeted mutagenesis) and also causes mutations that appear to be targeted at sites of DNA damage and that may be associated with DNA repair (4–6).

Certain mutations in the *lexA* gene inactivate the repressor function of LexA (7), causing constitutive expression of all genes belonging to the LexA regulon, including *umuDC*. In *recA*⁺ strains, presence of such a defective *lexA* [*lexA*(Def)] allele does not result in elevated spontaneous mutability, indicating that derepression of *umuDC* and other genes normally repressed by LexA is not sufficient for SOS mutator activity (8). However, the mutator activity promoted by

recA441, which encodes a RecA capable of acquiring the ability to promote cleavage of LexA without DNA damage (e.g., at 42°C in the presence of adenine), is strongly enhanced by a *lexA*(Def) allele. This and other observations by Blanco *et al.* (8) imply that, for SOS mutagenesis, RecA* is required to perform a function other than its known role as antirepressor against LexA.

Additional activities of RecA* that may be required for SOS mutagenesis could include (i) cleavage of one or more repressors other than LexA, resulting in induction of at least one additional gene and synthesis of a protein necessary for SOS mutagenesis; (ii) proteolytic processing of one or more proteins as a prerequisite for their participation in SOS mutagenesis; or (iii) mechanistic interaction with one or more proteins, or with DNA, via an activity that does not involve proteolysis but does require the activated form of RecA. In this report, we describe experiments designed to test the first of these possibilities.

MATERIALS AND METHODS

Bacterial Strains. Table 1 lists and describes the strains used in this study.

Culture Media. The liquid medium used in mutagenesis experiments was Difco nutrient broth with 0.5% NaCl added (NB). MCHT is minimal medium E (4) supplemented with 0.4% Casamino acids and 50 µg of tryptophan per ml. Mcht is minimal medium E supplemented with 0.04% Casamino acids and 0.1 µg of tryptophan per ml. Solid media were unsupplemented minimal E, MCHT and Mcht, each solidified with 1.5% Bacto Agar. Glucose (0.4%) was the carbon source in all minimal media. Where indicated, adenine, cytidine plus guanosine (each at 100 µg/ml), and/or chloramphenicol (CAP) (25 µg/ml) were added to the liquid medium or to the agar medium just before the plates were poured.

Preparation of Cultures. Cultures to be used in mutagenesis experiments were grown in NB at 30°C on a shaker to log-phase titers of 2–3 × 10⁸ cells per ml. These cultures were centrifuged, and the pellets were resuspended in 0.75 volume of saline solution (0.9% NaCl). Cultures to be used to examine stable DNA replication were grown to log-phase titers of ≈2 × 10⁸ cells per ml in MCHT.

UV Irradiation. Saline suspensions prepared as described above were divided into two portions, one of which was exposed to UV as described (4).

Bacterial Assays. Viable cell titers of the Trp[−] cultures were determined by plating aliquots of appropriate dilutions on MCHT agar. In some experiments, adenine or cytidine plus guanosine was added to the MCHT agar. These additives made no difference in the viable titer. Colonies were counted after 2 days of incubation at 30°C.

Abbreviations: RecA*, activated form of RecA; NB, nutrient broth; MCHT, minimal medium supplemented with 0.4% Casamino acids and 50 µg of tryptophan per ml; Mcht, minimal medium supplemented with 0.04% Casamino acids and 0.1 µg of tryptophan per ml; CAP, chloramphenicol.

Table 1. Bacterial strains

Strain	Relevant genotype*	
	<i>recA</i>	<i>lexA</i>
SC30	730	+
SC30-RP	+	+
SC30-TF	441	+
SC30-SP	730	71::Tn5
SC30-RP-SP	+	71::Tn5
SC30-TF-SP	441	71::Tn5

All hyphenated strains were constructed in one or two steps of P1 transduction from strain SC30. Strains SC30, SC30-RP, and SC30-TF have been described (9). The *lexA71::Tn5* allele was transduced from *E. coli* K12 strain GW2707 (obtained from G. Walker). Note: We have transduced several strains both to *lexA51(Def)* and to *lexA71::Tn5* and have detected no difference between these alleles in their ability to cause a LexA(Def) phenotype with respect to mutator activity.

*All strains also *trpE65 uvrA155*.

Selection of Trp⁺ Revertants. Aliquots (0.1 ml) of saline cell suspensions were plated either on minimal E agar (which selects Trp⁺ revertants already present and phenotypically expressed in the plated inoculum but does not allow new revertants to occur or to become expressed on the plate) or on Mcht agar (which selects both Trp⁺ mutants already expressed in the inoculum and mutants arising or becoming phenotypically expressed during the limited number of cell divisions that occur on the plate before the small tryptophan supplement is exhausted). When the tryptophan level is 0.1 µg/ml, the final population ceiling for the *trpE65* strains used in this study is $2-3 \times 10^9$ cells per plate, as determined by harvesting the cells from the surface after 6 hr of incubation and assaying the wash fluid (data not shown). By this time, division of Trp⁻ cells has essentially stopped and Trp⁺ colonies have not yet appeared. Trp⁺ colonies were counted after 3 days of incubation at 30°C.

Harvesting Bacteria from the Surface of Agar. After incubation as described in Table 3, plates were chilled for 10 min in the freezer compartment of a refrigerator, then transferred to the refrigerator for no longer than 30 min. Plates (12 at a time) were removed from the refrigerator and the bacteria were harvested by pipetting 2 ml of cold saline solution onto the surface of each plate, spreading the solution vigorously with a glass spreader, and recovering 1.5 ml of the wash fluid. Wash fluids from each set of 12 identical plates were pooled to yield a total of 18 ml. The pooled suspensions were centrifuged and the pellets were resuspended in 6 ml of saline solution. These suspensions were assayed to determine viable cell titers, and 0.1-ml aliquots were plated on minimal E agar to determine the number of phenotypically expressed Trp⁺ revertants per aliquot. The suspensions were then used as sources of inocula for NB cultures, as described in the legend of Table 3.

RESULTS

Effect of a *lexA(Def)* Allele on Spontaneous Mutability. Table 2 shows the effect of replacing *lexA*⁺ by *lexA71::Tn5* in strains carrying three different *recA* alleles, each of which causes a different constitutive level of SOS expression indicative of a different level of constitutive RecA⁺ activity. In the *recA*⁺ strain, the *lexA(Def)* allele does not increase the low spontaneous mutability characteristic of *recA*⁺ strains, showing that derepression of the LexA regulon is not sufficient for SOS mutator activity. In the *recA441* strain, *lexA71::Tn5* strongly enhances the modest mutator activity expressed by strain SC30-TF at 42°C, but causes only a slight increase in mutability at 30°C. These results support the conclusions of Blanco *et al.* (8) that RecA⁺ activity is required for SOS mutagenesis even in LexA(Def) strains and that the magnitude of the mutagenesis expressed is correlated with the degree of RecA⁺ activity. Additional evidence for these conclusions is provided by the results with the *recA730* strains (Table 2). In the *lexA*⁺ *recA730* strain SC30 (9), SOS functions are expressed constitutively at 30°C as well as at 42°C, presumably because of constitutive RecA⁺ activity. Replacement of *lexA*⁺ by the *lexA(Def)* allele has relatively little effect on the already high spontaneous mutability of strain SC30, indicating that RecA730⁺ activity is constitutively strong enough to cause virtually complete derepression of the LexA regulon in *lexA*⁺ strains.

In *lexA*⁺ strains carrying *recA441* or *recA730*, expression of SOS mutagenesis and of other facets of the SOS response is inhibited by cytidine plus guanosine and enhanced by adenine (4, 9, 10). Table 2 shows that the presence of cytidine plus guanosine depresses SOS mutator activity in the *lexA*⁺ *recA441* and *lexA*⁺ *recA730* strains, as expected, but not in their *lexA(Def)* derivatives. This suggests that cytidine plus guanosine inhibits the RecA⁺-mediated cleavage of LexA but not the formation of RecA⁺ or its activity in SOS mutagenesis. Adenine increases SOS mutator activity in all four of these strains (Table 2), implying that its stimulatory effect is exerted on both RecA⁺ activities, possibly by promoting the conversion of RecA to RecA⁺.

The response of strain SC30-TF-SP (Table 2) suggested a way to test the possibility that RecA⁺ activity is needed to inactivate an unknown repressor—that is, to induce a gene not derepressed by the presence of the *lexA71::Tn5* allele. Although RecA synthesis is derepressed at both 30°C and 42°C in this *lexA(Def)* strain (an expectation that we confirmed by gel electrophoresis, not shown), Trp⁻ to Trp⁺ mutability is ≈ 10 times higher at 42°C with adenine present than at 30°C without adenine. Whatever the unknown function of RecA441⁺ in SOS mutagenesis may be, it is apparently performed more efficiently at 42°C with adenine than at 30°C without adenine. The experimental strategy adopted took advantage of this difference to ask whether the presence of CAP during a limited incubation at 42°C with adenine would prevent the enhancement of spontaneous and UV-induced

Table 2. Spontaneous mutability (Trp⁻ to Trp⁺) in strain SC30 and derivatives

Strain	Genotype		Trp ⁺ colonies per plate*					
	<i>recA</i>	<i>lexA</i>	30°C			42°C		
			Mcht	+ C,G	+ Ade	Mcht	+ C,G	+ Ade
SC30-RP	+	+	3	2	7	2	4	7
SC30-RP-SP	+	71::Tn5	2	0	3	5	2	3
SC30-TF	441	+	7	4	15	21	8	90
SC30-TF-SP	441	71::Tn5	14	25	40	147	152	264
SC30	730	+	112	54	215	156	69	295
SC30-SP	730	71::Tn5	134	145	266	199	216	298

*Numbers are averages of four plates; average number of cells seeded per plate was 2.3×10^7 . + C,G: Mcht medium with cytidine and guanosine (each at 100 µg/ml). + Ade: Mcht medium with adenine (100 µg/ml).

mutability normally caused by these conditions in strain SC30-TF-SP. If not, it could be concluded that protein synthesis during the heat-plus-adenine treatment is not required for the elevated mutability and, therefore, that *RecA441*⁺ probably does not increase SOS mutagenesis at 42°C in this strain by increasing the derepression of one or more genes outside the LexA regulon.

Effect of CAP on *RecA*⁺-Dependent SOS Mutagenesis. Table 3 shows results of a typical experiment in a series designed to determine whether the presence of CAP during incubation of strain SC30-TF-SP at 42°C with adenine would prevent this treatment from increasing spontaneous and UV-induced mutability. Trp⁺ mutants were not quantified by the conventional method—that is, plating on minimal agar supplemented with a low level of tryptophan—because of differences expected to develop between populations incubated for 70 min with and without CAP. In CAP-containing medium, DNA replication continues (see Fig. 1) but the viable cell titer remains nearly unchanged (Table 3), whereas without CAP present both DNA replication and cell division continue, resulting in a 3-fold increase in viable cell titer. By the end of the incubation, therefore, the two populations would differ in the number of genome copies per cell, complicating direct quantitative comparison between them. Furthermore, phenotypic expression of newly arisen Trp⁺ mutations would not develop in CAP and might require more than the limited time permitted by immediate plating on the selective agar. Therefore, equal volumes of the treated suspensions were inoculated into NB and incubated overnight. Large inocula were used so that each inoculum would contain a representative proportion per nucleoid of genetically fixed (albeit not necessarily phenotypically expressed) mutations to

Trp⁺. The cultivation in NB had two purposes: to permit full phenotypic expression of the Trp⁺ mutations present in the inoculum and/or in their descendents and to allow normalization of the number of genome copies per cell in the cultures inoculated with CAP-treated bacteria, an expectation that was confirmed by microscopic observation of samples of the NB cultures (not shown). The frequency of Trp⁺ mutants per 10⁸ cells in each NB culture was determined by plating aliquots of washed-cell suspensions on unsupplemented minimal agar. If the heat-plus-adenine treatment increases the frequency of spontaneous and UV-induced mutations during the 70-min incubation, an elevated frequency of Trp⁺ mutants in the inocula taken from these suspensions should be reflected in elevated Trp⁺ frequencies in the corresponding NB cultures compared to the frequencies in those inoculated with cells incubated only at 30°C without adenine.

The data for strain SC30-TF-SP in Table 3 show that NB cultures inoculated with cells treated for 70 min at 42°C with adenine contain significantly higher frequencies of Trp⁺ mutants than NB cultures inoculated with cells incubated only at 30°C without adenine and that the presence of CAP during the heat-plus-adenine treatment neither abolishes nor diminishes this effect. In the *lexA*⁺ strain SC30-TF, in contrast, CAP totally prevents the heat-plus-adenine enhancement of Trp⁺ mutation frequencies in the corresponding NB cultures. In the *lexA*⁺ strain, the LexA regulon is repressed at 30°C, and its derepression at 42°C requires protein synthesis during the incubation at the higher temperature for thermal enhancement of both spontaneous and UV-induced SOS mutagenesis to occur, as has been shown in other *lexA*⁺ *recA441* strains (4). The lack of a similar effect of CAP in the

Table 3. Frequency of Trp⁺ revertants in NB cultures inoculated with cells given various treatments

											Trp ⁺ revertants per 10 ⁸ viable cells in final NB cultures	
70-min treatment			Trp ⁺ colonies per minimal agar plate									
Ade	CAP	°C	No UV				UV				No UV	UV
SC30-TF-SP (<i>lexA71::71Tn5 recA441</i>)												
–	–	30	7	2	2	6	42	50	63	46	1.5 ± 1.1	16.2 ± 2.9
–	+	30	4	4	5	8	52	39	48	43	1.3 ± 0.7	13.8 ± 2.4
+	–	42	24	14	20	13	150	201	181	185	7.3 ± 2.9	66.4 ± 7.9
+	+	42	22	18	16	24	157	171	196	179	8.3 ± 1.6	62.7 ± 5.8
SC30-TF (<i>lexA⁺ recA441</i>)												
–	–	30	3	7	1	0	15	16	9	9	1.1 ± 1.5	3.7 ± 1.6
–	+	30	2	8	6	0	17	12	8	11	1.3 ± 2.3	3.8 ± 1.2
+	–	42	12	14	9	16	72	64	68	47	4.7 ± 1.2	25.1 ± 2.8
+	+	42	4	0	1	3	15	12	13	8	0.7 ± 0.7	3.8 ± 0.9
SC30-RP (<i>lexA⁺ recA⁺</i>)												
–	–	30	2	2	0	1	8	11	8	7	1.2 ± 1.3	2.7 ± 0.6
–	+	30	3	0	2	8	14	9	9	8	1.0 ± 1.7	3.3 ± 0.9
+	–	42	0	1	6	4	6	4	4	7	0.8 ± 0.8	1.7 ± 0.6
+	+	42	2	2	0	1	2	5	5	6	0.4 ± 0.4	1.3 ± 0.5

Aliquots (0.4 ml, $\approx 10^8$ cells) of saline suspensions of log-phase cells, UV-irradiated or not, were spread on four kinds of plates: MCHT, MCHT + CAP, MCHT + Ade, and MCHT + Ade + CAP. Plates were incubated for 70 min, those without Ade at 30°C and those containing Ade at 42°C. The plates were then chilled, and the bacteria from each set of plates were harvested, pooled, centrifuged, and resuspended in saline solution. The saline suspensions were assayed and used to inoculate sets of NB cultures. In the experiment shown above, 1 ml of each suspension was inoculated into each of four tubes containing 9 ml of NB. The NB cultures were shaken overnight at 30°C. Bacteria were then collected by centrifugation and resuspended in 10 ml of saline solution. Viable cell titers of these suspensions were determined (average titer, 3.1×10^9 cells per ml), and a 0.1-ml aliquot of each suspension was spread on unsupplemented minimal E agar to yield the Trp⁺ colony counts shown above. The data are representative of 12 similar experiments, in which treatment time varied from 60 to 120 min and in which as many as 10 NB cultures were inoculated with cells given each treatment. In no single experiment did the presence of CAP during the incubation at 42°C with Ade prevent this treatment from increasing the frequency of Trp⁺ mutants in the corresponding NB cultures. The frequency of Trp⁺ mutants was increased an average of 4.1-fold by the heat-plus-Ade treatment with no CAP present, and 4.6-fold with CAP present during the treatment. The UV dose used (0.2 J/m²) caused no detectable killing, nor did it affect the extent of cell division that occurred during the 70-min incubation; assays of SC30-TF-SP cells harvested from the plates showed that viable cell titer on plates without CAP increased an average of 3.1-fold, with no significant difference between those incubated at 30°C and at 42°C; viable cell titer on plates containing CAP increased an average of 1.1-fold during the 70-min incubation.

lexA71::Tn5 strain, in which synthesis of LexA-repressed gene products is constitutively derepressed, means that no new gene product(s) need be synthesized during the incubation at 42°C for thermal enhancement of spontaneous and UV-induced mutability to occur in strain SC30-TF-SP.

DNA Replication in the Presence of CAP. Since SOS mutagenesis requires DNA replication, our results imply that strain SC30-TF-SP synthesizes DNA in the presence of CAP. In uninduced cells, inhibition of protein synthesis prevents reinitiation of DNA synthesis at the chromosomal origin and, therefore, permits replication to continue only until the round in progress terminates (11). One facet of the SOS response, however, is "stable DNA replication" (12), the ability to replicate DNA continuously in CAP. Fig. 1 shows that, at 42°C with adenine present, strain SC30-TF-SP expresses stable DNA replication, continuously incorporating labeled thymidine; the amount of DNA in the culture, as determined by a chemical method (13), increases more than 4-fold during the experimental period (data not shown). At 30°C without adenine, the strain does not express stable DNA replication. Thus, the heat-plus-adenine treatment results in the acquisition of the stable DNA replication capability, and this property is acquired in the absence of protein synthesis.

Fig. 1 also shows data for DNA replication in CAP for a *lexA71::Tn5 recA⁺* strain, which, like uninduced wild-type strains (12), does not express stable DNA replication at 42°C with adenine or at 30°C without adenine. Like SOS mutagenesis, therefore, stable DNA replication requires RecA⁺ activity even in a strain in which all LexA-controlled genes are constitutively derepressed.

Results qualitatively similar to those shown in Table 3 were obtained when protein synthesis was inhibited by starvation for tryptophan instead of by CAP or when the various treatments were administered in liquid medium, duplicating the conditions shown in Fig. 1. However, the enhancement of mutagenesis by the heat-plus-adenine treatment was less

FIG. 1. DNA replication in the presence of CAP in *lexA::Tn5* strains. Cultures of SC30-TF-SP (*lexA::Tn5 recA441*) (●, ○) and SC30-RP-SP (*lexA71::Tn5 recA⁺*) (▲, △) were grown at 30°C to titers of 2.3×10^8 and 2.6×10^8 cells per ml, respectively. Adenine (100 μ g/ml), CAP (25 μ g/ml), 3 H-thymidine (10 μ Ci/ml; specific activity 1 μ Ci/ μ g; 1 Ci = 37 GBq), and deoxyadenosine (300 μ g/ml) were added to one portion of each culture (●, ▲), and all the above except adenine were added to another portion of each (○, △). The cultures containing adenine were incubated at 42°C, and those not containing adenine, at 30°C. Samples (0.1 ml) were withdrawn, and radioactivity in acid-insoluble fractions was determined as described (12).

pronounced in liquid than on solid medium. Under the liquid-medium conditions, amounts of DNA per culture of SC30-TF-SP, determined by a chemical method (13), increased 4-fold and 1.6-fold in the absence and the presence of CAP, respectively, during a 70-min incubation at 42°C.

DISCUSSION

We have shown that SOS mutator activity can be enhanced by a 70-min heat-plus-adenine incubation in a *recA441 lexA*-(Def) strain whether or not CAP is present during this treatment. This result is consistent with the hypothesis that the additional RecA⁺ function in SOS mutagenesis is not the inactivation of an unknown repressor—i.e., that no RecA⁺-inducible gene outside the LexA regulon has an essential role in SOS mutagenesis. Such a conclusion is valid, however, only if the additional mutations caused by the thermal treatment, with CAP present, can be shown to have occurred during the 70-min incubation rather than later, after the removal of CAP and return to 30°C. A delayed effect could result, for example, from unusually slow replacement of an unknown repressor inactivated at 42°C, permitting synthesis of a necessary gene product to begin, and to continue for some time, after the removal of CAP and transfer to 30°C. Several considerations argue against this possibility. When the populations of SC30-TF-SP cells harvested from plates incubated at 42°C without CAP were assayed immediately after the 70-min treatment, the frequencies of Trp⁺ mutants already phenotypically expressed (as determined by plating on unsupplemented E agar) were actually higher than those later observed in the NB cultures inoculated with these cells. In the experiment described in Table 3, these frequencies were 12.2 and 93.4 mutants per 10^8 cells for unirradiated and irradiated populations, respectively, as compared with the final frequencies (7.3 and 66.4) for the corresponding NB cultures. Reconstruction experiments (data not shown) revealed that the proportion of Trp⁺ revertants in artificial mixtures with Trp⁻ cells was reduced to about two-thirds of the initial value during growth in NB cultures under conditions comparable to those described in Table 3. Correcting for this degree of selection against Trp⁺ revertants, we conclude that all or most of the enhanced yields of Trp⁺ mutants in this population were already phenotypically expressed (and, therefore, necessarily already genetically fixed) by the end of the heat-plus-adenine treatment and that no important part of the enhanced yields could be ascribed to persistent effects of this treatment after the transfer to 30°C.

The evidence against persistent expression of the heat-plus-adenine-enhanced mutagenic activity when CAP was not present during the treatment argues against unusually slow replacement of an unknown repressor. However, since phenotypic expression requires some protein synthesis, it cannot be directly demonstrated that the enhanced yield of Trp⁺ mutations was genetically fixed in the presence of CAP. In fact, no elevation of the mutant frequency was observed when the SC30-TF-SP cells were plated on unsupplemented E agar immediately after the heat-plus-adenine-plus-CAP incubation, in contrast to the result when CAP was absent during the heat treatment. Nevertheless, unless the inocula taken from the two populations already contain similarly elevated frequencies per nucleoid of genetically fixed Trp⁺ mutations, regardless of phenotypic expression, they would be unlikely to generate NB cultures with such similar final Trp⁺ mutant frequencies. A delay of one generation in the occurrence of a mutation reduces by half the size of the final clone produced. We cannot exclude the possibility that CAP delays the expression of a gene induced at 42°C while also increasing the magnitude of the delayed mutagenesis sufficiently to compensate for the delay, a combination of effects that could explain our results. The simplest interpretation of

our data, however, is that the unknown role of RecA* in SOS mutagenesis is not the derepression of any gene(s) outside the LexA regulon.

It could be argued that any DNA replication occurring in the presence of CAP might be characterized by reduced fidelity and, therefore, possibly result in a mutator activity quite different from the SOS mutator activity that occurs when CAP is not present. In that case, CAP might actually prevent heat-enhancement of SOS mutator activity (e.g., by preventing expression of a gene outside the LexA regulon) but could replace it by another kind of mutator activity, requiring RecA* activity only to permit enough DNA replication for its expression. Fig. 1 shows that about the same amount of DNA is synthesized in CAP by strain SC30-TF-SP in a 70-min incubation at 30°C as at 42°C with adenine, although only the latter condition, which increases RecA441* activity, promotes both stable DNA replication and enhancement of mutagenesis. Thus, DNA replication in CAP is not necessarily more mutagenic than normal replication. Additional evidence for this conclusion is the failure of a heat-plus-adenine-plus-CAP incubation to increase the yield of Trp⁺ mutants when the experiment in Table 3 is repeated with a *umuC* derivative of a *lexA(Def) recA441* strain, although stable DNA replication occurs under these conditions (data not shown). The enhanced mutagenesis observed in the CAP-treated populations is not only RecA*-dependent but also UmuDC-dependent, thus fulfilling the requirements that define SOS mutagenesis.

Both stable DNA replication and SOS mutator activity require RecA* not only for LexA cleavage (in *lexA*⁺ strains) but also for another function. In SOS mutagenesis, the second role of RecA* is probably not cleavage of another repressor, although our data do not rigorously exclude this possibility. In the case of stable DNA replication, our results permit the conclusion that the additional role of RecA* is not another antirepressor activity. This follows from the acquisition and expression of stable DNA replication capability by the *lexA71::Tn5 recA441* strain during incubation at 42°C

with adenine and CAP (Fig. 1), conditions that increase RecA* activity but inhibit protein synthesis. It is possible that a single RecA* activity modifies the replisome of SOS-induced cells so as to reduce both the fidelity of DNA replication and its requirements for reinitiation (12, 14). Such a modification might involve direct participation of RecA* as part of the replisome or proteolytic processing of one or more replisome proteins. Alternatively, two distinct activities of RecA* could cause these changes independently.

Note Added in Proof. D. G. Ennis and D. W. Mount (personal communication) have obtained evidence that SOS mutagenesis in bacteriophage λ requires RecA* activity even in LexA (Def) host strains.

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