

# Identification of the *recA* (*tif*) gene product of *Escherichia coli*

(protein X/SOS functions/two-dimensional gel electrophoresis/*lexA* gene)

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**ABSTRACT** Treatments that inhibit DNA synthesis in *recA*<sup>+</sup> *lexA*<sup>+</sup> *Escherichia coli* stimulate synthesis of a 40,000 molecular weight protein species (protein X). The protein X molecules produced by wild-type and mutant *E. coli* strains have been compared by two-dimensional gel electrophoresis. One *recA* mutant (DM1415 *spr recA1*) produced a protein X with a more acidic isoelectric point than protein X from the wild type, demonstrating that protein X is probably the product of the *recA* gene. Additional mutants carrying the *recA*-linked *tif-1* mutation yielded a protein X that was more basic than the wild-type protein, indicating that the *tif-1* mutation also alters the *recA* protein. Protein X molecules from the above mutants and wild-type *E. coli* have been shown to yield similar partial products upon limited proteolysis in sodium dodecyl sulfate, indicating they are the same protein species. These and additional studies suggest that (i) the *tif-1* mutation alters a site on the *recA* protein that is sensitive to DNA synthesis inhibition, (ii) synthesis of *recA* protein is self-regulated, and (iii) synthesis of *recA* protein is also regulated by the *lexA* product with *lexA*-suppressor mutations such as *spr* resulting in constitutive synthesis of *recA* protein.

The rates of synthesis of various membrane and cytoplasmic proteins of *Escherichia coli* are altered when DNA synthesis is inhibited or DNA is damaged (1). One protein, the 40,000 molecular weight (*M<sub>r</sub>*) protein X, is induced by inhibition of DNA synthesis in wild-type *E. coli* but is not induced in *recA* and *lexA* mutants of *E. coli* (2-5). These mutants are extremely sensitive to UV light and to various treatments that damage DNA; *recA* mutants are also deficient in genetic recombination. Both mutants also fail to induce normally other important cellular functions including those for prophage induction, inhibition of cell division, and induced mutagenesis. These have been collectively named "SOS functions" because they are thought to be a coordinated response for improved cellular survival (for review, see ref. 6). *recA* and *lexA* mutations map at 58 and 90 min, respectively, on the *E. coli* linkage map (7,8). McEntee *et al.* (9) recently identified the *recA* protein in cells infected with  $\lambda$ *precA* transducing phage. The *lexA* gene product has not yet been identified but it could be a regulatory protein because *lexA* mutations are dominant in F-*lexA*<sup>+</sup>/*lexA* heterozygous strains (10).

In the *tif* mutant of *E. coli*, the SOS functions appear to be expressed constitutively at 40-42° in the absence of an inducing treatment (11, 12). *tif* maps at or near the *recA* gene and has been proposed to influence the expression of *recA* (11, 13). Derivatives of *lexA* mutants resistant to UV light and mitomycin C have been isolated that also show constitutive expression of certain inducible processes. These have been shown to carry an additional mutation that maps at or near the *lexA* gene (13, 14). These mutations appear to alter the regulatory

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properties of the *lexA* product. One class of derivatives (*tsl*) forms filaments and synthesizes protein X at a high rate at 40-42° (14, 15). Another mutation (*spr*), which maps near the *lexA* gene, results in a high rate of constitutive expression of the SOS functions at 30-42° in a *tif sfi* genetic background (13). The *sfi* mutation in this strain stops filamentous growth of the cells but does not influence the constitutive expression of the other SOS functions (16).

This report describes the synthesis of protein X in a number of these *recA*, *tif*, and *lexA* derivatives. Using two-dimensional electrophoresis, we show that both *tif* and a certain missense *recA* mutation (*recA1*) alter the charge on protein X. This and other observations have led us to conclude that protein X is the *recA* gene product, that *tif* alters this product structurally, and that the *lexA* gene regulates the rate of synthesis of the *recA* product.

## MATERIALS AND METHODS

**Bacterial Strains.** The properties of the bacterial strains and their origins are listed in Table 1.

**Media.** The bacteria were grown at 37° in M-9 minimal medium plus 0.4% glucose, thiamin, and the appropriate amino acids as described (1). Temperature-sensitive mutants were grown at 30°; the nonpermissive temperature was 41°.

**Labeling with [<sup>35</sup>S]Methionine.** Exponentially growing bacteria were labeled with [<sup>35</sup>S]methionine (5  $\mu$ Ci/1-2 ml of sample) for 10 min at the appropriate temperature. The bacteria were then poured onto frozen M-9 medium containing a 1000-fold excess of nonradioactive methionine. The incorporation was linear over the labeling period. The bacteria were harvested by centrifugation at 8000 rpm in a Sorvall centrifuge at 4°, resuspended in 0.01 M Tris-HCl (pH 7.0), and recentrifuged.

**One-Dimensional Gel Electrophoresis.** Sodium dodecyl sulfate (NaDodSO<sub>4</sub>/) slab gel electrophoresis was performed as described (17).

**Two-Dimensional Gel Electrophoresis.** Electrophoresis was performed according to the procedure of O'Farrell (18) with a number of modifications.

**First dimension.** Ampholines of pH range 5-8 replaced those of pH range 5-7. The lysis buffer contained 9.5 M urea, 2% (wt/vol) Nonidet P-40, 2% Ampholines (1.6% pH 5-8, 0.4% 3.5-10), 5% 2-mercaptoethanol, and 10% glycerol. Bacteria were resuspended in 100  $\mu$ l of this buffer, sonicated for 10 sec with a Biosonik III (Bronwill) sonicator, and loaded onto the first-dimension isoelectric focusing gel. Cell extract in lysis buffer (10-20  $\mu$ l) containing ~500,000 cpm of [<sup>35</sup>S]methionine was loaded on each gel. The isoelectric focusing gels were poured as described (18) and overlaid with 8 M urea. It is difficult to determine the pH gradient and the isoelectric point

Abbreviations: *M<sub>r</sub>*, molecular weight; NaDodSO<sub>4</sub>, sodium dodecyl sulfate; pI, isoelectric point.

Table 1. Properties of bacterial strains

Strain	Relevant genotype					Origin
	<i>recA</i>	<i>tif</i>	<i>lexA</i>	<i>spr</i>	<i>sfiA</i>	
JM1	+	+	+	+	+	J. George
JM12	+	1	+	+	+	J. George
GC3217	+	1	+	+	11	J. George
DM1180	+	1	3	+	11	Ref. 13
DM1187	+	1	3	51	11	Ref. 13
DM1420	+	+	3	51	11	Ref. 13
DM505	56	+	+	+	+	*
DM1415	1	+	3	51	11	†

\* JC5088 Hfr KL16 *thr-300 ilv-318 recA56 spec-300* × JM1 *his-4 strA31*; His<sup>+</sup> [Str<sup>R</sup>] recombinants selected. JC5088 was obtained from A. J. Clark.

† KL16-99 Hfr KL16 *recA1* × DM1187 *his-4 strA31*; His<sup>+</sup> [Str<sup>R</sup>] recombinants selected. KL16-99 was from K. B. Low.

(pI) of protein X precisely because of the high concentration of urea (18). After polymerization, the urea was removed and the samples were applied, covered with overlay solution according to O'Farrell (18), and focused for 10,000 V-hr. No prerun of the gels was performed.

**Equilibration.** The samples were equilibrated according to the method of O'Farrell (18) except that the equilibration period was 5–10 min.

**Second dimension.** A 12% acrylamide gel was used, and the length of the second-dimension NaDodSO<sub>4</sub>/slab gel was increased to 228 mm.

**Peptide Mapping by Limited Proteolysis in NaDodSO<sub>4</sub>.** This was performed according to the method of Cleveland *et al.* (19), except that spots were cut from two-dimensional gels

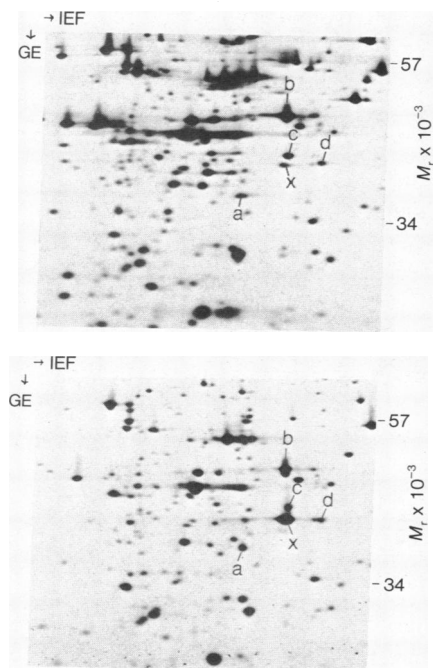


FIG. 1. Proteins of wild-type *E. coli* JM1 in the absence (Upper) and presence (Lower) of the DNA synthesis inhibitor nalidixic acid. A culture growing exponentially at 30° was labeled with [<sup>35</sup>S]methionine before and after addition of nalidixic acid (40 μg/ml) and harvested, and total cell proteins were subjected to two-dimensional electrophoresis (IEF, isoelectric focusing; GE, NaDodSO<sub>4</sub> gel electrophoresis). Only a portion of the gel autoradiogram is shown here. Protein X is indicated by the X on the gel; the other labeled proteins are reference proteins to aid in comparisons.

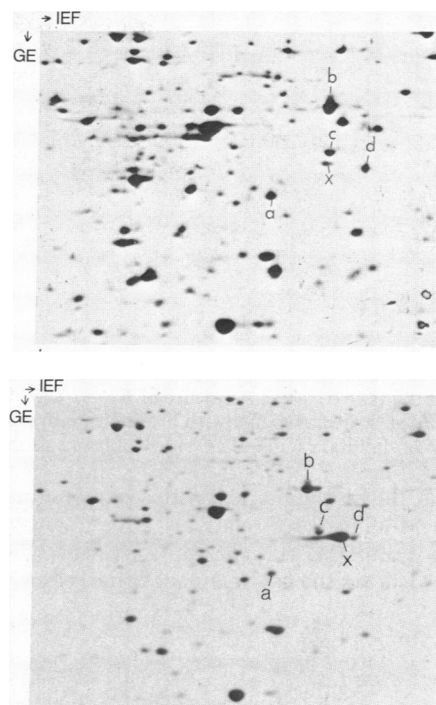


FIG. 2. Two-dimensional gel electrophoresis of total proteins from *E. coli* strains carrying a *recA* mutation. All *E. coli* strains were grown at 30° and labeled with [<sup>35</sup>S]methionine 40 min after the addition of nalidixic acid (40 μg/ml). Notations as in Fig. 1. (Upper) *E. coli* DM505 (*recA56*). (Lower) *E. coli* DM1415 (*spr tif+ recA1 sfi*).

after staining and destaining. After staining with Coomassie blue dye, the protein spots of interest were cut out of the gels, washed three times with distilled water and placed in Tris buffer (pH 6.8) and 0.1% NaDodSO<sub>4</sub> for 30 min at room temperature. The pieces of gel containing the protein of interest were then loaded into wells of a one-dimensional NaDodSO<sub>4</sub>/polyacrylamide slab gel, overlaid with 0.1 μg of α-chymotrypsin, and digested by turning off the current for 30 min just prior to the entrance of the dye front into the running gel.

**Staining and Drying Gels, Autoradiography, and Densitometry.** These procedures were performed as described (1).

**Chemicals.** [<sup>35</sup>S]Methionine (100–400 Ci/mmol) was from New England Nuclear, Boston, MA. Nalidixic acid and mitomycin C were from Sigma, St. Louis, MO. Ampholines were obtained from LKB. Acrylamide, *N,N'*-methylene bisacrylamide, and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were from Eastman Kodak. All other electrophoresis chemicals were purchased from described sources (18).

## RESULTS

Fig. 1 shows an enlarged portion of the two-dimensional protein pattern containing protein X, from a wild type *E. coli* strain (JM1) labeled with [<sup>35</sup>S]methionine in the absence or presence of nalidixic acid as an inducing agent. A similar pattern was obtained after treatment with mitomycin C (data not shown). Although minor variations in spot intensities were observed from one experiment to another, the protein X spot consistently showed the changes in position and intensity described below. As observed previously (1), protein X was induced by nalidixic acid; without it, only a slight trace of protein X was seen. The pI of protein X under denaturing conditions (urea and Nonidet P-40) was between pH 6.1 and 6.3.

Although little protein X was induced in a *recA* mutant—see,

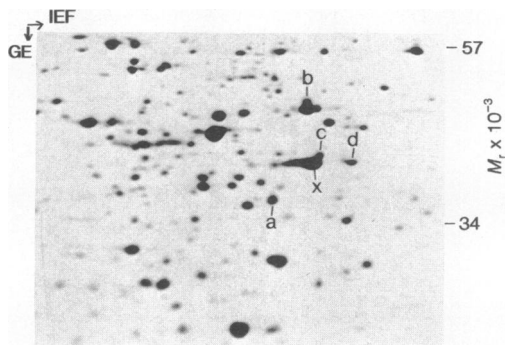


FIG. 3. Two-dimensional electrophoresis of total proteins from *E. coli* DM1420 (*spr tif<sup>+</sup> sfi*). A culture of *E. coli* DM1420 growing exponentially at 30° was labeled with [<sup>35</sup>S]methionine 40 min after the addition of nalidixic acid (40 μg/ml). Notations as in Fig. 1.

for example, the result obtained with a *recA56* mutant (Fig. 2 upper)—we found that a relatively large quantity was synthesized in strain DM1415 (*spr recA1 sfi*) with (Fig. 2 lower) or without (data not shown) treatment of the cells with nalidixic

acid. A large quantity was also obtained with a *spr recA56* mutant carrying the same *recA* mutation as the strain shown in Figure 2 upper (data not shown). We conclude that the *spr* mutation allows a high rate of synthesis of protein X in these *recA* mutants. By comparing the position of the protein X made by strain DM1415 with respect to nearby reference proteins, we conclude that protein X from DM1415 has a more acidic pI than does the wild-type protein X. This charge difference is not due to the presence of the *spr* mutation because the protein X from another *spr* mutant DM1420 (*spr recA<sup>+</sup> sfi*) was also produced either with or without an inducing treatment but in this case the pI of the protein was the same as in the wild-type strain (Fig. 3, and other data not shown).

We conclude that the *recA1* mutation is probably a missense mutation that alters the charge of the *recA* protein and, thus, that protein X is likely to be the product of the *recA* gene. The presence of the *spr* mutation appears to lead to a high rate of constitutive synthesis of the protein, even when it is functionally inactive in the *spr recA1* mutant.

The protein X synthesized by various mutants carrying the *tif-1* mutation (strains JM12, GC3217, and DM1187) had a

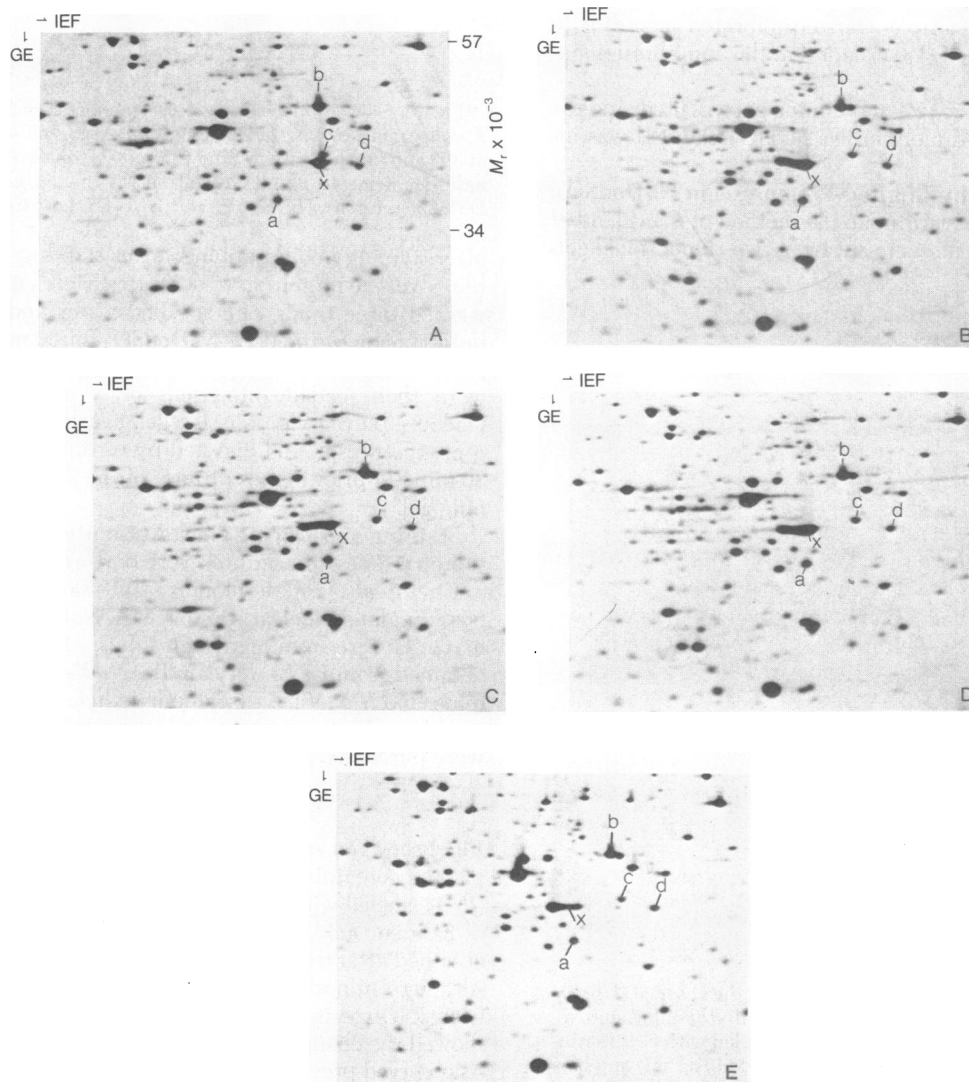


FIG. 4. Two-dimensional gel electrophoresis of total proteins from *E. coli* strains carrying the *tif-1* mutation. All *E. coli* strains were growing exponentially at 30° and were labeled with [<sup>35</sup>S]methionine 40 min after the addition of nalidixic acid (40 μg/ml). Notations as in Fig. 1. (A) *E. coli* wild-type JM1. (B) JM12 (*spr<sup>+</sup> tif sfi<sup>+</sup>*). (C) DM1187 (*spr tif sfi*) but labeled with [<sup>35</sup>S]methionine before addition of nalidixic acid. (D) DM1187 (*spr tif sfi*). (E) GC 3217 (*spr<sup>+</sup> tif sfi*).

more basic pI compared to the wild-type protein (Fig. 4). The position of protein X was the same whether or not the cells were treated with an inducing agent (Fig. 4, and other data not shown). A similar observation has been made in other laboratories (P. T. Emmerson and S. C. West; K. McEntee, personal communications). This change likely indicates that *tif* also alters the charge of the protein X by an amino acid substitution. Because protein X appears to be the *recA* product (see above), this result shows further that *tif* alters the structure of the *recA* protein.

The *tif* mutant JM12 (*spr*<sup>+</sup> *tif sfi*<sup>+</sup>) showed the pattern of protein X synthesis described previously (15). Only a small amount of protein X was synthesized at 30° unless nalidixic acid was added; at 41°, protein X was synthesized constitutively (data not shown), as described above for two strains carrying *spr*. A similar result was obtained with strain GC3217 (*spr*<sup>+</sup> *tif sfi*) (Fig. 4 D). These *tif* mutants express SOS functions constitutively at 41°; this expression is apparently related to extensive synthesis of the *tif*-modified *recA* protein at 41°. Strain DM1180 (*lexA tif sfi*) did not produce protein X after growth at 41° or after addition of nalidixic acid (data not shown). This result is consistent with the known inability of the strain to induce the SOS functions at 41° because the *lexA* mutation blocks the expression of *tif* (13). In strain DM1187 (*spr tif sfi*), protein X was synthesized constitutively at both 41° and 30° (Fig. 4 C); addition of nalidixic acid stimulated synthesis (Fig. 4 D) about 3-fold as judged by densitometric measurements of films exposed to one-dimensional NaDodSO<sub>4</sub> gels (data not shown). This strain is a UV- and mitomycin C-resistant derivative of DM1180 and carries an additional mutation *spr* that maps at or near the *lexA* locus and blocks *lexA* expression. It expresses the SOS functions constitutively at 30° and 41° (13) and, as in the case of the *tif* mutants JM12 and GC3217, this expression is related to the constitutive production of *tif*-modified *recA* protein.

To establish that the apparent charge alterations were actually in protein X, peptide mapping in a one-dimensional NaDodSO<sub>4</sub>/acrylamide gel was performed according to Cleveland *et al.* (19). The results of this experiment are shown in Fig. 5. The protein X spot from each of the different mutants showed a similar pattern of peptide fragments. The 45,000 *M<sub>r</sub>* protein from each of the different mutants gave a strikingly different pattern of fragments. The similarity of the peptide fragments generated from protein X molecules from different mutants mapping at the *recA* locus strongly suggests a common identity for these mutant proteins.

## DISCUSSION

We have shown that various *E. coli* mutants that map in the *recA* region produce protein X molecules with altered charges (Figs. 1–4). Preliminary peptide mapping indicates that these protein X molecules with different net charges yield similar peptides when treated with chymotrypsin (Fig. 5). The most plausible explanation for this result is that the mutations studied alter an amino acid that in turn changes the pI of protein X. We conclude that protein X is the product of the *recA* gene. A similar conclusion has been reached by K. McEntee (personal communication) based upon similar studies with a different *recA* mutant. Moreover, Little and Kleid (20) have found that purified protein X and the radiochemically pure *recA* protein induced by infection with λ*precA* (9) yield similar peptides.

We have not ruled out the possibility that the charge differences in protein X are due to different degrees of modification of protein X in the various strains. However, we find this explanation unlikely because both a more acidic pI and a more

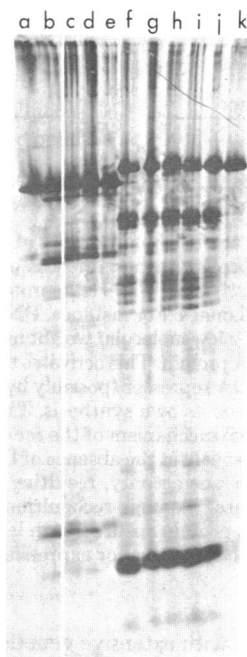


FIG. 5. Peptide mapping by limited proteolysis in NaDodSO<sub>4</sub>. Stained protein X spots and spots from a 45,000 *M<sub>r</sub>* protein for comparison were cut from the two-dimensional gels shown in Figs. 1–4 and subjected to α-chymotrypsin proteolysis in a one-dimensional NaDodSO<sub>4</sub> gel. After electrophoresis, the gel was dried and autoradiographed. Tracks are: a–e, protein X; f–k, 45,000 *M<sub>r</sub>* protein; a, JM1 protein X, no α-chymotrypsin; k, JM1 45,000 *M<sub>r</sub>* protein, no α-chymotrypsin; b–j, with α-chymotrypsin; b, JM1; c, DM1420; d, DM1415; e, DM1187; f, JM1; g, JM12; h, DM1420; i, DM1415; j, DM1187.

basic pI relative to the pI of wild-type protein X were detected in the mutants. In addition, protein X from both mutant and wild-type strains is phosphorylated (data not shown). It should be possible to determine how many charge units separate the mutant and wild-type proteins by the carbamylation technique of Steinberg *et al.* (21).

The identity of protein X as the *recA* protein should lead to an improved understanding of the mechanism of induction of the SOS functions. McEntee *et al.* (9) have shown that the *recA* protein may exist in the cell as a tetramer, although its subunit *M<sub>r</sub>* under denaturing conditions is 40,000. It appears to be synthesized in small quantities constitutively as judged by the presence of a weak spot at the protein X position (Fig. 1 upper). This weak spot appears to be protein X because the various *tif* mutants produce a protein X with a different pI at a different location on the gels and there is no spot found at the wild-type protein X position on these same gels (Fig. 4 B–E). A low level of *recA* protein synthesis is presumably sufficient to allow recombination in a *recA*<sup>+</sup> strain. Its rate of synthesis is greatly increased in wild-type *E. coli* treated with an inducing agent but not in *lexA* or *recA* mutants. Synthesis of the *recA* protein is constitutive in strains carrying *spr* mutations which are closely linked suppressors of *lexA* mutations. Moreover, this constitutive synthesis does not depend upon a functional *recA* product because DM1415 *spr recA1* double mutants still produce a missense product (ref. 15; above results). To account for these observations, we propose that the *lexA* gene product is a repressor of *recA* protein synthesis. In cells with damaged DNA, this repressor is inactivated, leading to induction of *recA* protein. *lexA* mutants make a repressor that cannot be inactivated; *tsl* suppressor mutations make it temperature-sensitive; and *spr* mutations decrease its activity. This repressor role of the *lexA*

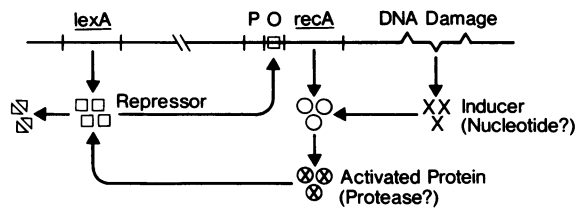


FIG. 6. Model for the biochemical roles of *recA* protein X in induction. In uninduced cells, synthesis of *recA* protein is repressed by the product of the *lexA* gene. The *recA* protein has a recognition mechanism for DNA damage. For instance, DNA damage might lead to the accumulation of a low molecular weight inducer molecule which combines with the *recA* protein. This activates the *recA* protein which in turn destroys the *lexA* repressor (possibly by proteolytic cleavage) and thereby derepresses its own synthesis. The *tif* mutation alters the damage-recognition mechanism of the *recA* protein so that activation occurs spontaneously in the absence of DNA damage. The *spr* mutation lowers repressor activity, resulting in constitutive *recA* protein synthesis. These damage-recognition and autoregulatory properties of the *recA* protein ensure a high level of the protein in a form that is biochemically active for expression of the various SOS functions of the cell.

product is consistent with extensive genetic studies (10, 13, 14, 22).

We have shown that the *tif* mutation alters the *recA* protein and the result of this change is constitutive expression of SOS functions and synthesis of *recA* protein at 41° in the absence of DNA damage. We conclude that wild-type *recA* protein must possess a mechanism for recognition of DNA damage. It appears that the protein must be activated in response to DNA damage in order to carry out its function. The *recA* protein could bind to a low molecular weight inducer molecule such as a nucleotide or DNA fragment. Alternatively, *recA* protein X binds to DNA (1), and contact of the protein with damaged DNA could activate it. The *tif* mutation could affect a site on the *recA* protein that recognizes DNA damage such that spontaneous activation occurs. For instance, its affinity for the low molecular weight inducer might be increased, particularly at 41°. After this activation step, the biochemically active protein could stimulate further synthesis of itself which would also be activated by inducer. The mutant DM1187 synthesizes the *tif*-modified form of the *recA* protein at a high rate, and this strain expressed SOS functions constitutively with high efficiency. This result is further evidence that a large amount of activated *recA* protein is necessary for induction. In addition, extracts of this strain contain an ATP-dependent protease that cleaves the  $\lambda$  repressor *in vitro* (ref. 23; unpublished data). This activity could clearly be that of the *tif*-modified *recA* protein itself.

Fig. 6 describes a model for the induction of *recA* protein, in which activated *recA* protein attacks the *lexA* coded repressor, allowing further synthesis of *recA* protein. This autoregulatory mechanism supplies an abundance of *recA* protein in a form that is active biochemically for induction of the various SOS functions. *recA* protein from induced cells binds to single-stranded DNA (1) and it could thereby modulate mutagenic DNA repair, inhibition of DNA degradation, UV-reactivation, and recombination. Moreover, 10% of the *recA* protein X is found associated with the cell membrane (1) and could be involved in the attachment of DNA to the membrane and in the regulation of membrane functions related to cell division (24).

In support of our model of transcriptional regulation of the

*recA* protein, it has been shown that low concentrations of rifampicin (2  $\mu$ g/ml) inhibit protein X synthesis without significantly affecting total protein synthesis (G. Satta, L. J. Gudas, and A. B. Pardee, unpublished data). In addition, in the presence of rifampicin plus nalidixic acid, all the pleiotropic effects of the *recA* deficiency occurred in wild-type *E. coli*, suggesting that the *recA* protein alone is responsible for these effects.

It appears likely that many of the pleiotropic effects of *recA* and *lexA* mutations can be explained by modifications in regulation of *recA* protein synthesis or its basic biochemical properties. We have evidence that the phenotypes of partial revertants of *lexA* mutants which show increased expression of some but not all SOS functions (see ref. 6) can be accounted for by variations in the extent of activation, the rate of synthesis, and the duration of synthesis of the *recA* protein after DNA damage, as altered by various *recA* and *lexA* mutations (unpublished data).

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