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Protein X is the product of the *recA* gene of *Escherichia coli*

(specialized transducing phages/gel electrophoresis/isoelectric focusing/*tsl*, *recA12*, and *tif*-1 mutations/SOS functions)

KEVIN MCENTEE*

Department of Biochemistry and of Biophysics and Theoretical Biology, The University of Chicago, Chicago, Illinois 60637

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ABSTRACT **The inducible protein X of *Escherichia coli* has been compared to the *recA*$^+$ protein made by specialized *recA* transducing phages. The molecular weights and isoelectric points of these proteins are identical. Two mutations located in the *recA* gene that alter the electrophoretic mobility or the isoelectric point of protein X have been studied. A *recA12* mutant strain, deficient in homologous recombination and repair, produces a smaller-than-normal protein X. A transducing phage carrying the *recA12* allele directs the synthesis of a smaller *recA* protein after infection of irradiated cells. A transducing phage carrying the *recA* region of a *tif-1* mutant strain codes for a *recA* protein with an isoelectric point more basic than that of the λ*precA*$^+$ product. The protein X of a *tif-1* mutant strain shows an identical shift in its isoelectric properties. Examination of several *tsl*$^-$ *recA*$^-$ strains indicates that protein X can be induced in several missense *recA* mutants but is not detected in *tsl*$^-$ strains carrying amber or deletion mutations of the *recA* gene. These results demonstrate that protein X is the product of the *recA* gene and that the *tif-1* mutation alters the properties of the *recA* protein. A model is suggested for autoregulation of the *recA* protein in the induction of functions expressed in response to DNA damage (SOS functions).**

In *Escherichia coli* a complex set of responses is observed after treatments that disrupt DNA synthesis or damage DNA. UV irradiation of cells produces mutations, induces prophage λ in lysogens, and stimulates reactivation and mutagenesis of phage containing DNA damage (Weigle or W-reactivation and W-mutagenesis) (see ref. 1 for review). Witkin (2) and Radman (3) have presented evidence that expression of these and other diverse processes (SOS functions) results from their activation by a common regulatory signal. SOS functions are not expressed constitutively in cells but are induced by treatments that inhibit DNA synthesis, such as UV or X irradiation, thymine starvation, and nalidixic acid or mitomycin C treatment (see ref. 1).

The principal evidence that mutagenesis, prophage induction, and W-reactivation share a common pathway is the isolation of pleiotropic mutations that alter the cells' response to inducing treatments. In *recA*$^-$ or *lexA*$^-$ strains, expression of SOS functions is not observed. These mutants display no UV-induced mutagenesis, W-reactivation, or prophage induction (3). Another mutation, *tif-1,* is closely linked to the *recA* gene (refs. 4 and 5; unpublished data) and shows conditional induction of SOS functions in the absence of DNA damage. All of the processes observed after exposure of wild-type cells to UV light or nalidixic acid are observed after shift of the *tif-1* mutant to high temperature (4). Furthermore, *tif-1*-mediated induction of SOS functions is abolished by *recA*$^-$ or *lexA*$^-$ mutations (6). The inducibility of SOS functions is demonstrated by kinetic studies of *tif-1-* or UV-mediated bacterial mutagenesis and phage λ reactivation (7, 8) as well as the demonstration that *de novo* protein synthesis is required after inducing treatments for expression of these functions (2, 8, 9).

At least one protein that is induced in *E. coli* after treatments eliciting expression of SOS functions has been studied. Inouye and Pardee (10) and Gudas and Pardee (11) have demonstrated that a 40,000 molecular weight protein, called protein X, is induced in wild-type cells by UV or nalidixic acid treatments or in *tif-1* strains at 42°. Induction of protein X is abolished in *recA*$^-$ or *lexA*$^-$ mutants. However, large amounts of protein X are made in *recA*$^-$ strains carrying a *tsl*$^-$ mutation (12). The *tsl*$^-$ mutation is tightly linked to the *lexA* locus and appears to alter expression of SOS functions (13). Although the induction of protein X is usually correlated with expression of SOS functions, the precise relationship between protein X and the genes controlling these inducible functions has not been determined.

The recent isolation of λ transducing derivatives carrying the *recA* gene has made possible the identification of the *recA* protein (14). A preliminary characterization of this protein suggested that it might be related to the inducible protein X (14). In this paper, biochemical and genetic evidence is presented that protein X is the product of the *recA* gene. Furthermore, a specialized phage carrying the *tif-1* mutation has been used to demonstrate that this mutation alters the *recA* protein. These observations provide strong support for the inducible SOS hypothesis (1, 3) and suggest that the *recA* product may be directly involved in the biochemical events responsible for expression of SOS functions.

MATERIALS AND METHODS

Bacterial Strains and Bacteriophages. The bacterial strains used are listed in Table 1. The *tsl*$^-$ *recA*$^-$ double mutant strains were derived from strain KM1200 by transduction to *srlC*$^+$ with P1*kc* grown on the appropriate *srlC*$^+$ *recA*$^-$ donor. The (*srl-recA*) deletions, Δ7 and Δ21, were transduced into a *cysC*$^-$ *tsl*$^-$ derivative by selecting *cysC*$^+$ recombinants. The Δ7 and Δ21 mutations were isolated as prophage deletions in strain KM2136 (18) and extend into the *recA* gene or its control region (unpublished data).

The λ*precA*$^+$ transducing phage has been described (18). A λ*ptif-1* phage was isolated from a low-frequency transducing lysate prepared from strain KM2157 as described for λ*precA*$^+$ (17). The λ*drecA12 alaS* phage was obtained from low-frequency transducing lysate of strain KM2168 by selecting *alaS*$^+$ *srl*$^+$ transductants of strain KM2055 (λ$^+$). Strains KM2157 and KM2168 were prepared by integrating λcI857 into the *srlA* gene of the non-lysogen as described (18). Unlike λ*precA*$^+$, neither λ*ptif* nor λ*drecA12 alaS* can transduce the *tif-1* strain JM888 (λ$^+$) to *tif*$^+$.

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Abbreviations: PMSF, phenylmethylsulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate.

* Present address: Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

Table 1. Bacterial strains used

Strain	Relevant genotype*	Source or comment
KM1200	*tsl2 srlC1 recA*$^+$	This work
KM1299†	*tsl2 recA99*	[DM455]
KM1201†	*tsl2 recA1*	[KL1699]
KM1212†	*tsl2 recA12*	[AB2462]
KM1213†	*tsl2 recA13*	[AB2463]
KM1207†	*tsl2 recA*Δ*7*	This work
KM1221†	*tsl2 recA*Δ*21*	This work
DM1187	*tif-1 spr51 sfiA11*	D. Mount (15)
JM888	*tif-1*	B. Low
DM800	*recA*$^+$ *lexA*$^+$	D. Mount (16)
DM844	*recA*$^+$ *lexA1*	D. Mount (16)
DM959	*recA*$^+$ *tsl2(lexA1)*	D. Mount (16)
KM2157	*tif-1 (gal-bio)*Δ2134 λ in *srl*	This work
KM2168	*recA12 (gal-bio)*Δ2134 λ in *srl*	This work
KM2055 (λcI$^+$)	*srlC5 alaS5* (λcI$^+$)	This work
KM601	*recA1*	(17)

* All *tsl2* mutant strains may carry the original *lexA1* mutation of strain DM844 (16). In the text, *tsl*$^-$ is equivalent to the *tsl2* allele.

† Derived by transduction of strain KM1200 to *srlC*$^+$ with P1*kc* grown on the *recA*$^-$ strain listed in brackets under Source.

Phage Protein Labeling, Electrophoresis, and Isoelectric Focusing. Labeling of transducing phage proteins and polyacrylamide gel electrophoresis were performed as described (14). Whole-cell preparations were labeled with either $^{35}SO_4$ (ICN, carrier free, 20 μCi) in 1 ml of M9S medium (19) or with L-[*U*-^{14}C]isoleucine (1 μCi, 330 mCi/mmol) in 1 ml of K115 medium (20) supplemented with the appropriate amino acids (20 mg/liter) and glucose (2 g/liter). Protein X was induced in *tif-1* or *tsl*$^-$ mutant strains by growth for 45 min at 42° and labeling for 20 min at 42°. Nalidixic acid induction of protein X was accomplished by growing cells for 45 min in medium containing the drug at 40 μg/ml after the addition of label for 20 min. After the labeling period, cells were chilled, washed with a solution containing 10 mM Tris (pH 7.5) and 1 mM phenylmethylsulfonyl fluoride (PMSF), and prepared for polyacrylamide gel electrophoresis (21) or isoelectric focusing. Samples were prepared for isoelectric focusing essentially as described by O'Farrell (22) with the following modifications. Cells were lysed by freeze-thawing in the presence of lysozyme (20 μg/ml), RNAse (20 μg/ml), and PMSF (1 mM). Without PMSF present during the lysis and subsequent DNase treatments, significant degradation of *recA* was detected on the two-dimensional gel. Samples in lysis buffer were loaded onto 7.5-cm isoelectric focusing gels that had been poured and prerun as described by O'Farrell (22). After focusing for 4200–5000 V-hr, the gels were frozen without equilibration in sodium dodecyl sulfate ($NaDodSO_4$) or loaded onto the second dimension. Electrophoresis on slab gels was done as described (22) for nonequilibrated isoelectric gels except that a 15% acrylamide gel containing $NaDodSO_4$ was used to display proteins in the second dimension. Gels were prepared for autoradiography or fluorography as described (14).

RESULTS

Treatment of *recA*$^+$ *lexA*$^+$ cells with nalidixic acid induces synthesis of a 40,000 molecular weight protein that can be seen on polyacrylamide gels (ref. 11; Fig. 1). Although copiously synthesized in *recA*$^+$ *lexA*$^+$ cells, this protein, called protein X, is not induced by nalidixic acid, UV irradiation, or mitomycin C in either *recA*$^-$ or *lexA*$^-$ mutant strains (ref. 12; Fig. 1). Comparison of the mobility of protein X on polyacrylamide gels with that of the *recA* protein made by the λ*precA*$^+$ transducing phage (14) demonstrates that protein X and the *recA*$^+$ gene product have identical subunit molecular weights (Fig. 1).

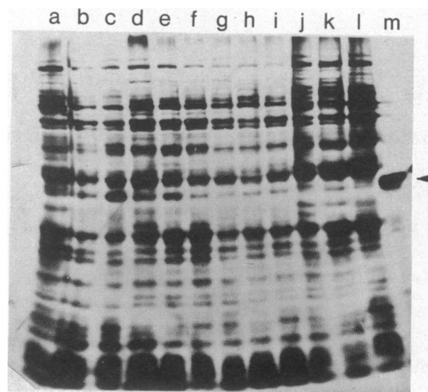

FIG. 1. Labeled whole-cell proteins showing induction of protein X in *recA* mutant strains. Cells were grown and labeled with L-[*U*-^{14}C]isoleucine, and samples (containing approximately 100,000 cpm) were electrophoresed in an 11% polyacrylamide gel. Migration was from top to bottom. Lanes: a, b, and c, strain JM888 (*tif-1*) grown at 30° (a), at 30° with nalidixic acid at 40 μg/ml (b), or at 42° with adenine (75 μg/ml) (c); d, e, and f, strain DM800 (*recA*$^+$ *lexA*$^+$) grown at 30° (d), at 30° with nalidixic acid (e), or at 42° (f); g, h, and i strain KM1299 (*tsl2 recA99*) grown at 30° (g), at 30° with nalidixic acid (h), at 42° (i); j and k, strain KM601 (*recA1*) grown at 30° (j) or at 30° with nalidixic acid (k); l, strain DM844 (*lexA1*) grown at 30° with nalidixic acid; m, *recA* protein (3000 cpm) made by λ*precA*$^+$ in strain 159 (λ*ind*$^-$).

Gudas (12) has shown that protein X can be induced in a *recA1* missense mutant if the strain also carries the *tsl*$^-$ mutation. This mutation causes conditional growth of cells and leads to high levels of protein X synthesis at both permissive and nonpermissive temperatures. The *tsl*$^-$ mutation, therefore, appears to bypass the requirement for the *recA*$^+$ product in the induction of protein X. It was of interest to examine the effects of other *recA* alleles upon protein X synthesis in a *tsl*$^-$ genetic background. Several nearly isogenic *recA*$^-$ derivatives of strain KM1200 were examined by polyacrylamide gel electrophoresis for protein X synthesis. A *recA1* as well as a *recA13* derivative of this *tsl*$^-$ strain showed protein X induction at 30° and 42° (data not shown). However, no protein X could be detected at any temperature or after nalidixic acid treatment of the *tsl*$^-$ *recA99* double mutant (Fig. 1). The *recA99* allele is an amber mutation in the *recA* gene (23). Furthermore, *tsl*$^-$ strains that carry deletion mutations into the *recA* gene (strains KM1207 and KM1221) did not show protein X synthesis at 30° or 42° or after exposure to nalidixic acid (Fig. 2). These results are consistent with the idea that the amber and deletion mutations are in the gene coding for protein X. No protein of molecular weight 40,000 would be produced in the amber *recA99* strain but a lower molecular weight amber fragment might accumulate. Although this amber fragment has not been detected in gel patterns of the *tsl*$^-$ *recA99* strain it might be rapidly degraded or, alternatively, it might comigrate with another cell protein in polyacrylamide gels.

Examination of cell proteins from a *tsl recA12* mutant strain (Fig. 2) indicates that protein X of this mutant has a slightly greater mobility in polyacrylamide gels. This smaller protein X (called protein X-12) is synthesized at high levels in the *tsl*$^-$ background. No protein of this molecular weight was induced in a *tsl*$^+$ *recA12* strain by nalidixic acid (data not shown).

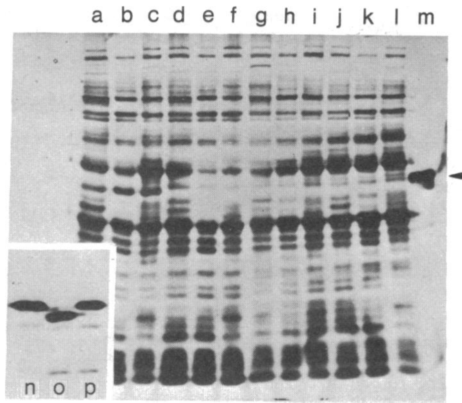

FIG. 2. Whole-cell proteins of *recA12* and *recA* deletion mutants labeled with L-[U-^{14}C]isoleucine and analyzed as in Fig. 1. Lanes: a, b, and c, strain KM1212 (*tsl2 recA12*) grown at 30° (a), at 30° with nalidixic acid (b), or at 42° (c); d, e, and f, strain KM2107 (*tsl2 recA*Δ*7*) grown at 30° (d), at 30° with nalidixic acid (e), or at 42° (f); g, h, and i, strain KM1221 (*tsl2 recA*Δ*21*) grown at 30° (g), 30° with nalidixic acid (h), or at 42° (i); j, k, and l, strain KM2103 (*tsl2 recA3*) grown at 30° (j), at 30° with nalidixic acid (k), or at 42° (l). The *recA3* mutation is a spontaneous UV-sensitive revertant of *tif-1* (unpublished data). A *recA* protein marker (3000 cpm) is shown in the last lane (m). (*Inset*) *RecA* proteins made by λ*precA*$^+$, λ*drecA12 alaS*, and λ*ptif-1*. Transducing phage proteins were labeled as described and analyzed by electrophoresis in an 11% polyacrylamide gel. Migration was from top to bottom. Lanes: n, λ*precA*$^+$; o, λ*drecA12 alaS*; p, λ*ptif-1*. Each lane contained approximately 5000 cpm. Upon longer exposure of this gel, the high molecular weight *alaS* proteins made by λ*drecA12 alaS* could be seen.

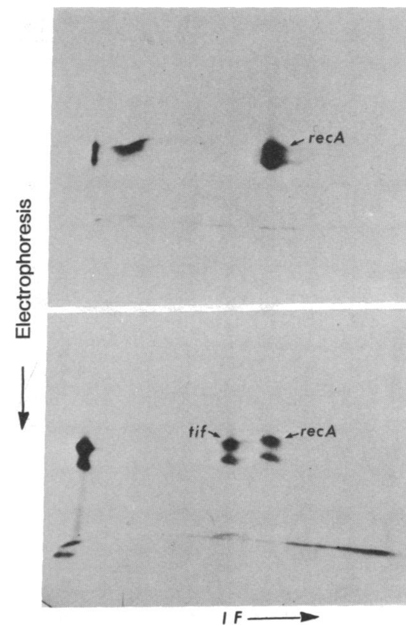

FIG. 3. Two-dimensional gel analysis of the *recA*$^+$ and *tif-1* proteins. Heavily UV-irradiated strain 159 (λ*ind*$^-$) was infected with λ*precA*$^+$ or λ*ptif-1* and the labeled proteins were analyzed. The direction of electrophoresis in polyacrylamide gels containing NaDodSO$_4$ is indicated by the arrow. The direction of the pH gradient is also indicated. (*Upper*) The *recA*$^+$ protein (arrow). (*Lower*) A mixture of extracts from λ*precA*$^+$ and λ*ptif-1* infected cells. The positions of the *tif-1* and *recA*$^+$ proteins are indicated.

A transducing phage carrying the *recA* region of a *recA12* strain was used to determine the electrophoretic properties of the *recA12* gene product. To ensure that the entire *recA* gene was carried by the phage, a transducing variant carrying the distal *alaS* gene in addition to the mutant *recA* gene and the *srlC* gene (14) was purified and used to infect heavily UV-irradiated cells. The *recA* protein made by the *recA12* transducing phage was smaller than the *recA*$^+$ product (Fig. 2 *inset*) and this change in mobility was identical to that observed for protein X-12 of the *tsl*$^-$ *recA12* strain. These results suggest that the *recA12* mutation may be a small deletion of the *recA* gene. The identical change in the electrophoretic mobility of protein X in the *tsl*$^-$ *recA12* strain KM1212 and the *recA* product coded by a transducing phage carrying the *recA12* allele argues strongly that protein X is the *recA* protein.

The *tif-1* mutation has been located extremely close to or within the *recA* gene by P1*kc* transduction (ref. 4; unpublished data). The conclusion that *tif-1* is allelic to *recA* is supported by studies with transducing phages for the *recA* region. The λ*precA*$^+$ phage complements a *tif-1* mutant host as judged by the ability of the lysogen to grow on minimal medium at 42°. Neither λ*psrl* (14) nor λ*precA99* (18) transduces *tif-1 sup*$^+$ strains to Tif$^+$ (unpublished data). These genetic results suggest that the *tif-1* mutation is in the *recA* gene. This possibility was investigated by isolating a λ transducing phage carrying the *recA* region of a *tif-1* strain and examining the *recA* protein of this variant on polyacrylamide gels containing NaDodSO$_4$. No difference in molecular weight was observed between the *recA* products of λ*precA*$^+$ and λ*ptif-1* (Fig. 2 *inset*). Furthermore, gel filtration experiments indicated that the undissociated *tif-1* protein is in a complex with a molecular weight of approximately 150,000 identical to the *recA*$^+$ product (ref. 14; unpublished data).

Analysis of the *recA*$^+$ product of λ*precA*$^+$ by the O'Farrell two-dimensional gel technique (22) revealed a single protein spot in the isoelectric dimension. Two protein spots were detected in the electrophoretic dimension in contrast to the single *recA* protein band observed on one dimensional gels (Figs. 1 and 3 *upper*). This lower molecular weight protein has an isoelectric point identical to that of the larger product. This smaller protein is believed to be a proteolytic fragment of the larger *recA*$^+$ protein because omission of the protease inhibitor PMSF during the preparation of cell extracts for isoelectric focusing significantly increased the intensity of the lower spot. A single isoelectric species also was seen when λ*ptif-1* infected cell extracts were analyzed by this technique (data not shown). A mixture of λ*ptif-1* and λ*precA* infected cell extracts revealed two distinct isoelectric species with identical molecular weights (Fig. 3 *lower*). One-dimensional isoelectric focusing of the *tif-1* and *recA*$^+$ products indicated that the *tif-1* product is more basic than the *recA*$^+$ protein (data not shown).

The protein X induced in *tif-1* strains has the same molecular weight as protein X induced in *recA*$^+$ cells (ref. 12; Fig. 1). Labeled whole-cell extracts of *tif-1* and *recA*$^+$ strains were compared by the two-dimensional gel system of O'Farrell (Fig. 4 *upper*). The identification of protein X in the two-dimensional gel patterns of *recA*$^+$ strain DM800 is based upon the following observations: (*i*) this protein was absent or decreased significantly in amount in extracts of cells not treated with nalidixic acid; (*ii*) this protein was made at high levels in the *tsl*$^-$ derivative DM959; and (*iii*) no protein at this position was made in *recA*$^-$ or *lexA*$^-$ strains after nalidixic acid treatment. Addition of a labeled extract of λ*precA*$^+$ infected cells to a labeled extract of uninduced strain DM800 resulted in the appearance of a protein at the same position as protein X in the induced cell pattern (data not shown). This result indicates that protein X from a *recA*$^+$ strain and the *recA*$^+$ product possess identical or extremely similar isoelectric points. A two-dimensional gel pattern of proteins from *tif-1* strain DM1187 is shown in Fig. 4 *lower*. This strain constitutively expresses SOS functions (15),

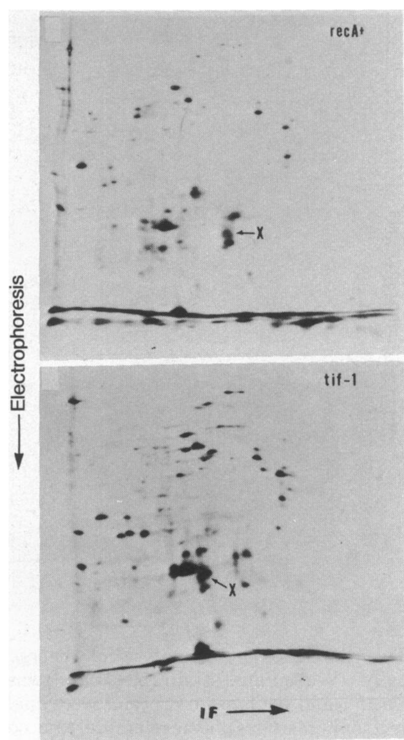

FIG. 4. Two-dimensional gel analysis of whole-cell proteins from *recA*+ and *tif-1* strains. The direction of electrophoresis in polyacrylamide gels containing NaDodSO$_4$ is indicated as well as the pH gradient. Protein X spot is indicated by the arrow. (*Upper*) Strain DM800 (*recA*+) was treated with nalidixic acid and labeled with $^{35}SO_4$ for 20 min. Samples containing approximately 100,000 cpm were analyzed and gels were exposed for 48–72 hr. (*Lower*) Strain DM1187 (*spr sfiA tif-1*) was treated with nalidixic acid and labeled with $^{35}SO_4$. Samples containing approximately 100,000 cpm were analyzed and gels were exposed for 48–72 hr. Although strain DM1187 makes high levels of protein X without nalidixic acid treatment, increased synthesis of protein X is seen after addition of the drug to this strain.

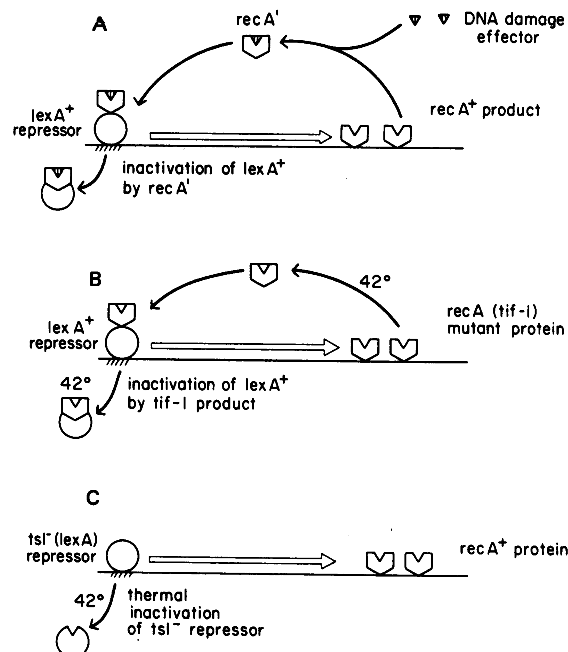

FIG. 5. Model for *recA* induction by UV-irradiation on *tif-1* or *tsl* expression. (*A*) The *recA*+ product interacts with an effector molecule produced after inducing treatment. This complex (*recA'*) inactivates the *lexA*+ repressor and allows transcription (large arrow) of the *recA* gene. (*B*) The *tif-1* allele of *recA* makes a protein which at 42° functions like *recA'* to inactivate the *lexA*+ repressor. (*C*) The *tsl*− mutation results in a partially defective repressor which is inactivated at 42° and allows transcription of the *recA* gene in the absence of any activated form of the *recA*+ protein.

carries a *tif-1* mutation, and is partially constitutive for protein X synthesis (unpublished data). The gel pattern of this *tif-1* mutant strain lacks a major protein spot at the location observed for protein X of induced *recA*+ strains. However, a major 40,000 molecular weight protein with a more basic isoelectric point was detected in the gel pattern of the *tif-1* mutant but was not observed in the *recA*+ protein pattern. This protein also was detected in gel patterns of another *tif-1* strain, JM888, grown at 42° (data not shown). The isoelectric point of protein X from this *tif-1* strain corresponded to the isoelectric point of the *recA* product made by λ*ptif-1*. The addition of a labeled λ*ptif-1* infected cell extract to a labeled extract of uninduced *recA*+ cells resulted in the appearance in the gel pattern of a protein at the position of protein X of the *tif-1* mutant (data not shown).

DISCUSSION

The identification of the inducible protein X of *E. coli* as the *recA* gene product has been demonstrated by the following observations: (*i*) the *recA*+ product made by a specialized transducing phage and protein X from a *recA*+ strain have identical molecular weights and isoelectric points; (*ii*) certain mutations in the *recA* gene alter the electrophoretic or isoelectric properties of protein X; and (*iii*) transducing phages carrying the mutant *recA* allele make an altered *recA* product with properties identical to those of the mutant protein X.

The observation that protein X is not induced in *tsl*− *recA99* or *tsl*− *recA*Δ*7* double mutants is consistent with this identification because a full-sized protein X would not be made in these strains. It is not unexpected that missense *recA* mutant strains would show induction of a protein with the same molecular weight as protein X because missense mutations do not generally alter the size of the mutant gene product. Some missense mutations of *recA*, including the *tif-1* allele, do alter the isoelectric properties of the protein. Recently, several additional *recA* mutations have been analyzed on transducing phages including a spontaneous *tif-1* revertant that is cold sensitive for recombination and expression of SOS functions. The isoelectric point of this mutant *recA* protein differs from that of the parental *tif-1* product. This is additional evidence that the *tif-1* mutation is in the *recA* structural gene.

The synthesis of high levels of the *recA* protein after inducing treatments of *tif-1* expression can be depicted as a positive feedback loop (Fig. 5 *A* and *B*). In the absence of an inducing agent, the *recA* gene is expressed at low levels. This basal level of *recA* protein is sufficient to promote homologous recombination. The inducing treatment may cause derepression of the *recA* gene by converting the *recA*+ protein to an altered form, *recA'*, which would activate the *recA* gene by inactivating the repressor molecule (*lexA* product) (24). The dominance of *lexA*− mutations would be explained if the mutant *lexA*− repressor were poorly recognized by the *recA'* activator (25). In this scheme, the *tsl*− mutation, a radiation-resistant revertant of *lexA*−, codes for a temperature-sensitive repressor of *recA* which allows a high level of the protein to be made at permissive temperature and full derepression of the gene at 42° in the absence of the *recA'* protein (Fig. 5*C*) (25). This model for regulation of the *recA* gene by *lexA* is also consistent with the

observation that *lexA*$^+$ and *lexA*$^-$ strains carrying hybrid *ColE1* plasmids in which the *srl recA* region is attached to the *ColE1* replisome synthesize the *recA* protein at high levels in the absence of inducing treatment (unpublished data). In such strains, the high dosage of the *recA* operator might titrate out the *lexA* coded repressor and permit overproduction of the *recA* product. Titration of *lac* repressor has been observed in strains carrying multiple copies of the *lac* operator region (26). Alternatively, the *recA* gene may be transcribed from a strong *colE1* promotor which is insensitive to the *lexA* repressor. The *tif-1* mutation would result in an altered form of the *recA* protein which would function like *recA'* in this induction pathway at 42° (Fig. 5*B*) (24). This model for *tif-1* action suggests that the *tif-1* allele would be dominant. However, Castellazzi *et al.* (5) have provided evidence that the *recA*$^+$ and *tif-1* alleles are codominant and that *tif-1* shows gene dosage effects when present on an episome. This codominance might be due to competition between *recA*$^+$ and *tif-1* subunits in the tetrameric *recA* protein (14). Similar codominance between *recA*$^+$ and *tif-1* has also been observed with λ*precA*$^+$ lysogens of a *tif-1* host. The conversion of *recA* to *recA'* in *recA*$^+$ strains could be an allosteric change in this protein due to binding of an effector produced by the inducing treatment. Gudas and Pardee (25) have suggested that a DNA breakdown product might be the inducer of SOS functions. This effector might be a digestion product derived from the hydrolysis of damaged or abnormal DNA by the *recBC* nuclease. Little and Hanawalt (27), however, have demonstrated that DNA degradation by the *recBC* enzyme is not required for induction of *recA* (protein X).

High levels of the 40,000 molecular weight *recA* product do not imply that the SOS pathway is expressed in the cell. For example, neither the *tsl*$^-$ *recA*$^+$ strain nor the *colE1-recA*$^+$ plasmid strain is phenotypically similar to strain DM1187 which expresses these SOS functions constitutively. This result demonstrates that derepression of the *recA* gene is not by itself sufficient for expression of the SOS induction pathway.

The *zab53* (6) and *lexB30* (28) mutations have been mapped close to or in the *recA* gene. These mutations, which abolish induction of SOS functions but are recombination proficient, interfere with protein X induction by nalidixic acid or mitomycin C (ref. 29; unpublished data). Transducing phages carrying these mutations have been isolated and the synthesis of the mutant *recA* protein after phage infection of heavily UV-irradiated cells has been examined by gel electrophoresis. Phages carrying these mutations synthesize only 1–5% of the amount of *recA* protein made by λ*precA*$^+$ under identical conditions (unpublished data). These results suggest that the *lexB30* and *zab53* alleles block expression of SOS functions by preventing derepression of *recA* by (*i*) preventing conversion of *recA* to *recA'*, (*ii*) decreasing the affinity of the mutant *recA'* inducer for the *lexA* repressor, or (*iii*) acting as a down-promoter-type mutation that would decrease the expression of the *recA* gene. Further experiments are needed to distinguish among these possibilities.

The results presented here point to a new functional complexity of the *recA* protein—this protein is a regulator of its own synthesis after treatments that induce SOS functions. This autoregulatory aspect of *recA* function is independent of its role in recombination but is intimately associated with its participation in mutagenesis, reactivation, and prophage induction in which derepression of the *recA* gene appears to be obligatory. Although complex, the molecular features of the regulation of the *recA* region should be elucidated by *in vitro* experiments using purified *recA* DNA and *recA* protein. Such systems should also be extremely useful in the purification of additional components involved in controlling expression of the SOS pathway.

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