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Genetics

A mutant of *Escherichia coli* showing constitutive expression of the lysogenic induction and error-prone DNA repair pathways

(phage λ repressor/mutator/*lexA*/UV repair)

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ABSTRACT **A mutant of *E. coli* (designated the STS mutant) has been isolated in which the phage induction and error-prone DNA repair pathways appear to be expressed constitutively without the cells having received an inducing signal. Phage λ was not able to lysogenize this mutant, whereas a noninducible mutant of λ, λcI*ind*$^-$, known to synthesize a repressor that is insensitive to the induction mechanism, lysogenized it normally. This result suggested that normal phage repressor was synthesized in the STS mutant but was then inactivated by the induction mechanism. The STS strain also had mutator characteristics, and showed spontaneous, error-prone repair of UV-damaged phage λ. Derived from a *lexA tif sfiA* parent strain, the STS mutant carried an additional mutation *spr* at the *lexA* locus that resulted in a high level of expression of the induction pathways. The properties of this and related strains provide additional evidence that induction of phage and induction of error-prone DNA repair occur by a similar mechanism, and further suggest a model for the regulation of these pathways.**

Exposure of λ lysogenic strains of *Escherichia coli* to an inducing agent such as ultraviolet light leads to phage lytic development (1). In uninduced cells, transcription of phage lytic functions is blocked by phage repressor (2), whereas in induced cells, a cell mechanism is expressed that inactivates repressor. Roberts and Roberts (3) found a fragment of repressor in an induced λ lysogen and proposed that proteolytic cleavage of repressor could be the primary inducing event. Treatments that induce lysogenic bacteria also inhibit cell division and are thought to induce an error-prone DNA repair pathway in *E. coli* (4–9). Witkin (4) has proposed that the mechanism that induces the prophage also acts against cellular repressors leading to the transcription of additional cellular proteins.

The effects of inducing treatments are considerably reduced in *recA*, *lexA* and certain other mutants of *E. coli* (4–8, 10–12), implying that the affected gene products play a primary role in the induction pathway; these products have not yet been identified. Roberts and Roberts (3) suggested that *recA* could be the structural gene for a protease that cleaves phage repressor. Genetic studies have shown that a diffusible product blocks induction in *lexA* mutants (11, 12), and it has been suggested that this product is a cellular repressor that regulates induction of DNA repair enzymes (13, 14).

In the *tif* mutant of *E. coli*, induction of phage, error-prone DNA repair, and inhibition of cell division occur spontaneously, in the absence of an inducing treatment. Induction of the *tif* mutant occurs when it is grown at 40–42°, but is not observed at 30° (15, 16). *tif* maps at the *recA* locus, along with several classes of mutations, including *recA*, that block either *tif* promoted induction or induction resulting from treatment with an inducing agent (11). *tif* induction at 40° is also fully blocked by *lexA* mutations which map (11, 12) at a different place on the *E. coli* linkage map (17), and by phage mutations that alter the repressor so that it is insensitive to the induction mechanism (3, 18). This implies that *tif* induction and induction after treatment with an inducing agent involve the same molecular interaction between phage repressor and the induction system. Other experiments with the *tif* mutant have failed to detect any defect in DNA synthesis or structure that might provide an endogenous inducing signal (15, 16). Moreover, the close linkage of *tif* to *recA*, whose normal function is required for induction, strongly suggests that this mutation regulates the expression of the induction mechanism (16).

George *et al.* (19) have recently described *tif* derivatives that can be grown indefinitely at 40–42°, allowing continuous expression of the inducible functions. These derivatives carry mutations in so-called *sfi* (suppressor of filamentation) genes that block inhibition of cell division without affecting phage induction or the induction of error-prone DNA repair.

The following report describes the isolation and characterization of a mutant of *E. coli* in which spontaneous induction is observable at 30° through 42°. Derived from a *tif sfi* strain, it carries an additional mutation (*spr*) at the *lexA* locus that results in increased expression of the induction pathway. Of particular interest is the inability of λ$^+$ to lysogenize this derivative, resulting in clear plaque development on indicator assay plates due to spontaneous inactivation of phage repressor and resulting failure of the phage to lysogenize the mutant cells.

MATERIALS AND METHODS

Bacterial and Bacteriophage Strains. The properties of the bacterial strains and their origins are listed in Table 1. Wild-type λ phage was obtained from R. Devoret, λcI*ind*$^-$ and λi^{434}T from D. Kaiser. Antiserum against λ was obtained from C. Fuerst.

Media. For phage experiments, tryptone broth containing 8 g of Bacto-tryptone, 5 g of NaCl, and 200 μg of thiamine per liter was used. It was solidified by 1% Bacto-agar for plates and 0.6% for overlay agar. For bacterial crosses and measurement of cellular mutation rates, the minimal media contained per liter: 12.1 g of Tris salts, 1.0 g of NH_4Cl, 22 mg of KH_2PO_4, 1.49 g of KCl, 4.68 g of NaCl, 220 mg of Na_2SO_4, 2.03 g of $MgCl_2 \cdot 6H_2O$, 147 mg of $CaCl_2 \cdot 2H_2O$, 8.1 mg of $FeCl_3 \cdot 6H_2O$, 200 μg of thiamine, 2 g of glucose, 200 mg of arginine and proline, 100 mg of histidine, isoleucine, valine, threonine, and leucine, 70 mg of methionine, 20 mg of cysteine, and 100 mg of streptomycin. This was solidified by 1.5% Difco agar. HCA minimal medium is the above minus histidine plus 40 mg of Difco casamino acids per liter.

UV Irradiation. The procedure has been described previously (12).

Transduction and Mating Procedures. Transduction procedures with phage P1 *vira* and mating procedures are described elsewhere (12, 20, 21). The phage lysate was exposed to a UV dose of 150 J/m^2.

Table 1. Bacterial strains

Strain no.	Relevant genetic markers*	Origin or derivation†
JM1	F⁻ *argE3 his-4 strA31*	J. George
GC3217	As JM1, also *tif-1 sfiA11*	J. George
DM972	Hfr Ra-2 *lexA3*	This laboratory
DM1180	As GC3217, also *argE*⁺ *lexA3*	DM972 × GC3217 (Arg⁺Str^R selection)
DM1187	As DM1180, also *spr-51*	Mitomycin-C resistant derivative of DM1180
F118/KL132	F' *pyrB*⁺/*pyrB31 recA1 strA31*	K. B. Low
F118/DM1187‡	F' *lexA*⁺ *spr*⁺/*lexA3 spr-51*	F118/KL132 × DM1187 (no selection used)
DM960	Hfr Ra-2 *metA28*	This laboratory
DM1196	As GC3217, also *metA28*	DM960 × GC3217 (Arg⁺Str^R selection)
JC5029	Hfr KLI6	A. J. Clark
DM1202	As GC3217, also *metA32 tif*⁺	JC5029 × DM1196 (His⁺Str^R selection)
DM1420	As DM1187, also *tif*⁺	JC5029 × DM1187 (His⁺Str^R selection)
DM837	F⁻ *malB32 lexA*⁺	This laboratory
DM1405	As GC3217, also *malB32*	P1·DM837 → DM1196 (Met⁺ selection)
DM1414	As GC3217, also *malB32 tif*⁺	P1·DM837 → DM1202 (Met⁺ selection)
JM12	As JM1, also *tif-1*	J. George
DM1201	As JM12, also *metA28*	DM960 × JM12 (Arg⁺Str^R selection)
DM1412	As JM12, also *malB32*	P1·DM837 → DM1201 (Met⁺ selection)
DM1408	As JM1, also *metA28*	DM960 × JM1 (Arg⁺Str^R selection)
DM1413	As JM1, also *malB32*	P1·DM837 → DM1408 (Met⁺ selection)
AB 2480	F⁻ *recA13 uvrA6*	P. Howard-Flanders

* The nomenclature is that followed by Bachmann *et al.* (17).
† The presence of *tif*, *sfiA*, and *spr* in the strains prepared in this study has been confirmed by appropriate backcrosses.
‡ This strain segregated clones that have lost F118.

RESULTS

Isolation of mutants

We have been analyzing the nature of the diffusible product that prevents expression of the phage induction and error-prone DNA repair pathways in *lexA* mutants of *E. coli* (12, 22). Since this product renders the mutant strain sensitive to treatments that damage DNA, e.g., exposure to mitomycin C, a useful approach has been to obtain resistant derivatives in which the antirepair activity of this product appears to have been modified (23). One class, the so-called *tsl* derivatives, has partially restored inducibility for some cellular functions and appears to express others constitutively without an inducing treatment being given it (14, 22). A limitation to this procedure is that one of the results of induction is an inhibition of cell division, and constitutive expression of this particular function should be lethal to the cell. In order to isolate resistant *lexA* derivatives in which this function can be expressed without lethal effects, I have started with a *tif sfiA* mutant (19). This strain grows quite normally when the phage induction and inducible error-prone DNA repair pathways are expressed at 40–42° (19). A *lexA* mutation was therefore crossed into this strain (GC3217) and mitomycin C resistant derivatives isolated from the resulting *lexA tif sfiA* strain (DM1180).

Bacterial mutagenesis

A representative of one class of derivatives that is resistant to mitomycin C, strain DM1187, showed high spontaneous mutation rates for the cell *his-4* mutation (an ochre mutation) at both 30° and 40°, with the rate at 40° being somewhat more intensely stimulated (Table 2). I will refer to this and other associated properties described below as the STS phenotype. This mutator phenotype was expressed at 40° but not at 30° in the *lexA*⁺ *tif sfiA* parent (strain GC3217), and not at either temperature in the *lexA tif sfiA* parent (strain DM1180), in accord with the observations of George *et al.* (19). Since spontaneous induction of phage and of error-prone DNA repair is also observed under the same conditions as the *tif* mutator phenotype (19), this result suggested that these pathways might be active at both 30° and 40° in the STS strain.

Effects on phage repressors

Recognizing that the phage induction pathway might inactivate phage λ repressor and affect the choice between the lytic and lysogenic growth response in phage-infected cells, I measured the ability of phage λ to lysogenize the STS strain. The results of phage plaque-type tests and frequency of lysogenization measurements are shown in Fig. 1 and Table 3. Phage λ⁺ made clear plaques on strain DM1187 and there was a corresponding 5000-fold lower yield of lysogenic colonies per infected cell. λc1*ind*⁻, a noninducible mutant of λ, made turbid plaques on

Table 2. Mutator effect in mitomycin C-resistant derivatives

Strain no. and genotype	Temperature	His⁺ colonies/10^8 viable cells
JMI	30°	12
lexA⁺ *spr*⁺ *tif*⁺ *sfi*⁺	40°	25
GC3217	30°	17
lexA⁺*spr*⁺ *tif sfi*	40°	145
DM1180	30°	6
lexA spr⁺ *tif sfi*	40°	12
DM1187	30°	145
lexA spr tif sfi	40°	347

Exponential cultures growing at the indicated temperature in tryptone broth were centrifuged and resuspended and diluted in saline. Approximately 2 × 10^7 cells were spread on HCA agar plates, which are selective for His⁺ revertant colonies. Viable cell counts were obtained on the same plates. Plates were incubated at the same temperature for 54 hr.

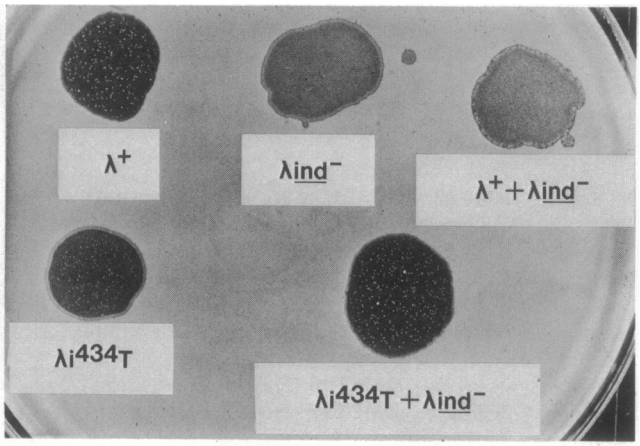

FIG. 1. Appearance of phage spots grown on strain DM1187. Drops of phage suspensions (10^9 plaque-forming units/ml) were placed on tryptone agar plates that had been seeded with indicator bacteria in overlay, dried, and incubated for 18 hr at 37°. Areas of the bacterial lawn infected by the noninducible phage mutant λc*Iind*$^-$ or by the mixture of λ$^+$ and λc*Iind*$^-$ contain large numbers of lysogenic survivors and appear turbid. Single plaques of λc*Iind*$^-$ have a corresponding turbid appearance on this bacterial indicator (24). The inducible phage strains, λ$^+$ and λi^{434}T, and the mixture of strains λi^{434}T and λc*Iind*$^-$ do not lysogenize this indicator so that the respective areas are relatively clear due to the absence of bacterial growth.

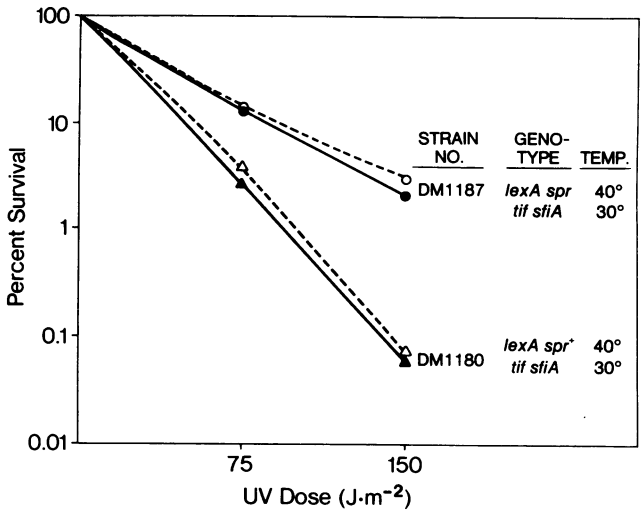

FIG. 2. Survival of UV-irradiated phage λ on strains DM1180 and DM1187. Cultures were grown to exponential phase in tryptone broth containing 10 mM $MgSO_4$ and 2 mg of maltose per ml at the temperature indicated, centrifuged, resuspended and concentrated 5-fold in 10 mM $MgSO_4$, infected at a multiplicity of infection of <0.001 with UV-irradiated phage suspensions for 15 min at 30° or 40° (>90% absorption), diluted, and assayed for plaque-forming infective centers on indicator strain AB2480. This indicator strain was used because it is deficient in both host-cell reactivation and UV-reactivation and therefore does not repair unabsorbed phage particles efficiently.

strain DM1187 and lysogenized the strain at a normal frequency. This phage mutant has been shown to make an altered repressor that is insensitive to induction (3, 18). When the same host strain was coinfected with λ$^+$ and λc*Iind*$^-$, the λc*Iind*$^-$ mutant was dominant to wild-type λ in the establishment of lysogeny. Results of 37° plaque-morphology tests with the inducible heteroimmune phage λi^{434}T (25) shown in Fig. 1 were similar to those for λ. A phage mixture of λi^{434}T and λc*Iind*$^-$ produced a clear spot, indicating that the dominance of λc*Iind*$^-$ to λ$^+$ is immunity-specific. In these same experiments, phage λ made clear plaques at 30–42° on strain DM1187, whereas λi^{434}T was observed to make turbid plaques at 30–32° and clear plaques at 37–42°, suggesting that the phage induction pathway may be more active at higher temperatures. Phage λ made turbid plaques at 30°–41° on *lexA*$^+$ *tif sfiA* (GC3217) and *lexA tif sfi* (DM1180) indicator strains, and the frequency of lysogenization of these strains at 37° was normal, indicating the results were specific for the STS strain.

Phage repair and mutagenesis

The results given earlier suggested that the error-prone DNA repair pathway may be expressed constitutively in strain DM1187. The increased ability of UV-irradiated cells to repair UV-irradiated phage λ and introduce mutations into the extra survivors is thought to result from induction of this pathway (5). If it is expressed spontaneously in strain DM1187 but not its parent DM1180, there should be a difference in the survival and mutagenesis of UV-irradiated λ grown on these host strains. This expectation was fulfilled by the results given in Figs. 2 and 3, which show that strain DM1187 repaired λ more efficiently than strain DM1180 at both 30° and 40°, and there was a high yield of clear plaque mutants among the survivors, particularly at 40°. There was no increase in phage mutants per survivor when irradiated phage were grown on strain DM1180 because any expression of *tif* is blocked by the *lexA* mutation present in this strain. The efficiency of phage repair in strain DM1187

Table 3. Lysogenic response of infected cells

Strain no.	Genotype	Percent lysogenization after infection with λ$^+$	λ c*Iind*$^-$	λ$^+$ + λ c*Iind*$^-$
JM1	*lexA*$^+$ *spr*$^+$ *tif*$^+$ *sfiA*$^+$	100	66	54
DM1202	*lexA*$^+$ *spr*$^+$ *tif*$^+$ *sfiA*	57	100	85
GC3217	*lexA*$^+$ *spr*$^+$ *tif sfiA*	67, 32	72	75
DM1180	*lexA spr*$^+$ *tif sfiA*	30, 59	16, 16	30, 46
DM1420	*lexA spr tif*$^+$ *sfiA*	49	31	100
DM1187	*lexA spr tif sfiA*	0.06*, 0.02	24, 20	20, 17
F118/DM1187	*lexA*$^+$ *spr*$^+$/*lexA spr tif sfiA*	32, 45	12	16

Cells grown to stationary phase at 37° in tryptone broth containing 10 mM $MgSO_4$ and 2 mg of maltose per ml were concentrated and infected with each phage at a multiplicity of approximately 10 infective particles per viable cell. After 15 min for adsorption (>80%), antiserum against λ was added to neutralize unadsorbed phage and the infected cells were incubated an additional 60 min. They were then diluted and spread on plates with antiserum against λ. After incubation on plates at 37° for 42 hr, colonies were tested for their immunity to λ.

* The few surviving colonies obtained usually lysed spontaneously when regrown in tryptone broth or on tryptone agar plates. These are not stable lysogens and were not scored.

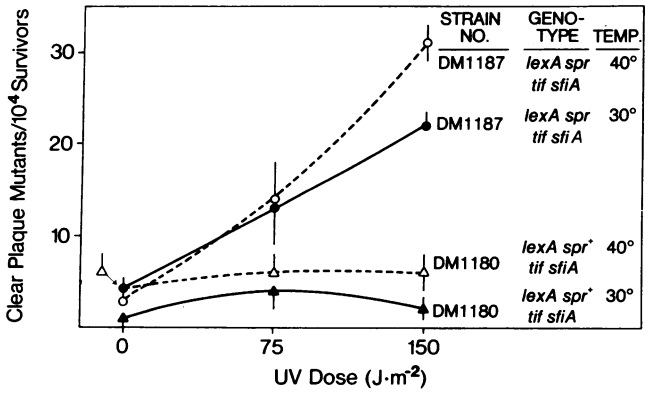

FIG. 3. Mutagenesis of UV-irradiated phage λ reactivated in strains DM1180 and DM1187; data are from the same experiments as shown in Fig. 1. Vertical bars give one-half the 95% confidence interval for the estimate of mutant yield.

approaches that observed in wild-type strains that have been treated with an inducing agent. Error-prone DNA repair of phage λ was observed in *lexA*$^+$ *tif sfi*$^{+\text{ or }-}$ strains when these were grown at 40° (19).

Genetic analysis

The inability of strain DM1187 to become lysogenized by λ$^+$ compared with the near normal capability of the parent *lexA*$^+$ *tif sfiA* strain made it possible to map the mutation that results in increased expression of the phage induction pathway. The crosses shown in Table 4 established that this mutation, designated *spr-51* (*sp*ontaneous *r*epressor inactivation), maps at the *lexA* locus. They also showed that this phenotype was observed only when the recipient strain carried both *tif* and *sfiA*. The requirement for *sfiA* is likely due to the induction-related block in cell division, preventing growth of *spr tif sfiA*$^+$ transductants into colonies. Lysogenization experiments using *sfiA* host strains having various combinations of these genetic markers (Table 3) confirmed the requirement for *tif* for the nonlysogenic response. I conclude that *tif* and *spr* act in concert to allow a high degree of expression of the phage induction pathway in strain DM1187. Other experiments (not shown) have indicated that both mutations are also necessary for full expression of the error-prone DNA repair pathway. Moreover, the expression of the phage induction pathway in the STS mutant is abolished by an additional *recA* mutation (data not shown), indicating that the *recA*$^+$ function is essential.

Rare *lexA* transductants were found in a transduction cross from strain DM1187 to a different *malB* recipient strain, indicating the presence of *lexA3* in strain DM1187 and proving that the deficiency in DNA repair due to *lexA3* is suppressed by *spr* in this strain. The rarity of such transductants further indicated that *spr-51* and *lexA3* are closely linked.

The above results suggest that the diffusible product that stops induction in *lexA* mutants has been modified by *spr*. To obtain additional information as to the nature of this modification, I introduced episome F118, known to carry *lexA*$^+$ *spr*$^+$ (12), into strain DM1187. The resulting strain F118/DM1187 (genotype *lexA*$^+$ *spr*$^+$/*lexA spr tif sfiA*) had the same properties as the *lexA*$^+$ *tif sfiA* parental strain with regard to sensitivity, ability to become lysogenized by λ$^+$ (Table 3), repair of UV-irradiated phage λ, and host sensitivity to UV. *spr* is therefore recessive to *spr*$^+$ carried on an episome.

DISCUSSION

When wild-type phage λ infects the STS mutant, little, if any, lysogenization occurs, presumably because phage repressor is inactivated at a rate that is sufficiently rapid that the resulting phage growth is lytic. This interpretation is supported by the observation that infection with the phage mutant λc*Iind*$^-$, which synthesizes an altered repressor that is insensitive to the lysogenic induction mechanism, leads to stable repressor levels and lysogenization occurs at a normal frequency. The mechanism of inactivation of phage repressor in this host thus appears to be the same one that inactivates repressor in an induced lysogen, and this pathway, normally repressed in wild-type cells and induced when cell DNA is damaged, appears to be expressed constitutively. The STS mutant also repairs UV-irradiated λ phage as efficiently as UV-irradiated wild-type cells but without pretreatment with UV being necessary, and a high yield of mutants is found among the phage survivors. This result supports the hypothesis that an error-prone DNA repair pathway is induced concurrently with the phage induction pathway, and suggests further that this pathway is expressed constitutively in the STS mutant.

Table 4. Cotransduction of *spr* with *malB*

Recipient strain no.	Genotype *tif*	Genotype *sfiA*	No. of transductants analyzed	No. with STS phenotype
DM1405	−	−	89	34
DM1414	+	−	54	0
DM1412	−	+	38	0
DM1413	+	+	48	0

The donor strain was DM1187 *malB*$^+$ *lexA3 spr-51 tif-1 sfiA11*, and Mal$^+$ transductants of the recipient strains, which carry *malB32*, were selected. Transductant colonies were grown at 30° (recipient strain DM1412) or 37° and purified on selective medium prior to determination of their phenotype. The STS phenotype was scored by replica-plating a grid of colonies on tryptone agar plates to plates on which particles of λ$^+$ or λc*Iind*$^-$ had been previously deposited. Absence of growth on the λ$^+$ phage-containing plate after 18 hr indicated the STS phenotype. The frequency of cotransduction of *lexA3* with *malB32* under these conditions is 30%.

The STS strain was derived from the *tif sfiA* mutant previously described by George *et al.* (19), which itself shows constitutive expression of the phage and repair induction pathways when it is grown at 40–42° (15, 16, 19). In the STS derivative these are expressed at 30°–42°, with somewhat stronger effects being observed at higher temperatures. This increased expression is due to an additional mutation, *spr*, which maps at or near the *lexA* locus. *lexA* mutations were shown previously to lead to the synthesis of a diffusible product that blocks *tif* induction at 40–42° (11). The *spr* mutation appears to decrease the activity of this product with the result that the induction pathways are more fully expressed than in the original *lexA*$^+$ *tif* strain.

It was previously suggested that the *lexA*$^+$ product is a repressor that prevents the synthesis of DNA repair enzymes, and that in cells with damaged DNA, this repressor is inactivated, leading to enzyme induction (13, 14). It was further suggested that *lexA* mutations make this repressor noninducible and that suppressor mutations such as *spr* diminish the activity of repressor. This explanation could also account for the effects of *spr* mutations observed in this study, as described below.

Castellazzi *et al.* (11) have pointed out that the *tif* mutation could affect the regulatory site of a DNA repair protein that could be the *recA* protein itself. This protein could be *activated* for induction by interaction with damaged DNA or some small molecule generated from the associated inhibition of DNA replication, and then functions to initiate the expression of the

induction pathways. I propose that the *tif* mutant could produce an altered *recA* protein that is activated spontaneously in the absence of an inducing treatment to a certain extent at 30° but to a greater extent at 42°. This mechanism for *tif* induction is supported by the observations that *tif* is tightly linked to the *recA* locus and that the Tif phenotype is abolished in *tif recA* mutants (11, 16). Moreover, when *tif lexA*$^+$ lysogens are shifted from 30° to 42°, inactivation of phage repressor proceeds in the presence of chloramphenicol, suggesting that the induction proteins were already present at 30° in a less active form (26, 27).

I further propose, as did Clark (28), that the *lexA* repressor could regulate the level of the *recA* protein in response to the needs of normal DNA metabolic processes and cell growth. *lexA* mutations could lower the level of the *recA* protein below normal levels, and *spr* mutations could result in abnormally high levels of the protein. If the *recA* protein is spontaneously activated by the expression of *tif*, this high level could increase the overall activity of the induction pathway and lead to an increased rate of inactivation of phage repressor that accounts for failure of phage to lysogenize the STS mutant. Moreover, this overall increase in activity should be more pronounced at 42° since the *recA* protein itself would be more active at this temperature, and this was observed to be the case. A different *lexA* suppressor (*tsl*) restored thermal inducibility to *lexA tif* strains, bu not the increased expression observed with *spr* (unpublished observations). Some repressor activity may remain in such derivatives thereby maintaining the quantity of *recA* protein at approximately that in *lexA*$^+$ strains.

These controls on the level and activity of the *recA* protein predict that it supplies a key function for expression of the phage induction and error-prone DNA repair pathways. In its activated form, *recA* protein could clearly be a protease that attacks phage repressor (3) and a similar cellular repressor that regulates an error-prone DNA repair pathway.

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