

The *lexA* gene product represses its own promoter

(SOS responses/*recA* gene/*tif* mutagenesis)

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ABSTRACT The products of the *lexA* and *recA* genes of *Escherichia coli* regulate the cellular response to DNA damage (the SOS response). Here we describe the cloning of the wild-type *lexA* gene and the identification of its 24,000-dalton protein product. We also describe construction, by recombination *in vitro*, of a phage that bears the *lexA* promoter fused to the *lacZ* gene. Experiments with this fusion phage and with multicopy plasmids that carry the *lexA* gene showed that the *lexA* gene product represses expression of its own promoter. This repression occurs even if the cell has no *recA* gene, showing that the *lexA* protein need not be complexed to the *recA* protein for activity. Moreover, the presence of multicopy plasmids that carry the *lexA* gene blocks expression of all SOS responses tested. This presumably results from two effects: (i) repression of the *recA* gene, the product of which is required to activate many of these responses; and (ii) direct repression of other functions involved in the SOS response.

Damage to the DNA of an *Escherichia coli* initiates a battery of responses (for reviews, see refs. 1 and 2). These have been called "SOS responses"; some of the better defined of these are the induction of synthesis of large amounts of *recA* protein, the induction of filamentous growth, the induction of a new DNA repair capability, and the induction of lambdaoid prophages. The latter is the best understood biochemically. Roberts and coworkers (for a review, see ref. 3) have shown that the probable cause of the induction of phages λ and P22 is the cleavage of their repressor proteins by the *recA* product.

The SOS responses mentioned above can be elicited without overt damage to cellular DNA. This requires a mutation in *recA* called *tif* (4, 5). Cells that bear the *tif* mutation manifest all SOS functions at high temperature, but not at low temperature. Filamentous growth, which is lethal if it continues for more than a few hours, can be specifically blocked by a *sfi* mutation (6). A *tif sfi* cell at high temperature displays all the other SOS functions.

The conditional expression of SOS functions in a *tif sfi* cell can be made constitutive by endowing it with a third mutation, called *spr*, which abolishes the function of a gene called *lexA* (7). In one simple view, the *lexA* product is a repressor of the *recA* gene (8, 9), and a *spr* mutation, which destroys *lexA* function, allows production of large amounts of the *recA* protein. According to this view, overproduction of the *tif* mutant form of the *recA* protein induces the other SOS functions. However, production of large amounts of the wild-type *recA* protein does not (unpublished data; J. Salstrom, personal communication). We argue below that removal of active *lexA* product from a cell, even in the absence of *recA* protein, is sufficient to induce some SOS functions.

Here we describe the cloning of the wild-type *lexA* gene and

the identification of its protein product, and we present evidence that the *lexA* product represses its own expression as well as expression of other functions involved in the cellular response to DNA damage. Little and Harper (10), using a different methodology, have also concluded that the *lexA* product regulates its own expression.

MATERIALS AND METHODS

Bacterial and bacteriophage strains, listed in Table 1, were constructed by published genetic techniques (13-15). ColE1 and plasmids derived from it were mated by using F⁺ or F' (*lac-pro*) essentially as described (12). Assay of β -galactosidase was as described in ref. 13.

Methods for the isolation of plasmid DNA, its manipulation *in vitro*, and transformation of cells have been described (refs. 16 and 17, and references therein). Techniques for the construction of fusion phages *in vitro* have been described (18, 19).

Plasmid-encoded proteins were visualized on 12% polyacrylamide sodium dodecyl sulfate gels (20) by using the "maxicell" technique (21) with the following modifications: a *uvrA recA phr*⁺ cell was used as the plasmid host, cells were UV irradiated at lower total doses (A. Sancar and W. D. Rupp, personal communication) to a maximum of 60 J/m², D-cycloserine was added after irradiation to a concentration of 100 μ g/ml, and [³⁵S]methionine (>400 Ci/mmol; 1 Ci = 3.7 \times 10¹⁰ becquerels) was used at a concentration of 70 μ Ci/ml. Gels were either dried and exposed to Ultrofilm ³H (LKB) or treated with the fluorographic agent En³Hance (New England Nuclear), dried, and exposed to XR-5 x-ray film (Kodak) at -80°C. In later experiments, the starvation for sulfur prior to labeling was omitted with no apparent effect. These modifications have the cumulative effect of making the procedure faster and more reliable.

Tests for SOS responses were done on agar plates that contained antibiotics to ensure, when relevant, the continued presence of plasmids. UV sensitivity was measured as described (22). *tif* mutagenesis was measured on appropriately supplemented minimal E plates as described (11, 23). *tif* filamentous death measured the survival of plasmid-carrying *tif sfi*⁺ strains on prewarmed minimal A plates (13) with 75 μ g of adenine per ml at 42.5°C. The plating behavior of wild-type λ on a *tif sfi spr* strain that bore a plasmid was used to ascertain whether or not the plasmid bore the *lexA* gene (8). "Lexless" filamentous death measured the survival of strains bearing either the *tsl-1* mutation or an amber mutation in *lexA* and a temperature-sensitive amber suppressor [*spr-55 (lexA3) supD*^{ts74}] on LB plates prewarmed to 42.5°C.

Abbreviation: STS phenotype, ability of λ ⁺ to make clear plaques on *spr tif sfi* bacteria (8).

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Table 1. Bacterial strains

Strain	Description or relevant genotype	Source
RB113	<i>thr-1 leu-6 proA2 argE3 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37 recA13 uvrA6</i>	P. Howard-Flanders
RB142	WP-44 ₉ -NF-50. A nonfilamenting <i>trp tif-1 uvr B/K-12</i> hybrid	E. Witkin (11)
RB397	RB142 Δ(<i>lac-pro</i>) _{XIII} <i>mal</i> ⁺	Unpublished construction
RB400	RB142 Δ(<i>lac-pro</i>) _{XIII} <i>mal</i> ⁺ <i>recA</i> ⁺	Unpublished construction
RB404	<i>dam-3 dcm-6 metB1 galK2 galT22 his-4 thi-1 tonA31 tsx-78 mtl-1 supE44</i>	M. Marinus via M. Meselson
RB481	Δ(<i>lac</i>) _{X74} <i>galISII::OP308 strA tif-1 srl::Tn10</i>	Unpublished experiments
DM1420	<i>his-4 strA31 tif⁺ sfiA11 (lexA3) spr-51</i>	D. Mount (8)
JS511	As DM1420 but <i>recA</i> Δ ₃	J. Salstrom
RB653	Δ(<i>lac</i>) _{U169} <i>strA thi relA malE7::Tn5 lexA⁺</i>	L. Guarante
RB672	As DM1420 but Δ(<i>lac-pro</i>) _{XIII} <i>srl::Tn10 tif-1</i>	Unpublished construction
DM511	<i>thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37 (lexA3) tsl-1</i>	D. Mount
XAC	<i>ara thi nalA argEam rif Δ(lac-pro)</i>	J. Miller
RB669	As XAC but <i>spr-55(am) supD74 arg^{ts}</i>	Unpublished construction
JA200/pLC44-14	F ⁺ Δ(<i>trpE5</i>) <i>recA thr leu lacY</i> containing a ColE1-derived plasmid that carries <i>dnaB⁺</i> and <i>lexA⁺</i>	L. Clarke and J. Carbon (12)
NK 5031	Δ(<i>lac</i>)M5265 <i>nal^r supF</i>	N. Kleckner
F118/KL132	F ⁺ <i>lexA⁺/F⁻ pyrB31 recA1 strA31</i>	B. Bachmann via J. Salstrom
AB1157	<i>thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 sup-37</i>	G. Walker

All strains are F⁻ *E. coli* K-12 derivatives unless otherwise noted.

RESULTS

Phenotypes conferred by the plasmid pLC44-14

pLC44-14 is a plasmid that was reported to carry the *dnaB* gene (reported in ref. 12). Because *dnaB* had been mapped close to the *lexA* gene (24), it seemed possible that this plasmid also carried the *lexA* gene. That the plasmid indeed carried *dnaB*⁺

Table 2. Sensitivity to UV irradiation conferred by various plasmids

Strain	Survival, %
AB1157	95
AB1157/ColE1	91
AB1157/pLC44-14	0.2
AB1157/pBR322	90
AB1157/pRB160	0.08

Strain AB1157 was transformed with the plasmids indicated and the UV sensitivity of the resulting strains was measured (22). Numbers given refer to the percent of cells surviving a UV dose of 10 J/m².

Table 3. Suppression of *tif* mutagenesis by *lexA*⁺ plasmids

Strain	<i>trp</i> ⁺ revertants	
	32°C	42.5°C + adenine
RB400	20	36
RB397	20	300
RB397/ColE1	22	280
RB397/pLC44-14	24	20
RB397/pBR322	20	310
RB397/pRB160	18	20

Plasmids bearing *lexA*⁺ or not bearing it were introduced into the *tif sfi* strain RB397. Transformed strains were then grown at 32°C or at 42.5°C in the presence of adenine as described by Witkin (11, 23). The otherwise isogenic *tif*⁺ *sfi* strain RB400 served as one control. Numbers given refer to the absolute number of *trp*⁺ revertant colonies scored after 72 hr at the indicated temperature.

was confirmed by its ability to complement a conditionally lethal mutation in *dnaB*, *dnaB107* (not shown).

The plasmid pLC44-14 was then tested to see if it carried the *lexA* gene. pLC44-14 was mated into the *spr tif sfi* strain RB672 and phage λ was plated on the resulting strain. The *spr* allele is recessive to *lexA*⁺, and the STS phenotype—that wild-type phage λ makes clear plaques when plated on a *spr tif sfi* strain—is suppressed by introduction into the strain of an F' factor that bears *lexA* (8). If pLC44-14 carried *lexA*, it would also be expected to suppress the STS phenotype because the wild-type *lexA* gene would again be expected to be dominant *in trans* to the chromosomal *spr* allele. Indeed, λ⁺ makes turbid plaques on a *spr tif sfi* strain carrying pLC44-14, but the phage makes clear plaques on the same strain without the plasmid or with the control plasmid ColE1.

Wild-type cells harboring pLC44-14 manifest a substantial degree of UV sensitivity (Table 2), and *tif sfi* cells bearing the plasmid show complete suppression of *tif* mutagenesis (Table 3). Both of these effects are presumably the consequence of the increased cellular amounts of *lexA* gene product conferred by the plasmid.

tif (but *sfi*⁺) cells that bear pLC44-14 exhibit substantially reduced *tif* filamentous death (Table 4), and this phenotype was used initially as a selection for the cloned *lexA*⁺ gene.

Subcloning of the *lexA*⁺ gene

A small fragment of bacterial DNA carrying the *lexA* gene was subcloned in a series of steps from pLC44-14 to yield pRB160. The starting plasmid, pLC44-14, was first cut with a variety of restriction enzymes and the fragments thus generated were inserted into appropriate plasmid vehicles; plasmids that carried *lexA*⁺ were selected by their ability to spare the *tif sfi*⁺ cell

Table 4. Suppression of *tif* filamentous death by *lexA*⁺ plasmids

Strain	Relative survival at 42.5°C with adenine
RB481	0.0001
RB481/ColE1	0.0003
RB481/pLC44-14	0.06
RB481/pBR322	0.0008
RB481/pRB160	0.12

Control plasmids or plasmids carrying the *lexA* gene were introduced into the *tif sfi*⁺ strain RB481 and dilutions of the resulting transformed strains were plated on unsupplemented minimal A medium plates at 32°C and at 42.5°C in the presence of adenine at 75 μg/ml. The number given is the ratio of the number of surviving colonies at the high temperature relative to the number of surviving colonies at the low temperature.

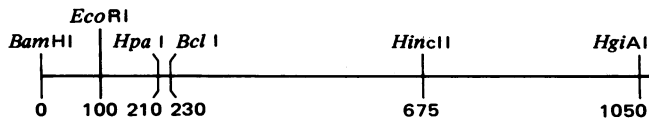


FIG. 1. Restriction endonuclease map of the cloned *lexA* gene and contiguous DNA. Distances are given in base pairs from the *Bam*HI site designated as zero.

RB481 from filamentous death. Plasmids that conferred the ability to survive the selection all suppressed the STS phenotype and rendered the *spr tif sfi* or wild-type cells that bore them sensitive to UV. DNA inserted into the plasmid vehicles was mapped with a large number of restriction endonucleases, and the knowledge gained from mapping was used to construct plasmids with smaller insertions, which again were screened for their ability to confer UV sensitivity and their ability to suppress the STS phenotype. This process of mapping, deletion, and screening for *lexA* function eventually yielded a short *Eco*RI/*Hgt*AI fragment on which *lexA* resided. In a final step the *Hgt*AI site shown at 1050 on the map of the gene (Fig. 1) was treated with S1 nuclease, then with T4 DNA ligase in the presence of synthetic *Hind*III linkers to convert it to a *Hind*III site, and the resulting *Eco*RI/*Hind*III fragment was inserted into pBR322 (25) to create pRB160.

No rearrangement of the fragment that carries *lexA*, either internal to it or affecting its termini, occurred during the sub-cloning process. This was established by demonstrating the presence of the *Eco*RI/*Hgt*AI 950 fragment, as well as restriction sites internal to it, in pLC44-14. That no rearrangement of the fragment that carries the chromosomal *lexA* gene occurred during the construction of pLC44-14 was shown by demonstrating the existence of the same *Eco*RI/*Hgt*AI 950, as well as restriction sites internal to it, in a Southern blot (26) of wild-type *E. coli* DNA (not shown).

As a final independent check for *lexA* function, pRB160 and pLC44-14 were checked for their abilities to complement conditionally lethal mutations in *lexA*. *tsl-1* (probably a *lexA*^{ts}) and the amber mutation *spr-55* (in a temperature-sensitive amber suppressor strain) cause lethal filamentation in *sfi*⁺ bacteria at high temperature, whether the cell has a functional *recA* gene or not (refs. 24 and 27 and unpublished). This observation suggests that the wild-type *lexA* product directly represses a function that causes filamentation (or blocks septum

formation) when expressed. A single copy of *lexA*⁺ in *trans* blocks this lethal filamentation in all members of the population, as do both pLC44-14 and pRB160 (see Table 5).

Identification of the *lexA*⁺ gene product

The maxicell technique was used to examine plasmid-encoded proteins. This technique (ref. 21 and *Materials and Methods*) makes use of the fact that UV irradiation of a *recA* cell initiates massive degradation of chromosomal DNA while the smaller plasmid DNA molecules remain intact. pRB160 encodes a single new protein with a mobility consistent with a molecular weight of 24,000, in addition to the β -lactamase and the tetracycline-resistance proteins contributed by the plasmid vehicle (Fig. 2, lane 3). Removal of the insertion or its disruption by manipulations *in vitro* abolished *lexA* function, by any genetic test used, and abolished production of the 24,000-dalton protein in maxicells. One such experiment is described below.

Inactivation of the cloned *lexA* gene

pRB160 was cut at the *Bcl*I site (Fig. 1) in the presumptive *lexA* coding sequence, and treated with T4 DNA ligase to recircularize the linear molecules; strain RB404 was transformed with DNA from this reaction. We reasoned that head-to-head dimer plasmids, which should arise frequently from this construction, should have suffered a disruption of the cloned *lexA* gene. Two

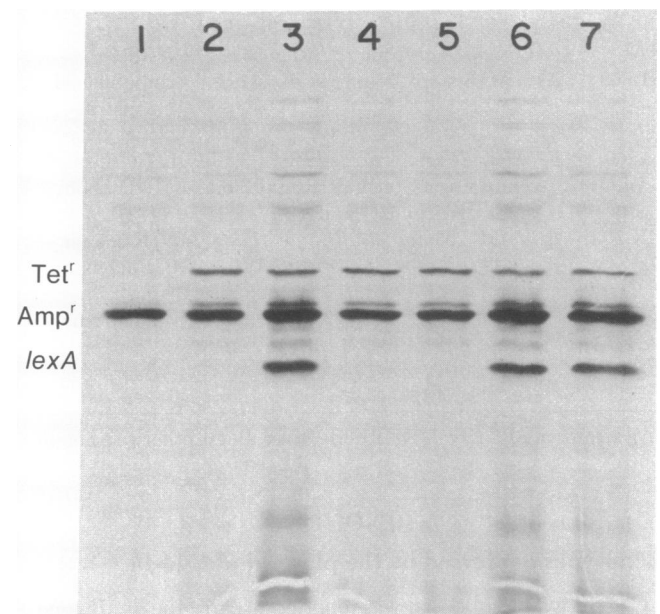


FIG. 2. Identification of the wild-type *lexA* gene product. The *recA uvrA* strain RB113 was transformed with the indicated plasmids and plasmid-encoded proteins were visualized as described in *Materials and Methods*. Lane 1, the 29,000-dalton β -lactamase produced by pGL101 (G. Lauer, unpublished), a derivative of pBR322 that no longer confers tetracycline resistance. Lane 2, β -lactamase and tetracycline-resistance proteins produced by pBR322. Lane 3, pRB160, which encodes the 24,000-dalton *lexA*⁺ polypeptide as well as proteins specifying tetracycline and ampicillin resistance. Lanes 4 and 5, head-to-head dimer derivatives of pRB160 that have lost *lex* function and no longer produce the 24,000-dalton *lexA*⁺ product. Lanes 6 and 7, monomer derivatives of the previous plasmids that have regained *lex* function and the ability to produce the 24,000-dalton protein. Molecular weights of labeled proteins in bands were estimated from comparison of their mobility with that of unlabeled proteins of known molecular weight. Amp^r indicates the β -lactamase made by all the plasmids used; Tet^r indicates one prominent protein band made by plasmids that confer resistance to tetracycline; and *lexA* indicates the 24,000-dalton wild-type *lexA* gene product.

Table 5. Suppression of *lex*less filamentous death by *lexA*⁺ plasmids

Strain	Relative survival at 42.5°C
DM511	5×10^{-6}
DM511/F118	0.88*
DM511/ColE1	1.2×10^{-5}
DM511/pLC44-14	0.92
DM511/pBR322	4×10^{-6}
DM511/pRB160	1.03
RB669	1×10^{-5}
RB669/F118	0.85*
RB669/ColE1	1×10^{-5}
RB669/pLC44-14	1.01
RB669/pBR322	1.5×10^{-5}
RB669/pRB160	0.98

The indicated plasmid was introduced into the *tsl* strain DM511 or the *spr-55*(*supD*^{ts}) strain RB669, and dilutions of the transformed strains were plated on LB plates at 32°C and 42.5°C. Survival of strains at 42.5°C is given as the relative number of survivors at 42.5°C. F' 118 is an F' factor that carries *lexA* (8).

* These strains tended to lose the F'.

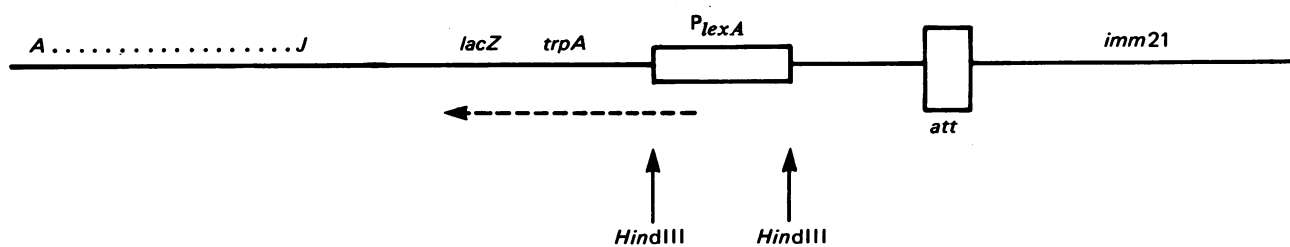


FIG. 3. Structure of the *lexA* promoter-*lacZ* fusion phage λ RB200, a derivative of λ 132 (19). Transcription *in vitro* (28) of fragments of the cloned *lexA* gene (not shown) revealed a single strong promoter. Transcription proceeds rightward from its startpoint at 150 in Fig. 1. The *Bam*HI/*Hpa*I fragment indicated in Fig. 1 was isolated and rendered flush (16), the flush ends were converted to *Hind*III ends by using synthetic oligonucleotide linkers (as in ref. 17), and the resulting *Hind*III/*Hind*III fragment was inserted into pTR262 (T. Roberts, unpublished) and cloned to yield pRB141. Purified *Hind*III-ended promoter fragment from pRB141 was ligated with λ 132 DNA to yield λ RB200. This phage was detected by its ability to form red plaques on lawns of the indicator strain NK 5031 on lactose MacConkey agar plates (19). Control experiments (not shown) demonstrated that the presence and proper orientation of the promoter fragment were necessary for efficient production of β -galactosidase from the phage.

hundred independent transformants were screened for UV sensitivity. Fifteen transformants were UV resistant. The structures of these plasmids were determined, and they were used to transform RB672. All 15 recombinant plasmids had the head-to-head structure and had lost *lexA* function, as measured by the loss of their ability to confer UV sensitivity and to suppress the STS phenotype, and no longer produced the 24,000-dalton protein (see Fig. 2, lanes 4 and 5). Function was restored by cutting the dimer plasmids again with *Bcl*I and religating: all monomer plasmids arising from this step had regained *lexA* function and the ability to produce the 24,000-dalton protein (Fig. 2, lanes 6 and 7).

Control of the *lexA* promoter *in vivo*

In order to study the activity of the *lexA* promoter *in vivo*, we have fused it to the *lacZ* gene by using recombination *in vitro*. This fusion is carried on a phage that readily forms lysogens. Lysogens of this phage synthesize β -galactosidase under control of the *lexA* promoter. The method used to construct this *lacZ* fusion phage, λ RB200, diagrammed in Fig. 3, was similar to that used by other workers in this laboratory (18, 19, 29).

Single lysogens of this phage in *spr sfi recA*⁺ and *spr sfi recA* Δ ₃ were either transformed or transduced with the sources

of *lexA*⁺ indicated in Table 6. As can be seen, the introduction into either strain of a plasmid that bears *lexA*⁺ is sufficient to repress the amount of β -galactosidase made from the phage more than 10-fold.

DISCUSSION

The *lexA* gene has been cloned in a manner that has facilitated its mapping and the study of its control. The 24,000-dalton protein identified as the *lexA*⁺ gene product is almost certainly the same as the 24,000-dalton protein that Little and Harper find produced by a λ *plexA*⁺ transducing phage (10). A protein of about 85,000 molecular weight, possibly identical to the one previously identified as the *lexA* gene product (24), was found to be encoded on a fragment of DNA contiguous to the *lexA* gene. Plasmids that produced only this 85,000-dalton protein and no other had no *lexA* activity, by any test used (not shown), and the fragment that encoded it was jettisoned during the subcloning process.

Although we cannot prove that the promoter we observe is the promoter that directs transcription of the *lexA* gene *in vivo*, several lines of evidence argue to this point.

(i) This is the only promoter on the fragment that is active *in vitro*.

(ii) It is located near the *lexA* gene. The nucleotide sequence of the fragment (not shown) reveals, downstream from the startpoint of transcription, what we believe to be the start of the *lexA* coding sequence.

(iii) Expression from this promoter-bearing fragment is regulated *in vivo* in response to changes in the amount of active *lexA* gene product.

We could recapitulate our working model by stating that the *lexA* gene product turns off the functions involved in the cell's response to DNA damage: directly, by repressing some of them (refs. 10 and 31 and this paper), and indirectly, by repressing synthesis of the *recA* protein (8, 9), which, when derepressed, turns on other genes involved in the SOS response by inactivating their specific repressors. We suppose, as have others (2, 5), that the *recA* protein, upon induction, becomes competent to cleave cellular repressors of SOS functions, including the *lexA* protein. According to this view, the *tif* mutant form of the *recA* protein is competent to cleave cellular repressors when it is produced in large quantities, without requiring whatever change in the state of the cell is necessary to convert the wild-type *recA* product into an efficient protease *in vivo*.

What aspects of the role of the *lexA* product in the cellular response to DNA damage have been clarified by our experiments? We have found that the *lexA* promoter, fused to the *lacZ* gene, is apparently repressed by the *lexA* gene product (see also

Table 6. Effect of wild-type *lexA* product on β -galactosidase synthesized from λ RB200

Source of <i>lexA</i> in strain lysogenized with λ RB200	β -Galactosidase in lysogenized strain, units	
	<i>recA</i> ⁺	<i>recA</i> ⁻
<i>spr</i> -51 on chromosome	2500	2000
<i>lexA</i> ⁺ on chromosome	600	—*
<i>spr</i> -51 on chromosome, pRB160 in cytoplasm	250	150

The *recA*⁺ *sfi spr* strain DM1420 or the isogenic *recA* deletion strain JS511 was lysogenized with λ RB200, and *ter* excision tests (as in ref. 31, as modified by J. Salstrom, personal communication) were performed on resulting lysogens to determine which were monolysogens. Monolysogens were then transformed with pRB160 or transduced to *lexA*⁺ and single colonies of the strains that resulted were grown in LB medium containing 0.2% glucose to minimize expression from the chromosomal *lacZ* gene. Under these conditions, either strain lysogenized with λ RB200 without the promoter-bearing fragment, or with the promoter-bearing fragment in an orientation opposite to the correct one, produced about 50 units of β -galactosidase, whereas the same strains without the fusion phage produced less than 5 units.

* Any marker other than *recA*⁺ cannot be stably transduced into the *recA*⁻ strain.

ref 10). It has been variously proposed that the *lexA* and *recA* products form a complex that serves to repress some cellular SOS functions (2), or that the *lexA* product binds cellular repressors induced by UV, protecting them from *recA*-mediated cleavage (1). It is significant that the apparent repression of transcription we observe occurs even if the *recA* gene is deleted in the cell. The finding that *lexA* protein acts without *recA* product to repress transcription of its own gene lends credence to the notion that the *lexA* protein can act alone to repress other functions involved in the SOS response. We have argued that the *lexA* gene product also acts alone to repress a function that causes lethal filamentation when expressed. In addition, C. Kenyon and G. Walker (personal communication) have identified several loci in *E. coli* that appear to be repressed by the *lexA* product acting alone, and there is at least a hint that other cellular functions may be controlled in the same way (31–34).

In distinction to the SOS functions that may be repressed by the *lexA* product itself, some SOS functions do not seem to be directly repressed by *lexA*, that is, do not occur in undamaged *recA*⁺ or *recA*⁻ cells when the *lexA* protein is removed. Numbered in this class are some of the most interesting SOS functions, including induction of prophages and induction of mutagenesis. We presume that these functions are repressed by other repressors also susceptible to proteolytic cleavage by the *recA* product.

We thank Anthony Poteete, Tom Roberts, and Barbara Meyer for valuable discussions and John Salstrom and Leonard Guarente for unpublished strains and advice. We thank David Mount and members of his laboratory for strains and advice, particularly for the suggestion that pLC44-14 carried the *lexA* gene; John Little and Joan Harper for making their results available before publication; and Peter Lomedico for kindly performing the Southern hybridization.

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