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Identification of the *lexA* gene product of *Escherichia coli* K-12

(autoregulation/λ*lexA* transducing phages/*lexA* plasmids/*recA* regulation)

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ABSTRACT **The *Escherichia coli lexA* gene encodes a product important in induction of the *recA* gene and the expression of various cellular functions, including mutagenesis and prophage induction. As a start in a biochemical analysis of the *lexA* function, a family of λ transducing phages carrying *lexA*$^+$, *lexA3*, *lexA3 spr-54*, and *lexA3 spr-55* alleles of the *lexA* gene was isolated and characterized. Polypeptides synthesized by these phages were examined. λ*lexA*$^+$ made a distinctive protein 24 kilodaltons (kd) in size. λ*lexA3*, which encodes an active mutant form of the protein dominant to wild-type function, made a slightly larger protein 25 kd in size. The latter protein was shown to be the mutant *lexA3* gene product by the fact that λ*lexA3 spr-55*, which carries an amber mutation in *lexA3*, made the 25-kd protein in hosts with an amber suppressor but not in a suppressor-free host. In hosts carrying a multicopy *lexA3* plasmid, neither the 25-kd nor the 24-kd protein was made. This result suggests that *lexA* is autoregulated and that expression of the 24-kd protein made by λ*lexA*$^+$ is subject to the same controls. This and other evidence argues that the 24-kd protein is the product of the wild-type *lexA*$^+$ gene.**

After DNA damage or inhibition of DNA replication, *Escherichia coli* shows a number of new responses, such as prophage induction, mutagenesis, and new DNA repair capacity, whose expression appears to be coordinately controlled (1). These processes are often called "SOS functions," because many of them are believed to aid cell survival. Their expression is controlled by a regulatory system involving at least two genes: *recA* and *lexA*. The *recA* protein is made at high levels under conditions that induce SOS functions (2–5), and its various activities (6–8) may account directly for a number of these responses. Genetic studies (3, 9–11) suggest that *lexA* product regulates the expression of the *recA* gene, perhaps as a simple repressor, implying that a primary function of *lexA* protein in the induction of SOS functions is to regulate the level of *recA* protein in response to the needs of the cell.

The phenotypes of strains carrying several mutant alleles of the *lexA* gene are consistent with this model. First, *lexA*$^-$ mutants actually make an active product which is dominant to wild-type function (12); *lexA*$^-$ strains induce *recA* at greatly reduced levels after DNA damage (3). The model asserts that the *lexA* mutant repressor is resistant to inactivation, accounting for its dominance. Second, certain recessive mutants in *lexA*, termed *spr* (13), appear to inactivate its function, because they express *recA* and several SOS functions constitutively, that is, in the absence of DNA damage (9, 13, 14).

This model makes several testable predictions about the biochemical functions of the *lexA* gene product. Our goal is to test these predictions, and those of other models, *in vitro* with purified components. A first step in this direction is to identify the product of the *lexA* gene. We have used recombinant DNA techniques to isolate a transducing phage, λ*lexA3*, which carries the *lexA3* allele of the *lexA* gene (unpublished data). This phage contains a 2.9-kilobase (kb) insert of *E. coli* DNA that includes the *lexA3* gene but no other genes to our knowledge. We expected, therefore, that it should make the *lexA3* protein and few, if any, other bacterial polypeptides. The present paper describes the isolation and characterization of derivatives of λ*lexA3* which carry *lexA*$^+$ or one of several *spr* amber alleles. We then use this family of phages to identify the *lexA3* gene product and to study regulation of the *lexA* gene.

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MATERIALS AND METHODS

Strains. Some of the bacterial strains used are described in Table 1; phage lysogens and plasmid transformants of these strains were used as indicated. Phage strains were λ$^+$, λ*ind*$^-$, λ*cI857ts ind*$^-$, λ*cI857ts ind*$^-$ *Sam7* (the latter two strains are termed simply "λ" in the text), λ*lexA3 cI857ts ind*$^+$ (unpublished data), and its derivative λ*lexA3 cI857ts ind*$^-$ (termed λ*lexA3* for brevity), which was isolated by a cross with λ*cI857ts ind*$^-$; for isolation of λ*lexA*$^+$, λ*lexA3 spr-54*, and λ*lexA3 spr-55* (all of which carry the *cI857ts ind*$^-$ markers), see next section. λ*lexA3* carries a segment of *E. coli* DNA 2.9 kb in length from plasmid pJL26, and the other λ*lexA* phages are believed to differ from it only by their *lexA* alleles. All the λ*lexA* phages form plaques and lysogenize normally. Plasmids pJL21 *lexA*$^+$ and pJL26 *lexA3* were made by *in vitro* combination (unpublished data) using as vector the multicopy plasmid pBR322 (20); pJL30 *lexA3 spr-54* and pJL31 *lexA3 spr-55* arose by *in vivo* recombination between pJL26 and hosts with these markers. All these pJL plasmids carry a 2.9-kb insert, and their structures are believed to be the same except for the mutations in *lexA*. pMB9 *su7*, carrying the amber suppressor *su7* (21), was the gift of L. Soll; we shall term it "pMB9 *supU*."

Isolation of λ*lexA* Derivatives. Phage strain λ*lexA3 cI857ts ind*$^-$ was propagated in AB1157 *lexA*$^+$/pJL21, DM2000 *lexA3 spr-54*/pJL30, or DM2001 *lexA3 spr-55*/pJL31, and plated at 34°C on the same host strain lacking plasmid on tryptone plates containing mitomycin C (Sigma, 0.15 μg/ml). Lysogens arising in the center of a λ*lexA3* plaque cannot grow, because they are phenotypically LexA$^-$ and sensitive to mitomycin C, and so the plaques are clear; by contrast, λ and derivatives of λ*lexA3* which no longer carry the dominant *lexA3* allele form turbid plaques, because lysogens in the plaque are resistant to mitomycin C and are able to grow. Turbid plaque variants were present in stocks made as described above at a frequency of about 10^{-3}, and were purified and characterized.

Other Techniques. Cells were grown for labeling in M9 medium (22) containing threonine (50 or 80 μg/ml), leucine, arginine, proline, histidine, isoleucine, and valine (40 or 50 μg/ml each), thiamine (1 μg/ml), and either 0.4% glucose (for Fig. 1) or 0.2% maltose and 0.2% glycerol (for Figs. 2 and 3). Cultures were treated with nalidixic acid, labeled, irradiated with ultraviolet (UV) light, and analyzed by electrophoresis in sodium dodecyl sulfate/polyacrylamide gels as described (5, 23), with minor variations as indicated. Tryptone plates containined 1% Bactotryptone, 0.5% NaCl, 1% Difco agar, and

Abbreviations: kb, kilobase pairs; kd, kilodaltons.

Table 1. Bacterial strains employed

Strain	Relevant genotype	Source, reference, or derivation
AB1157	*recA*$^+$ *lexA*$^+$ *sup-37*	(15)
DM49	*recA*$^+$ *lexA3 sup-37*	(12)
DM511	*recA*$^+$ *lexA3 tsl-1 sup-37*	(16)
DM1187	*spr-51 tif-1 sfiA11 sup-37*	(13)
DM1590	As DM1187, but *sup*$^+$	D. Mount
DM1621	As DM1590, but *recA*$^+$	D. Mount
DM1631	As DM511, but *sup*$^+$	D. Mount
DM1675	*spr-51 recA*$^+$ *sfiA11 supD* (as DM1621 but *supD*)	D. Mount
DM2000	*lexA3 spr-54 tif-1 sup*$^+$	(14)
DM2001	*lexA3 spr-55 tif-1 sup*$^+$	(14)
JC10236	*recA*$^+$ *srl-300*::Tn10	M. Volkert
JL390	DM1590 (λ*ind*$^-$)	This work
JL391	*spr-51 recA*$^+$ *sup*$^+$ (λ*ind*$^-$) *srl-300*::Tn10	Transduce JL390 with P1 grown on JC10236
JL393	*spr-51 zab-53 tif-1 sup*$^+$ (λ*ind*$^-$) *srl*$^+$	Transduce JL391 with P1 grown on JM1253
JM1253	*zab-53 tif-1 srl*$^+$	(17)

Nomenclature is that of ref. 18 with these exceptions: *tif-1* and *zab-53* are alleles of the *recA* gene; *spr* and *tsl* are alleles of the *lexA* gene, originally derived from *lexA3*; *spr-54* and *spr-55* are amber mutations, but *spr-51* is not. In this nomenclature, *sup*$^+$ signifies the absence of a nonsense suppressor. We use *supU* instead of *trpT* to emphasize the suppressor activity of the pMB9 *supU* plasmid carrying this allele. Tn10 confers resistance to tetracycline; in JC10236, it is inserted into the *srl* gene cluster adjacent to *recA* (cf. 19).

thiamine at 1 μg/ml. Transformation and plasmid DNA purifications were as described (24) with minor modifications. This work was conducted under P1, EK1 conditions as specified in the 1976 National Institutes of Health Guidelines until it became exempt under the Revised Guidelines.

RESULTS

Isolation and Characterization of λ*lexA* Transducing Phages. Phages carrying various alleles of the *lexA* gene were derived by *in vivo* recombination between λ*lexA3* and a particular host *lexA* allele. Progeny phage that had acquired the host *lexA* allele were isolated by screening for variants that did not transduce *lexA3*, followed by characterization to distinguish phage that had rescued the host allele from those that were no longer *lexA3* due to a new mutation. These recombinants were shown by several criteria (summarized in Table 2) to carry the *lexA* genotype of the host in which they had been grown.

Three tests were used to characterize the *lexA* allele on each transducing phage. First, to test whether phage expressed a functional *lexA* product, their capacity to complement the recessive cell division defect of *tsl-1* strains was measured. *tsl-1* is a mutation in or very near *lexA* that may encode a temperature-sensitive *lexA* protein (3, 16); complementation signifies that the phage strain can provide *lexA* function but does not distinguish *lexA*$^+$ from *lexA3*. Second, lysogens of the various transducing phages were tested for the pattern with which they regulated the *recA* gene in a host otherwise lacking *lexA* function (Fig. 1). In this *recA*$^+$ *spr-51 sup*$^+$ host, DM1621, wild-type *recA*$^+$ is expressed at high levels in the absence of treatments that induce *recA* in wild-type hosts (not shown), as expected from the same property of the *spr-51 tif-1* strain from which it was derived (9, 13); treatment with nalidixic acid increased this level considerably. In all the λ*lexA* lysogens studied, the regulation of *recA* followed the pattern expected from studies of strains carrying *lexA*$^+$, *lexA3*, or *lexA3 spr* amber alleles on the chromosome (see Introduction) (3, 9, 14).

Table 2. Biological properties of λ*lexA* transducing phages

	Phage strain				
				λ*lexA3 spr-54* and λ*lexA3 spr-55*	
	λ	λ*lexA*$^+$	λ*lexA3*	*sup*$^+$ host	*sup*$^-$ host
Plaque morphology*	Turbid	Turbid	Clear	Turbid	Clear
tsl complementation†	−	+	+	−	+
In *spr recA*$^+$ hosts:					
recA regulation‡	Not done	LexA$^+$	LexA$^-$	Spr	LexA$^-$
UV sensitivity§	Resistant	Resistant	Very sensitive	Resistant	Sensitive

In all cases, λ*cI857ts ind*$^-$ (termed "λ"), λ*lexA*$^+$, and λ*lexA3* gave the responses listed in cells both with and without a suppressor.

* On mitomycin C plates. See *Materials and Methods* for rationale of this test; suppressor strains DM1675 (*supD*) and DM1621 carrying pMB9 *supU* were used.

† *tsl* complementation was done in λ$^+$ lysogens of DM511 and DM1631; stocks were spotted onto lawns of these strains in top agar, followed by incubation at 42.5°C; "+" indicates heavy growth in the spot, "−" means no growth.

‡ See Fig. 1; phenotypes listed are those shown by DM1187 (Spr), AB1157 (LexA$^+$), and DM49 (LexA3); see Introduction for references.

§ Strains are described in Fig. 1; in addition, lysogens of DM1675 *supD* were tested. Data for chromosomal *lexA*$^+$, *lexA3*, and *lexA3 spr-54* and *lexA3 spr-55* strains are in refs. 12 and 14, except for the intermediate sensitivity of the latter alleles in *supU* hosts (this work, not shown), also seen in DM2000 and DM2001 carrying pMB9 *supU*. The intermediate sensitivity of λ*lexA3 spr-54* and λ*lexA3 spr-55* lysogens in DM1675 is not understood.

Third, the sensitivity of these same lysogens to UV irradiation was measured. Again, the phenotypes of the lysogens were the same (except as noted) as those of strains carrying *lexA*$^+$, *lexA3*, *lexA3 spr-54*, or *lexA3 spr-55* at the normal chromosomal site.

Identification of the *lexA3* Gene Product. We examined the polypeptides synthesized by the set of λ*lexA* transducing phages with the use of UV-irradiated cell system (25). In this system, cells are heavily irradiated with UV light, essentially destroying their DNA coding capacity, then multiply infected with phage of a particular genotype. Proteins expressed by the infecting phage are detected by radiolabeling and electrophoretic analysis in polyacrylamide gels containing sodium dodecyl sulfate. The hosts we used carried a λ*ind*$^-$ prophage to repress phage lytic genes, so the observed bands should primarily represent products of genes in the bacterial insert and the phage immunity region.

We chose a host with little or no *recA* and *lexA* function, hoping to minimize effects of wild-type or higher levels of these gene products on regulation of *lexA* (see below). This host, JL393, had the *spr-51* allele at *lexA* and *zab-53 tif-1* mutations at *recA*, and as expected from studies with λ*zab-53* phages (26) it synthesized very low levels of *recA* protein during exponential growth or after treatment with nalidixic acid (not shown). In this host, λ*lexA3* produced several bands, including a band 25 kilodaltons (kd) in size (Fig. 2, lanes 3 and 8) that was not synthesized in cells infected with λ*lexA3 spr-55* or with λ (lanes 4 and 2, respectively). In the same strain carrying in addition an amber suppressor, however, the λ*lexA3 spr-55* amber phages did synthesize this 25-kd band (lane 8). Because the *spr-55* mutation was derived from a *lexA3* background, we conclude that the 25-kd band is the product of the *lexA3* gene.

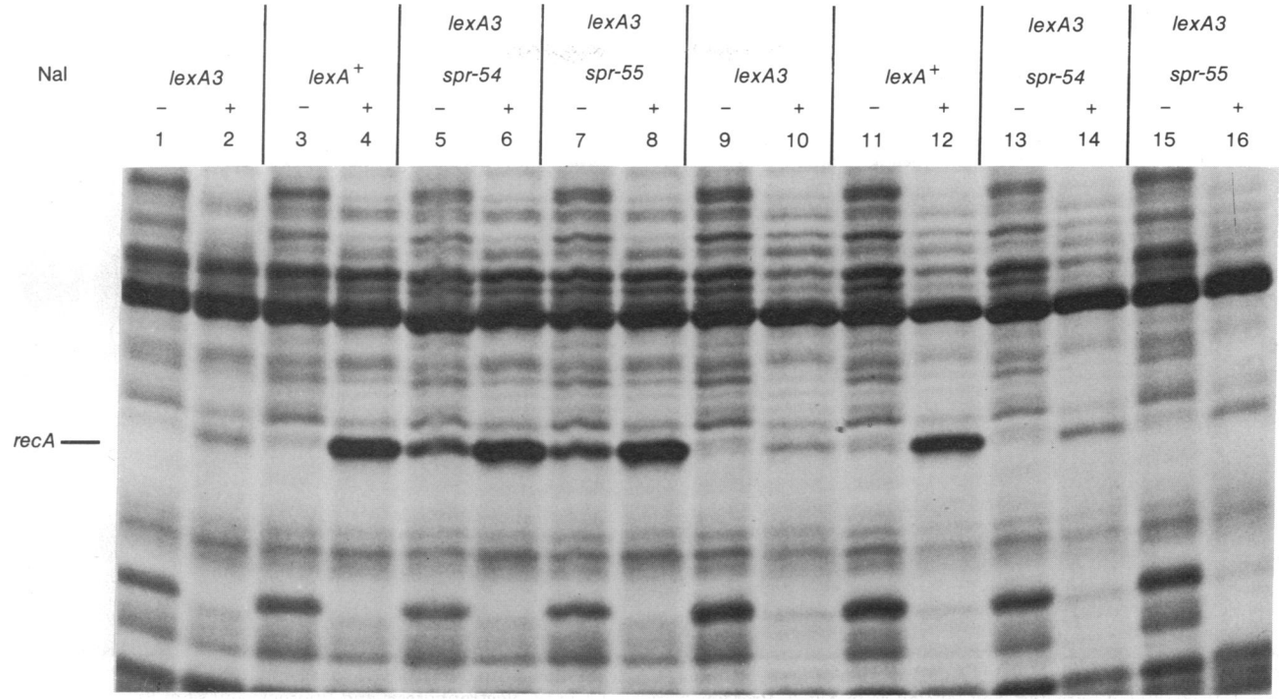

FIG. 1. Regulation of *recA* in lysogens of λ*lexA* transducing phages. Strain DM1621 was lysogenized with λ*lexA*+, λ*lexA3*, λ*lexA3 spr-54*, or λ*lexA3 spr-55*. Plasmid pMB9 *supU* was introduced by transformation. Cultures were grown at 32°C; portions were treated with nalidixic acid (Nal) (50 μg/ml) for 30 min, and aliquots of treated and untreated cultures were pulse-labeled for 5 min with [^{35}S]methionine (New England Nuclear) and analyzed by electrophoresis (11% gel) and autoradiography as described (5). Equal numbers of counts were added to each lane. Only the central portion of the pattern is shown; the position of *recA* protein is indicated. The prophage corresponding to each pair of lanes is indicated. Even-numbered lanes were from cultures treated with nalidixic acid; lanes 1–8, hosts without pMB9 *supU*; lanes 9–16, hosts with pMB9 *supU*.

λ*lexA*+ also made a distinctive band, but it was slightly smaller, 24 kd, in size than that made by λ*lexA3* (Fig. 2, lanes 5 and 10). Because we have neither amber mutants nor insertions in the *lexA*+ gene, however, we cannot yet be certain that this band is the *lexA*+ gene product (see *Discussion*). We shall describe its properties later.

λ*lexA3 spr-55* made a small polypeptide (Fig. 2, lane 4, indicated by "A"), whose size we have not measured accurately; it may be an amber fragment, because it is made at a lower rate in a *sup*− host than in a *sup*+ host (lanes 4 and 9). λ*lexA3 spr-54*, which carries a different *spr* amber mutation, made a polypeptide 22 kd in size (see Fig. 3, lanes 11 and 15) in a *sup*+ host. This band may also be an amber fragment of the *lexA3* protein, because it was not made by λ*lexA3 spr-55* and it appears to be regulated in parallel with the *lexA3* protein (next section).

Autoregulation of *lexA*. It has been found (D. Mount, J. W. Little, and S. Edmiston, unpublished data) that cells carrying the plasmids pJL21 *lexA*+ and pJL26 *lexA3* (multicopy plasmids derived from pBR322) behave as if they overproduce their respective *lexA* proteins. To determine whether the presence of these plasmids affects the expression of the *lexA* gene, we carried out infection experiments like those described above by using the same host as before but carrying in addition either pJL21, pJL26, pJL31 *lexA3 spr-55*, or pBR322. Because the host carried the *zab-53* allele at *recA*, the rate of expression of *recA*

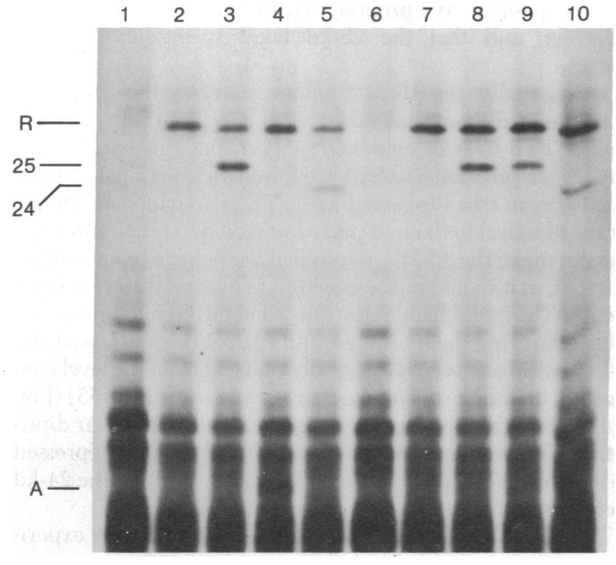

FIG. 2. Identification of *lexA3* gene product. Strains JL393 and JL393/pMB9 *supU* were grown at 37°C, concentrated, suspended in medium supplemented with 10 mM $MgSO_4$, and irradiated with UV light (1800 J/m^2). Portions were infected with the indicated phage at a multiplicity of 10–15, labeled with [^{35}S]methionine (600 Ci/mmol, 20 μCi/ml) (1 Ci = 3.7×10^{10} becquerels), and analyzed by electrophoresis (15% gel) as described (23), followed by fluorography (27). Only the bottom half of the pattern is shown; no bands were visible in the top half. The position of λ repressor, identified in other experiments with extracts of cells carrying pKB280 (28), is indicated by "R"; those of the 25-kd, 24-kd, and amber fragment bands discussed in the text are indicated by "25", "24", and "A", respectively. The sizes of the 25-kd and 24-kd bands were determined in another gel (not shown) by using as size markers carbonic anhydrase (29 kd), λ repressor (26.2 kd), and soybean trypsin inhibitor (21.5 kd). Lanes 1–5: JL393 host infected with no phage (lane 1), λ*cI857ts ind*− *Sam7* (lane 2), λ*lexA3* (lane 3), λ*lexA3 spr-55* (lane 4), or λ*lexA*+ (lane 5). Lanes 6–10: JL393/pMB9 *supU* infected with no phage (lane 6), λ*cI857ts ind*− *Sam7* (lane 7), λ*lexA3* (lane 8), λ*lexA3 spr-55* (lane 9), or λ*lexA*+ (lane 10).

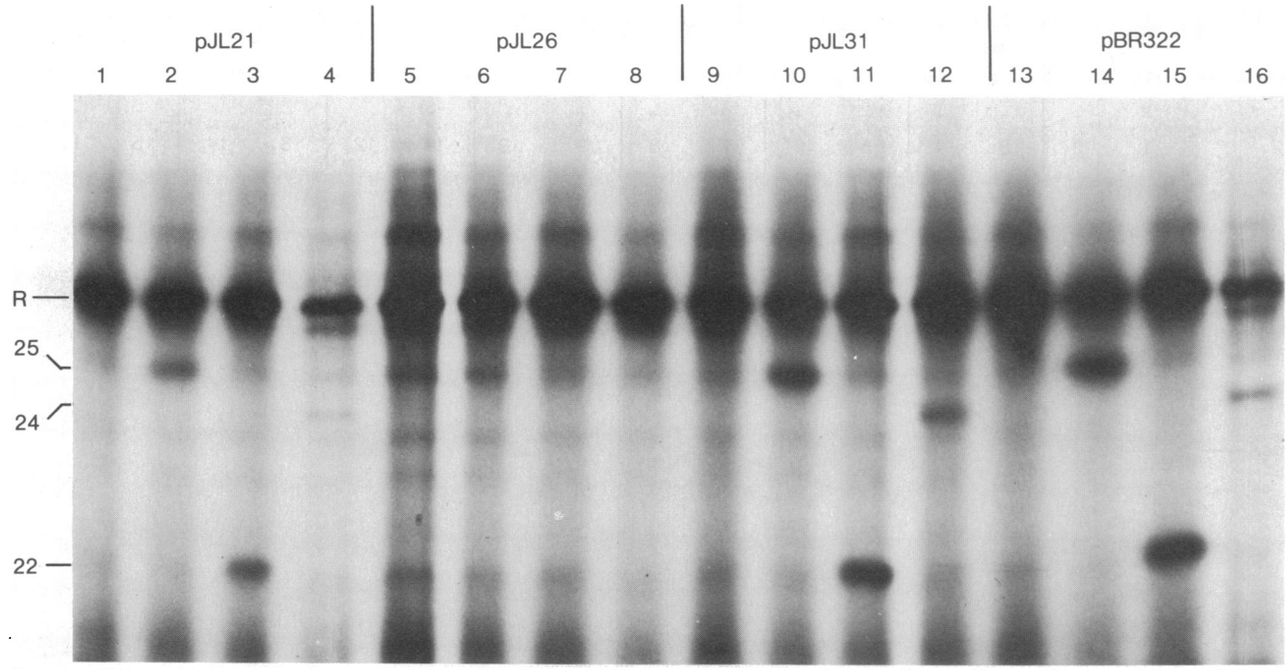

FIG. 3. Effect of *lexA* plasmids on expression of *lexA*. Strain JL393 was transformed with pJL21, pJL26, pJL31, or pBR322. Cells were grown, UV-irradiated, infected, and labeled as in Fig. 2. Proteins were analyzed by electrophoresis and fluorography as in Fig. 2. Cells carry the indicated plasmid; the position of λ repressor (R) and the 25-kd, 24-kd, and 22-kd bands described in the text is indicated. Only the central portion of the pattern is shown. Infection was with λ*cI857ts ind*$^-$ *Sam7* (lanes 1, 5, 9, and 13), λ*lexA3* (lanes 2, 6, 10, and 14), λ*lexA3 spr-54* (lanes 3, 7, 11, and 15), or λ*lexA*$^+$ (lanes 4, 8, 12, and 16).

was low in all these strains (not shown; cf. ref. 26). Any changes observed in the presence of the *lexA* plasmids should not therefore be due to major changes in the level of the *recA* protein in response to the LexA phenotype.

Hosts carrying these plasmids were infected with λ, λ*lexA*$^+$, λ*lexA3*, or λ*lexA3 spr-54*. In the hosts carrying pJL31 or pBR322, the transducing phages made the respective 24-kd, 25-kd, and 22-kd bands described above (Fig. 3, lanes 10–12 and 14–16). In contrast, none of these bands was seen in a host carrying pJL26 *lexA3* (lanes 6–8), suggesting roughly that they are expressed at less than 10–20% of the rates in the hosts with pJL31. These findings suggest that *lexA3* regulates itself and that the 24-kd and 22-kd bands made by λ*lexA*$^+$ and λ*lexA3 spr-54* are regulated in the same fashion (see *Discussion*).

In a separate experiment (not shown), the *lexA3* band was observed after infection of irradiated lysogens of the *lexA*$^+$ strain *AB1157* or the *lexA3* strain DM49 at levels similar to that seen in strain JL393 (Fig. 2), suggesting that repression depends upon overproduction of the *lexA3* protein by the multicopy plasmid pJL26. Finally, in hosts carrying pJL21 *lexA*$^+$, a decrease of roughly 50% was observed in the levels of the *lexA* bands (Fig. 3, lanes 2–4), suggesting that the *lexA*$^+$ product exerts an autoregulatory effect, but to a considerably lesser degree than the *lexA3* product does.

DISCUSSION

We have isolated and characterized a set of transducing phages carrying *lexA*$^+$, *lexA3*, *lexA3 spr-54*, and *lexA3 spr-55* alleles of the *lexA* gene. Evidence that these phages carry the specified alleles is summarized in Table 2. We shall discuss studies of the polypeptides encoded by these phages and data that indicate that *lexA* regulates its own expression.

The λ*lexA3* phage encodes a distinctive 25-kd polypeptide (Fig. 2, lane 3), which is also made by λ*lexA3 spr-55* in a *supU* host (lane 9) but not in a *sup*$^+$ (suppressor-free) host (lane 4). No other new polypeptides were observed, though ones about 26 kd in size might be obscured by the λ repressor, and ones smaller than about 20 kd might be lost in small host material. In order to conclude that the 25-kd protein is the *lexA3* mutant gene product, we must assume that the *spr-55* amber mutation actually lies in the same gene as *lexA3*, which (with other *lexA*$^-$ alleles) defines the *lexA* gene. Extensive genetic evidence from this laboratory supports this assumption (14), as does the fact that both mutations lie on the same 2.9-kb DNA fragment that is cloned into these phages. This fragment could, however, carry a few small genes in addition to the 700 base pairs or so needed for a 25-kd polypeptide. We cannot rule out the formal possibility that the amber mutations are in a gene encoding a 25-kd polypeptide and lying upstream from *lexA3* in a multicistronic operon; in this model, polarity would prevent functional expression of the actual *lexA3* gene in the absence, but not in the presence, of nonsense suppression. Recent findings that UV light can relieve polarity in some systems (29) argue against this model. Although it would be interesting if true, there are no data to support it. We propose that the simple interpretation is correct, and that the 25-kd band is the *lexA3* gene product.

Unexpectedly, the λ*lexA*$^+$ phage, carrying the wild-type *lexA*$^+$ gene, did not make this 25-kd band, but one of 24 kd instead. Two lines of evidence suggest that this band is the *lexA*$^+$ gene product: (*i*) λ*lexA*$^+$ expresses a functional activity or activities *in vivo* (Table 2; Fig. 1). These activities are similar but not identical to those displayed *in vivo* by λ*lexA3*; λ*lexA*$^+$ does not make the 25-kd protein that we believe to have these activities, so it should make something new and different from λ*lexA3*. The 24-kd band is the only such difference we see. (*ii*) In cells carrying the *lexA3* plasmid pJL26, the amount of the 25-kd *lexA3* band seen is drastically reduced from the level seen in cells without plasmid or carrying pJL31 (*lexA3 spr-55*) (Fig. 3). Levels of the 24-kd band suffer the same or a similar diminution. These data suggest (see below) that *lexA3* is repressed by high levels of *lexA3* protein and that synthesis of the 24-kd protein is regulated by the same mechanism.

The 25-kd and 24-kd bands were also detected in experi-

ments (unpublished data) with minicell strains (cf. ref. 30) containing plasmids pJL26 *lexA3* and pJL21 *lexA*$^+$, respectively. The plasmids contain the same fragment of bacterial DNA as do the λ*lexA* transducing phages but contain no other common sequences. In minicells, plasmid pJL30 *lexA3 spr-54* also encoded the same 22-kd band (believed to be an amber fragment) seen in Fig. 3. These results suggest that the bands observed are encoded entirely in the bacterial insert, rather than being read-through products. Moreover, because the bands can be detected in two quite different systems, we conclude that they are not artifacts of the UV-irradiated cell system.

If the 24-kd band is the product of the *lexA*$^+$ gene, why is it smaller than the *lexA3* mutant protein? Several possibilities are that one or both bands is a processed form; transcription or translation start or stop signals could be different; or the *lexA3* mutation might be a small insertion, perhaps resulting in addition of extra amino acids in a normally protease-sensitive region of the protein (cf. ref. 31) and altered interaction with a specific protease. We are currently testing these possibilities and examining the products of other *lexA*$^-$ alleles.

It was recently reported that the *lexA* protein is 85 kd in size (26). This conclusion was based not on studies with altered mutant proteins but on analysis of proteins encoded by a set of transducing phages that carried bacterial DNA extending to various distances clockwise from the gene *malE* (18). We did not observe this protein as a band in our experiments with λ*lexA*$^+$ and λ*lexA3*, and we believe that it is encoded in a gene outside of *lexA* and not carried *in toto* on our phage strains. We did observe a large polypeptide made in minicells (umpublished data) by a *ubiA*$^+$ plasmid, pJL22, which carries the insert in λ*lexA*$^+$ plus an additional 5.5 kb of DNA towards *malE*.

We also have used the λ*lexA* phages to study the *in vivo* function of *lexA* protein. Evidence that one such function is autoregulation came from experiments with host cells containing multicopy *lexA* plasmids. The presence of the *lexA3* plasmid pJL26 prevented or greatly decreased the appearance of the 24-kd, 25-kd, and 22-kd bands made by λ*lexA*$^+$, λ*lexA3*, and λ*lexA3 spr-54*, respectively (Fig. 3). This effect results specifically from the *lexA3* allele on the plasmid, because a *lexA3 spr-55* plasmid did not exert the effect. We believe it is due to elevated levels of the *lexA3* protein in this strain, because the effect was not seen in a host with a single chromosomal *lexA3* gene (not shown); we have not measured the levels of the *lexA3* protein in a strain carrying pJL26, however.

A similar but weaker effect was observed in cells containing the *lexA*$^+$ plasmid pJL21 (Fig. 3). The presence of a background and of faint host bands makes it difficult to quantitate this effect, but the levels of the *lexA*-specific bands are decreased roughly 50% compared to those in cells carrying pJL31 or pBR322. Why *lexA3* and *lexA*$^+$ products should differ in the severity of this effect is unclear at present.

Although it is conceivable that the *lexA*-specific bands are absent in cells with pJL26 because these cells contain a specific protease, the *in vivo* properties of *lexA3* strains do not suggest that *lexA3* protein, at least, would be susceptible to such cleavage. Instead, we suggest that the *lexA3* band is not seen because the *lexA3* gene product negatively regulates its own expression.

Data from minicell experiments (unpublished) support this suggestion. Minicells containing pJL26 are expected to contain high levels of *lexA3* protein; they express the 25-kd band at a very low rate compared with that at which minicells containing pJL30 (*lexA3 spr-54*) express the 22-kd band believed to be a *lexA3 spr-54* amber fragment. This comparison suggests, again, that high levels of the *lexA3* product substantially repress the *lexA* gene.

An autoregulatory mechanism (32) has at least two features that might play important roles in the *recA–lexA* regulatory cycle. First, autoregulation would maintain a constant level of *lexA* protein in exponentially growing cells; and second, it would allow this level to reach its optimal value quickly when cells were making a transition from a state with no *lexA* function (such as when the SOS functions are induced) to a normal growth state.

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