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Phenotypes of *lexA* Mutations in *Salmonella enterica*: Evidence for a Lethal *lexA* Null Phenotype Due to the Fels-2 Prophage

Kim Bunny,† Jing Liu,‡ and John Roth*

Department of Biology, University of Utah, Salt Lake City, Utah 84112

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The LexA protein of *Escherichia coli* represses the damage-inducible SOS regulon, which includes genes for repair of DNA. Surprisingly, *lexA* null mutations in *Salmonella enterica* are lethal even with a *sulA* mutation, which corrects *lexA* lethality in *E. coli*. Nine suppressors of lethality isolated in a *sulA* mutant of *S. enterica* had lost the Fels-2 prophage, and seven of these (which grew better) had also lost the Gifsy-1 and Gifsy-2 prophages. All three phage genomes included a homologue of the *tum* gene of coliphage 186, which encodes a LexA-repressed cI antirepressor. The *tum* homologue of Fels-2 was responsible for *lexA* lethality and had a LexA-repressed promoter. This basis of *lexA* lethality was unexpected because the four prophages of *S. enterica* LT2 are not strongly UV inducible and do not sensitize strains to UV killing. In *S. enterica*, *lexA*(Ind$^-$) mutants have the same phenotypes as their *E. coli* counterparts. Although *lexA* null mutants express their error-prone DinB polymerase constitutively, they are not mutators in either *S. enterica* or *E. coli*.

The LexA-repressed SOS regulon includes genes that encode DNA repair enzymes (19). This regulon is induced when DNA damage produces single-stranded DNA, which interacts with RecA protein to form a complex that stimulates autocleavage of the LexA repressor protein (55). A mechanistically similar system regulates lysogeny of phage lambda (in *Escherichia coli*) and P22 (in *Salmonella enterica*) without direct involvement of LexA. For these phages, single-stranded DNA bound to RecA protein causes autocleavage of the major phage repressor protein instead of LexA (41, 42). In contrast, lysogens of coliphage 186 can be maintained only in the presence of LexA protein, which represses the phage *tum* (antirepressor) gene (27, 44). Phage 186 prophages are induced when cleavage of LexA allows *tum* expression and thereby upsets prophage repression.

The SOS system of *S. enterica* seems similar to that of *E. coli* (4). In both bacteria, *lexA* is the first gene in a two-gene operon (with *dinF*) that is repressed by LexA protein. In *S. enterica*, a series of genes (*din*) have been identified that show RecA-dependent induction by mitomycin C and are repressed by overproduction of the LexA protein (48). In *E. coli*, a *lexA* null mutation causes lethal cell filamentation, which can be prevented by a *sulA* mutation.

Evidence is presented that *lexA* null mutations in *S. enterica* are lethal even in the presence of a *sulA* mutation and that this lethal phenotype is due to induction of genes within resident prophages. Surprisingly, these prophages are not strongly UV inducible, and strains of *S. enterica* carrying all three LexA-regulated phages are not more sensitive to UV killing than is *E. coli* or isogenic *Salmonella* strains lacking the prophages. Induction of LexA-regulated genes of the Fels-2 and Gifsy prophages may inhibit cell growth without causing full prophage induction. Loss of prophages may be the most frequent event that provides resistance to this inhibition. Noninducible *lexA*(Ind$^-$) mutants of *S. enterica* behave like their *E. coli* counterparts. Although *lexA* null mutations increase expression of the error-prone DinB polymerase in *Salmonella* spp., as in *E. coli* (53, 54), they do not cause a mutator phenotype. Thus, SOS induction of DinB appears to be necessary but not sufficient for mutagenesis (as is known for the error-prone UmuCD polymerase).

MATERIALS AND METHODS

Strains. Except where noted, all strains used (Table 1) were derived from *S. enterica* serovar Typhimurium strain LT2. Key mutations are diagrammed in Fig. 1. The insertion mutations *lexA40*::Kan (Fig. 1B) and *sulA46*::Spc were constructed and provided by Montserrat Llagostera and Xavier Garriga (15). The reported structures were confirmed by sequencing. This *lexA* insertion proved to be lethal in our strains, so the received strains must carry a suppressor of *lexA* lethality; their genetic background is referred to as SLT2 (Spanish LT2).

Plasmids pJWL26 and pJWL21, carrying the *lexA3*(Ind$^-$) and *lexA*$^+$ alleles of *E. coli*, were provided by John Little (28, 33). A strain cured for Gifsy-1 (TT20492; was MA3408) and another lacking both Gifsy-1 and -2 (TT20493; was MA4587) were obtained from Lionello Bossi and Nara Figueroa, as were insertion mutations in the prophages of Fels-1 and Fels-2. Two strains lacking Fels-2 TR17000 (= DB7000) and TT10910 (= DB9071) were obtained from David Botstein; the second of these was found to also lack the Gifsy-1 and -2 prophages (see Results). The element Tn*10*dT-POP is a transposition-defective derivative of Tn*10* that directs tetracycline-inducible transcription out of both ends (39). The *recN*::MudJ insertion was isolated and characterized by David Thaler (40). It is inserted at nucleotide 95 of the *recN* gene (Julianne Grose, personal communication).

Media and chemicals. The minimal medium was NCE (6) containing glucose or lactose (0.2%) plus nutrient supplements at the concentrations recommended previously (17). Liquid rich medium was Luria-Bertani broth (LB), and the solid rich medium was nutrient broth (NB; Difco Laboratories) supplemented with 5 g of NaCl per liter and solidified with 1.5% BBL agar. Final concentrations of antibiotics in rich medium were: kanamycin sulfate (Kan), 50 μg/ml; tetracycline (Tet), 20 μg/ml; chloramphenicol (Cam), 25 μg/ml; rifampin (Rif; supplied by ICN), 80 μg/ml; spectinomycin (Spc), 150 μg/ml; and zeocin (Zeo; supplied by Invitrogen), 50 μg/ml. The chromogenic β-galactosidase substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Diagnostic Chemicals) was used at 25 μg/ml in NB for the colony sectoring tests. Restriction enzymes were

* Corresponding author. Present address: Microbiology Section of DBS, Center for Genetics and Development, University of California, Davis, Davis, CA 95616-8665. Phone: (530) 752-6679. Fax: (530) 752-7663. E-mail: jrroth@ucdavis.edu.

† Present address: Center for Genetics and Development, University of California, Davis, Davis, CA 95616-8665.

‡ Present address: Stowers Institute for Medical Research, Kansas City, MO 64110.

TABLE 1. Strain list

Strain	Background if other than TR10000	Genotype[a]	Source or reference
TR10000	Wild type	*S. enterica* serovar Typhimurium strain LT2	Laboratory collection
TR17000	=DB7000	Wild type (LT2 lacking Fels-2)	David Botstein
TR7178	*E. coli* K-12	*ara thiA* Rif^r Δ13 *lac proB*/F′128 *lacI*^{q} *lacI33*(fs) *lacIZ*(Ω fusion) *pro′*	Cairns (14)
TR7235		/pJWL21 *lexA*$^+$ from *E. coli*	This study
TR7236		/pJWL26 *lexA3*(Ind$^-$) from *E. coli*	This study
TR7237	*E. coli*	/pJWL21 *lexA*$^+$ from *E. coli*	John Little
TR7238	*E. coli*	/pJWL26 *lexA3*(Ind$^-$) from *E. coli*	John Little
TT8371		*thr-469*::MudA (blue)	Laboratory collection
TT9048		*recA1*	Laboratory collection
TT10268		*zai-808*::Tn*10 proC150*	C. Miller
TT10725		*metE205 ara-9 metH2357*::MudA (blue)	Laboratory collection
TT10910	DB9071	*hisO1242* Δ(Fels-2 Gifsy-1 Gifsy-2)	David Botstein
TT16803		*hisC1264*::MudJ *argR*::Tn*10*dTet *srl*::Tn*10*dCam Δ*hisG203*	Laboratory collection
TT17200		*sulA zaj-9222*::Tn*10 din-9*::MudJ	C. Mark Smith
TT17202		*sulA zaj-9222*::Tn*10 din-80*::MudJ	C. Mark Smith
TT17207		*leuD21 din-84*::MudJ	C. Mark Smith
TT17212		*leuD21 din-10*::MudJ	C. Mark Smith
TT17213		*leuD21 din-11*::MudJ	C. Mark Smith
TT17214		*leuD21 din-14*::MudJ	C. Mark Smith
TT17215		*leuD21 din-220*::MudJ	C. Mark Smith
TT17216		*leuD21 din-240*::MudJ	C. Mark Smith
TT17217		*leuD21 din-243*::MudJ	C. Mark Smith
TT17218		*leuD21 din-292*::MudJ	C. Mark Smith
TT17499		*hisG618 cysA1585*::MudA	Laboratory collection
TT17652	SLT2	Rif^r *sulA446*::Spc (was UA1615)	Montserrat Llagostera and Xavier Garriga
TT17653	SLT2	Rif^r *sulA446*::Spc *lexA40*::Kan (was UA1685)	Montserrat Llagostera and Xavier Garriga
TT18829		*recN557*::MudJ	David Thaler
TT20492	ATCC 14028	*S. enterica* serovar Typhimurium ΔGifsy-1	N. Bossi and N. Figueroa
TT20493	ATCC 14028	Δ(Gifsy-1 Gifsy-2)	N. Bossi and N. Figueroa
TT21685		*malB661*::Tn*10*dTet	Laboratory collection
TT22236		/pTP223	Tony Poteete
TT22613	SLT2	*sulA446*::Spc *lexA41*::Cam(sw)	This study
TT22614	SLT2	*sulA446*::Spc *lexA41*::Cam(sw)/pTP223	This study
TT22885		/pCP20 lambdaC1857 FLP (Amp^r Cam^r)	Laboratory collection
TT22888		DUP1995[(*metH235*) ∗MudA∗ (*thr-469*)]	This study
TT22964		*lexA33*::[Cam *lexA3*(Ind$^-$)](sw)	This study
TT23151		*zgc-9189*::Cam *recA281*(O^c) *recA*$^+$	This study
TT23199	SLT2	*sulA446*::Spc *lexA42*::(FRT-Cam-FRT) (sw)	This study
TT23200	SLT2	*sulA446*::Spc *lexA42*::FRT(sw) *malB661*::Tn*10*dTet	This study
TT23203		*sulA446*::Spc DUP1995[(*metH235*) ∗MudA∗ (*thr-469*)]	This study
TT23204	SLT2	*sulA446*::Spc *malB661*::Tn*10*dTet	This study
TT23205		*sulA446*::Spc	This study
TT23206		*zgc-9189*::Cam *recA281*(O^c) *recA*$^+$/pJWL21 plasmid (*lexA*$^+$)	This study
TT23207		*zgc-9189*::Cam *recA281*(O^c) *recA*$^+$/pJWL26 plasmid [*lexA3*(Ind$^-$)]	This study
TT23252		*lexA33*::[Cam *lexA3*(Ind$^-$)](sw)	This study
TT23255		*sulA446*::Spc *lexA41*::Cam(sw) Slx1 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23256		*sulA446*::Spc *lexA41*::Cam(sw) Slx2 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23257		*sulA446*::Spc *lexA41*::Cam(sw) Slx3 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23259		*sulA446*::Spc *lexA41*::Cam(sw) Slx5 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23260		*sulA446*::Spc *lexA41*::Cam(sw) Slx6 ΔFels-2	This study
TT23261		*sulA446*::Spc *lexA41*::Cam(sw) Slx7 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23262		*sulA446*::Spc *lexA41*::Cam(sw) Slx8 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23263		*sulA446*::Spc *lexA41*::Cam(sw) Slx9 ΔFels-2	This study
TT23264		*sulA446*::Spc *lexA41*::Cam(sw) Slx10 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23276		*sulA446*::Spc *lexA41*::Cam(sw) *recN557*::MudJ Slx2 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23280		*sulA446*::Spc *lexA41*::Cam(sw) *recN557*::MudJ Slx6 ΔFels-2	This study
TT23283		*sulA446*::Spc *lexA41*::Cam(sw) *recN557*::MudJ Slx9 ΔFels-2	This study
TT23296	DB9071	*sulA446*::Spc *hisO1242* ΔFels-2	This study
TT23298	ATCC 14028	*sulA446*::Spc ΔGifsy-1	This study
TT23299	ATCC 14028	*sulA446*::Spc Δ(Gifsy-1 Gifsy-2)	This study
TT23309		*sulA446*::Spc *malB661*::Tn*10*dTet *lexA*$^+$ Slx8 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23315		*recN557*::MudJ	This study
TT23317		*sulA446*::Spc *recN557*::MudJ	This study
TT23320		*sulA446*::Spc *malB661*::Tn*10*dTet *recN557*::MudJ Slx2 Δ(Gifsy-1 Gifsy-2 Fels-2)	This study
TT23324		*sulA446*::Spc *malB661*::Tn*10*dTet *recN557*::MudJ Slx6 ΔFels-2	This study

Continued on following page

TABLE 1—*Continued*

Strain	Background if other than TR10000	Genotype[a]	Source or reference
TT23327		*sulA46*::Spc *malB661*::Tn*10*dTet *recN557*::MudJ Slx9 ΔFels-2	This study
TT23351		*recN557*::MudJ *zgc-9189*::Cam *recA281*(O^c) *recA*$^+$	This study
TT23353		*sulA46*::Spc *recN557*::MudJ *zgc-9189*::Cam *recA281*(O^c) *recA*$^+$	This study
TT23355		*recN557*::MudJ/pJWL21 *lexA*$^+$	This study
TT23357		*sulA46*::Spc *recN557*::MudJ/pJWL21 *lexA*$^+$	This study
TT23361		*sulA46*::Spc *recN557*::MudJ/pJWL26 *lexA3*(Ind$^-$)	This study
TT23379	DB7000	*sulA46*::Spc ΔFels-2	This study
TT23381		*recN557*::MudJ *lexA33*::[Cam *lexA3*(Ind$^-$)](sw)	This study
TT23383		*sulA46*::Spc *recN557*::MudJ *lexA33*::[Cam *lexA3*(Ind$^-$)](sw)	This study
TT23388		*sulA46*::Spc *recN557*::MudJ *zgc-9189*::Cam *recA281*(O^c) *recA*$^+$/pJWL21 *lexA*$^+$	This study
TT23392		*recN557*::MudJ *zgc-9189*::Cam *recA281*(O^c) *recA*$^+$/pJWL21 *lexA*$^+$	This study
TT23393		*recN557*::MudJ *zgc-9189*::Cam *recA281*(O^c) *recA*$^+$/pJWL26 *lexA3*(Ind$^-$)	This study
TT23465		*recN557*::MudJ *recA462*::T-POP	This study
TT23489		*recN557*::MudJ/pJWL26 *lexA3*(Ind$^-$)	This study
TT23491		*sulA46*::Spc *recN557*::MudJ *zgc-9198*::Cam *recA281*(O^c) *recA*$^+$/pJWL26*lexA3*(Ind$^-$)	This study
TT23515		*sulA46*::Spc/pBADhisC*dinF*	This study
TT23522	Wild type	/pJWL26Cam derivative of pJWL26 *lexA3*(Ind$^-$) (Cam^r cassette inserted 97 bp upstream of *lexA* start codon)	This study
TT23563		*gin-48*(Fels-2)::Kan(sw) (was MA7273)	N. Bossi and N. Figueroa
TT23569		*sulA46*::Spc/pTP223	This study
TT23570	SLT2	*sulA46*::Spc/pTP223	This study
TT23571	SLT2	*sulA46*::Spc *lexA40*::Kan/pTP223	This study
TT23647		*trpB9 hisC9955*::MudJ::TPOP/pKD46	This study
TT23649		*tum*$^{Fels-2}$::Cam(sw)	This study
TT23650		*sulA46*::Spc *tum*$^{Fels-2}$::Cam(sw)	This study
TT23656	Wild type	Made by making TT23205 *sulA*$^+$	This study
TT23657		Δ(Fels-2 Gifsy-1 Gifsy-2)	This study
TT23764		*proB1657*::Tn*10 sulA46*::Spc Δ(Fels-2 Gifsy-1 Gifsy-2)/F′128 (P$_{tum}$::*lacZ*$^+$)	This study
TT23765		*proB1657*::Tn*10 sulA46*::Spc Δ(Fels-2 Gifsy-1 Gifsy-2) *lexA41*::Cam(sw)/F′128 (P$_{tum}$::*lacZ*$^+$)	This study
TT23770		*metA22 metE551 trpD2 ilv-452 leu pro* (leaky) *hsdLT6 hsdSA29 hsdB strA120*/pKD46/F′128 *mhpC31*::Tn*10*d-Tet	This study

[a] (sw) indicates a swap mutation for which the target gene has been deleted and replaced with a resistance gene.

from New England Biolabs. Unless otherwise specified, chemicals were obtained from Sigma Chemical Company.

Transduction and transformation methods. Transduction crosses were mediated by the high-frequency generalized transducing mutant of phage P22 HT105-1 (43). Transductant clones were purified and freed of phage on green indicator plates (10). Phage sensitivity of cells was tested by cross-streaking with the P22 clear-plaque mutant H5 on green indicator plates. For transduction tests of a bacterial strain's ability to inherit a particular donor marker, the efficiency of the transducing lysate was demonstrated by its ability to correct the *hisG* mutation in strain TT17499 (*hisG618 cysA1585*::MudA). All lysates used produced greater than 1,000 His$^+$ transductants per minimal cysteine plate under the conditions of our crosses.

For most of the linear transformation experiments (16, 61), the recipient strain carried plasmid pTP223, which includes genes for tetracycline resistance (Tet^r) and expresses the recombination functions of phage lambda (*red*, *gam*, and *exo*) from a *lac* promoter. This plasmid was obtained from Tony Poteete (34, 35). To delete the *tum* gene of Fels-2 by linear transformation, the recipient strain (TT23647) carried plasmid pKD46, obtained from Barry Wanner (16). Plasmid pKD46 was also carried in the recipient strain used to create the Fels-2 *tum* regulatory fusion to *lacZ* (TT23770). Both methods were adapted for use in *S. enterica* by Eric Kofoid (personal communication).

Replacement of *S. enterica lexA*$^+$ gene with *E. coli lexA3*(Ind$^-$) allele. The chloramphenicol resistance determinant (Cam^r) from plasmid pACYC184 was amplified by PCR and introduced by linear transformation just upstream of the *E. coli lexA3*(Ind$^-$) gene in plasmid pJWL26. The resulting plasmid (pJWL26Cam; strain TT23522) was used as the template for PCR amplification of a fragment carrying both Cam^r and the *E. coli lexA3* gene. The primers for this PCR had sequence at their 5′ ends that was homologous to the ends of the *S. enterica lexA* gene. The PCR product was introduced into the *Salmonella* chromosome by linear transformation. Transformants acquired Cam^r and the mutant *lexA3* allele from *E. coli* in place of the *S. enterica lexA*$^+$ gene. The structure of the final mutation, *lexA33*(Ind$^-$) (Fig. 1A), was verified by sequencing.

Construction of an *S. enterica lexA* swap mutation. The Cam^r gene from pACYC184 was PCR amplified with primers whose 5′ ends included sequence homologous to the ends of the *S. enterica lexA* gene. This product was introduced by linear transformation into an SLT2 strain (TT23570) which has *sulA46*::Spc in its chromosome and harbors plasmid pTP223 (encoding recombination functions of phage lambda). This genetic background (SLT2) carried an unknown suppressor of *lexA* lethality (later shown to be the lack of prophages). Transformants inherited Cam^r in place of the chromosomal *lexA*$^+$ coding sequence; one of these, *lexA41*::Cam(sw), was used in subsequent experiments (Fig. 1B).

Construction of *lexA* in-frame deletion. The Cam^r gene from pACYC184 was amplified by PCR. The 5′ ends of both primers contained FLP recognition target (FRT) sites and sequences that were homologous to the ends of the *S. enterica lexA* gene. The PCR product was introduced into the chromosome of an *S. enterica* (SLT2) strain by linear transformation, selecting Cam^r; transformants acquired a Cam^r determinant flanked by FRT sites in place of the *lexA*$^+$ gene—an allele designated *lexA42*::(FRT-Cam-FRT; strain TT23199). A deletion between FRT sites generates an in-frame *lexA* deletion allele that encodes a 31-amino-acid product containing the first 10 and the last 9 amino acids of the LexA protein and 12 intervening amino acids encoded by the single remaining FRT sequence.

After moving the *lexA42*::(FRT-Cam-FRT) allele into a strain carrying insertion *malB661*::Tn*10*dTet (25 to 30% cotransducible with *lexA*), the Cam^r determinant was removed by Flp recombinase (16). Recombinase was provided by plasmid pCP20 (obtained from Barry Wanner), which is temperature sensitive for replication and encodes a heat-inducible Flp recombinase (12). At 30°C, this plasmid replicates without expressing Flp recombinase; at 42°C, the plasmid expresses the Flp recombinase and ceases replication. Strains carrying this plasmid were streaked on NB and incubated at 42°C overnight. The streaks were replica plated to NB-ampicillin (to score the presence of the plasmid), to NB-chloramphenicol (to score the presence of the Cam^r gene within *lexA*), and to NB. Strains were recovered with the FRT deletion and without the plasmid. The

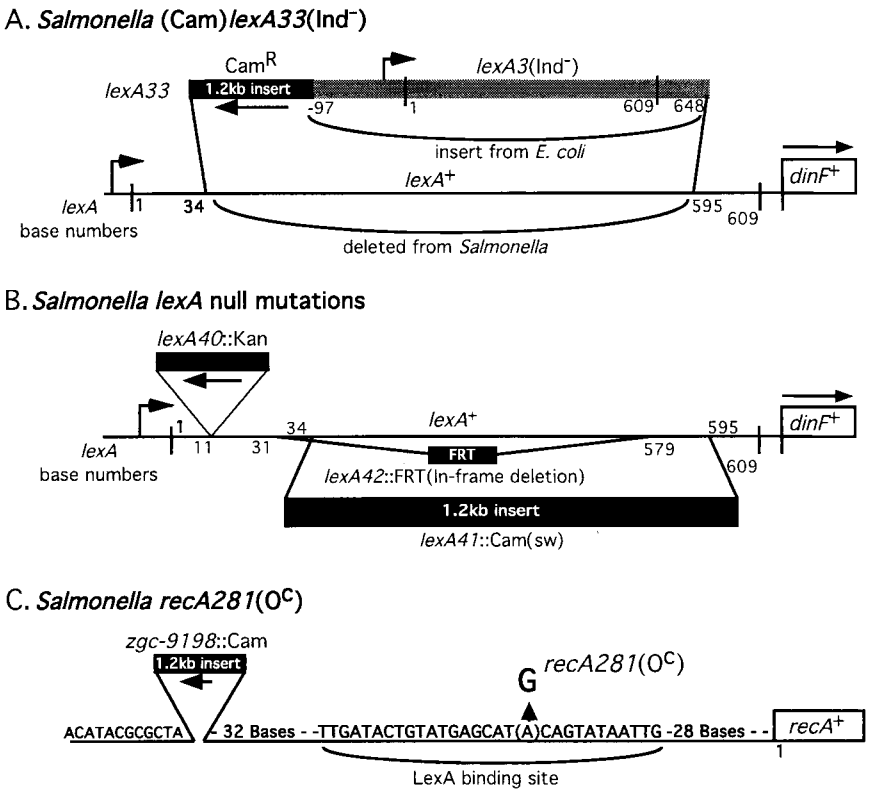

FIG. 1. Structure of constructed *lexA* and *recA* mutations. (A) For the *lexA33*(Ind$^-$) allele, bases 35 to 594 of the *Salmonella lexA* gene were replaced with a Cam^r gene and the complete *lexA3*(Ind$^-$) allele of *E. coli*. (B) The *lexA* null mutation *lexA40*::Kan is an insertion of a kanamycin resistance gene after base 11 of *lexA* and was constructed by Montserrat Llagostera and Xavier Garriga (15). The *lexA41*::Cam(sw) allele is a replacement of *lexA* bases 35 to 594 with the above Cam^r gene. The *lexA42*::FRT mutation is an in-frame deletion that removes bases 32 to 578. (C) In the *recA281*(O^c) mutation, a G residue replaces A in the *lexA* binding site upstream of *recA*. The Cam^r gene was inserted upstream to allow selective transduction of this mutation into new strains. Construction methods are described in Materials and Methods.

in-frame deletion (*lexA42*::FRT; strain TT23200) was verified by sequencing a PCR-amplified *lexA* fragment and is diagrammed in Fig. 1B.

The lethal phenotype of the in-frame deletion was demonstrated by a transduction cross with a donor carrying the insertion *malB661*::Tn*10*dTet closely linked to *lexA42*::FRT. The recipient carried a *lexA33*(Ind$^-$) allele with its associated Cam^r marker and *sulA* but no suppressor of *lexA* lethality. None of the Tet^r transductants lost Cam^r; that is, it was impossible to replace the recipient *lexA*(Ind$^-$)-Cam^r allele with the donor *lexA* in-frame deletion. Without the *lexA* deletion in the donor, 30% of the transductants from this cross are Cam^s. This method was also used to show that the *lexA* in-frame deletion is lethal in strains carrying a Fels-2 prophage.

Construction of *recA* operator constitutive (O^c) mutation. A previously characterized *recA* operator constitutive mutant of *E. coli* (*recAo281*) carries a point mutation in the LexA binding site between the −35 and −10 regions of the *recA* promoter region (51). This mutational change, an A/T to G/C transition at position −40, was introduced into the *S. enterica* genome by linear transformation (Fig. 1C). Primers for PCR were designed to amplify the pACYC184 Cam^r gene. At the 5′ end of one primer was the sequence −91 to −120 upstream of *recA*; at the 5′ end of the second primer were bases −1 to −39 upstream of *recA*, and then the 40th base of this primer introduces the point mutation within the LexA binding site. The PCR product was introduced into an SLT2 *lexA40*::Kan strain (TT23571) by linear transformation, selecting Cam^r. The presence of the *recA* O^c mutation (*recAo281*) was confirmed by sequencing.

Construction of chromosomal *lexA*$^+$ duplication. The chromosomal region between the *metH* and *thr* genes was transduced by two-fragment transduction (23). A P22 lysate was prepared on mutant *metH2357*::MudA(Amp Lac) (at 91.3 min) and another on mutant *thr469*::MudA(Amp Lac) (at 0 min). A 1:1 mixture of these lysates was used to transduce Amp^r into strain LT2. While most transductants inherit one or the other of the donor insertions, some acquire a hybrid insertion at the joining point of a duplication that includes *lexA* as part of the region between the two insertions. This occurs if an appropriate pair of cotransduced fragments recombine with each other before recombining with the chromosome (23). Such duplication-bearing transductants were identified as Amp^r clones with an unstable Lac$^+$ phenotype (sectored blue colonies on NB–X-Gal). The presence of the duplication was confirmed by transducing the hybrid Mud-*lac* joining point into LT2 and observing that every transductant was unstably Lac$^+$.

Cloning *dinF* under arabinose control. The *dinF* gene of *S. enterica* was PCR amplified with one primer with an *Nco*I site near its 5′ end, the first 20 bp of *dinF* at its 3′ end and a second primer with 61 bp just downstream of *dinF* at its 3′ end. A 1.4-kb *dinF*$^+$ sequence was amplified with these primers, digested with the endonucleases *Nco*I and *Eco*RI (which cuts 41 bp downstream of *dinF*), and ligated into the *Nco*I and *Eco*RI sites of the plasmid vector pBADhisC (Invitrogen) to produce plasmid pBAD*hisCdinF*. The cloned *dinF* gene is controlled by the arabinose-inducible pBAD promoter. The integrity of the gene was confirmed by sequencing. This *dinF*-bearing plasmid did not correct the lethality of *lexA* null mutations with or without induction by L-arabinose (0.2%). Thus, the lethal phenotype of *lexA* null mutations is not due to lack of DinF.

Construction of a *dinF*::Zeo swap mutation. The zeocin resistance gene (Zeo^r) from pCR-BluntII-Topo (Invitrogen) was PCR amplified with primers whose 5′ ends included sequence homologous to the ends of the *S. enterica dinF* gene. This linear product was introduced by electroporation, and transformants that carried the Zeo^r determinant in place of most of the *dinF* gene were selected; only 13 amino acids of the DinF protein sequence are left, and the *lexA* gene is intact. The *dinF1012*::Zeo swap is 100% linked to *lexA*$^+$ in crosses with a recipient strain carrying the *lexA41*::Cam swap mutation.

Deleting the *tum* homolog of the Fels-2 prophage. The Cam^r gene from pACYC184 was PCR amplified with primers whose 5′ ends contained sequence

that is homologous to the ends of the Fels-2 *tum*-like gene. Linear transformation was used to introduce this fragment into the chromosome of the LT2-derived strain TT23647. The swap was confirmed by PCR and by linkage with another Fels-2 marker, *gin-48*::Kan(sw), obtained from Bossi and Figueroa.

Fusing the Fels-2 *tum* promoter to the *lac* operon. A sequence including 282 bp immediately upstream of the *tum* gene and 126 bp of the gene itself was PCR amplified and placed immediately upstream of the *lacZ* gene on an F′ *lac* plasmid to create a *tum-lacZ* fusion. This was done by the two steps diagrammed in Fig. 2. First, the region was cloned upstream of *lacZ* in a high-copy-number cloning plasmid, pTrchis2-Topo-*lacZ* (Invitrogen) (Fig. 2A). The *lacZ* gene in this recombinant plasmid (Fig. 2B) was constitutively expressed, suggesting that the plasmid copy number was so high (about 200) that it exceeded the repression capacity of the LexA protein produced by a single chromosomal *lexA* gene.

Therefore, the *tum* control region and part of the *lacZ* gene from the first plasmid were PCR amplified to produce a fragment with ends homologous to sites in a region near the upstream end of the *lac* operon in F′128. Selection was made for loss of tetracycline resistance (7) from the recipient strain, which carried a Tn*10*dTet insertion near *lac* in the *mphC* gene of F′128. This required that the *tum* control region be inserted in place of about 6 kb of plasmid sequence (including *mphC*::Tn*10*dTet) immediately upstream of the *lacZ* gene (Fig. 2C).

Assay of β-galactosidase. Overnight cultures were diluted 100-fold into LB and grown with shaking to an optical density at 650 nm (OD_{650}) of 0.6. Where appropriate, tetracycline (20 μg/ml) was added to maintain plasmids, and mitomycin C (1 μg/ml) was added to liquid growth medium. β-Galactosidase activity was determined in chloroform-permeabilized cells as described by Miller (32). Enzyme activity was expressed as nanomoles of nitrophenol produced per minute per OD_{650} unit of cell culture.

UV survival assay. Overnight cultures grown in LB medium were diluted 100-fold and grown to an OD_{650} of 0.6 (in duplicate). Serial dilutions of these cultures were spread on NB plates and irradiated with UV light. UV fluence was measured with a short-wave UV meter (model J-225; Ultra-Violet Products, Upland, Calif.). Plates (NB) spread with the irradiated cells were incubated for 48 h at 37°C, and cells were counted.

Preparation of DNA for pulsed-field gel electrophoresis. Cells were grown in 1.0 ml of LB broth, harvested by centrifugation, and treated as follows. After washing twice in TEN buffer (10 mM Tris [pH 7.5], 100 mM EDTA [pH 8.0], 250 mM NaCl) and resuspension in 0.5 ml of TEN, cells were mixed with 0.75 ml of 0.9% SeaKem agarose (suspended in TEN) and dispensed into 100-μl plug molds (Bio-Rad). Agarose plugs were treated for 2 h at 65°C in lysing solution (0.2% sodium dodecyl sulfate, 0.5% Sarkosyl, 10 mM Tris [pH 7.2], 100 mM EDTA [pH 8.0], 50 mM NaCl) and then at least 8 h in lysing solution with 0.1% lysozyme at room temperature. Plugs were subsequently treated with 1 mg of proteinase K per ml in wash solution (10 mM Tris [pH 8], 50 mM EDTA [pH 8.0]) for 48 h at 42°C. To inactivate the proteinase K, plugs were treated with 0.01 mM phenylmethylsulfonyl fluoride in wash solution for 1 h, washed five times, and stored in 0.1× wash solution; this method was adapted from that of Bergthorsson and Ochman (5). Before digestion, agarose plugs were washed seven times in 3 ml of sterile water, cut into fragments, and incubated for 30 min in 2 volumes of the appropriate buffer. Plugs were digested with 30 U of *Xba*I or *Bln*I.

Procedure for pulsed-field gel electrophoresis. Electrophoresis was performed in a noncommercial apparatus with 0.5× Tris-borate-EDTA (TBE) buffer at 14°C. Approximately 15 μl of a plug was loaded into a 0.9% agarose gel. Electrophoresis was run for 24 h at 150 V in a noncommercial apparatus with hexagonally arrayed electrodes. To resolve high-molecular-weight bands, pulse times were initially 60 s and decreased linearly with time to 30 s over the 24-h period of electrophoresis. To resolve low-molecular-weight *Xba*I bands, a constant 7-s pulse time was used over the entire 24-h period. Fragments of phage lambda (New England Biolabs) were used as low-range molecular weight standards.

Determining the insertion sites of *din*::MudJ elements. DNA was isolated from insertion mutants grown overnight in 1 ml of NB broth. Cells were pelleted and resuspended in 200 μl of Quick DNA buffer (10 mM Tris-HCl [pH 8.5], 1 mM EDTA, 0.2% sodium dodecyl sulfate) and incubated for 5 min at 100°C. Debris was removed by centrifugation (10 min at 13,000 rpm), and the supernatants were stored at −20°C. These DNA samples were diluted 100-fold and used in single-primer PCRs (see below).

The region adjacent to one end of the inserted element was PCR amplified with a single primer carrying sequence at the *attL* end of MudJ (21). Initial cycles are performed at high stringency to linearly amplify the junction region, and later cycles are performed at low stringency to allow adventitious initiation on the chromosomal sequences and subsequent logarithmic amplification. Amplification conditions (in an Idaho Technologies air cycler) were 20 cycles of <1 s at 94°C, <1 s at 55°C, and 1 min at 72°C, followed by 30 cycles of <1 s at 94°C, <1 s at 40°C, and 1 min at 72°C, followed by 30 cycles of <1 s at 94°C, <1 s at 55°C, and 1 min at 72°C. The primer TP251 (5′-GCAAGCCCCACCAAATCTAATC CCA-3′) directed replication outward from the *attL* end of MudJ into the adjacent chromosomal region. PCR products were treated with single-strand exonuclease (1 μl of a 1-U/μl solution of exonuclease I) for 1 h at 37°C to remove excess primers, purified with a Qiaquick PCR purification kit, and sequenced with a nested primer, TP240 (5′-CCGAATAATCCAATGTCC-3′).

RESULTS

Characterization of a *lexA*::Kan insertion mutation. A strain carrying a *lexA* insertion mutation after base 11 of the coding sequence (*lexA40*::Kan; Fig. 1B) was constructed in vitro and kindly provided to us by Llagostera and Garriga (15). This strain (TT17653; = UA1685) also carries a constructed *sulA46*::Spc (spectinomycin resistance) insertion that was rendered essential by the *lexA* defect and could not be removed by transduction. The original *sulA lexA* mutant shows no growth defect. This behavior is similar to that of *E. coli lexA* null mutants, whose lethal phenotype is corrected by a *sulA* mutation (22, 26).

Unlike *E. coli*, our wild-type *S. enterica* LT2 strain could not inherit the *lexA40*::Kan insertion whether or not the recipient carried a *sulA* mutation. Our *sulA*$^+$ wild-type LT2 strain (TR10000), when used as a transduction recipient for the *lexA40*::Kan insertion, gave about 1% of the expected transductant number (Table 2). These few transductants were found to carry a duplication of the *lexA* region, based on the fact that they gave rise to frequent kanamycin-sensitive segregants and showed both a *lexA*$^+$ and a *lexA40*::Kan allele by PCR analysis. Apparently a cell with an existing *lexA*$^+$ duplication inherited the donor *lexA40*::Kan allele by recombination with one copy and retained a *lexA*$^+$ allele in the other copy. This suggests that duplications were present in about 1% of the cells in the recipient population, a fraction that is typical of genes in this region of the chromosome (2). The *lexA* null mutation appears to cause a recessive lethal phenotype in our wild type and could be maintained only by cells with a second wild-type *lexA*$^+$ allele.

In support of this conclusion, the *lexA40*::Kan insertion could be efficiently transduced into a strain that carried a constructed *lexA*$^+$ duplication (Table 2) (see Materials and Methods for duplication construction). Duplication strains that inherited the *lexA40*::Kan insertion gave rise to segregants that lost the join-point markers (Lac$^+$ and Amp^r); all such segregants also lost the Kan^r phenotype associated with the *lexA* mutation. That is, no haploid segregants that maintained the *lexA*::Kan insertion were recovered. This demonstrated that a *lexA* null mutation had a lethal phenotype in a SulA$^+$ background of *S. enterica* (as known for *E. coli*) and that that this lethality is recessive to a wild-type *lexA* allele.

Unexpectedly, recipients carrying a *sulA* mutation also yielded few *lexA40*::Kan transductants (TT23205 [Table 2]). Some of the few transductants carried a spontaneous *lexA* duplication, as described above. However, a slightly greater number formed small flat colonies that were not seen for the SulA$^+$ recipient. When these small colonies were streaked on rich medium containing kanamycin, they gave rise mostly to small, flat, single colonies like the original transductants, but a small percentage of the new colonies were large and appeared

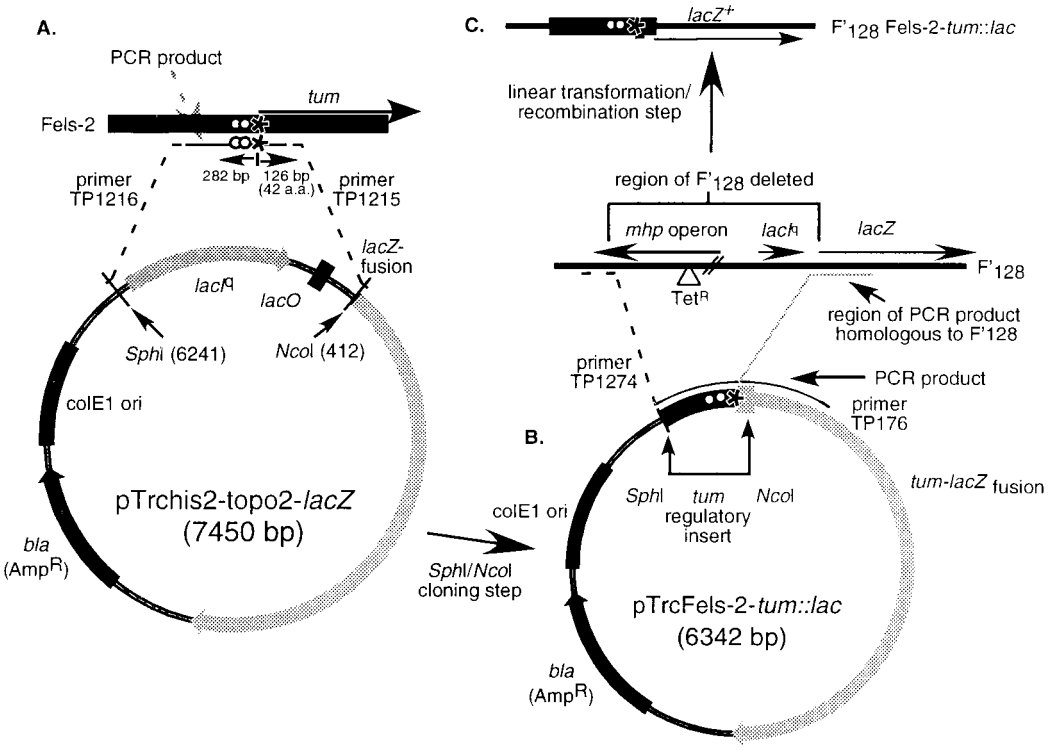

FIG. 2. Construction of a *tum* promoter-*lacZ* reporter construct. (A) The control region of the Fels-2 *tum* gene was PCR amplified and cloned adjacent to the *lacZ* gene of the Topo cloning vector (Stratogene). Strains with this high-copy-number plasmid show constitutive *lacZ* expression. The *tum* control region was amplified from the above plasmid (B) and inserted (by recombination following linear transformation) adjacent to the *lacZ* gene of the low-copy-number plasmid F′128 (C). Strains with this plasmid show LexA-controlled expression of LacZ.

healthy. The fast-growth phenotype was stable and was not due to a *lexA* duplication; the donor *lexA40*::Kan allele had been inherited in place of the recipient *lexA*$^+$ allele (determined by PCR). This suggested that *lexA* mutations in *S. enterica* (unlike those in *E. coli*) were lethal even in a *sulA* background and that suppressors of this lethality arose at high frequency.

Phenotype of a *lexA* deletion (swap) mutation. To be sure that the behavior of *lexA40*::Kan was not a unique property of this insertion, a deletion of the *S. enterica lexA* gene was constructed by linear transformation (16, 38, 61). A chloramphenicol resistance gene (Cam^r) was used to replace 561 of the 609 bases of the *lexA* coding sequence in the SLT2 background, received from Llagostera and Garriga (see Materials and Methods). When transduced into our genetic background, the new mutation, *lexA41*::Cam, behaved exactly as did the *lexA40*::Kan mutation described above (Table 2). Thus, both the deletion and insertion alleles of *lexA* had a recessive lethal phenotype that was corrected, in a *sulA* background, by frequent suppressors. Neither these suppressors nor the *sulA* mutation was separately sufficient to correct lethality.

Lethality of *lexA* null mutations is not due to a polar effect on *dinF*. In *E. coli*, where *lexA* lethality was not seen, most work employed point mutations that may be less strongly polar than the insertion or swap alleles used here (26, 56). The possibility that lethality in *S. enterica* was due to stronger polarity on the *dinF* gene was eliminated because an in-frame *lexA* deletion, expected to be nonpolar, was also lethal in *S. enterica*. In addition, overexpression of *dinF* from a plasmid did not allow inheritance of *lexA* null mutations (see Materials and Methods).

Isolation of suppressors of *lexA* null mutations. Ten independent *lexA* suppressor strains (Slx) were isolated in a *sulA* LT2 strain by transducing in the *lexA41*::Cam swap mutation, selecting for chloramphenicol resistance on NB plates. The rare flat colonies that arose were restreaked on selective medium and (as seen before) gave rise primarily to flat colonies plus an occasional healthy colony. It is presumed that the initial transductants arose from recipient cells that happened to carry a weak suppressor of *lexA* lethality and that secondary growth-enhancing suppressors arose during restreaking. Both primary and secondary suppressor strains were included in our set of 10.

Preliminary characterization of suppressors. All 10 suppressor strains were prototrophic. Two strains (Slx6 and Slx9) formed flat, slower-growing colonies and were expected to carry only the primary weak suppressor of *lexA*. Seven strains (Slx1, -2, -3, -5, -7, -8, and -10) formed healthy colonies and are inferred to carry both primary and secondary suppressors. (The Slx4 strain proved phage resistant and was not studied further.) None of the nine characterized suppressor strains contained a duplication of the *lexA* region; PCR amplification of *lexA* showed only the swap mutant allele.

All nine suppressor strains expressed the SOS-induced *recN*::MudJ operon fusion constitutively, as expected for strains lacking LexA repressor (Table 3). Thus, the suppressors correct the lethal phenotype without restoring repression of

TABLE 2. Lethality of *lexA* null mutations in strain LT2

Transductional recipient			No. of selected transductants obtained with indicated donor (constant phage input, 3×10^9 PFU)		
Strain	Relevant genotype	Genetic background if other than LT2	*lexA440*::Kan (TT17653)[a]	*lexA441*::Cam (TT22613)[b]	(Cam)*lexA33*(Ind$^-$) (TT22964)[b]
TR10000	Wild type (LT2) *recA*$^+$ *lexA*$^+$		1[c]	3[c]	444
TT23205	*sulA46*::Spc		12[d]	22[d]	524
TT23204	*sulA46*::Spc (no Fels-2 or Gifsy-1 or -2)	SLT2	476	368	492
TT22888	Duplication (*lexA*$^+$/*lexA*$^+$)		348	396	532
TT23203	*sulA46*::Spc duplication (*lexA*$^+$/*lexA*$^+$)		460	364	428
TT23296	*sulA46*::Spc (no Fels-2 or Gifsy-1 or -2)	DB9071	480	512	500
TT23298	*sulA46*::Spc (no Gifsy-2)	ATCC 14028	43[d]	6[d]	552
TT23299	*sulA46*::Spc (no Gifsy-1 or -2)	ATCC 14028	20[d]	10[d]	548
TT23379	*sulA46*::Spc (no Fels-2)	DB7000	340[e]	516[e]	515
TT23649	*tum*(Fels-2)::Cam(sw) *sulA*$^+$		3[c]		(656)[f]
TT23650	*tum*(Fels-2)::Cam(sw) *sulA46*::Spc		306[e]		(678)[f]

[a] Selection was for resistance to kanamycin on NB containing 50 μg of kanamycin per ml.
[b] Selection was for resistance to chloramphenicol (Cam) on NB containing 25 μg of chloramphenicol per ml.
[c] Healthy colonies that contained a duplication of the *lexA* gene.
[d] Mixture of healthy colonies and flat, small colonies. Healthy colonies contained a duplication of the *lexA* gene.
[e] Colonies were virtually all flat and small.
[f] Since this recipient was already Cam^r, a *hisC*::Kan (TT16803) marker strain was used as the donor instead of (Cam)*lexA33*; transductants were selected on NB containing kanamycin (50 μg/ml).

the SOS regulon. When the *lexA*$^+$ allele was reintroduced into the Slx strains (by cotransduction with *malB661*::Tn*10*dTet), the resulting *lexA*$^+$ suppressor strains again repressed the *recN*::*lac* fusion. These *lexA*$^+$ suppressor strains (like the SLT2 background) supported efficient introduction of a *lexA* null allele.

Difficulty in mapping these suppressors and the high frequency of new suppressor mutations suggested that they might reflect gene duplication or deletion. Pulsed-field gel electrophoresis revealed the prophage loss described below.

Suppression of *lexA* null mutations by loss of prophages. Strain LT2 of *S. enterica* carries four active phages—Fels-1, Fels-2, Gifsy-1, and Gifsy-2. All nine suppressor strains lacked the Fels-2 prophage; the seven healthier suppressor strains had also lost the Gifsy-1 and Gifsy-2 prophages. The Fels-1 prophage remained in all strains.

The Fels-2 prophage was contained within an 80.9-kb *Xba*I fragment that was absent from both the healthy and slow-growing suppressor strains. All of the healthy suppressor strains showed a 40-kb decrease in the size of an *Xba*I fragment that, in the wild type, is 794.7 kb and includes both the Fels-1 and Gifsy-2 prophages. (Representative strain Slx10 is shown in Fig. 3A.) Strains with this deletion produced a large *Bln*I fragment (data not shown; diagrammed in Fig. 4) whose size demonstrated removal of two *Bln*I sites known to be within the Gifsy-2 prophage (18). The deletion size (~40 kb) and the loss of these *Bln*I sites suggested that Gifsy-2 (45.8 kb) is missing and Fels-1 was still present in the healthy suppressor strains. The Gifsy-1 prophage spans two *Xba*I fragments (37.9 kb and 31.6 kb), both of which were missing from the healthy suppressor strains (Fig. 3B). The restriction map in Fig. 4 indicates the fragments described above.

The slow-growing suppressor strains TT23260 (Slx6) and TT23263 (Slx9) lacked only the Fels-2 prophage (Fig. 3). The 37.9- and 31.6-kb bands indicative of Gifsy-1 were present but faint. This proved to reflect frequent loss of Gifsy-1 from the slow-growing suppressor strains. This instability is consistent with the high frequency with which these strains gave rise to healthy derivatives that have lost Gifsy-1 and -2. The Gifsy-2 prophage seemed to be present but was particularly unstable in Slx9. This conclusion is based on the fact that both the 794.7-kb *Xba*I band containing the Gifsy-2 insertion and the smaller derived deletion ~755-kb band were present. This behavior was seen for multiple single-colony isolates of strain TT23263. Thus, strains lacking Gifsy-2 arose and overgrew when the suppressor strain was cultured to make the pulsed-field gel electrophoresis sample. The original slow-growing suppressor strains appeared to have lost only Fels-2. Data below suggest that both Gifsy-1 and -2 are unstable (or are counterselected) in *lexA* null strains lacking Fels-2. It is unclear why Gifsy-2 appears to be more unstable in Slx6 than in Slx9.

Wild-type *S. enterica* strain LT2 and several derived strains known to lack particular prophages were used as controls for the pulsed-field gel electrophoresis analysis. Strain DB7000 (TR17000) from David Botstein is known to lack Fels-2; the derived strain TT10910 (DB9071) was found, in the course of these studies, to lack three phages, Fels-2, Gifsy-1, and Gifsy-2, although it was never placed under selection to maintain *lexA* null (Fig. 3). Strain TT20492 (= MA3408 from Bossi and Figueroa) lacks Gifsy-1, and strain TT20493 (= MA4587) lacks both Gifsy-1 and -2.

The above conclusions were verified by PCR amplification of the bacterial attachment sites of the Fels-2 and Gifsy-1 and -2 phages and amplification of both of the phage-bacterium junctions of Gifsy-1 and -2. Strains inferred above to lack Fels-2, Gifsy-1, and Gifsy-2 all showed bacterial attachment site fragments of a size expected if the phage was perfectly excised. Slow-growing suppressor strains inferred to still possess Gifsy prophages gave amplification of both the empty attachment site and the phage-bacterial junction fragments, suggesting that some cells in the culture retained the Gifsy phages while others excised them. This is consistent with the frequent loss of Gifsy prophages in *lexA* null strains lacking Fels-2 (data not shown).

TABLE 3. Effects of *lexA* alleles on induction of a *recN*::*lac* fusion[a]

Strain	Relevant genotype	β-Galactosidase in *recN*::*lac* strains grown without (−) or with (+) mitomycin C (1 μg/ml)			
		sulA$^+$		*sulA46*::Spc	
		−	+	−	+
TR10000	*recA*$^+$ *lexA*$^+$ (without *recN*::*lac*)	0	0	0	0
TT23315	*recA*$^+$ *lexA*$^+$ (with *recN*::*lac*)	13.1	268.5	9.8	162.8
TT23465	*recA643*::Tn*10*T-POP	2.7	2.2	ND	ND
TT23381	*lexA33*(Ind$^-$)	2.2	1.9	2.3	2.0
TT23351	*recA281*(O^c)	9.5	368.2	10.8	197.0
TT23355	/pJWL21 *lexA*$^+$	3.7	4.0	4.3	4.5
TT23489	/pJWL26 *lexA3*(Ind$^-$)	3.8	4.4	4.2	21.9
TT23392	*recA281*(O^c)/pJWL21 *lexA*$^+$	5.2	56.5	5.3	54.7
TT23393	*recA281*(O^c)/pJWL26 *lexA3*(Ind$^-$)	4.2	4.0	4.2	4.6
TT23276	Slx2 *sulA46*::Spc *lexA41*::Cam	ND	ND	215.5	289.1
TT23320	Slx2 *sulA46*::Spc *lexA*$^+$	ND	ND	5.9	202.9
TT23280	Slx6 *sulA46*::Spc *lexA41*::Cam	ND	ND	144.8	173.5
TT23324	Slx6 *sulA46*::Spc *lexA*$^+$	ND	ND	2.8	133.1
TT23283	Slx9 *sulA46*::Spc *lexA41*::Cam	ND	ND	149.1	175.8
TT23327	Slx9 *sulA46*::Spc *lexA*$^+$	ND	ND	3.6	89.1

[a] All strains except TR10000 (top line) contained the *recN551*::MudJ insertion. Assays were done with at least two cultures, each tested in duplicate. Units are nanomoles of nitrophenol produced per minute per unit of optical density at 650 nm of cell suspension. ND, not done.

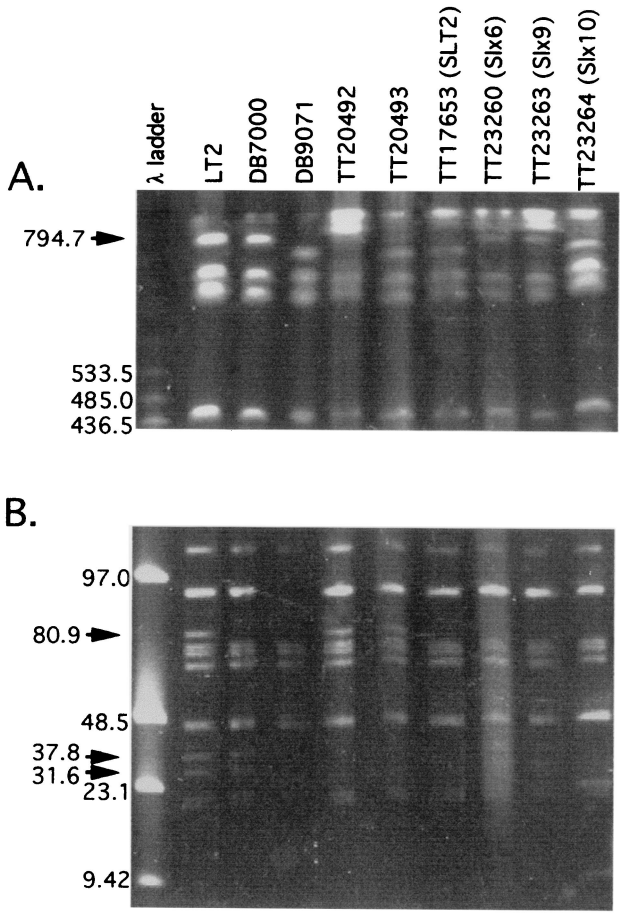

FIG. 3. Pulsed-field gel electrophoresis of genomic *Xba*I fragments from various strains of *S. enterica*. Arrows indicate bands of interest. For DNA preparation and electrophoresis conditions, see Materials and Methods. (A) High-molecular-weight *Xba*I bands were separated with pulse times decreasing from 60 to 30 s over a 24-h period. (B) Low-molecular-weight *Xba*I bands were separated with 7-s pulses over a 24-h period. Strain DB7000 lacks the 90-kb band (due to plasmid pSLT) and also the chromosomal fragments indicated by arrows (see text).

The fully viable original *lexA40*::Kan insertion mutant received from Llagostera and Garriga (SLT2; = TT17653) was assumed to carry some suppressor of *lexA* lethality. These strains showed a pulsed-field gel electrophoresis pattern identical to that of the healthy suppressor strains described above (Fig. 3) and therefore, like them, appears to have lost Fels-2 and both Gifsy phages. Either the SLT2 wild type had lost these phages prior to construction of the *lexA40*::Kan insertion, or these phages were lost in the process of that construction.

Genetic evidence that suppressors of *lexA* null mutations are prophage deletions. Transposon insertions are available for all four of the prophages present in the genome *S. enterica* (LT2)—Fels-1, Fels-2, Gifsy-1, and Gifsy-2 (1, 18, 59). The Gifsy-1 and Gifsy-2 insertions (*din-11*::MudJ and *din-243*::MudJ, respectively) are described below. The Fels-1::MudJ and the Fels-2::Kan insertions were provided by Bossi and Figueroa.

One expects it to be difficult to transduce an insertion mutation within a donor prophage into a recipient strain lacking that prophage. If the recipient possesses the prophage, the transducing phage is required to package only the insertion and some flanking material to support recombination. However, if the recipient lacks the target prophage, then the donated fragment must include all of the donor prophage with the inserted resistance determinant and material to support recombination. In addition, there is the possibility that introduction of a prophage into a recipient that lacks it (or its immunity region) will cause zygotic prophage induction and consequent lethality.

Fels-1::MudJ(Kan^r) could be transduced with high efficiency into all of the suppressor strains, indicating that the Fels-1 prophage was present in all suppressor strains. The Gifsy-1::MudJ and Gifsy-2::MudJ elements could not be transduced into any of the healthy suppressor strains Slx1, -2, -3, -5, -7, -8, or -10, indicating that these suppressor strains lacked both of these prophages and supporting the conclusions based on pulsed-field gel electrophoresis. Both Gifsy phage insertions could be transduced into the unhealthy suppressor strains Slx6 and Slx9, though the Gifsy-1 insertion transduced at a lower efficiency, probably due to loss of the phage during growth of the cultures. This result is consistent with the pulsed-field gel electrophoresis and PCR results, suggesting that these suppressors retained the Gifsy prophages, albeit unstably. Consistent with the pulsed-field gel electrophoresis results, the SLT2 background, in which the original *lexA40*::Kan insertion was received, proved to be a poor recipient for insertions in Fels-2, Gifsy-1, and Gifsy-2 (Table 4 and Fig. 3).

Loss of prophages is sufficient to correct lethality of *lexA* null mutations. The *lexA* null mutations were transduced into the four control strains that had lost various combinations of these prophages but had not previously been under selection to

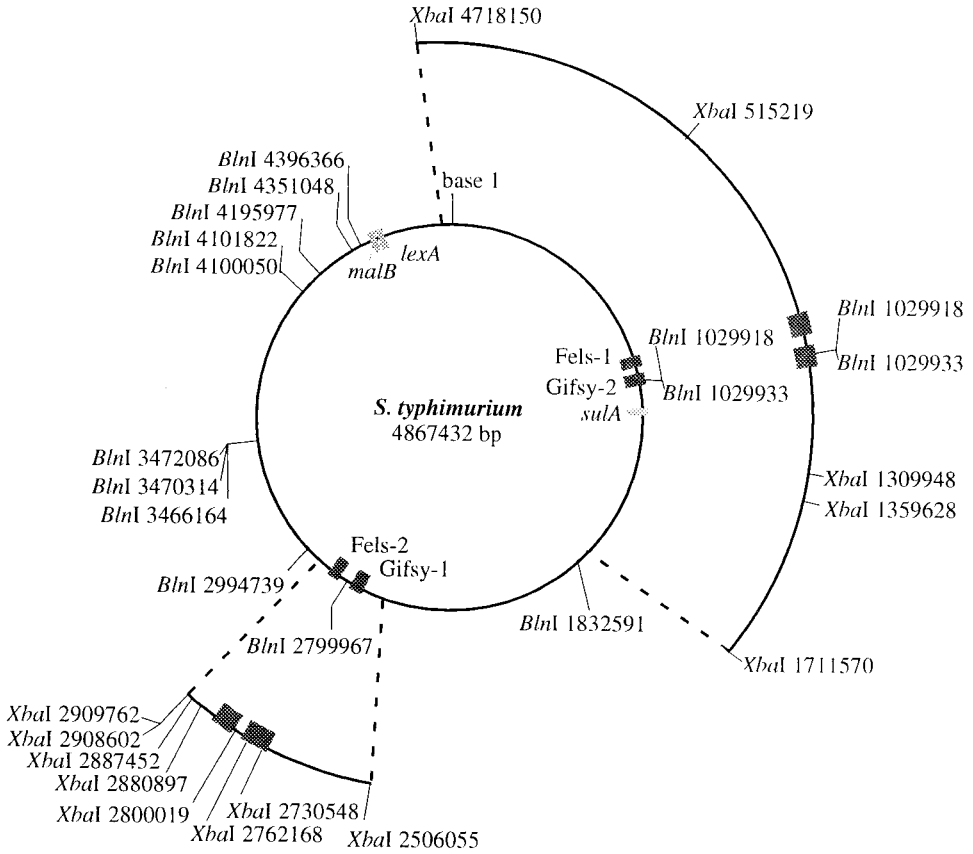

FIG. 4. Genome map of *S. enterica*, indicating locations of prophages and *Xba*I and *Bln*I restriction sites. Boxes represent complete prophage inserted in the *Salmonella* genome and genes relevant to this study. The published sequence of *S. enterica* serovar Typhimurium strain LT2 was used to construct this map; sequence coordinates are for a genome that includes all four active prophages (29).

grow with a *lexA* null mutation. The *sulA46*::Spc mutation was introduced into these strains prior to testing (Table 2). The *lexA* null mutations are lethal in strains that lack only the Gifsy prophages. The strain lacking only Fels-2 inherited the *lexA* null mutations efficiently, but all recombinants formed the small flat colonies characteristic of *lexA* mutants carrying Gifsy-1 and -2. The strain lacking Fels-2 and both Gifsy phages (TT23296) was comparable to the SLT2 strain in which the *lexA* mutation was received; both strains inherited *lexA* insertion mutations efficiently and gave healthy recombinant strains. Since these strains were never under selection to carry a *lexA* null mutation, we conclude that loss of the prophages (in addition to a *sulA* mutation) is sufficient to suppress the lethal phenotype of a *lexA* null mutation.

The nonpolar, in-frame deletion mutation *lexA42*::FRT showed the same behavior as the insertion and swap alleles. (See Materials and Methods for a description of the crosses.) The deletion was efficiently inherited by all of the suppressor (Slx) strains tested and also by tester strains lacking the Fels-2 prophage; it could not be inherited by strains lacking only Gifsy prophages. Coinheritance of *lexA* null with *mal*::Tn*10*dTet was lower in a recipient lacking only Fels-2 (15%) than was seen for the *lexA*$^+$ allele (27%) or for the *lexA* null allele in any of the healthy suppressor backgrounds (24 to 29%), probably due to poor growth of *lexA* strains lacking only Fels-2.

P22 prophage does not cause *lexA* lethality. A *lexA*$^+$ allele and a P22 prophage were introduced into all seven strong suppressor strains (lacking *sulA*, Fels-2, Gifsy-1, and Gifsy-2). The introduced P22 phage carried an *sie* mutation, which allows the lysogen to serve as a transductional recipient. Each such lysogen (e.g., TT23340 to TT23343) efficiently inherited either the *lexA40*::Kan insertion or the *lexA41*::Cam swap deletion. Thus, a P22 prophage does not cause *lexA* to show a lethal phenotype. This confirms previous conclusions that P22 regulation is independent of LexA (49).

Fels-2, Gifsy-1, and Gifsy-2 but not Fels-1 or P22 encode homologues of the coliphage 186 cI antirepressor Tum. Phage 186, unlike its close relative P2, forms lysogens that are UV inducible (58). Inducibility is due not to direct RecA-mediated cleavage of the phage repressor (as in phage lambda and P22), but to induction of a LexA-repressed phage gene for an antirepressor (Tum) that binds reversibly to the phage 186 phage repressor protein and prevents it from binding to its operator (8, 27, 44). Homologues of the phage 186 *tum* gene are found in all three of the phages Fels-2, Gifsy-1, and Gifsy-2 that contribute to *lexA* lethality in *S. enterica*. Homologs of *tum* were not found in the genomes of Fels-1, P22, or P2.

The *tum* gene of Fels-2 is similar in length to that of coliphage 186 but includes a frameshift mutation (Fig. 5A); alternatively, this region may include two genes, each encoding a

TABLE 4. Transduction tests for presence of Gifsy and Fels prophages[a]

Transductional recipient		No. of selected transductants obtained with indicated donor (constant phage input)			
Strain	Relevant genotype	Gifsy-1 (*din-9*)::MudJ (TT17200)	Gifsy-2 (*din-243*)::MudJ (TT17217)	Fels-1::MudJ (TT23252)	Fels-2 (*gin-48*)::Kan (TT23563)
TT23205	*sulA46*::Spc *lexA*$^+$	488	180	544	87
TT23204	*sulA46*::Spc *lexA*$^+$ (SLT2)	0	0	346	0
TT23255	Slx1	0	0	612	0
TT23256	Slx2	0	1	284	0
TT23257	Slx3	0	3	412	0
TT23259	Slx5	0	0	500	0
TT23260	Slx6[b]	48	255	66	0
TT23261	Slx7	0	1	404	0
TT23262	Slx8	0	0	508	0
TT23263	Slx9[b]	33	266	90	0
TT23264	Slx10	0	0	416	0

[a] All strains carried a *sulA46*::Spc insertion, and all but the first two carried the *lexA41*::Cam deletion/insertion. Selection was for resistance to kanamycin on NB.
[b] The growth rate of these strains was often considerably lower than for any of the other suppressor strains.

protein homologous to part of Tum. The upstream region (or gene) encodes the actual antirepressor function, while the second region (or gene) encodes a homologue of the *E. coli* DinI protein (see Discussion) (Gail E. Christie, personal communication). The *tum* region of the Gifsy phages is smaller, encoding a protein that is homologous to DinI and the carboxy-terminal portion of coliphage 186 Tum.

Potential LexA binding sites were identified immediately upstream of all of these genes (Fig. 5B). This suggested that *lexA* null mutations might cause expression of the Fels-2 *tum* antirepressor function, leading to phage induction or expression of phage proteins that inhibit bacterial growth. In either case, removal of the Fels-2 *tum* region should eliminate the *lexA* lethal phenotype. The genome of P22 lacks a *tum* homologue but encodes another antirepressor (*ant*) that does not appear to be regulated by LexA (49).

Fels-2 *tum* deletion suppresses the lethal phenotype of *lexA* null mutations. A deletion of the entire *tum* region from the Fels-2 prophage (see Materials and Methods), together with a *sulA* mutation, allowed efficient inheritance of the original *lexA40*::Kan insertion mutation by strains possessing all four of the LT2 prophages. The transduction frequency was comparable to that seen for recipients with a *lexA*$^+$ duplication (TT22888 [Table 2]) and that seen for the SLT2 suppressor background. Transductants of the *tum sulA* mutant formed small flat colonies that threw off healthy suppressor strains upon restreaking (presumably due to the subsequent loss of the Gifsy prophages). This supports the model above and suggests that *lexA* null mutations cause expression of the Fels-2 *tum* antirepressor, which either causes production of phage proteins that inhibit cell growth or causes cell lysis by at least occasional full induction of the Fels-2 prophage.

We have not demonstrated that the residual growth defect in the presence of Gifsy prophages is due to induction of their *tum* genes. The *tum* gene homologue in these prophages does not include the portion of the phage 186 *tum* gene thought to encode the antirepressor, only the DinI homologue (see Discussion).

Control region of Fels-2 *tum* gene includes a LexA-repressed promoter. To further test the idea that LexA regulates the Fels-2 *tum* promoter, we placed this promoter region adjacent to the *lacZ* gene on plasmid F′128 (see Materials and Methods). Strains with this fusion showed induction by mitomycin C if the strain was *lexA*$^+$ and constitutive high expression if the strain carried a *lexA* null mutation (Table 5). By similar methods, we fused the promoter region of both Gifsy phages to a *lac* reporter and saw similar evidence for LexA-mediated induction by mitomycin (data not shown).

Most of the known SOS-regulated (*din*) genes in *S. enterica* are within prophages. If phage transcription is controlled in part by the LexA repressor, one might expect that the set of damage-inducible (*din*) genes in *S. enterica* (48) would include some within the affected prophages. These *din* genes were identified as MudJ(Lac) insertions that showed mitomycin-inducible levels of LacZ. They were inferred to be LexA (SOS) regulated because their induction is prevented by a *recA* mutation and induced by overexpression of LexA (48).

Eight of the 12 available *S. enterica din*::MudJ insertions (48) were sequenced as described in Materials and Methods. Seven of the sequenced insertions lay within the prophage of either Gifsy-1 or Gifsy-2 and only one lay in a standard chromosomal gene (Table 6). We failed to obtain sequence for four of the other insertions, but two of these (*din-220*::MudJ and *din-292*::MudJ) are also inferred to lie within prophages because they could not be transduced into a strain (TT23309) that lacked the Gifsy-1 and -2 and Fels-2 prophages (Shawn Gerum, personal communication). Thus, 9 of the 10 *din* insertions tested appeared to be within prophages.

***lexA*(Ind$^-$) mutation in *S. enterica* prevents induction of SOS-regulated genes.** The above results suggested that the lethality of *lexA* null mutations in *S. enterica* (LT2) is due to prophages carried by that strain and does not reflect a difference between the SOS control systems of *S. enterica* and *E. coli*. To verify this, a constructed *lexA*(Ind$^-$) mutation (see Materials and Methods) was tested for its effect on the SOS-controlled *recN* gene (Table 3). In *lexA*$^+$ strains, the *recN*::*lac* fusion was induced by mitomycin C, and that induction was prevented by a *recA* (TT23465) mutation. Induction was also prevented by the *lexA*(Ind$^-$) allele placed either in the chromosome or on a plasmid (strains TT23381 and TT23489, respectively). The plasmid *lexA*(Ind$^-$) allele prevented *recN* induction even when RecA protein (the LexA coprotease) was

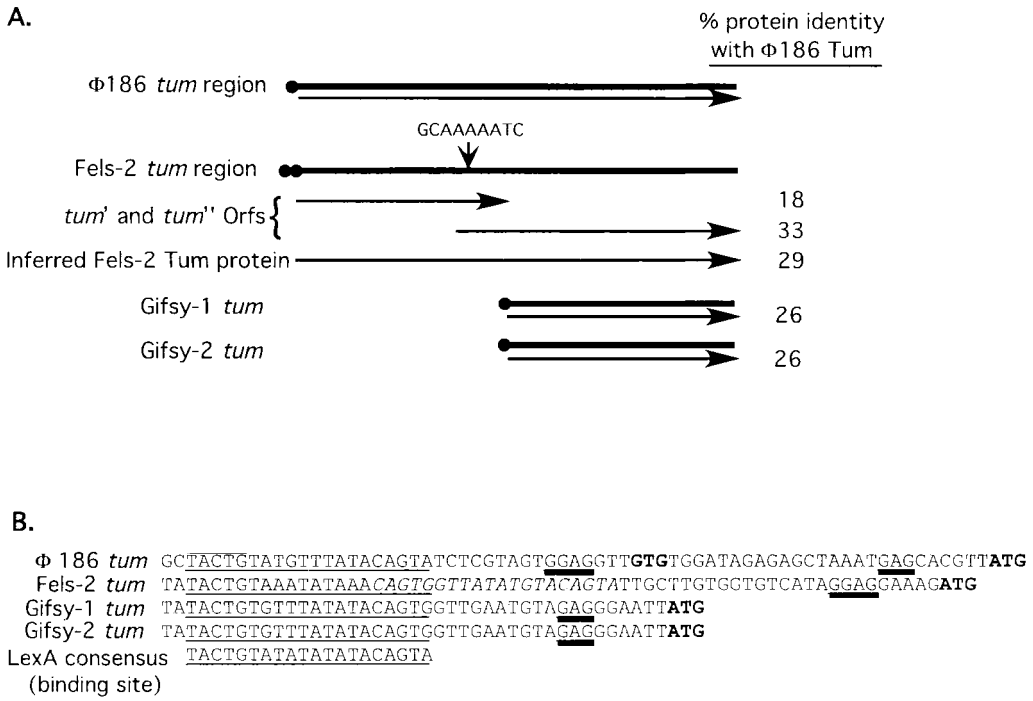

FIG. 5. Comparison of the *tum* region of coliphage 186 with those from Fels-2, Gifsy-1, and Gifsy-2. (A) Schematic representation of the *tum* genes of these prophages. Black circles indicate potential LexA binding sites. Arrows indicate the predicted open reading frame(s) for each gene. The potential site for frameshifting in the Fels-2 *tum* gene is indicated. (B) Potential LexA binding sites (underlined) upstream of the Fels-2, Gifsy-1, and Gifsy-2 *tum* genes. Sequences are compared to the demonstrated LexA binding region of coliphage 186 *tum* (8) and the LexA binding site consensus sequence from the review by Walker (56). Italicized bases in the Fels-2 sequence indicate a second potential LexA binding site. Potential start sites for translation are in bold. Predicted Shine-Dalgarno sequences are double underlined. Overlines denote the −10 promoter region identified by Brumby et al. (8).

expressed constitutively (TT23393 [Table 3]). Overexpression of *lexA*$^+$ (from a plasmid) also prevented induction of *recN* by mitomycin C (TT23355) in an otherwise wild-type background and partially repressed *recN* in a strain carrying a *recA* operator mutation (TT23392). The SOS response of the *recN* fusion was not altered by the presence of a *sulA446*::Spc mutation. This behavior is like that shown previously for the SOS system of *E. coli* (55) A chromosomal *lexA*(Ind$^-$) mutation also prevented mitomycin induction of *din*::*lac* fusions in Gifsy prophages (Table 6).

Plasmids carrying the *lexA*$^+$ or *lexA*(Ind$^-$) gene reduce recombination by superrepression of the *recA* gene. The RecA protein of *E. coli* is superrepressed by overexpression of the LexA$^+$ or LexA(Ind$^-$) repressor protein (14, 52), and this repression is sufficient to reduce recombination rates (55). In *S. enterica*, plasmids carrying either *lexA*$^+$ or *lexA33*(Ind$^-$) reduced recombination at least 500-fold, as judged by transductional inheritance of a drug resistance marker (recipients TR7235 and TR7236 [Table 7]). The recombination defect in these plasmid-bearing strains was increased about 200-fold by a *recA* operator constitutive mutation (TT23206 and TT23207) and thus appeared to be due to superrepression of *recA*. This operator mutation (51) is known to reduce the apparent affinity of LexA for the *recA* control region by a factor of 10 (13). Residual ability of LexA to bind the mutant *recA* operator may explain the slight decrease in recombinant yield caused by the plasmids in a *recA*(O^c) strain. A single chromosomal copy of the *lexA*(Ind$^-$) allele caused no defect in recombination that could be detected by the methods used (TT22964 [Table 7]). These results suggest that the SOS system of *S. enterica* is like that of *E. coli.* Construction of the *recA*(O^c) and *lexA*(Ind$^-$) mutations is described in Materials and Methods.

LexA-regulated prophages do not make *S. enterica* sensitive to UV killing. Wild-type strains of *S. enterica* are not notably sensitive to UV despite the fact that they carry the phages discussed here. This was retested directly, comparing *E. coli* K-12 to *S. enterica* LT2 strains with and without these prophages (Fig. 6). Strains of *S. enterica* with the prophages were very slightly more sensitive to UV than were either *E. coli* or an

TABLE 5. LexA-dependent control of a Fels-2 *tum*::*lac* fusion[a]

Strain	Relevant genotype	β-Galactosidase activity in F′128-Fels-2 *tum*::*lac* strains	
		LB	LB + mitomycin C (1 μg/ml)
TT23764	*recA*$^+$ *lexA*$^+$	3.6	95.2
TT23765	*lexA41*::Cam	168.2	180.3

[a] Both strains contained the *proB1567*::Tn*10* and *sulA446*::Spc insertions and lacked prophages Fels-2, Gifsy-1, and Gifsy-2. They also contained the plasmid F′128 (P_{tum}::*lacZ*$^+$), which encodes a *lacZ* gene regulated by the control region of the Fels-2 *tum* gene. Assays were done with at least two cultures, each tested in duplicate. Units are nanomoles of nitrophenol produced per minute per optical density unit at 650 nm of cell suspension.

TABLE 6. Sequenced damage-inducible (*din*) gene insertions

Damage-inducible gene insertion[a]	Gene containing the MudJ insertion[b]	Location of identified gene	Repression by LexA overexpression[c]	Inducibility by mitomycin C[d]	
				lexA$^+$	*lexA*(Ind$^-$)
din-240::MudJ	*recE*	Prophage Gifsy-2[e]	Weak	Yes	None
din-11::MudJ (identical to *din-80*::MudJ)	*recE*	Prophage Gifsy-2[e]	Weak	Yes	±
din-14::MudJ	Gene for putative T4-like terminase large subunit	Prophage Gifsy-2	ND	Yes	None
din-243::MudJ[f]	Gene resembling the H gene of phage lambda (tail component)	Prophage Gifsy-2	Strong	Yes	None
din-10::MudJ	ORF between genes that resemble the J and tail fiber genes of phage lambda	Prophage Gifsy-2	Strong	Yes	None
din-9::MudJ	Between genes that resemble the "*gpshp*" and *gp-7* genes of phage 21	Prophage Gifsy-1	Strong	Yes	None
din-84::MudJ	*ybhA*	Chromosome, 18.3 min	Weak	Yes	None

[a] Isolated by Smith et al. (48).
[b] Determined by single-primer PCR and sequencing (see Materials and Methods). ORF, open reading frame.
[c] Data from Smith et al. (48). ND, not done.
[d] Determined by replica plating duplicate patches of strains to NB–X-Gal plates containing 0, 50, 100, 150 and 200 ng of mitomycin C per ml.
[e] The *recE* genes of Gifsy-1 and -2 are identical, and therefore linkage (or lack thereof) with an *nadB* insertion (*nadB215*::Tn*10*) approximately 11 kb from *recE* in Gifsy-1 was used to determined if the *recE* insertions were in Gifsy-1 or -2.
[f] With no DNA damage and a wild-type LexA protein, this gene is fully repressed; in a *lexA*(Ind$^-$) background, the gene is transcribed at a low level but does not appear to be upregulated in the presence of DNA damage.

S. enterica strain lacking the phages. Apparently UV irradiation does not effectively induce these prophages. Thus, *lexA* null mutations may reduce the growth rate of lysogens rather than inducing lysis.

LexA null mutations do not cause a mutator phenotype. The error-prone polymerase DinB is part of the LexA-controlled SOS regulon (24, 50). The DinB polymerase contributes to *lac* reversion under selective conditions in the Cairns experiment (9, 30, 31) and is responsible for the associated general mutagenesis suffered by *lac* revertants in that experiment (E. S. Slechta, K. L. Bunny, E. Kofoid, K. Sivaraman, D. I. Andersson, and J. R. Roth, unpublished data). This associated mutagenesis appears to requires SOS induction (presumably of DinB) because it is eliminated by a *lexA*(Ind$^-$) mutation as well as by a *dinB* mutation (47). However, this induction was not sufficient for mutagenesis, as judged by mutation rates measured in a *lexA* null mutant.

Seven suppressor strains carrying the *lexA41*::Cam null mutation were compared to isogenic *lexA*$^+$ strains for the effect of the mutations on both base substitution and frameshift mutation rate. The base substitution rate was scored by selecting rifampin-resistant (Rif^r) mutants. Frameshift mutation (-1) rate was scored by assessing reversion of a *lac* +1 frameshift allele (*lacIZ33*) inserted in the *Salmonella* chromosome as described previously (46). Ten independent cultures of each strain were grown in LB and plated on selective medium (LB with rifampin or NCE with 0.2% lactose). The number of mutations per 10^8 plated cells varied from culture to culture, as expected, but *lexA*$^+$ and *lexA* strains showed no detectable difference in the median number of either base substitution or frameshift mutants (data not shown). This experiment could have detected a twofold increase in the frequency of either mutation type. Thus, DinB-dependent mutagenesis appears to require some second factor in addition to SOS (DinB) induction.

DISCUSSION

The SOS regulon of *S. enterica* (LT2) appears to be controlled by the LexA protein as originally described for *E. coli* (K-12). The lethal phenotype of *lexA* null mutations in *sulA* mutants of *S. enterica* (LT2) is inferred to be a consequence of prophages carried in the genome of *S. enterica*. Lethality is caused primarily by the Fels-2 prophage, and growth is secondarily impaired by the Gifsy-1 and -2 prophages, which appear to be destabilized (or counterselected) in the absence of LexA.

All three of these *S. enterica* prophages carry homologues of the *tum* cI antirepressor gene of phage 186, and in all three phages, the promoter region of the *tum* homologue includes an apparent LexA binding site. Thus, the *tum* antirepressor gene of the Fels-2 prophage is necessary for the observed lethal phenotype of *lexA* null mutations, and the *tum* gene is regu-

TABLE 7. Recombination reduction by plasmids carrying *lexA*$^+$ or *lexA3*(Ind$^-$)

Transductional recipient		No. of Kan^r transductants per plate (constant phage input)[b]
Strain	Relevant genotype	
TR10000	*recA*$^+$ *lexA*$^+$, wild type	>1,500
TT9048	*recA1*	0
TR7235	*recA*$^+$ *lexA*$^+$/pJWL21 *lexA*$^+$	31
TR7236	*recA*$^+$ *lexA*$^+$/pJWL26 *lexA3*(Ind$^-$)	12
TT23151	*recA281*(O^c)	>1,500
TT23206	*recA281*(O^c)/pJWL21 *lexA*$^+$	624
TT23207	*recA281*(O^c)/pJWL26 *lexA*(Ind$^-$)	422
TT22613	*sulA46*::Spc *lexA41*::Cam(sw) (SLT2)[a]	>1,500
TT22964	*lexA33*(Ind$^-$)	>1,500

[a] SLT2 background from Montserrat Llagostera and Xavier Garriga.
[b] Donor strain TT10286 carries a *hisD*::Kan marker; transduction was done on NB containing kanamycin.

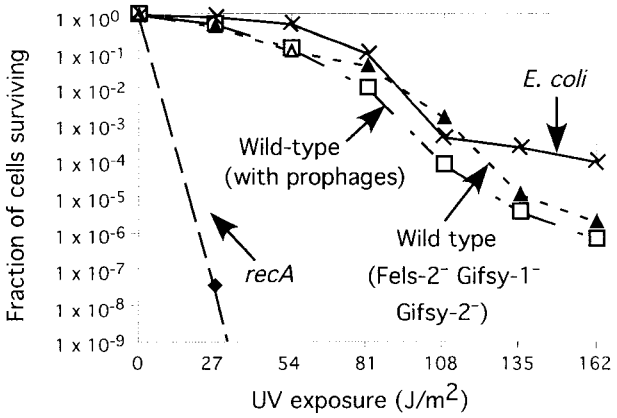

FIG. 6. UV killing of *E. coli* and *S. enterica*. Strains tested were as follows: wild-type *S. enterica* (*recA*$^+$ *lexA*$^+$ *sulA*$^+$, with all prophages present [TT23656]); *S. enterica* Fels-2$^-$ Gifsy-1$^-$ Gifsy-2$^-$ (*lexA* suppressor strain Slx10 made *lexA*$^+$ and *sulA*$^+$ [TT23657]); *S. enterica recA* (*recA1* [TT9048]); *E. coli* K-12 strain, *lexA*$^+$ *sulA*$^+$ *recA*$^+$, without a lambda lysogen [TR7178]).

lated by LexA. The growth rate of *lexA* null mutants lacking Fels-2 was improved by loss of the Gifsy phages.

It seems reasonable that lack of LexA protein might cause full induction of Fels-2 and thereby lead to cell killing. However, since this prophage is not strongly induced by UV irradiation, it seems more likely that the lack of LexA causes expression of some subset of phage genes (perhaps mediated by the Tum antirepressor) whose products restrict cell growth without causing full phage induction. Cells that inherit the *lexA* null mutation might be able to grow only if they have spontaneously lost their Fels-2 prophage prior to acquiring the *lexA* null mutation. In either case, UV irradiation (and consequent LexA cutting) appears to be less effective at inducing the phage or its host-inhibitory gene products than is complete loss of the LexA protein. The same considerations apply to the Gifsy phages. Expression of genes from these phages (in a *lexA* mutant) could destabilize the prophages or cause expression of phage genes that inhibit cell growth and the consequent positive selection for spontaneous prophage loss.

The Fels-2 prophage is related to phages P2 and 186 of *E. coli*. P2 lacks a *tum* gene and is not induced by UV; phage 186 is induced by UV, and this induction is mediated by derepression of its *tum* gene following LexA inactivation (44). While removal of the Fels-2 *tum* gene (plus a *sulA* mutation) corrects the lethal phenotype of *lexA* null mutations in *S. enterica*, the phage *tum* gene sequence appears to carry a frameshift mutation that divides the reading frame (as seen in phage 186) into two approximately equal-sized parts. It is not clear whether these represent distinct genes or require spontaneous frameshifting in order to produce a functional product. Genes for transposases have also been found to include a frameshift and a "shifty" sequence that is thought to make transposition depend on occasional spontaneous frameshifting during translation (11). Fels-2 may hedge its bets in this way and cause a low-probability induction during a standard UV exposure. Deletion of the *lexA* gene may allow more expression and thereby lead to more frequent induction.

An alternative explanation is that the two open reading frames seen in Fels-2 actually encode two distinct proteins with independent functions. This interpretation is supported by the recent finding in the P2-related phage PSP3 that the sequence corresponding to the phage 186 *tum* gene encodes two distinct genes (G. E. Christie, personal communication). The upstream gene encodes the antirepressor activity, and the downstream gene encodes a homologue of the DinI protein of *E. coli*, which is SOS induced and may limit the consequences of an SOS

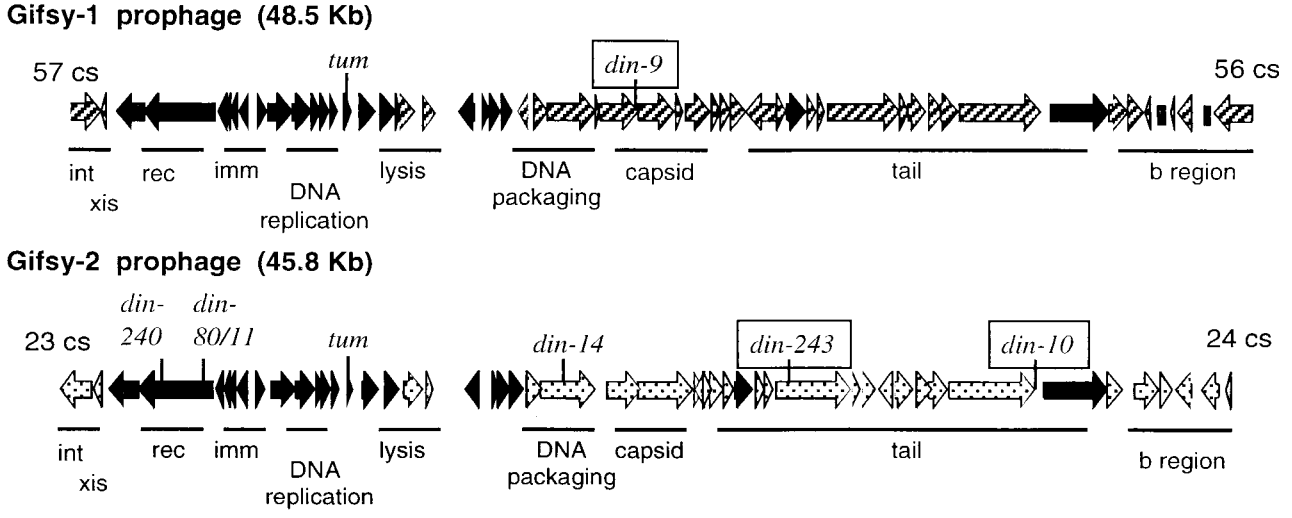

FIG. 7. Genetic map of the Gifsy phages, with the damage-inducible (*din)* insertions indicated. Allele numbers point to the site of a *din*::MudJ insertion. Alleles that are strongly repressed by overexpression of LexA are boxed. Open reading frames in black are nearly identical in both phages, patterned open reading frames show little similarity between Gifsy-1 and Gifsy-2. Nomenclature is as for lambda-like phages: *int*, integration; *xis*, excision; *red*, recombination; *imm*, immunity; b region, nonessential genes. The positions of the phages in the *Salmonella* genome are shown in centisomes (cs). (Basic diagram kindly provided by Lionello Bossi and Nara Figueroa.)

induction (60). If the two portions of the *tum* region have different functions, Gifsy prophages have only the distal (DinI-like) gene and may lack an antirepressor activity (Fig. 5). The Gifsy phages are inefficiently induced by UV, and the weak observed induction appears to depend on RecA function (L. Bossi and N. Figueroa, personal communication).

Fusions of *lac* on the right side of the Gifsy prophage (Fig. 7) are those reported previously to be strongly induced by mitomycin C and strongly repressed by overproduction of the LexA protein (48). It seems likely that these Gifsy transcripts are induced following DNA damage and by *lexA* null mutations. In contrast, *lac* fusions on the left side of these prophages are only weakly induced by mitomycin, and that induction is only partially prevented by overproduced LexA. It is possible that induction of Gifsy phages may require two signals for full induction. The system-repressing genes on the left side in Fig. 7 may not be induced in a LecA-dependent manner.

Null *lexA* mutations are not strong mutators even though they do cause induction the SOS regulon and thereby the error-prone polymerases DinB and UmuC. This suggests that both polymerases require an additional second factor to activate their mutagenic ability. For UmuCD, the second factor is known to be activation of the UmuCD enzyme by RecA-mediated cleavage of the UmuD protein (3, 25, 37, 57). The second factor for DinB must be different, since *umuCD* mutations prevent virtually all UV mutagenesis in both *E. coli* (36, 45) and *S. enterica* (25). The second factor required by DinB-dependent mutagenesis is provided when a leaky *lac* mutant grows under selection to amplify its *lac* operon (20, 46, 47). Our preliminary results suggest that the second factor for DinB-dependent mutagenesis is coamplification of the *dinB* gene with *lac* (20; Slechta et al., unpublished); these genes are located close together on the F′128 plasmid (E. Kofoid, U. Bergthorsson, E. S. Slechta, and J. R. Roth, submitted for publication).

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