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Direct Selection of Mutations Reducing Transcription or Translation of the *recA* Gene of *Escherichia coli* with a *recA-lacZ* Protein Fusion

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When a *recA-lacZ* protein fusion was cloned into phage λ, the resulting transducing phage grew normally on wild-type *Escherichia coli*, but its growth was severely inhibited in *lexA*(Def) mutant strains that express *recA* constitutively at high levels. Mutants of the transducing phage that grew on the *lexA*(Def) strains were isolated and were found to affect production of the RecA–β-galactosidase hybrid protein. Most mutants, including a number of nonsense mutants, were phenotypically LacZ$^-$. LacZ$^+$ mutants were also isolated; most of these expressed lower basal and induced levels of β-galactosidase activity. DNA sequence analysis revealed that some of the LacZ$^+$ mutations were in the *recA* promoter. One of these was found to prevent induction. Unexpectedly, three of the mutations that reduced expression were located in the *recA* structural gene, at codons 10, 11, and 12. Further analysis of the codon 10 mutant showed that it most likely affected translation since it had little effect on transcription as measured by β-galactosidase synthesis from a *recA-lacZ* operon fusion. This expression defect was not limited to the protein fusion, since the codon 10 mutation also reduced synthesis of RecA protein when present in a complete *recA* gene. Analysis of the *recA* DNA sequence in the fusion revealed that each of the mutations at codons 10, 11, and 12 increases the homology between this region of the mRNA and a sequence found at codons 1 to 4. Thus, the secondary structure of the mutant *recA* mRNAs may be affecting translation.

RecA protein provides essential functions for homologous recombination and inducible DNA repair in *Escherichia coli* (21). The intracellular level of RecA protein appears to be important in these processes since the basal synthesis of RecA is relatively high and can be increased further as part of the SOS response (18). A primary control of *recA* gene expression is exerted by the *lexA* gene product, a repressor that binds to the operator site (SOS box) of the *recA* gene and limits its transcription (4, 19). Under normal cellular conditions, when the *recA* gene is repressed, the basal level of RecA protein is maintained at about 1,000 molecules per cell (15, 26). After a DNA-damaging treatment, the repressor cleavage function of RecA (9) becomes activated, leading to proteolytic cleavage of the LexA repressor and induction of the *recA* gene (12, 17, 19). As a result, the level of RecA protein in the cell increases by as much as 20-fold (6, 15, 26, 34). This regulation of *recA* expression poses an interesting problem: how is the cell able to maintain such a high basal level of RecA protein under conditions in which the gene is expressed at only a small fraction of its capacity? The fact that the *recA* promoter is one of the strongest in *E. coli* (24) undoubtedly contributes to this phenomenon. However, the possibility that the *recA* gene can also be expressed from a second promoter or that it is subject to additional regulation has not been ruled out. No *recA* promoter mutations have been definitively identified to test this possibility. The *recA453* allele (formerly *zab-53*; see reference 7), whose site of mutation in *recA* is not known, acts as a promoter mutation in that it reduces expression of the *recA* gene (14, 20). However, this mutation also reduces the induction ratio of RecA synthesis (14, 20); thus, its interpretation is not straightforward.

We have constructed *recA-lacZ* fusions and used them to study the expression of the *recA* gene in vivo (34). Fusions to *lacZ* allow a variety of methods to be used to select for mutations affecting expression of the hybrid gene (33). In some cases, the hybrid protein produced by a *lacZ* protein fusion causes a novel phenotype, and this can be exploited to select for mutants. This approach has been widely used to analyze exported proteins (30) and has yielded mutations affecting regulatory sequences as well as functional domains involved in secretion. In this report, we describe and exploit a novel phenotype of *recA-lacZ* protein fusions. We have found that a lambda phage carrying *recA-lacZ* protein fusion will not grow on a *lexA*(Def) mutant strain in which constitutive high-level expression of the fusion occurs due to a mutation that inactivates the LexA repressor. We have used this phenotype to select for mutations in the *recA-lacZ* hybrid gene that allow phage growth on a *lexA*(Def) host. The mutations isolated in the *recA* sequence include nonsense mutations, promoter-down mutations, a mutation in the translation initiation codon, and single-base changes within the *recA* structural gene that reduce the amount of hybrid protein produced. Our results indicate that the *recA* gene uses a single strong promoter but may be subject to additional modes of regulation.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. Bacteria, bacteriophages, and plasmids are listed in Table 1. The *recA* deletion in strain GE643 was constructed in vitro and extends from the *Sst*II site 108 nucleotides upstream from the *recA*

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TABLE 1. Bacteria, bacteriophages, and plasmids

Name	Description[a]	Origin (reference)
Bacteria		
MC4100	*araD139 Δ(argF-lac)U169 rpsL150 relA1flbB5301 ptsF25 deoC1*	(5)
GE152	*thr-1 leu-6 his-4*(Oc) *rpsL1 supE galK2 sulA11 lexA51*(Def) *Δ(argF-lac)U169*	This work
GE643	GE152 *ΔrecA1398 srl*::Tn*10*	This work
GE2265	*thr-1 leu-6 his-4*(Oc) *argE3 ilv*(Ts) *galK2 supE mtl ara sulA11 Δ(argF-lac)U169*	This work
DM2001	*his-4 proA2 sulA11 tif-1 rpsL1 lexA55*(Def, Am)	(25)
CA7027	HfrH *Δ(argF-lac)U169 Δ(ara-leu) thi*	M. Berman
GE439	CA7027 *ΔmalB1*	This work
LE30	F$^-$ *mutD5 rpsL azi galU95*	(31)
Bacteriophages		
IRI	fl derivative	(10)
λGE190	*c*I (Ind$^-$) φ(*recA-lacZ*)*1*(Hyb)	(34)
λGE272	*c*I (Ind$^-$) φ(*recA-lacZ*$^+$)*1*	(34)
λGE285	λGE190 Δ(*recA-lacZ*)*2*	λGE190 × pGE244
λGE287	*c*I (Ind$^-$) φ(*recA-lacZ*)*2*(Hyb)	λGE285 × pGE251
λGE289	*recA*$^+$	Insertion of a *Bam*HI fragment from pGE226 into the vector λD69 (31)
λGE292	λGE190 *recA1399*	λGE190 × pGE248
λGE294	λGE190 Δ(*recA-lacZ*)*3*	λGE190 × pGE246
Plasmids		
pMLB524	pBR322 Δ*tet* '*lacZ lacY*'	Carries codons 1007 through 1023 of *lacZ* (1)
pMLB1034	pBR322 Δ*tet* '*lacZ lacY*'	Carries codons 9 through 1023 of *lacZ*
pMLB1060	pBR322 Δ*tet* '*lacZ lacY*'	Carries codons 651 through 1023 of *lacZ* (1)
pMLB1097	pBR322 Δ*tet* '*lacZ lacY*'	Carries codons 280 through 1023 of *lacZ* (1)
pGE113	pBR322 Δ*tet* φ(*recA-lacZ*)*1*(Hyb) *lacY*'	(34)
pGE226	pBR327 Δ(*Hin*dIII-*Ava*I) *recA*$^+$	This work
pGE244	pGE113 Δ(*recA-lacZ*)*2*	*Nco*I-*Sst*I deletion (34)
pGE245	φ(*recA-lacZ*)*1*(Hyb) M13 IG region	This work
pGE246	pGE245 Δ(*recA-lacZ*)*3*	*Sst*II-*Cla*I deletion
pGE248	pGE245 *recA1399*	Frameshift mutation at *recA* codon 36
pGE249	pGE226 *recA1400*	*Eco*RI linker inserted at *Nco*I site in *recA*
pGE251	pBR322 Δ*tet* φ(*recA-lacZ*)*2*(Hyb)	Insertion of *Eco*RI fragment from pGE249 into pMLB1034
pGE256	pGE245 *recAM1301*	λGE190 *recAM1301* × pGE246
pGE216	pBR322 Δ*tet* φ(*recA-lacZ*$^+$)*1 lacY*'	(34)
pGE284	pGE216 *recAM1301*	Insertion of *Bam*HI fragment from pGE256 into pMLB1010 (8)
pGE321	pGE226 *recAM1301*	Insertion of *Sst*II-*Nco*I fragment from pGE256 into pGE226

[a] Hyb indicates that the fusion makes a hybrid protein.

promoter to the *Eco*RI site 282 nucleotides from the end of the *recA* gene.

Media and chemicals. L, MacConkey, and M63 minimal media were described previously (22, 31). Ampicillin was used at 150 μg/ml. The indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) was used to score LacZ$^+$ cells and phage (31).

General methodology. Standard techniques (22, 31) were used for λ growth, λ induction, and construction of λ lysogens. Crosses between λ phages and plasmids, in which recombinants were identified by their Lac phenotype, were performed by preparing a λ plate stock on a strain harboring the plasmid and then plating the resulting lysate on a Δ*lac* strain on L agar containing XG. Assays of β-galactosidase activity were performed on permeabilized cells as described by Miller (22). The methods used for constructing recombinant DNA molecules in vitro have been described previously (34).

DNA sequencing. The DNA sequence of mutant *recA-lacZ* fusions was determined by the chain termination sequencing method (29) as described by P-L Biochemicals, Inc. The templates used in all cases were single-stranded DNAs made from pBR322-derived plasmids that contain the intergenic origin of replication region (IG region) of phage M13. Growth of strains and extraction of single-stranded DNA from phage particles was done by the methods of Zagursky and Berman (35).

Mutations were crossed from λ phages into the plasmid pGE246 for DNA sequence determination. This plasmid carries the sequences flanking φ(*recA-lacZ*)*1*(Hyb) but has been deleted for the *recA* portion of the fusion; the deletion extends from the *Sst*II site upstream from the *recA* promoter to the *Cla*I site in *lacZ*. The cross was performed by infecting the M13-sensitive strain CA7027, harboring plasmid pGE246, with the phage fl derivative IR1 (10) and plating it on L agar. This phage, which is closely related to M13, is not sensitive to the replication interference caused by the cloned IG region and gives higher yields of phage particles (35). The mutant λGE190 phages were then spotted on these cells, and the plate was incubated overnight at 37°C. During this incubation, some of the λ phages recombined with pGE246 to produce a plasmid carrying the mutant

TABLE 2. Growth of lambda transducing phages in *lexA*(Def) strains

Bacteriophage	Genes carried	Efficiency of plating[a]	Plaque size on *lexA*(Def)	LacZ phenotype on *lexA*(Def)[b]	Phage yield[c]	
					lexA$^+$	*lexA*(Def)
λ wild type		0.42	Normal	−	18	7
λGE289	*recA*$^+$	0.55	Normal	−	ND	ND
λGE190	φ(*recA-lacZ*)*1*(Hyb)	7.7×10^{-5}	Heterogeneous	Mixed	41	0.005
λGE287	φ(*recA-lacZ*)*2*(Hyb)	0.51	Small	+	61	0.15
λGE272	φ(*recA-lacZ*$^+$)*1*	0.42	Small	+	24	0.18
λGE292	φ(*recA-lacZ*)*1*(Hyb) with frame-shift mutation in *recA*	0.62	Normal	+/−	28	6

[a] Strains GE2265 (*lexA*) and GE152 [*lexA*(Def)] were grown in L broth to mid-log phase. The cultures were then centrifuged and suspended in 10 mM $MgCl_2$. Dilutions (0.1 ml) of phage lysates were added to 0.1 ml of cells, allowed to adsorb for 20 min at room temperature, plated in soft L agar with XG, and incubated overnight at 37°C. Efficiency of plating is the ratio of phage titer on GE152 to that on GE2265.

[b] Scored as follows on L agar containing XG: +, blue plaques; −, white plaques; +/−, light blue plaques; mixed, both blue and white plaques.

[c] Strains GE2265 and GE152 were grown in L broth to mid-log phase and then centrifuged and suspended in 10 mM $MgCl_2$. Phage was added at a multiplicity of infection of 0.1 and allowed to adsorb for 20 min at room temperature. The cells were then diluted into L broth to a concentration of about 2×10^5 cells per ml and grown for 2 h at 37°C. Numbers represent phage produced per input phage. ND, Not determined.

fusion. This plasmid was then packaged into an IR1 phage particle. Recombinants were isolated by scraping phage from the λ spots into 2 ml of phage buffer and heating this lysate at 65°C for 30 min to inactivate cells. The lysate (1 to 10 μl) was then used to infect the λ^r M13^s strain GE439 (100 μl), and Ap^r Tc^r LacZ$^+$ transductants were selected on L agar containing ampicillin, tetracycline, and XG. Since strain GE439 is λ^r M13^s, only those plasmids packaged into IR1 particles were recovered. Furthermore, since the starting plasmid, pGE246, gives a LacZ$^-$ phenotype, only plasmids that had received the fusion from the λ phage gave LacZ$^+$ transductants. The desired transductants were then purified, and their structure was verified by restriction enzyme analysis.

Characterization and mapping of LacZ$^-$ mutations. To determine whether LacZ$^-$ mutants of λGE190 were nonsense mutants, we spotted the phages on a set of strains carrying various nonsense suppressors. The β-galactosidase indicator XG was added to lawns of these strains and the extent of suppression was judged by the darkness of blue in the phage spot.

LacZ$^-$ mutations were mapped by spotting the phages on lawns of strain MC4100 or on lawns of MC4100 strains that harbored plasmids containing either *recA* (pGE226) or various parts of *lacZ* (pMLB524, pMLB1034, pMLB1060, pMLB1097). Lawns were made on lactose-MacConkey plates. A mutation was judged as mapping to a particular region if Lac$^+$ colonies, which resulted from homologous recombination between phages and plasmids, grew up in the spot after 1 or 2 days of incubation at 37°C.

Construction of φ(*recA-LacZ*)*2*(Hyb). Plasmid pGE226 is a derivative of pBR327 (32) in which the *Hin*dIII-*Ava*I fragment encoding the *tet* gene has been replaced with a 3-kilobase *Bam*HI fragment carrying the *recA* gene. This plasmid was opened at the single *Nco*I site at codon 35 of *recA*. The ends of the cut were filled in with T4 DNA polymerase and then ligated with an octanucleotide *Eco*RI linker to give plasmid pGE249. An *Eco*RI fragment from pGE249, extending from a site upstream from *recA* to the new site after *recA* codon 35, was then inserted into the *Eco*RI site of the *lacZ* protein fusion vector pMLB1034 to give plasmid pGE251. In this construction, the *recA* sequence is fused in frame with *lacZ* to give φ(*recA-lacZ*)*2*(Hyb). This fusion was then crossed from the plasmid into the phage λGE285 to give phage λGE287. Phage λGE285 is LacZ$^-$ and is the result of a cross of phage λGE190 with plasmid pGE244 in which a portion of φ(*recA-lacZ*)*1*(Hyb) extending from the *Nco*I site in *recA* to the *Sst*I site in *lacZ* has been deleted.

Construction of λGE292. Plasmid pGE245 is a derivative of pMLB1034 that carries the fusion φ(*recA-lacZ*)*1*(Hyb). It also contains the IG region from phage M13 inserted at an *Aha*III site located near the end of the *bla* gene of the plasmid. The M13 IG region was inserted in pGE245 in the same fashion as in pZ150 (35). Plasmid pGE245 was cleaved with *Nco*I, and the ends were filled in with T4 DNA polymerase and then religated to give plasmid pGE248. This added four nucleotides at the *Nco*I site and put the fusion out of frame with respect to *lacZ*. Strains with this plasmid were very light blue on XG indicator plates. Phage λGE292 was made by crossing λGE190 with pGE248 and isolating LacZ$^-$ plaques on a lawn of strain MC4100 on L agar containing XG.

RESULTS

Inhibition of growth of λ phages carrying *recA-lacZ* fusions in *lexA*(Def) strains. We previously described a *recA-lacZ* protein fusion containing the promoter and the first 47 codons of *recA* fused to a 117-base-pair open reading frame from the *S* end of the phage Mu chromosome, which is in turn fused to codon 8 of *lacZ* (34). The fusion is regulated in the same manner as *recA*, but produces a hybrid protein with β-galactosidase activity. This hybrid protein has 47 amino acids from RecA at its N terminus followed by 39 amino acids from Mu and 1,015 amino acids of β-galactosidase. The fusion was cloned into an *att*$^+$ *int*$^+$ *c*I(Ind$^-$) λ vector, resulting in phage λGE190. This phage grew normally in wild-type *E. coli* strains, but both the efficiency of plaque formation and the phage yield were severely reduced on the *lexA*(Def) strains DM2001 and GE152 (Table 2). The *lexA*(Def) mutation inactivates the LexA repressor, which normally controls the *recA* gene, and results in constitutive high-level expression from the *recA* promoter (23, 25). No defect in the growth of wild-type phage λ or a λ phage carrying a complete *recA* gene, λGE289, was observed in the *lexA*(Def) strains (Table 2). Thus, it appears that high-level expression of the fusion interfered with the growth of λGE190. The λGE190 plaques that did form on *lexA*(Def) strains were heterogeneous in size and also displayed different degrees of β-galactosidase activity on XG indicator plates. As shown below, these rare plaques were formed by phages with mutations in the hybrid gene.

Factors affecting the inhibition of λ growth. To investigate the basis for this novel phenotype, we used several other

TABLE 3. LacZ⁻ mutants of λGE190

Mutation type	No. of mutants mapping in the following region (base pairs)[a]:				
	recA	*lacZ* (20–828)	*lacZ* (828–1941)	*lacZ* (1941–end)	Undetermined
Amber	4	3	0	0	2
Ochre	0	2	4	0	0
Other	4	19	1	0	14

[a] Mapping of LacZ⁻ mutants of λGE190 was performed by the spot test for marker rescue described in the text. Mutations were localized by comparing the rescue of a Lac⁺ phenotype from cells (MC4100) containing plasmid pGE226, pMLB1034, pMLB1060, pMLB524, or pMLB1097.

recA-lacZ fusions. Phage λGE287 carries a fusion that was constructed in vitro by joining codon 8 of *lacZ* to codon 35 of *recA* with no intervening Mu DNA sequences. This is a protein fusion because translation initiates in *recA* to produce a hybrid RecA–β-galactosidase protein. Lysogens of phage λGE287 have basal and induced β-galactosidase activity levels similar to those in lysogens of phage λGE190 (data not shown). However, phage λGE287 did not display the severe growth defect of λGE190 on *lexA*(Def) strains, although it did make smaller plaques and had a reduced phage yield in these strains (Table 2).

Phage λGE272 carries a *recA-lacZ* operon fusion at the same site in *recA* as the protein fusion of λGE190. In the operon fusion, transcription is from the *recA* promoter, but *lacZ* is separated from the *recA* and Mu*S* sequences by a *trp* DNA sequence and has its own translation start site. Consequently, expression of the operon fusion produces a RecA-Mu*S* peptide, terminating in *trp*, and a separate β-galactosidasepolypeptide. This phage did not show the severe growth defect seen with the analogous protein fusion phage λGE190 (Table 2). Rather, λGE272 formed plaques on *lexA*(Def) strains with the same efficiency as did wild-type λ, but the plaques were small when compared with those formed on *lexA*⁺ strains, and the phage yield was reduced. Another phage, λGE292, was constructed by introducing a frameshift mutation at the *Nco*I site in *recA* in the protein fusion in λGE190. This mutation restored the growth of this phage to wild-type levels in the *lexA*(Def) strain (Table 2). From these results, it appears that neither the RecA-Mu polypeptide from the operon fusion nor the short RecA polypeptide from the frameshift mutant nor β-galactosidase alone is sufficient for the severe inhibition of phage growth. Rather, the RecA, Mu, and β-galactosidase sequences must be joined.

Finally, we found that the severe inhibition of lambda growth was not peculiar to the fusion of λGE190 but was characteristic of other *recA*-Mu*S*-*lacZ* protein fusions. A set of 24 fusions of *lacZ* to *recA*, made with the phage λp*lac*Mu3 (3), all displayed the same severe growth defect on *lexA*(Def) strains when crossed into the same λ vector as λGE190 (not shown). These fusions are similar to the fusion carried on λGE190 in that they are protein fusions of *recA* to *lacZ* and they contain the same amount of *lacZ* and the Mu*S* end. However, they have various amounts of *recA*: the shortest contains slightly more of the *recA* gene than the fusion in λGE190, and the longest contains 308 codons from *recA*. Thus, the amount of RecA in the hybrid protein, at least from 47 to 308 amino acids, does not make any difference in the plating phenotype of the phages.

Selection for mutations in the *recA*-Mu*S*-*lacZ* fusions that allow lambda growth. When λGE190 was plated on a *lexA*(Def) strain, about 1 phage in 10^4 made a plaque. The efficiency of plaque formation increased by about 30-fold when the phage was first mutagenized by growing it through a *mutD* strain. Because of this and because a significant proportion of the phages that did form plaques were LacZ⁻, it was apparent that the phages that formed plaques on the *lexA*(Def) strain were mutants. Phages were isolated from plaques on *lexA*(Def) strains and tested to determine the type and site of mutation that had occurred. An unmutagenized lysate of λGE190 was plated on the *lexA*(Def) strain DM2001 with no Lac indicator. Seventy randomly chosen plaques were analyzed further and found to plate with high efficiency on the *lexA*(Def) strain, unlike λGE190. Of these 70 phages, 69 were LacZ⁻ or produced reduced levels of β-galactosidase, indicating that mutations that relieve the phage growth defect occur predominantly, if not solely, in the gene fusion.

Of the phages, 53 were LacZ⁻, as judged by plaque color on plates containing XG (Table 3), including 15 nonsense mutants which were identified by using suppressor strains as described above. Four of the nonsense mutations and 4 of the other LacZ⁻ mutations, possibly deletion or other frameshift mutations, mapped in the *recA* portion of the fusion; 24 LacZ⁻ mutations mapped in the first 828 base pairs of *lacZ* after the *recA*-Mu sequence; and 5 mapped between base pairs 828 and 1941 of *lacZ*. No LacZ⁻ mutations were found to map in the last portion of the *lacZ* gene. Several mutations could not be mapped, and these were probably in the Mu DNA sequences or were deletions spanning the fusion joint. The asymmetric distribution of the LacZ⁻ mutations, especially the nonsense mutations, suggests that the shorter the polypeptide produced by the fusion gene, the less severe is the effect on phage growth. Furthermore, although all of the LacZ⁻ mutant phages had an efficiency of plating on *lexA*(Def) stains of about 0.5, a few formed very small plaques, and these were the phages carrying mutations that mapped later in *lacZ*.

Of the mutant phages, 17 were LacZ⁺. These were characterized by measuring the basal and UV-induced levels of β-galactosidase in lysogens (Table 4). Most of the LacZ⁺ phages showed both basal and induced levels of β-galactosidaseactivity below those of λGE190 (class I). Two mutants showed slightly reduced basal levels and normal induced levels (class II), and one mutant showed an elevated induced level (class III). All phages plated with an efficiency of about 0.5 on *lexA*(Def) strains, but, in general, the phages with the highest β-galactosidase levels formed very small plaques. The unusual class III mutant was found to have an insertion at the beginning of *lacZ*, which most likely accounts for its behavior. Since most mutations either reduced

TABLE 4. LacZ⁺ mutants of λGE190

Class	No. of isolates	β-Galactosidase (U) detected[a]	
		Basal level	UV-induced level
I	14	20–60	60–250
II	2	100–200	770
III	1[b]	200	2,000
λGE190		280	720

[a] Lysogens of GE2265 (*lexA*⁺) were grown in L broth at 37°C for 4 to 5 h. When cultures were growing exponentially, they were centrifuged and suspended in M63 minimal media. Samples were UV irradiated (254 nm) for 15 s at 1 J/m² per s. L broth was added to irradiated and unirradiated samples, and cultures were incubated for 2 h and then assayed.

[b] Restriction enzyme analysis of this mutant revealed the presence of an insertion at the beginning of the *lacZ* sequence.

A

-35 LexA Binding -10

ACTTGATACTGTATGAGCATACAGTATAATTGCTTCA-(48 bp)-ATG...

C CG G

B

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

ATG GCT ATC GAC GAA AAC AAA CAG AAA GCG TTG GCG GCA GCA CTG GGC ...

A C T T

FIG. 1. Sites of mutation of the *recA* gene. (A) Promoter region. The −35 and −10 promoter consensus sequences (24) are enclosed in boxes, as is the ATG translation initiation codon. The consensus sequence for binding of the LexA repressor (4, 19) is indicated by the dotted line over the sequence. The transcription start (arrow) is also shown (13, 27). (B) Beginning of the structural gene. The coding sequence corresponding to the N terminus of the RecA protein is shown, numbered from the N-terminal alanine residue (13, 27). Mutations described in the text are shown beneath the sequences. The *recA* DNA sequence obtained in these experiments corresponds to the sequence that was previously reported (13, 27).

the amount or size of the hybrid protein produced, we conclude that the high-level production of the complete hybrid protein is detrimental to phage growth.

Characterization and DNA sequence of LacZ$^+$ mutants. The LacZ$^+$ mutants that showed reduced β-galactosidase activity could have mutations affecting the expression of the fusion gene, the stability of the hybrid protein or its mRNA, or the β-galactosidase specific activity of the hybrid protein. In addition, LacZ$^+$ mutants that displayed normal levels of β-galactosidase activity (e.g., class II) may affect the functional domains of the hybrid protein that cause the growth defect. To distinguish these possibilities, we have begun to analyze these mutations in more detail. The site of mutation for nine LacZ$^+$ mutants has been determined by DNA sequencing (Fig. 1). All mutations were found to be single-base changes at different sites throughout the hybrid gene. Four mutations altered the *recA* promoter: one was in the −35 consensus sequence and three were in the −10 consensus sequence. One mutation altered the ATG translation initiation codon, and one mutation altered base pair 94 of the Mu*S* sequence. Three mutations altered bases in the *recA* structural gene. The phages that carried each of these nine mutations had a plating phenotype on *lexA*(Def) strains like that of wild-type phage λ.

All of the mutations except the one in Mu*S* reduced the basal levels of β-galactosidase activity by at least 10-fold (class I mutants) (Table 5). The mutation in the Mu sequence caused basal β-galactosidase activity to be about one-third of that of the parent fusion (class II mutant). All mutations also caused a reduction in induced β-galactosidase levels. After treatment with DNA-damaging agents, such as mitomycin C or UV radiation, the β-galactosidase activity of lysogens of λGE190 increased from about 5-fold to 15-fold. Eight of the mutants had lower induced β-galactosidase levels than the parent but showed the same induction ratio after treatment with mitomycin C or UV radiation (not shown) or when expressed in a *lexA*(Def) strain. However, in the promoter mutant *recAM1270*, there was no increase, or only a very slight increase, in β-galactosidase activity after inducing treatments or when expressed in the *lexA*(Def) strain.

The mutation in the structural gene at codon 10, *recAM1301*, was analyzed further to determine whether the mutation altered the amount or specific enzymatic activity of the hybrid protein. Whole-cell extracts of strains carrying plasmids with the mutant fusion were run on sodium dodecyl sulfate-polyacrylamide gels (data not shown). The amount of hybrid protein that was seen on these gels was greatly reduced in the mutant as compared with the wild type. Thus, it appears that the mutation has a strong effect on expression.

When the *recAM1301* mutation was introduced into a *recA-lacZ* operon fusion (plasmid pGE284), the level of β-galactosidase expression was almost the same as that of the wild-type operon fusion (plasmid pGE216) (Table 6). In

TABLE 5. β-galactosidase activities of φ(*recA-lacZ*)*1*(Hyb) mutants

Mutant	Altered sequence	β-Galactosidase detected [U (induction ratio)]		
		Basal rate[a]	With MC[a]	*lexA*(Def)[b]
λGE190	Wild type	298	2,691 (9.0)	966 (3.2)[c]
recAM1251	-35 region TTGATA → TCGATA	22	115 (5.2)	152 (6.9)
recAM1271	-10 region TATAAT → CATAAT	27	234 (8.7)	202 (7.5)
recAM1270	-10 region TATAAT → TGTAAT	18	38 (2.1)	18 (1.0)
recAS1272	-10 region TATAAT → TATAGT	31	446 (14.4)	436 (14.1)
recAM1300	Initiation codon ATG → ATA	3	36 (12.0)	43 (14.3)
recAM1301	codon 10 TTG → TCG Leu → Ser	22	116 (5.2)	215 (9.8)
recAS1302	codon 11 GCG → TCG Ala → Ser	22	140 (6.7)	409 (18.6)
recAS1303	codon 12 GCA → GTA Ala → Val	10	114 (11.4)	ND
Mu*S* 3-15	Mu codon 31 (base pair 94) GCT → GAT Ala → Asp	97	887 (9.1)	1,129 (11.6)

[a] Lysogens of GE2265 (*lexA*$^+$) were grown in L broth at 37°C to mid-log phase. Cultures were then diluted into fresh L broth with or without mitomycin C (MC) at 1 μg/ml, grown for 5 h at 37°C, and then assayed.

[b] Lysogens of GE152 [*lexA*(Def)] were grown in L broth at 37°C. Assays were done when cultures were in exponential growth. ND, Not determined.

[c] The frequency of lysogenization of GE152 by λGE190 was reduced about 100-fold from the frequency of lysogenization of GE2265. Lysogens that were isolated displayed various levels of β-galactosidase, usually much lower than expected.

TABLE 6. Effect of the *recAM1301* mutation on the β-galactosidase activity of *recA-lacZ* fusions

Plasmid[a]	Type of fusion	*recA* sequence	β-galactosidase (U) detected in[b]:	
			Uninduced culture	MC-induced culture
pGE113	Protein	Wild-type	2,900	6,790
pGE256	Protein	*recAM1301*	362	1,950
pGE216	Operon	Wild type	6,950	17,190
pGE284	Operon	*recAM1301*	5,090	13,660

[a] All plasmids were in strain MC4100 (*lexA*$^+$).
[b] Cultures were grown in L broth at 37°C for 2 h. Mitomycin C (MC) was added to half of the culture to a final concentration of 1 μg/ml, and cultures were incubated for 4 h and then assayed.

contrast, this mutation significantly reduced expression of the protein fusion in a plasmid, e.g., pGE113 versus pGE256 (Table 6). This suggests that the mutation has little effect on transcription of the gene fusion or the stability of the mRNA.

The *recAM1301* mutation was also introduced into a complete *recA* gene that had been cloned in a plasmid. As judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell extracts (Fig. 2), this mutation also reduced expression of the complete *recA* gene, analogous to its effect on the *recA-lacZ* protein fusion. Interestingly, in M63 medium, the effect of the *recAM1301* mutation on expression was partially suppressed (Fig. 2). Taken together, these results indicate that the *recAM1301* mutation affects the translation or the stability of the protein produced.

DISCUSSION

We have exploited the inability of a λ phage carrying a *recA-lacZ* protein fusion to grow on *lexA*(Def) strains to isolate mutations in the *recA* gene. Although the reason for the growth defect is obscure, analysis of other *recA-lacZ* fusions shows that it is clearly not due to the high level of transcription of the fusion per se but rather to some property of the hybrid protein that is produced. The portion of the protein encoded by Mu*S* DNA may be important for this phenotype, since a point mutation in the Mu sequence alleviates the growth defect without drastically affecting expression of the fusion. Thus, it is possible that this selection could be used with *lacZ* fusions to other highly expressed genes when this Mu*S* sequence is present. However, the observation that a fusion lacking the Mu sequence shows a partial impairment in phage growth suggests that the *recA* portion of the fusion may contribute to the defect as well.

Regardless of the mechanism, the growth defect of phage λGE190 on *lexA*(Def) strains has given us a strong selection for a variety of new and useful *recA* mutations. Four nonsense mutations in the *recA* sequence of the fusion, that is, in the first 47 codons of *recA*, have been isolated and identified. By using fusions with longer sections of *recA*, we should be able to isolate nonsense mutations throughout the gene. It should also be possible to identify deletion and frameshift mutations with this selection as LacZ$^-$ mutations mapping in *recA*.

We have also isolated four promoter mutations, all of which reduce the homology of the *recA* promoter with the consensus sequence for *E. coli* promoters (24) and are promoter-down mutations as judged by β-galactosidase levels. Since each of these mutations reduces both the basal and induced level of expression, it is apparent that *recA* has only a single strong promoter. This is in contrast to some other genes of the SOS system, such as *urvB* and *ssb*, which have separate constitutive and inducible promoters (2, 28).

The LexA protein represses *recA* expression by binding to a site at the *recA* promoter between the −10 and the −35 consensus regions (4, 19) (Fig. 1). Three of the promoter mutants appear to be subject to normal repression, since they show wild-type induction ratios after inducing treatments or in a *lexA*(Def) strain. The *recAM1270* mutant, however, appears to be poorly inducible since the level of expression of the fusion is the same in a *lexA*(Def) strain as in a *lexA*$^+$ strain and since this mutant is only slightly induced by mitomycin C. This effect was also observed when the mutation was crossed into a complete *recA* gene or when the mutant fusion was introduced into the *recA* locus in the *E. coli* chromosome (unpublished data). It is possible that this mutation is exceptional in that it so severely depresses transcription that expression from this promoter is below the limits of detection. In this case, the expression that is observed may originate at a different, weak promoter. Alternatively, the *recAM1270* mutation may have a direct effect on induction, for example, by affecting the structure of the LexA-binding site. This explanation seems unlikely since expression was not enhanced in the *lexA*(Def) mutant host in which the LexA repressor is inactive. It is also possible that this mutation affects the binding site for another, unknown, transcription factor.

Finally, we have isolated four mutations affecting the *recA*

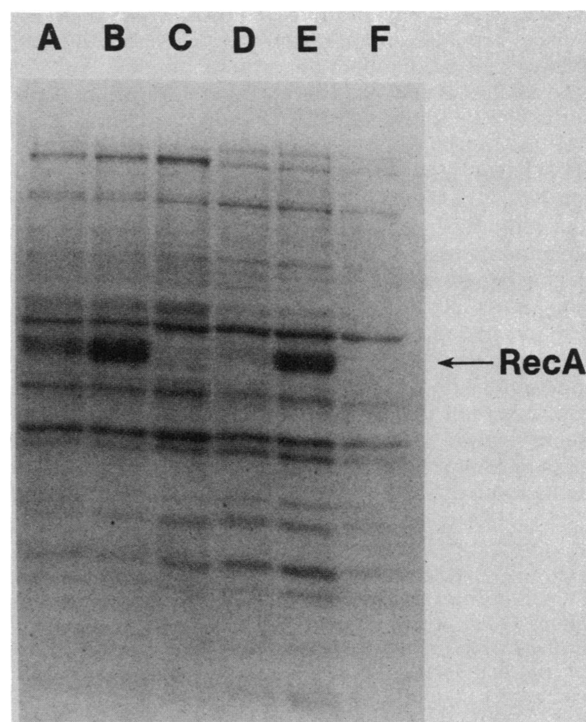

FIG. 2. Effect of the *recAM1301* mutation on production of RecA protein. The mutation was transferred from the *recA-lacZ* fusion into a complete *recA* gene by replacing the *Sst*II-*Nco*I fragment of *recA* in plasmid pGE226 with that from plasmid pGE256 to create plasmid pGE321. Cultures of GE643 carrying no plasmid (lanes C and F), pGE226 (lanes B and E), or pGE321 (lanes A and D) were grown in M63 (lanes A through C) or L (lanes D through F) medium, and whole-cell extracts were prepared, electrophoresed through sodium dodecyl sulfate-polyacrylamide gels, and stained with Coomassie blue as described previously (31).

structural gene. One of these affects the ATG translation initiation codon, showing that translation does in fact start at this position and that the RecA protein is not subject to N-terminal processing. We have also found three mutations that change codons 10, 11, and 12 of *recA* and unexpectedly reduce expression. The mutation in codon 10 had little effect on expression of a *recA-lacZ* operon fusion in a plasmid vector and hence does not reduce transcription. This result also suggests that the mRNA is not made more unstable by the mutation in codon 10. When this mutation was introduced into a complete *recA* gene, it also reduced synthesis of the RecA protein. Preliminary experiments indicate that the stability of the RecA protein produced from this mutant is not significantly reduced (unpublished data). This result is not surprising since we know of no case in which a mutation reduces the stability of a protein and a related hybrid protein. How, then, can mutations in the structural gene reduce expression? A preliminary search of the *recA* DNA sequence present in the fusion (from +1 to +191) for homologies to the region around codons 10, 11, and 12 revealed one region (+53 to +63) immediately after the initiation codon that could base pair with this region (+80 to +90). The resulting structure contains 5 GC, 2 AU, and 2 GU base pairs in the 11-nucleotide segment. However, more striking is the fact that each of the mutations isolated in codons 10, 11, and 12 results in the formation of an additional, or stronger, base pair in this structure. Other base-pairing schemes were observed that involved the region from codon 10 to codon 12; however, these structures were not stabilized by all three mutations. Thus, one possibility is that increased stability of an mRNA structure in this region decreases translation initiation in these mutants. An alternative possibility is that the mutations create rare codons. In fact, the UCG codons created by the mutations in codons 10 and 11 are considered to be rare codons in highly expressed genes (11, 16). However, this is not true for the mutation in codon 12. Thus, if these clustered mutations act by a similar mechanism, the rare codon model seems unlikely. Since these mutants show a normal induction ratio after DNA-damaging treatments or when expressed in a *lexA*(Def) mutant, it is apparent that any mechanism that affects translational efficiency does not play a part in the regulation of RecA synthesis in the induction of the SOS response. This is consistent with our earlier observation that translational control does not play a part in this induction (34). Further characterization of these and other mutations that lower expression and of revertants that increase expression should allow us to analyze the regulation of *recA* in greater detail.

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