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Signal Strains That Can Detect Certain DNA Replication and Membrane Mutants of *Escherichia coli*: Isolation of a New *ssb* Allele, *ssb-3*

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Mutations in several *dna* genes of *Escherichia coli*, when introduced into a strain with a *lac* fusion in the SOS gene *sulA*, resulted in formation of blue colonies on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). Unexpectedly, several lines of evidence indicated that the blue colony color was not primarily due to induction of the SOS system but rather was due to a membrane defect, along with the replication defect, making the cell X-Gal extrasensitive (phenotypically Xgx), possibly because of enhanced permeability to X-Gal or leakage of β-galactosidase. (i) In most cases, β-galactosidase specific activity increased only two- to threefold. (ii) Mutations conferring tolerance to colicin E1 resulted in blue colony color with no increase in β-galactosidase specific activity. (iii) Mutations in either the *dnaA*, *dnaB*, *dnaC*, *dnaE*, *dnaG*, or *ssb* gene, when introduced into a strain containing a *bioA*::*lac* fusion, produced a blue colony color without an increase in β-galactosidase synthesis. These *lac* fusion strains can serve as signal strains to detect *dna* mutations as well as membrane mutations. By localized mutagenesis of the 92-min region of the chromosome of the *sulA*::*lac* signal strain and picking blue colonies, we isolated a novel *ssb* allele that confers the same extreme UV sensitivity as a Δ*recA* allele, which is a considerably greater sensitivity than that conferred by the two well-studied *ssb* alleles, *ssb-1* and *ssb-113*. The technique also yielded *dnaB* mutants; fortuitously, *uvrA* mutants were also found.

Although many genes involved in DNA replication and modulation of DNA structure in *Escherichia coli* have been identified, several replication proteins have not yet been matched with any gene (21), suggesting that other DNA replication genes, designated *dna* genes, remain to be identified. Furthermore, several suppressors of mutations in *dna* genes are known, and these suppressors may represent new *dna* genes (1, 39).

Bacterial genes whose products are involved in DNA replication have been identified by several strategies that entail detection of mutants under nonpermissive growth conditions. Temperature-sensitive *dna* mutants have been selected by their resistance to UV irradiation resulting from their failure to incorporate 5-bromouracil into DNA at high temperatures (7, 8). Such *dna* mutants have also been selected by their resistance to thymine starvation, which does not affect nonreplicating DNA (44). A variation on the same theme is to take advantage of the inability of mutants to incorporate labeled thymine at high temperatures while remaining able to synthesize protein and RNA (30, 32, 37, 38).

Our isolation procedure attempts to identify bacterial genes whose products are involved in DNA replication. The method was designed to detect *dna* mutants by virtue of defects in the structure of their DNA, but it apparently succeeded for other reasons. Our original aim was to detect mutants having lesions in DNA by making use of the fact that damaged DNA activates RecA protein to the protease state, resulting in the induction of the expression of a large number of unlinked genes collectively called the SOS regulon (28). The promoters of a number of SOS genes have been fused to the *lac* operon (17); these *lac* fusion genes produce β-galactosidase in response to DNA damage. The presence of DNA defects in a *dna* mutant, particularly an excess of single-stranded DNA regions, would be expected to result in induction of an SOS fusion gene, causing a blue colony on a plate containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), a chromogenic substrate for β-galactosidase.

Evidence that some *dna* mutations can indeed induce the SOS system comes from the work of Schuster et al. (31), although induction was obtained only at temperatures nonpermissive for growth of the *dna* mutants. They found that strains mutated in *dnaB*, *dnaC*, *dnaE*, or *dnaG*, when incubated for 3 h at a nonpermissive temperature and then shifted to 30°C, produced an SOS response, namely induction of prophage lambda. Mutations in *dnaB* produced particularly strong induction of lambda, and shift-up of *dnaB* mutants to a nonpermissive temperature has proved to be a general method for inducing the SOS system (43). On the other hand, strains mutated in *dnaA* did not induce lambda.

In the work discussed here, we found that a number of *dna* mutations at temperatures permissive for growth caused a small induction of the SOS gene *sulA*::*lac* and also resulted in blue colonies. However, the same mutations also produced blue colonies without an increase in β-galactosidase in a strain containing a *bioA*::*lac* fusion gene, which is not an SOS gene; this suggested that in the case of the SOS fusion gene the blue colony color was not always caused just by induction of the *sulA*::*lac* gene. Rather, it is likely that the DNA replication defect was associated with a membrane defect.

By virtue of the presumed membrane defect and with the aid of a *sulA*::*lac* strain, we isolated a new *ssb* allele that confers exceptionally high sensitivity to UV light.

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TABLE 1. Bacterial and phage strains

Strain or phage	Relevant genotype	Source (reference)[a]
Strains		
AB1157	*thr-1 leuB6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44*	A. J. Clark
AB1899	As AB1157 but *lon* (nonmucoid)	A. McPartland
AM103	As AB1899 but *sulA*::Mu d1(Ap *lac*)*cts*	A. McPartland
EST955	As AM103 but Ts^+	
SG1030	Δ*lac-169 zai-403*::Tn*10*	S. Gottesman
EST960	As AM103 but Δ*lac-169 zai-403*::Tn*10*	P1(SG1030) × AM103
CS39	As EST960 but *sulA*::*lacZ*[λ p1(209)]	
CS45	As AB1157 but *lon* Δ*lac-169 sulA*::*lacZ*[λ p1(209)] Tet^s	CS39 Tet^s
CS52	As CS45 but Δ*recA srl-300*::Tn*10*	
CS50	As CS45 but *zjc-1*::Tn*10*	
CS57	As CS45 but *ssb-113 zjc-1*::Tn*10*	
CS66	As CS45 but *dnaA5 zic-501*::Tn*10*	
N4177	*gyrB203*(Ts) *gyrB221*(Cou^r)	B. J. Bachmann (22)
CS67	As N4177 but *zic-501*::Tn*10*	
CS68	As CS45 but *gyrB203*(Ts) *gyrB221*(Cou^r) *zic-501*::Tn*10*	P1(CS67) × CS45
CS72	As CS45 but *ssb-1 zjc-1*::Tn*10*	
CS96	As CS45 but *dnaA508 zic-501*::Tn*10*	
CS101	As CS45 but *dnaG3 zgh-2359*::Tn*10*	
CS115	As CS45 but *dnaE486 zae-502*::Tn*10*	
CS116	As CS45 but *dnaC2 zjj-512*::Tn*10*	
BM5078	*bioA*::*lacZ*[λ p1(209)]	M. Casadaban
CS142	As BM5078 but *dnaE486 zae-502*::Tn*10*	
CS143	As BM5078 but *dnaC2 zjj-512*::Tn*10*	
CS144	As BM5078 but *dnaA508 zic-501*::Tn*10*	
CS146	As BM5078 but *ssb-113 zjc-1*::Tn*10*	
CS147	As BM5078 but *ssb-1 zjc-1*::Tn*10*	
CS148	As BM5078 but *dnaG3 zgh-2359*::Tn*10*	
CS149	As BM5078 but *ssb-3 zjc-1*::Tn*10*	
CS162	As BM5078 but *gyrB203 gyrB221 zic-501*::Tn*10*	
NP29	*valS1*(Ts)	F. C. Neidhardt
NC32	B/r *hspM hspR lac valS*(Ts)	F. C. Neidhardt
CS175	As NP29 but *zji-80*::Tn*10*	
CS176	As NC32 but *zji-80*::Tn*10*	
CS179	As CS45 but *valS1 zji-80*::Tn*10*	
CS182	As CS45 but *valS zji-80*::Tn*10*	
EST2408	As BM5078 but *alaS*	
P2731	*tolC210*::Tn*10-48*	P. Reeves (25)
CS184	As CS45 but *tolC210*::Tn*10-48*	
CS185	As BM5078 but *tolC210*::Tn*10-48*	
CS186	As BM5078 but *valS zji-72*::Tn*10*	
CS189	As BM5078 but *valS1 zji-72*::Tn*10*	
CS196	As CS45 but *ssb-3 zjc-1*::Tn*10*	
CS54	As CS45 but *dnaB495 zjc-1*::Tn*10*	
CS222	As CS45 but *uvrA294 zjc-1*::Tn*10*	
CS223	As CS45 but *dnaB499 zjc-1*::Tn*10*	
CS300	As CS196 but P1 Cm *lxc-1*	
Phages		
P1 Cm		M. B. Yarmolinsky
P1 Cm *clr100*		M. B. Yarmolinsky
P1 Cm *lxc-1*		J. L. Rosner
P1 Cm *bac-1*		M. B. Yarmolinsky (10)
λ p1(209)		M. Casadaban

[a] A blank means this work.

MATERIALS AND METHODS

Bacteria and phages. The bacterial and phage strains are listed in Table 1; except for the *E. coli* B/r strain NC32, the bacteria were all derivatives of *E. coli* K-12. P1 was used for transduction as described previously (34). The *ssb* alleles linked to *zjc-1*::Tn*10* were derived from JGC175 (*ssb*$^+$), JGC197 (*ssb-1*), and JGC248 (*ssb-113*) (12). The *dnaA*, *dnaC*, and *dnaE* alleles with their linked Tn*10* (Tet^r) markers were derived from strains provided by J. A. Wechsler: JW396 (*dnaA5 zic-501*::Tn*10*), JW402 (*dnaA508 zic-501*::Tn*10*), JW407 (*dnaC2 zjj-512*::Tn*10*), JW357 (*dnaE486 zae-502*::Tn*10*), and JW355 (*zic-501*::Tn*10*). The *dnaG3* allele was linked to *zgh-2359*::Tn*10*. P1 Cm *lxc-1* and P1 Cm *bac-1* were used to identify *ssb* and *dnaB* mutants by complementation (10, 16). λ p1(209) was used for stabilization of Mu d1 (Ap, *lac*) fusions (9).

Media and reagents. The rich medium was LB (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter). The minimal media were M9-CAA and X-Gal-M9-CAA (24). Over the period of the experiments, X-Gal was used at 40, 60, or 80 μg/ml, depending on the potency of a particular batch. Biotin (1 mM) was added to the M9-CAA medium

when *bioA-lac* fusion strains were used in order to lower the basal level of β-galactosidase activity. Chloramphenicol, ampicillin, tetracycline, and mitomycin were used at 12.5 μg/ml, 50 μg/ml, 25 μg/ml, and 0.5 μg/ml, respectively. H and λ media were previously described (24). Tetracycline-sensitive cells were selected by the method of Maloy and Nunn (20).

Construction and stabilization of the *sulA*::*lac* signal strains. The structural gene for β-galactosidase was linked to the promoter of *sulA* by means of a Mu dl(Ap *lac*) operon fusion (A. McPartland, J. Yamashita, and M. Villarejo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K58, p. 147) to yield AM103, which produces increased amounts of β-galactosidase in response to agents that induce the SOS response. The chromosomal lactose operon was deleted from AM103 yielding EST960, so that only β-galactosidase produced from the *sulA*::*lac* fusion would be measured. Because Mu dl(Ap *lac*) transposes frequently, the fusion was stabilized with λ pl(209) (9).

Since the Mu phage encodes a temperature-sensitive repressor, strains carrying this phage will be killed at 42°C. Those cells that have lost the Mu phage by homologous recombination between the *c* ends will survive at high temperature. Temperature-resistant segregants were selected by streaking different dilysogens on LB plates prewarmed to 44°C for 2 h and then incubating the streaked plates at 44°C. A λ-immune, temperature-resistant, ampicillin-sensitive colony that exhibited induction of β-galactosidase by mitomycin was selected. The temperature resistance and ampicillin sensitivity indicated that the Mu had been replaced by the lambda. Induction with mitomycin indicated that β-galactosidase expression was still under the control of the *sulA* promoter.

Induction of the stabilized version of the *lac* fusion strain was tested by comparison of the colony color on plates containing X-Gal and 0.5 μg of mitomycin per ml versus those with just X-Gal, and then was confirmed by an enzyme assay. This strain, CS39, was then made into the Tet^s strain CS45 so that regions targeted for localized mutagenesis could then be marked by the insertion into that region of a selectable Tn*10* element, such as Tn*10*, into the desired region. For example, CS50, the starting strain for mutagenesis in the 92-min region, was constructed by transduction of the tetracycline resistance element *zjc-1*::Tn*10* into CS45.

Localized mutagenesis. A P1 Cm *clr100* lysogen of CS50, which contains a Tn*10* at the 92-min region of the chromosome, was selected at 30°C after cross-streaking the cells with the phage on LB plates containing chloramphenicol. The lysogen was grown at 30°C to early log phase (optical density at 600 nm [OD_{600}] = 0.2) and then mutagenized by adding 25 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) per ml, after which the culture was aerated for 30 min. The MNNG was removed by centrifugation, and the cells were washed and resuspended in an equal volume of LB. The culture was then incubated at 30°C for 30 min and made 0.2 M in $MgSO_4$, and the P1 was induced by raising the temperature to 42°C for 20 min, after which the culture was incubated at 30°C until cell lysis occurred. Cell debris was removed by centrifugation, and the mutagenized P1 was stored at 4°C.

The lysate was then used to transduce CS45 to Tet^r by standard methods with selection on plates containing X-Gal and 25 μg tetracycline per ml at 30°C. P1 *clr* lysogens, detected by their chloramphenicol resistance, were discarded (29). All blue-colony formers were retained and tested for temperature sensitivity and UV sensitivity by spot tests. These two phenotypes were chosen because they are associated with mutations in known *dna* genes (i.e., *dnaB* and *ssb*) in this region (2).

TABLE 2. Effect of known mutations on colony color and β-galactosidase activity of a *sulA*::λ*lac* signal strain[a]

Strain	Mutation	Effect at growth temperature (°C)					
		30		35		37	
		Color	β-gal	Color	β-gal	Color	β-gal
CS45	None (wild type)	PB	1.0	PB	1.0	PB	1.0
CS66	*dnaA5*	PB	1.0	PB	1.2	NG	NG
CS96	*dnaA508*	PB	1.5	PB	1.3	NG	NG
CS54	*dnaB495*	B	13	B		B	10
CS223	*dnaB499*	B	3.0	B		B	
CS116	*dnaC2*	PB	0.8	B	1.7	B	1.8
CS115	*dnaE486*	PB	1.2	B	2.7	NG	NG
CS101	*dnaG3*	PB	1.3	PB	1.2	NG	NG
CS72	*ssb-1*	B	1.5	B	2.0	B	2.8
CS196	*ssb-3*	B	3.0	B		B	2.4
CS57	*ssb-113*	B	3.2	B	3.7	B	2.0
CS68	*gyrB203 gyrB221*	W	0.9	PB	1.1	PB	1.2
CS184	*tolC210*::Tn*10-48*	B				DB	1.0
CS179	*valS1*	PB		W		NG	NG
CS182	*valS*	PB		PB		PB	
EST2408	*alaS*	PB		PB		NG	NG

[a] Colony color: DB, deep blue; B, blue; PB, pale blue; W, white; NG, no growth; blank, strain not tested. β-gal refers to units of β-galactosidase in the mutant relative to that in the wild type.

β-galactosidase assay. The β-galactosidase assay technique has been described (24).

Host cell reactivation. Host cell reactivation was used as a test for a mutant *uvrA* gene (13). The cells in question were grown in tryptone broth (13 g of tryptone and 7 g of NaCl per liter) containing 0.2% maltose and 0.01 M $MgSO_4$, and served as indicator for UV-irradiated λ *vir*. Plaques were counted after overnight incubation at 35°C.

Complementation tests. Mutants were lysogenized with P1 Cm, P1 Cm *bac-1*, and P1 Cm *lxc-1*. The latter two phages produce elevated levels of analogs of *E. coli* DnaB and SSB proteins, respectively (10, 16). Lysogens were selected by their resistance to chloramphenicol.

RESULTS

Effect of *dna* mutations on colony color of *lac* fusion strains. The effect of mutations in seven *dna* genes on the colony color of the *sulA*::*lac* strain CS45 on X-Gal plates was examined. Different temperatures were used in order to observe differences in *lac* expression due to the temperature-sensitive defects. The *sulA* gene is an SOS gene, inducible by DNA damage (14). At the highest permissive temperature, derivatives of CS45 containing either *dnaB*, *dnaC*, *dnaE*, or *ssb* mutations formed blue colonies, whereas the *dnaA*, *dnaG*, and *gyrB* derivatives formed pale colonies like the *dna*$^+$ control (Table 2). (The isolation of the two *dnaB* mutants and the *ssb-3* mutant, as well as of other mutants, is described below.) The mutant strains that formed blue colonies also showed small but reproducible increases in β-galactosidase specific activity relative to that of the wild type.

However, the two- to threefold increase in β-galactosidase specific activity seemed too small to account for the change in color, especially since several mutants that differed markedly in intensity of blueness nevertheless had similar specific activities (cf. *dnaG3*, *ssb-1*, and *gyrB203*). It seemed possi-

TABLE 3. Effect of known mutations on a colony color and β-galactosidase activity of a *bioA*::λ*lac* signal strain[a]

Strain	Mutation	Color	β-gal
BM5078	None (wild type)	PB	1.0
CS144	*dnaA508*	B	1.0
CS150	*dnaB499*	B	
CS143	*dnaC2*	DB	1.0
CS142	*dnaE486*	B	
CS148	*dnaG3*	DB	1.0
CS147	*ssb-1*	B	1.0
CS149	*ssb-3*	DB	1.0
CS146	*ssb-113*	B	1.0
CS162	*gyrB203 gyrB221*	W	
CS185	*tolC210*::Tn*10-48*	DB	
CS189	*valS1*	PB	
CS186	*valS*	PB	
EST2406	*alaS*	PB	

[a]Abbreviations as in Table 2, footnote *a*. Growth was at 37°C except for CS189 and CS142, which were grown at 35°C because they fail to grow at 37°C.

ble, therefore, that the blue colony color was due largely to a membrane defect that might, for example, increase the permeability for X-Gal, which would provide more substrate for the modest amount of β-galactosidase present in the cell. To test this idea, a mutation known to increase membrane permeability to many agents, namely *tolC210*::Tn*10-48* (26), was introduced into the *sulA*::*lac* strain. The resulting mutant formed deep blue colonies without any increase in β-galactosidase specific activity (Table 2). Spontaneous colicin E1-tolerant mutants also gave this result (data not shown). For this diverse group of mutants we use the phenotype symbol "Xgx," for "X-Gal-extra sensitive."

To examine further whether blue colony color could occur in the absence of an increase in β-galactosidase synthesis, the *dna* mutations were introduced into strain BM5078, which contains a *lac* fusion in the *bioA* gene (4). The DNA-damaging agent mitomycin, which strongly induces SOS genes, does not induce this *bio* gene (unpublished data). Mutations in six *dna* genes (including *ssb*) and *tolC* conferred blue colony color on the *bioA*::*lac* strain without any increase in β-galactosidase specific activity (Table 3).

The *sulA*::*lac* and *bioA*::*lac* strains will be called "signal strains," since they signal the presence of *dna* mutations and membrane mutations by causing a change in colony color.

Some *dna* mutations, such as *dnaA* and *dnaG*, conferred blue colony color on the *bioA*::*lac* strain but not on the *sulA*::*lac* strain. This may be explained, in part, by the different genetic backgrounds. It is possible the strains were being compared at different stages of expression of their defects; they could be tested at a higher temperature when the *bioA*::*lacZ* strains were used. Thus, the *dnaA* and *dnaG* mutants were viable in the *bioA*::*lac* strain but not in the *sulA*::*lac* strain at 37°C, the temperature at which the blue colony color could be observed. It is also possible that there are differences in the membrane proteins in the two genetic backgrounds.

To prove that blue colony color is not due simply to temperature sensitivity, *alaS*, *valS*, and *valS1* mutations, which make the cell temperature sensitive for protein synthesis, were introduced into the *bioA*::*lac* and *sulA*::*lac* strains. The resulting mutants did not produce blue colonies even at temperatures close to the upper limit for growth. Indeed, the *ssb-3* mutation discussed below produced deep blue colonies in both fusion strains at 35°C, far below the minimum lethal temperature of 43°C. We conclude that the blue colony color produced in *dna* strains that show no increase in the level of β-galactosidase activity can be attributed specifically to the *dna* mutations.

Reversion of colony color. For two strains defective in DNA synthesis, the *ssb-1 sulA*::*lac* mutant (CS72) and the *dnaA508 bioA*::*lac* mutant (CS144), spontaneous reversions from blue to pale colonies were observed to give the high frequency of about 10% revertants in an overnight culture. The pale revertants were just as temperature sensitive as the blue parents. Also, when temperature-resistant revertants were selected from other strains, including the *dnaB*, *dnaC*, and *ssb-113* derivatives of *sulA*::*lac*, both blue and pale colonies were found. The ability to suppress the association between the blue colony color and the replication defect is a notable example of a split mutant phenotype.

Noncorrelation of colony color with sensitivity to various agents. Because of the suspicion that the blue colony color was related in many cases to increased permeability of the cell to X-Gal, we looked for a relation of colony color to permeability to other agents. We tested the efficiencies of plating (EOPs) of the mutant derivatives of the *sulA*::*lac* strain on LB plates containing either EDTA, sodium dodecyl sulfate, ampicillin, crystal violet, or carbenicillin. No correlation was found between colony color and sensitivity to the agent (data not shown), but this does not rule out the possibility of a membrane defect (40).

Distinguishing between *dna* mutants and membrane mutants. Since both types of mutations produce blue colonies in the *sulA*::*lac* and *bioA*::*lac* strains, colony color cannot be used to distinguish *dna* mutants from primary membrane mutants. However, the small reproducible increase in β-galactosidase specific activity produced in the *sulA*::*lac* strain by mutations in genes *dnaB*, *dnaC*, *dnaE*, and *ssb* suggests that other *dna* mutations would also be distinguishable by increased enzyme activity.

Use of the *sulA*::*lac* signal strain to isolate *dnaB* mutants. To test whether the signal strain CS45 could be used to detect new mutations in known *dna* genes by their blue colony color, we carried out localized mutagenesis of the 92-min region of the *E. coli* genome, where at least two *dna* genes are located in the order *malB-lexA-dnaB-uvrA-ssb-zjc*::Tn*10* stretching from about 91.5 to 92.5 min (12). A P1 lysogen of CS50, which contains the Tn*10* element at the 92.5-min region of the genome but is otherwise isogenic with CS45, was mutagenized with MNNG (see Materials and Methods). The P1 lysate made by temperature induction of the mutagenized cells was used for transduction of CS45. Transductants were spread on X-Gal plates containing 25 μg of tetracycline per ml to enrich for mutants linked to the Tn*10* element. To screen for transductants that could produce blue colonies, the plates were incubated for 2 days at 30°C.

Out of a total of 3,000 Tet^r transductants, 50 made blue colonies, and those were tested for temperature sensitivity, cold sensitivity, and UV sensitivity. Four mutants were UV sensitive, and three others were temperature sensitive. The remaining 43 blue-colony mutants and one of the temperature-sensitive mutants were not studied further. Two of the blue temperature-sensitive colonies were presumed to contain *dnaB* mutations because they lost their blue color and became temperature resistant upon lysogenization with phage P1 Cm *bac-1*, which overproduces an analog of *dnaB* (17). One of the presumptive mutants, carrying *dnaB499* (CS223), was transductionally backcrossed with Tet^r into the parental CS45 strain, confirming the linkage of blue colony color and temperature sensitivity.

Use of the *sulA*::*lacZ* signal strain to isolate *uvrA* mutants.

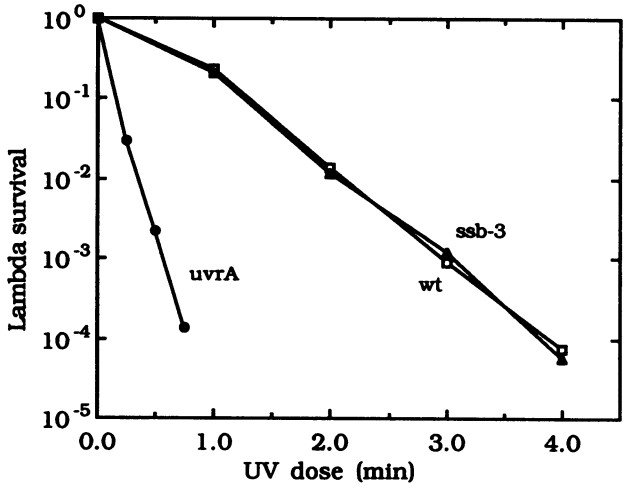

FIG. 1. Survival of UV-irradiated phage lambda on several hosts as a function of time of irradiation. This is a test for host cell reactivation. The strains used were CS45 (wild type), CS196 (*ssb-3*), and CS222 (*uvrA294*). The UV source was a 15-W germicidal lamp.

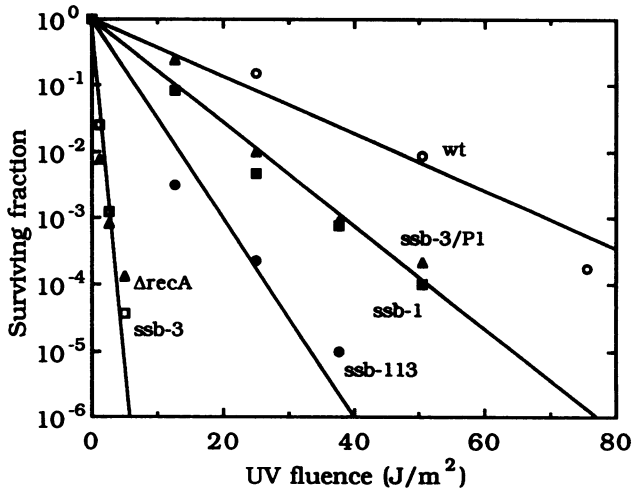

FIG. 2. UV survival curve of *E. coli* mutants. Strains used were CS45 (wild type), CS72 (*ssb-1*), CS196 (*ssb-3*), CS57 (*ssb-113*), CS300 (*ssb-3*/P1 Cm *lxc-1*), and CS52 (Δ*recA*).

Among the 50 blue mutants obtained by localized mutagenesis, four were extremely sensitive to UV light, which might be expected for mutations in the *uvrA* or *ssb* genes. Three of the four mutants were found to be defective in host cell reactivation of UV-irradiated phage lambda, which is consistent with a defect in the *uvrA* gene. The data are shown for one mutant, carrying *uvrA294*, as well as for the fourth mutant, which will be shown below to have a defective *ssb* gene (Fig. 1). The other two mutants, carrying *uvrA290* and *uvrA298*, showed the same Hcr$^-$ phenotype.

When the *uvrA* mutations were backcrossed into CS45 by P1 transduction, their UV sensitivity could be separated from the dark blue color. Thus we detected the *uvrA* mutants through the fortuitous production by MNNG mutagenesis of multiple mutations that accidentally created a blue-colony phenotype linked to, but separable from, the UV sensitivity. The signal strain does not directly score *uvrA* mutants.

Three-factor crosses involving the blue color (*xgx-1*), UV^s (*uvrA*), and Tet^r (*zjc-1*) from the *uvrA290* mutant showed that the gene order is *xgx-uvrA-zjc* (unpublished data). It is thus possible that the *xgx-1* mutation is in the *dnaB* or *lexA* gene.

Isolation of a new *ssb* allele, *ssb-3*. The fourth UV^s blue colony was very UV sensitive in semiquantitative spot test assays at all growth temperatures and was only moderately temperature sensitive for growth, giving an EOP of 10^{-3} at 43°C, at which temperature the parental strain, CS45, gave an EOP of 0.5. To determine whether blue colony color and UV sensitivity were caused by the same mutation, the mutant was transductionally backcrossed to CS45. Two types of blue colonies were obtained. One type of transductant formed large deep blue colonies at 30°C after overnight incubation and was Uvr$^+$ and Ts$^+$. It was not studied further. The second type of transductant, designated CS196, was very UV sensitive, grew slowly, and formed deep blue colonies at 30°C but only if incubated for 2 days. Incubation of the parent strain CS45 for the same length of time at 30°C resulted in pale blue colonies. At 35°C, strain CS196 formed deep blue colonies after a single overnight incubation. Thus the original transductant colony arising after localized mutagenesis contained two mutations that produced blue colony color, but only one of these mutations conferred UV sensitivity and slow growth. (The second blue-colony mutation could be the same as one of the *xgx* mutations that initially gave the *uvrA* mutants their blue color.)

The UV-sensitive transductant, CS196, was proven to contain a defective *ssb* allele. This proof was accomplished by testing complementation of the defective allele with P1 Cm *lxc-1*, a type of mutant P1 that overproduces an analog of *E. coli* SSB protein from its *lxc-1* gene (16). Strain CS196 was lysogenized with P1 Cm *lxc-1*; for comparison it was also lysogenized with P1 Cm *bac-1* (10) and P1 Cm (29). The CS196/P1 Cm *lxc-1* lysogen had the following properties. (i) It formed large pale-blue colonies at 35°C in contrast to the small deep-blue colonies characteristic of CS196 and the control lysogens; the presumed membrane alteration that causes the blue colony is therefore recessive to the Ssb$^+$ analog of P1. (ii) While CS196 was as UV sensitive as a Δ*recA* strain, the sensitivity was greatly relieved in the CS196/P1 Cm *lxc-1* lysogen (Fig. 2). (iii) Blockage of SOS induction, known to occur for the *ssb-1* and *ssb-113* mutants (3, 36) and similarly observed for the CS196 mutant by using mitomycin induction of the *sulA*::*lac* gene for the assay, was greatly relieved in the P1 lysogen (Fig. 3). Since complementation of these properties was not observed in CS196 lysogenized with the two P1 control phages, the positive complementation results obtained with P1 Cm *lxc-1* proved that CS196 contains an *ssb* allele; it is designated *ssb-3*. The *ssb-3* mutation showed 85% cotransduction with *zjc-1*::Tn*10*, which is the same close linkage found for *ssb-1* and *ssb-113* (12).

The fact that CS196 is so highly UV sensitive led us to wonder if the sensitivity might actually be due to a second mutation in CS196 that was in the *uvrA* gene, which is linked to the *ssb* gene, and that might not have been separated from the *ssb* mutation in the transduction. We were able to show, however, that CS196 was not defective in *uvrA* function because it exhibited normal host cell reactivation (Fig. 1). Therefore the extreme UV sensitivity appears to be due to the mutant *ssb-3* allele. The *ssb-113* and *ssb-1* alleles, like *ssb-3*, confer blue colony color on both the *sulA*::*lac* and *bioA*::*lac* strains (Tables 2 and 3).

The *ssb-3* mutant is considerably more UV sensitive than the isogenic *ssb-1* and *ssb-113* mutants, being as sensitive as the isogenic Δ*recA* strain CS52 (Fig. 2), and it is also much

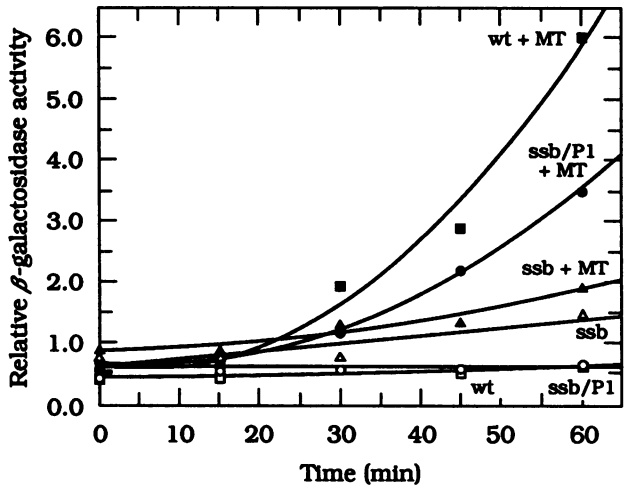

FIG. 3. Suppression of the *sulA* SOS response by the *ssb-3* mutation and partial restoration of function by P1 Cm *lxc-1*. The *sulA*::*lac* strains used were CS45 (wild type), CS196 (*ssb-3*), and CS300 (*ssb-3*/P1 Cm *lxc-1*). β-Galactosidase units were OD_{420}/OD_{600}, measured as a function of time of cell growth. MT, Mitomycin.

less temperature sensitive than these latter two *ssb* strains, having an EOP of 1 at temperatures up to 42°C. All three *ssb* alleles show a 2- to 3-fold increase in expression of the *sulA*::*lac* gene at 37°C (Table 2), whereas maximum induction by mitomycin of the *sulA*::*lac* gene gives a 20-fold increase in β-galactosidase activity above the basal level (unpublished data). Thus *ssb* mutations, which are defective in DNA replication (23), are able to induce the *sulA* gene to a small extent.

DISCUSSION

We have shown here that mutations in six *dna* genes of *E. coli* not only confer a replication defect but also an accompanying defect (Xgx) that results in blue colonies on X-Gal plates; this was found by introducing the *dna* mutations into a strain with *lac* genes fused to the SOS gene *sulA*. The blue color may be due to enhanced permeability to X-Gal, enhanced activity of cellular β-galactosidase, or release of β-galactosidase from the cells (possibly by lysis). This strain is called here a "signal strain," which detects *dna* mutants and also primary membrane mutants.

Release of β-galactosidase into the agar medium would result in a wide diffuse blue halo around the colonies. In a spot test, a 1-mm halo was in fact seen for the *dnaB495* and *dnaB499* mutants but not for the *ssb-3* and *tolC210* mutants; the selective release was confirmed by an assay of β-galactosidase following centrifugation of the cells, which showed for the *dnaB495* strain (CS54) that the supernatant contained 14% of the activity but less than 2% for *ssb-3* (CS149), *tolC210* (CS185), or the wild type (CS45 and CS128) (C.-T. Kuan, personal communication).

Since the signal strain contains a fusion in the *sulA* gene, which is expressed in response to DNA damage, it would also be expected to detect *dna* mutants that contained sufficient defects in DNA structure to strongly induce the *sulA* gene even if the mutants had no membrane defect. However, most of the known *dna* mutants tested here produced only a two- to threefold induction of *sulA* at permissive temperatures, so that induction of *sulA* is at most a minor cause of their blue colony color. In any case, induction of the *sulA*::*lac* gene is not needed for blue colony color: introduction of *dna* mutations into a strain containing a *bioA*::*lac* fusion, not inducible by DNA damage, also resulted in deep blue colonies.

If a cell contains *lac* genes fused to a gene with a moderate basal level of expression, the β-galactosidase present in the cell in combination with altered membrane permeability could result in a blue colony color. Thus many strains with *lac* operon fusions could be designated "signal strains" and could detect mutants with membrane defects. However, the presence of a fusion in the SOS gene *sulA* would be particularly helpful in distinguishing between a primary *dna* mutant and a primary membrane mutant because a number of *dna* mutants have been found to show a small but significant induction of the *sulA* gene, whereas the one membrane mutant tested did not.

The involvement of the cytoplasmic membrane in DNA replication was hypothesized many years ago (15), but proof of this involvement has been difficult to obtain. An in vitro system from *Bacillus subtilis* (5) has shown a requirement for membrane components in DNA synthesis; in contrast, an in vitro replication system from *E. coli* which uses as template a plasmid containing the cellular origin of replication has no absolute requirement for any membrane component (11). More-recent evidence does support the view that DNA synthesis in both *B. subtilis* (18) and *E. coli* (45) involves the membrane. Proof of interaction of the replication complex with membrane components can benefit from our genetic approach, which here implicates mutations in genes *dnaA*, *dnaB*, *dnaC*, *dnaE*, *dnaG*, *ssb*, and *gyrB* in a membrane defect.

Although in *E. coli* it has not yet been possible to demonstrate suppression of the replication defect of a *dna* mutation by a second mutation in a gene encoding a membrane protein, in the case of phage T4, mutations in gene 32, which encodes a protein functionally analogous to the SSB protein of *E. coli*, are suppressed by mutations in T4-encoded membrane proteins, namely the products of the *rIIA* and *rIIB* genes (27).

The apparent enhanced permeability to X-Gal of some *dna* mutants raises the question of how a conformational change presumably caused in proteins of the inner membrane by conformational changes in the DNA replication complex can affect the entry of solutes through the proteins of the outer membrane. We are thus led to postulate that conformational changes in inner membrane proteins can cause conformational changes in some of the proteins of the outer membrane, resulting in more-rapid entry of certain solutes. It is known that mutational changes in proteins can affect cell permeability (6, 26); a precedent for an interaction between the inner and outer membranes comes from the observation that an excess of phage capsid proteins in the inner membrane results in severe changes in cell permeability (6).

Up to now, *dna* mutations have been isolated in $sulA^+$ strains, in which DNA defects sufficient to activate RecA to a large extent would cause lethal filamentation by induction of the *sulA* gene product (7, 8, 30, 32, 37, 38, 44). It is possible, however, that mutant *sulA* strains may be able to tolerate more defects in their DNA than $sulA^+$ strains. Therefore, isolation of *dna* mutations in cells that would lethally filament in response to DNA damage would restrict the selection of *dna* mutants to those which would produce only a small induction of the *sulA* gene at permissive temperature. As done here, the isolation of new *dna* mutants in *sulA*(Def) cells, which do not lethally filament, will expand

the potential range of mutants as well as show us how much inducing signal is compatible with cell viability.

We have found that various mutations can be recognized by the blue colony color they confer on the signal strain. The three *uvrA* mutations did not themselves produce a blue colony color with the signal strain, but were observed coincidentally among the blue colonies at the high frequency of 3/50 either because they occurred at that frequency among all the transductants or because the MNNG mutations were so clustered that an Xgx mutation was likely to be accompanied by other mutations in the 92-min region. The remaining 43 mutations in the 92-min region of the genome, the only region we have studied so far, produced a blue colony color without producing UV sensitivity or conditional lethality. These mutations, which appear likely to occur over the entire genetic map, may represent genes encoding membrane proteins whose integrity is not necessary for cell viability.

The *ssb-3* mutation produced a blue colony on both signal strains. It also produced a definite, though small (threefold) increase of β-galactosidase activity in the *sulA*::*lac* strain, which may be puzzling in view of the fact that it partially blocked the SOS induction of the *sulA* gene that normally would occur with the DNA-damaging mitomycin treatment (Fig. 3); this block of SOS induction is like that shown by *ssb-1* and *ssb-113* (36). Confirmation that the mutation is in the *ssb* gene has been obtained by sequencing of the mutant gene (J. W. Chase, K. R. Williams, C. S. Schmellik-Sandage, and E. S. Tessman, unpublished data), and the finding of the single substitution Gly-15 → Asp in the DNA-binding domain (42) of the protein.

The temperature-sensitive replication defect of *ssb-3* was not observed below 43°C, a temperature higher than that needed for the other two *ssb* alleles; at 42°C it showed an EOP of 1. We have compared *ssb-3* with *ssb-1* and *ssb-113* in several other respects (unpublished data). The recombination frequency of the *ssb-3* mutant as measured in an Hfr mating is reduced to 25% that of the wild type, about the same reduction found for P1 *vir* transduction in an *ssb-1* recipient (12). The *ssb-3* mutant shows no increase in spontaneous mutagenesis over the wild type, which is unlike the behavior of the *ssb-113* mutant (19). UV-induced mutagenesis is blocked by the *ssb-3* mutation, which is also the case for
the *ssb-113* allele (19) as well as for Weigle mutagenesis of lambda in *ssb-1* and *ssb-113* strains (41); we observed the block even in the low fluence range where protease-constitutive, recombinase-negative *recA*(Prt^c Rec$^-$) mutants, which are almost as UV sensitive as *ssb-3*, give high frequencies of UV-induced mutagenesis (35). The high spontaneous mutation frequency of *his-4* → *his*$^+$ by the *recA1211*(Prt^c Rec$^+$) strain, an increase of 140-fold over the *recA*$^+$ strain (33), is not reduced by the substitution of the *ssb-3* allele.

The UV sensitivity of the *ssb-3* mutant is similar to that of a Δ*recA* strain, which is about as sensitive as any single mutant can be and far more sensitive than the other two *ssb* mutants (Fig. 2); substitution of the *recA*$^+$ allele by *recA1211*(Prt^c Rec$^+$) restored about 50% of the UV resistance of the wild-type strain (unpublished data).

The *ssb-3* mutant is also notable for its clear-cut split phenotype: the defects in UV repair and UV mutagenesis occur at temperatures far below the lethal temperature of 43°C. The technique described here provides us with a selective way to easily increase the currently small variety of defective *ssb* alleles.

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