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Mutant Single-Strand Binding Protein of *Escherichia coli*: Genetic and Physiological Characterization

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A mutation in the *Escherichia coli* gene for single-strand binding protein results in temperature-sensitive deoxyribonucleic acid replication (R. R. Meyer, J. Glassberg, and A. Kornberg, Proc. Natl. Acad. Sci. U.S.A. **76**:1702-1705, 1979). The mutant (*ssb-1*) is also more sensitive to ultraviolet irradiation and about one-fifth as active in recombination. Single-strand binding protein is thus implicated in repair and recombination as well as in replication. The mutation in *ssb* is located between *uvrA* and *melA* at 90.8 min on the genetic map. The *ssb* gene appears to be allelic with *lexC*, a gene with a proposed role in regulating inducible deoxyribonucleic acid repair.

The *Escherichia coli* single-strand binding protein (SSB; deoxyribonucleic acid [DNA]-binding protein) (18, 24) is essential for DNA replication in vitro (17, 22, 26) but, for lack of a mutant or specific inhibitor, its role in vivo had been uncertain.

We have found mutant SSB in *E. coli* SG1635 (15), a strain isolated after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis and characterized as temperature sensitive for cell growth and DNA synthesis (23). A mutation causing temperature-sensitive cell growth was mapped at about 75 min and named *dnaM710* (23). We found that strain SG1635 carries two mutations: one (*dnaM710*) causing temperature-sensitive cell growth and the other (*ssb-1*) causing temperature-sensitive DNA replication in vivo and in vitro. In this report we characterize further the effects of mutant SSB, report evidence that SSB is involved in the processes of DNA repair and recombination, and show that the *ssb* gene is located between *uvrA* and *melA*, at 90.8 min on the genetic map.

MATERIALS AND METHODS

Bacteria and phages. The bacterial strains used in this study are listed in Table 1. Phage P1*vir* was obtained from R. Calendar (University of California, Berkeley) and was used for transduction as described previously (16). ST-1 (3), lambda *wt*, and lambda NK155 with a Tn*10* transposon inserted in the immunity region (10) were gifts from J. Scott, D. Kaiser, and G. Weinstock, all of this department.

Media. L broth-thymidine contained (per liter): NaCl, 5 g; yeast extract, 5 g; tryptone, 10 g; 1 M NaOH, 1.1 ml; and thymidine, 50 mg. M63 minimal medium was as used previously (16). Bottom agar for plates contained 1.2% agar and 0.7% soft agar.

Marker selection and identification. Tetracycline-resistant (Tet^r) isolates were selected or identified on L broth agar containing 25 μg of tetracycline per ml. *ubiA*$^+$ isolates were selected on M63 minimal agar with 0.2% succinate as the carbon source (28); *melA*$^+$ isolates were identified on MacConkey agar containing 2% melibiose; *uvrA* isolates were identified by failure to grow on L broth agar plus 0.02% methyl methane sulfonate; *ssb* isolates were generally identified by failure to grow at 42°C on L broth agar plates lacking NaCl.

***E. coli* C600 pool with randomly inserted Tn*10* transposons.** The Tn*10* insertion procedure was basically that of Kleckner et al. (11). Strain C600 was grown in L broth-thymidine to a density of 5×10^8 cells per ml and infected with lambda NK155 at a multiplicity of infection of 100. The infected culture was incubated at 37°C for 15 min, and 0.1-ml portions were plated on L broth agar plus tetracycline, using L broth soft agar. After incubation at 37°C overnight, the top agar (containing ~2,000 Tet^r colonies on eight plates) was scraped into a flask containing 50 ml of L broth-thymidine plus 25 μg of tetracycline per ml and incubated overnight at 37°C.

RESULTS

Strain SG1635 is defective in SSB. Strain SG1635 is temperature sensitive for cell growth and DNA synthesis (23). Extracts prepared from this strain were deficient in supporting conversion of phage G4 single-stranded DNA to the duplex replicative form. The deficiency could be overcome by adding purified SSB, but not primase or holoenzyme, to the extract (15) (Table 2).

Mutation *dnaM710* is not responsible for

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TABLE 1. *E. coli strains used in this work*

Strain	Genotype/phenotype[a]	Source or reference[b]
DG17	*argG6 metB his-1 leu-6 thyA3 mtl-2 xyl-7 malA1 gal-6 lacY1 rpsL104 tonA2 tsx-1* $\lambda^r \lambda^-$ *supE44*	D. Glaser
SG1635	*dnaM710 ssb-1*, DG17	NG mutagenesis of DG17 Glaser (28)
AN385(λ)	*ubiA402 gal* $str^r \lambda^+$	
JGC109	*ssb-1 zhg-1*::Tn*10*, SG1635	P1(C600 with random Tn*10* insertions) → SG1635, selection for Tet^r Ts^+ at 41°C
JGC115	*ssb-1 zhg-1*::Tn*10 dnaM710* SG1635	P1(JGC109) → SG1635, selection for Tet^r
JGC127	*dnaM710 zhg-1*::Tn*10*, DG17	P1(JGC115) → DG17, selection for Tet^r
JGC155	*ssb-1 gal* $Str^r \lambda^+$	P1(JGC109) AN385(λ), selection for *ubiA*$^+$
JGC158	*ssb*$^+$ *gal* $Str^r \lambda^+$	P1(JGC109) → AN385(λ), selection for *ubiA*$^+$
JGC175	*ssb*$^+$ *zjb-1*::Tn*10 gal* $Str^r \lambda^+$	P1(C600 with random Tn*10* insertions) → JGC155, selection for Tet^r Ts^+ at 42°C
JGC178	*ssb*$^+$ *zjb-2*::Tn*10 gal* $Str^r \lambda^+$	Same as JGC175
JGC197	*ssb-1 zjb-1*::Tn*10 gal* $Str^r \lambda^+$	P1(JGC175) → JGC155, selection for Tet^r
JGC200	*ssb-1 zjb-2*::Tn*10 gal* Str^r, λ^+	P1(JGC178) → JGC155, selection for Tet^r (11)
C600	*thr leu lac* $T1^r$ *supE*	
JGC206	*ssb-1 zjb-1*::Tn*10*, C600	P1(JGC197) → C600, selection for Tet^r
JGC208	*ssb*$^+$ *zjb-1*::Tn*10*, C600	P1(JGC197) → C600, selection for Tet^r
AB1927	Hfr *argG* → *metC*; *metA28 purF1*	CGSC 1927
AB468	F$^-$ *purD13 thr-1 his-4 proA2*	CGSC 468
JGC215	*ssb-1 zjb-1*::Tn*10 purD13*, AB468	P1(JGC197) → AB468, selection for Tet^r
JGC217	*ssb*$^+$ *zjb-1*::Tn*10 purD13*, AB468	P1(JGC197) → AB468, selection for Tet^r
M2508	Hfr *purE* → *lip melA7 metB1 relA1*	CGSC 4926
KL209	Hfr *argE* → purA *malB16 thr-1* $\lambda^r \lambda^-$ *supE44*	CGSC 4315
JGC225	*ssb-1 zjb-2*::Tn*10 melA7*, M2508	P1(JGC200) → M2508, selection for Tet^r
AB2500	*uvrA6 leu arg* $T6^r$ *lac thi his thy mtl thr pro ara gal* Str^r *supE*	A. Ganesan
JGC231	*uvrA6 ssb-1 zjb-2*::Tn*10 melA7*; not tested for other markers	JGC225 × AB2500, selection for Tet^r Str^r (15)
GR7	*groPB558 galK*	

[a] For gene symbols see Bachmann et al. (2). Nomenclature for the Tn*10* transposon follows the system of Hong and Ames (8) as adapted by Kleckner et al. (11).

[b] For P1*vir* transduction, "P1 (X) → (Y), selection for Tet^r" means that Tet^r transductants were selected from strain Y after infection with P1*vir* grown on strain X. CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn. NG, Nitrosoguanidine.

deficiencies in SSB, DNA replication, or single-stranded phage growth. The *dnaM710* mutation was moved from strain SG1635 back to the parental strain by P1*vir* transduction. A Tn*10* transposon was first inserted near the *dnaM* locus by phage P1*vir* transduction, using an *E. coli* C600 pool containing randomly inserted Tn*10* transposons as donors (see Materials and Methods) and strain SG1635 as the recipient. Transductants were selected for growth at 41°C on plates containing L agar plus tetracycline. One such transductant, JGC109, has a Tn*10* transposon about 80% linked to *dnaM*$^+$ by P1*vir* transduction. The Tn*10* marker was then used to backcross the *dnaM710* mutation into the parental strain, DG17. Tn*10* was transduced from strain JGC109 into strain SG1635, and a Tet^r/*dnaM710* strain was isolated (JGC115). This strain was used to construct JGC127 by transducing Tn*10* into strain DG17 and scoring growth at 41°C. The mutation causing temperature-sensitive growth

TABLE 2. *Effect of purified replication proteins on G4 DNA synthesis in a crude enzyme fraction*[a]

Strain	Addition	Nucleotide incorporated (pmol)
DG17 parent of SG1635	None	24
	Primase	34
	Holoenzyme	25
	SSB	31
SG1635 (*dnaM710 ssb-1*)	None	<0.1
	Primase	<0.1
	Holoenzyme	<0.1
	SSB	31
JGC109 (*ssb-1*)	None	<0.1
	Primase	<0.1
	Holoenzyme	<0.1
	SSB	19
JGC127 (*dnaM710 ssb*$^+$)	None	20
	Primase	40
	Holoenzyme	28
	SSB	36
JGC155 (*ssb-1* λ^+)	None	<0.1
	Primase	<0.1
	Holoenzyme	<0.1
	SSB	30
JGC158 (*ssb*$^+$ λ^+)	None	30
	Primase	36
	Holoenzyme	32
	SSB	35

[a] Extracts and complementation assays were as described previously (15). A concentrated cell culture in Tris-sucrose was lysed by addition of lysozyme and Brij 58. After centrifugation, the supernatant was brought to 40% saturation with saturated ammonium sulfate solution, and the precipitate was collected by centrifugation and suspended in I buffer (50 mM imidazole·HCl [pH 6.8], 5 mM dithiothreitol, 1 mM EDTA, 20% [vol/vol] glycerol) plus 40% ammonium sulfate. The precipitate, collected again and redissolved in I buffer, was the complementation extract. Complementation assays were in 25-μl amounts containing: 20 mM Tris-hydrochloride (pH 7.5); 5 mM dithiothreitol; 1 mM EDTA; 100 μM each ATP, CTP, GTP, and UTP; 50 μM each dATP, dCTP, dGTP; 15 μM [^{3}H]dTTP (1,000 cpm/pmol); 0.2 mg of bovine serum albumin per ml; 5 mM $MgCl_2$; 5 mM spermidine; 2.5% sucrose; G4 DNA (230 pmol of nucleotide); and 1.2 μl of complementation extract (55 to 65 μg of protein). Incubation was for 10 min at 30°C. Supplementations with purified replication proteins were as indicated. DNA polymerase III holoenzyme (14), primase (21), and SSB (26) were purified as described previously.

of strain JGC127 was mapped by Hfr crosses (12) at 75 to 79 min (data not shown), in agreement with the map position previously reported (23).

If the *dnaM710* mutation were responsible for the SSB defect in strain SG1635, an extract of strain JGC109 (*dnaM*$^+$) should support phage G4 DNA replication, and an extract of strain JGC127 (*dnaM710*) should not. However, Table 2 shows the opposite result, which indicates that the *dnaM710* mutation is not the cause of the SSB defect. Although SG1635 is known to be temperature sensitive for DNA synthesis (23) and growth of single-stranded phage (25), we have found that strain JGC127 (*dnaM710*) at 42°C can synthesize DNA and support growth of single-stranded phage (data not shown).

SG1635 carries a second mutation (*ssb-1*) causing temperature sensitivity. Strain JGC109 had been selected as a Tet^r transductant able to grow at 41°C. At 42°C, however, strain JGC109 was still temperature sensitive. The temperature sensitivity lesion in strain JGC109, *ssb-1*, was mapped by Hfr crosses to between 90 and 98 min. It was cotransducible with *ubiA* (located at about 90 min) by phage P1*vir* transduction. Using phage P1*vir* transduction and strain JGC109 as the donor, we transduced strain AN385 (λ^+ *ubiA*) to *ubiA*$^+$; *ssb-1* (JGC155) and *ssb*$^+$ (JGC158) transductants were isolated. This temperature sensitivity lesion could then be correlated with the binding protein defect (Table 2).

Map position of *ssb*. We performed a series of phage P1*vir* transductions and Hfr conjugations involving *ssb-1* and various markers in the *malB-melA* region of the chromosome. Preliminary two- and three-factor crosses established relative gene orders. Conjugation with strain JGC225 (Hfr *pur*E→*lip ssb zjb-2*::Tn*10 melA*) as the donor and strain AB2500 (*uvrA*) as the recipient yielded the gene order *uvrA ssb zjb-2*::Tn*10 melA*. The results of a five-factor cross are shown in Table 3. An analysis of this and other data (not shown) establishes the gene order shown in Fig. 1.

Mutation *ssb-1* results in a defect in SSB and temperature-sensitive growth of single-stranded phage. Extracts of strain JGC155 are defective in SSB (Table 2) (15); SSB purified from strain SG1635 is temperature sensitive in vitro (15). Since extracts of strain JGC155 were deficient in supporting G4 DNA replication in vitro, we wished to know whether strain JGC155 was temperature sensitive in vivo for growth of single-stranded phage. We used single-stranded phage ST-1 (3) because, unlike closely related phage G4, it grows on *E. coli* K-12 strains. A deficiency in SSB led to temperature-sensitive growth of single-stranded phage in vivo (Table 4). We have shown previously that *ssb-1* causes temperature-sensitive cellular DNA synthesis (15).

TABLE 3. *Genetic mapping of ssb-1 by P1vir transduction*[a]

Genotype of transductants				No. of transductants
uvrA	*ssb*	*tet*	*melA*	
−	+	S	+	86
−	−	R	+	68
+	+	S	+	48
−	−	S	+	33
+	−	R	+	21
−	+	R	+	19
+	−	S	+	18
+	+	R	+	6
−	−	S	−	1

[a] JGC231 (*uvrA ssb zjb-2*::Tn*10 melA*) served as the donor; AN385 (*ubiA*) served as the recipient. *ubiA*$^+$ transductants were selected as described in Materials and Methods. Isolates containing the *ssb* mutation failed to grow on L broth agar plates at 42°C. From data in this table, cotransduction frequencies of *ubiA* and other markers are: *uvrA*, 0.69 (207/300); *ssb*, 0.47 (141/300); *zjb-2*::Tn*10*, 0.38 (114/300); *melA*, 0.003 (1/300).

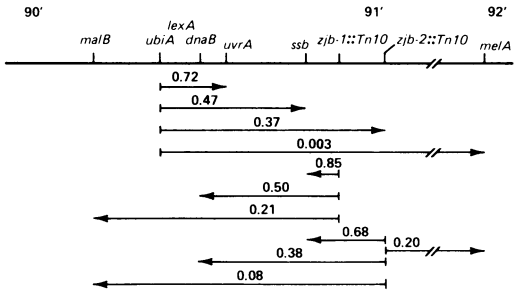

FIG. 1. *Genetic map of the E. coli chromosome containing ssb in the malB-melA region. Map distances were calculated from P1 transduction data (Table 3) and unpublished data. Bars indicate selected markers; arrows point to unselected markers. Strains KL209 and GR7 were recipients in P1vir transduction experiments positioning malB and dnaB, respectively. Numerical values are direct cotransduction frequencies. The position of the malB locus is taken from Bachmann et al. (2); that of the lexA locus is from McEntree (13). P1vir cotransduction frequencies were converted to distance in minutes as described by Bachmann et al. (2).*

An *ssb-1* strain is UV sensitive even at 37°C. To determine whether SSB was involved in the repair of UV-induced damage, we irradiated an otherwise isogenic *ssb ssb*$^+$ pair of strains. Survival of the *ssb-1* strain (JGC206) was greatly reduced compared with that of the *ssb*$^+$ strain (Fig. 2).

An *ssb-1* strain is less active in recombination. A pair of *pur*D strains, *ssb*$^+$ and *ssb-1*, was constructed and used as recipients during P1*vir* transduction. Strain AB1927 (Hfr*argG*→*metC metA*) was the donor (the *metA* locus is closely linked to the *purD* locus) (2), and the frequency of *purD*$^+$ and *purD*$^+$ *metA*$^+$ recombinants was determined. The *ssb-*

TABLE 4. *Growth of phage ST-1 on ssb E. coli*[a]

Strain	Yield of ST-1 at:	
	36°C	43°C
JGC158 (*ssb*$^+$)	160	352
JGC155(*ssb*)	158	34

[a] Cultures were grown in L broth-thymidine to a cell density of 2×10^8/ml. $CaCl_2$ was added to 10^{-1} M, and 0.1-ml portions were transferred to pairs of tubes and incubated at 36 or 43°C. Ten minutes later, cultures were infected with phage ST-1 at a multiplicity of infection of about 0.025. Five minutes after infection, cultures were diluted 1,000-fold into L broth-thymidine that had been preheated to the appropriate temperature. At 60 min after infection, cells were lysed by the addition of chloroform and left at room temperature for 20 min. Lysates were titrated for plaque-forming units with C600 on L broth agar plates and L broth soft agar plus 5×10^{-2} M $CaCl_2$. Phage yield was computed as the final number of plaque-foming units divided by the input.

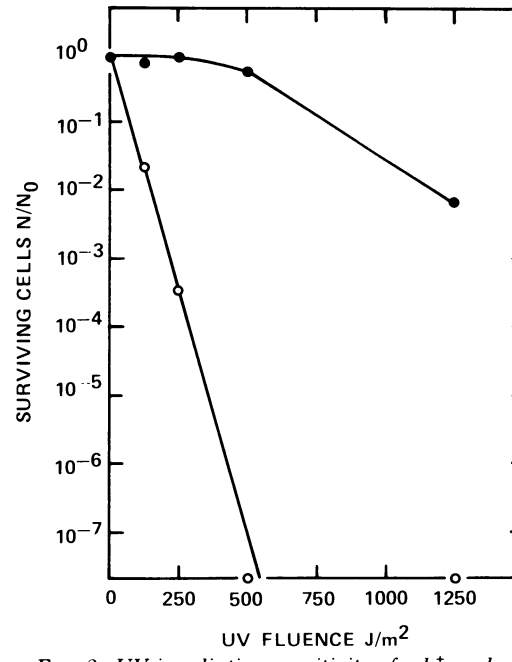

FIG. 2. *UV irradiation sensitivity of ssb*$^+$ *and ssb strains. Strains JGC206 (ssb) and JGC208 (ssb*$^+$*) were grown in L broth-thymidine to a cell density of* 2.5×10^8*/ml, centrifuged at 7,000 rpm for 10 min, and suspended in M63 minimal medium. Cultures were poured into glass petri plates. After exposure to the indicated amounts of UV irradiation, 0.1-ml portions were removed to determine the number of surviving cells. Symbols: ●, ssb*$^+$*; ○, ssb.*

TABLE 5. *Effect of ssb-1 on recombination*[a]

Recipient strain	Frequency of *purD*$^+$ recombinants	No. of *purD*$^+$ recombinants tested	No. of *purD*$^+$ *metA*$^+$ *recombinants*
Expt 1			
JGC217 (*ssb*$^+$)	2.5×10^{-5}	300	58
JGC1 (*ssb*)	0.4×10^{-5}	300	28
Expt 2			
JGC217	1.6×10^{-5}	300	62
JGC215	0.3×10^{-5}	300	21

[a] AB1927 (Hfr *argG* → *metC metA*) was the donor for P1*vir* transduction of either JGC217 (*purD ssb*$^+$) or JGC215 (*purD ssb-1*) at 37°C. *purD*$^+$ recombinants were selected on M63 agar plates with 0.2% glucose as the carbon source and supplementd with B_1, Thy, Thr, His, Pro, and Met. *purD*$^+$ recombinants were scored for the ability to grow on M63 agar plates lacking Met but containing all the other above-mentioned additions. Frequency of *purD*$^+$ recombinants is the number of *purD*$^+$ recombinants per milliliter divided by the number of recipient cells per milliliter.

1 mutation reduced recombination about fivefold (Table 5).

DISCUSSION

The identification of mutant SSB (15) allowed us to examine the physiological role played by this protein. Mutant SSB affected the replication of DNA, as seemed plausible, since it is required for in vitro DNA replication (17, 22, 26). At 42°C, *ssb* mutants exhibited a fast-stop phenotype with respect to *E. coli* DNA replication (15) and restricted the growth of single-stranded phages (25; Table IV) and phage lambda (unpublished data).

The effect of mutant SSB on recombination was not dramatic—about a fivefold reduction at 37°C. Mutations in other genes, e.g., *recA* or *recBC*, have a much greater effect on recombination (4). Thus, our data do not support a model directly involving SSB as an essential element in recombination (20), although such a model is not excluded. Although *ssb* strains were UV sensitive at 37°C, mutant SSB may not have been completely inactive in recombination at this temperature. Alternatively, SSB, like the analogous T4 gene 32 protein (19), may have more than one functional domain, with the *ssb-1* mutation affecting these domains differently.

The UV sensitivity of *ssb-1* strains indicated an important role for SSB in DNA repair synthesis. That this sensitivity was exhibited even at a temperature which allowed DNA synthesis to proceed normally (compare Fig. 3, this paper, with Fig. 4 of Meyer et al. [15]) suggests that SSB may play different roles in these processes.

Mutations in certain *E. coli* genes which affect UV sensitivity, e.g., *recA*, *lexA*, and *lexC*, also affect induction by UV of lambda prophage. *ssb* mutants are deficient in this induction (as measured by phage yield) at temperatures at which lytic growth is only slightly impaired (unpublished data). The UV sensitivity and impairment of lambda induction in *ssb* cells raise the possibility that SSB has a role in regulation of the inducible repair system of *E. coli* (27). Consistent with this view is the probable identity of *ssb* and *lexC*, the latter a gene proposed as a regulator of inducible repair (9). Evidence for the identity of *ssb* and *lexC* is as follows. (i) Both *lexC* (9) and *ssb* (this paper) have been mapped to the right of *uvrA*, at about 90.8 min on the genetic map. After conjugation, recombination between *ssb-1* and *lexC113* occurs only about once in 4×10^6 times, in crosses in which the *zjb-z*::Tn*10* marker is received by 1% of the recipient cells (unpublished data). (ii) Although *lexC-113* was discovered as a mutation conferring sensitivity to X-irradiation (6), it also causes UV sensitivity, blocks lambda induction, and is responsible for temperature-sensitive DNA replication (7, 9), the same phenotypes we find for *ssb* mutants. (iii) Preliminary results with extracts of a *lexC* strain indicate that, like the *ssb* strain, it is defective in SSB and that the binding protein purified from the *lexC* strain is temperature sensitive for phage G4 replication, as described for the *ssb* strain (15).

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