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The *ssb* Gene of Plasmid ColIb-P9

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The IncI1 plasmid ColIb-P9 was found to carry a single-stranded DNA-binding (SSB) protein gene (*ssb*) that maps about 11 kilobase pairs from the origin of transfer in the region transferred early during bacterial conjugation. The cloned gene was able to suppress the UV and temperature sensitivity of an *ssb-1* strain of *Escherichia coli* K-12. The nucleotide sequence of the ColIb *ssb* gene was determined, giving a predicted molecular weight of 19,110 for the SSB protein. Sequence data show that ColIb *ssb* is very similar to the *ssb* gene on plasmid F, which is also known to map in the leader region. High-level expression of *ssb* on ColIb required derepression of the transfer (*tra*) genes and the activity of the positive regulatory system controlling these genes, suggesting that the SSB protein contributes to the conjugative processing of DNA. A mutant of ColIb*drd-1* carrying a Tn*903*-derived insertion in *ssb* was constructed, but it was unaffected in the ability to generate plasmid transconjugants and it was maintained apparently stably in donor cells both following mating and during vegetative growth. Hence, no biological role of ColIb SSB protein was detected. However, unlike the parental plasmid, such ColIb *ssb* mutants conferred a marked Psi$^+$ (plasmid-mediated SOS inhibition) phenotype on *recA441* and *recA730* strains, implying a functional relationship between SSB and Psi proteins.

Single-stranded DNA-binding proteins (SSBs) are a class of proteins that have a high affinity for single-stranded DNA and bind with no sequence specificity. SSB encoded by *Escherichia coli* K-12 functions in a variety of DNA metabolic processes, as shown by properties of temperature-sensitive mutants (9). The phenotypes conferred by one well-characterized mutation, *ssb-1*, include rapid cessation of DNA replication at high temperature and UV sensitivity at 30°C resulting from defective recombinational repair (16, 28, 30, 41). Both of these phenotypes are suppressible by a range of conjugative plasmids. This phenomenon was originally discovered with plasmid F and shown to be caused by a plasmid-encoded gene, designated F *ssb*, that is homologous to *E. coli ssb* (8, 26). Conjugative plasmids representing 12 of 23 incompatibility groups, including IncI1 plasmids and the IncP plasmid RP4, were also reported to suppress the *ssb-1* mutation and to carry sequences that hybridize to F *ssb*, implying that a diverse set of self-transmissible plasmids carry related *ssb* genes (18). Such plasmids also carry sequences homologous to the *psiB* gene of R6-5 (17; E. Golub, personal communication). The Psi function (plasmid-mediated SOS inhibition) interferes with induction of the cellular SOS response, possibly by inhibiting activation of RecA protein (2, 3).

The function of plasmid-encoded SSBs has yet to be established. However, enhanced suppression of *ssb-1* by plasmids containing a *drd* mutation, which derepresses expression of the conjugative transfer genes, suggests that plasmid SSBs may participate in the metabolism of plasmid DNA during bacterial conjugation (19). This process involves transfer of a single strand of DNA from the donor to a recipient cell and is associated with synthesis of a replacement DNA strand in the donor and a complementary strand in the recipient (43).

In this paper, we show that the IncI1 (i.e., IncIα) plasmid ColIb-P9 carries a gene homologous to the *ssb* determinant of F, whereas, in contrast to the previous report, the IncP plasmid RP4 lacks such a gene. The location of the ColIb *ssb* gene, its nucleotide sequence, and aspects of its regulation are reported. Construction and properties of a ColIb*drd-1* plasmid carrying an insertion in *ssb* are described, including the finding that the mutation conferred a Psi$^+$ phenotype on the host cell.

MATERIALS AND METHODS

Plasmids and bacterial strains. Plasmids are described in Table 1. Plasmids pLG221, pLG252, pLG274.2, and pLG276.3 have been described previously (10, 34). Plasmid pLG273 was constructed by infection of a strain containing ColIb-P9*drd-1* with bacteriophage λ carrying the Tn*10del4*-HH104 element (40), with selection for tetracycline resistance and subsequent screening for the loss of colicin Ib activity. pLG272 was isolated by the same procedure, involving λ::Tn*5* infection of a ColIb-P9-containing strain and selection for kanamycin resistance. Other plasmid constructions are described in the text. Vector plasmids were pACYC184 (7), pBR328 (36), and pLG339 (37). pKAC50 is a pBR322 derivative carrying *ssb* of plasmid F (8, 26). *E. coli* K-12 F$^-$ derivatives and their relevant genotypes included KL450, *ssb-1 gyrA* (26), provided by E. Golub; KL450R, a spontaneous rifampin-resistant (Rif^r) derivative; BW97, *gyrA* (10, 34); GC4415 *rec*$^+$ Δ*lac sfiA99*::Mu d(Ap *lacZ*$^+$) (23); GC4597, *recA441* (λ *cI ind1 sfiA*::*lacZ*$^+$) (24); and GY7221, *recA730* Δ*lac sfiA211* (λ *cI ind1 sfiA*::*lacZ*$^+$), provided by R. Devoret (3).

Media. Media, culture conditions, and antibiotic concentrations were as described previously (10, 34). Rifampin was added to 20 μg ml^{-1}.

DNA manipulations. Isolation of plasmid DNA, transformation procedures, and conditions used for DNA analysis and ligation have been described previously (34).

Qualitative tests for *ssb-1* suppression by plasmids. Suppression of temperature sensitivity was determined by plating dilutions of overnight cultures onto prewarmed nutrient agar plates, followed by incubation overnight at 30 and 44°C. UV survival was measured by using overnight cultures diluted to an A_{600} of 0.35 (~2.5 × 10^8 cells per ml). A further

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10-fold dilution in phosphate buffer was irradiated at 0.5 J m^{-2} s^{-1}. Samples were taken after appropriate doses and assayed for CFU. Nutrient agar contained suitable antibiotics to select plasmid-containing cells. Incubation was at 30°C.

Bacterial conjugation. In tests of conjugative efficiency, bacteria from overnight cultures were grown in nutrient broth for approximately three mass doublings to an A_{600} of 0.35. Cultures were prewarmed at the mating temperature for 5 min, and the donor and recipient strains were then mixed in a 1:9 (vol/vol) ratio. After 30 min, mating was interrupted in a mechanical agitator, and transconjugants were selected at 30°C.

Assay of β-galactosidase. Induction and measurement of β-galactosidase expression from *sfiA::lacZ*$^+$ fusion strains were as described elsewhere (6), except that strains were grown in SGC (M9 salts containing 0.4% [wt/vol] glucose and 0.2% [wt/vol] Casamino Acids; Difco Laboratories).

Southern hybridization. DNA fragments used as probe sequences were isolated from 0.6% (wt/vol) low-gelling-temperature agarose gels and radiolabeled by using random oligodeoxyribonucleotides as primers (14). Restriction fragments were transferred to Hybond N nylon membrane (Amersham International) by the method of Reed and Mann (33). Hybridization was carried out at 65°C, and stringency was defined by a final wash of 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate (SDS).

Nucleotide sequence determination. About 2 μg of DNA, prepared by a rapid boiling method (21), in 8 μl of water was denatured by the addition of 2 μl of 1 M NaOH and 1 mM EDTA. After 5 min at room temperature, 3 μl of 3 M sodium acetate, 17 μl of water, and 30 μl of isopropanol were added, and the mixture was centrifuged for 15 min. The dried pellet was dissolved in 7.5 μl of water. To this was added 1.5 μl of K buffer (100 mM Tris hydrochloride, pH 8.0, 50 mM $MgCl_2$) and 1 μl of primer oligomer (25 to 50 μg ml^{-1}). Following heating to 103°C and annealing at 37°C for 20 min, 1 μl of DNA polymerase I (Klenow fragment, 1 U ml^{-1}; Boehringer Mannheim), 1 μl of *E. coli*: single-stranded-DNA-binding protein (SSB) (0.6 mg ml^{-1} ; Pharmacia), and 3 μl of [α-^{35}S]dATPαS (370 MBq μl^{-1}; 44 TBq $mmol^{-1}$; Amersham) were added. The dideoxy reactions contained 3 μl of this mixture and 2 μl of 37.5 μM ddATP and 60 μM dCTP, dGTP, and dTTP (A); 10 μM ddCTP, 4 μM dCTP, and 80 μM dGTP and dTTP (C); 25 μM ddGTP, 4 μM dGTP, and 80 μM dCTP and dTTP (G); and 125 μM ddTTP, 4 μM dTTP, and 80 μM dCTP and dGTP (T). Polymerization was at 37°C for 15 min. Chase reactions (10 min, 37°C) were started by the addition of 2 μl of a mixture of 0.5 mM dATP, dCTP, dGTP, and dTTP. Reactions were stopped by the addition of 4 μl of formamide-dye mix (1 mg of xylene cyanol FF, 1 mg of bromophenol blue, 40 μl of 0.5 M disodium EDTA per ml of formamide). After being heated to 103°C, 3-μl samples were loaded per lane of a hot 6% polyacrylamide–7 M urea gel. Gels were run at 80 W for 1.5 to 5 h, fixed in 10% acetic acid, washed in water, and dried prior to autoradiography.

Minicells and SDS-polyacrylamide gel electrophoresis. Minicells were purified from plasmid-containing derivatives of strain DS410 and processed as described previously (42), except that proteins were labeled with [^{35}S]methionine (1 MBq ml^{-1}; 50 TBq $mmol^{-1}$; Amersham) for 30 min followed by a 5-min chase with unlabeled methionine. SDS-polyacrylamide gel electrophoresis was performed using gels containing 18% (wt/vol) acrylamide (monomer:bis ratio, 44:0.8). [^{14}C]-methylated proteins (Amersham) were used as molecular weight markers.

Computer analysis. Programs obtained from the University of Wisconsin Genetics Computer Group (12) were used for the prediction of secondary structure based on the method of Chou and Fasman (11) and for generating hydropathy profiles using Kyte and Doolitte values (27).

TABLE 1. Plasmids used and their abilities to suppress the temperature sensitivity of KL450 *ssb-1*

Plasmid (s) present	Description	KL450 survival[a]
None		6.8×10^{-5}
pACYC184	Cm^r Tc^r	2.9×10^{-5}
pBR328	Ap^r Cm^r Tc^r	4.2×10^{-4}
pLG221	ColIb*drd-1 cib*::Tn*5* Km^r	0.74
pLG252	pBR325 Ω(ColIb *exc*$^+$ *Eco*RI 3.5 kb) Ap^r Tc^r	NT
pLG272	ColIb *cib*::Tn*5* *ssb*$^+$ Km^r	1.4×10^{-4}
pLG273	ColIb*drd-1 cib*::Tn*10* *ssb*$^+$ Tc^r	0.76
pLG274.2	ColIb*drd-1 tra*::Tn*1723* *ssb*$^+$ Km^r	3.8×10^{-5}
pLG276.3	ColIb*drd-1 tra*::Tn*5* *ssb*$^+$ Km^r	7.2×10^{-5}
pLG283	pBR328 Ω(ColIb *ssb*$^+$ *Sal*I 10.1 kb Δ2.35 kb) Ap^r Cm^r	0.30
pLG284	pACYC184 Ω(ColIb *ssb*$^+$ *Cla*I-*Sal*I 6.3 kb Δ2.35 kb) Cm^r	0.96
pLG286	pLG284 *ssb*::(*aphA-I Pst*I 1.2 kb) Cm^r Km^r	1.3×10^{-4}
pLG288	pLG273 *ssb*::(*aphA-I Pst*I 1.2 kb) Km^r Tc^r	8.6×10^{-6}
pLG289	pLG339 Ω(ColIb *ssb*$^+$ *Cla*I-*Sal*I 6.3 kb Δ2.35 kb) Km^r	0.11
pLG339	Km^r Tc^r	2.1×10^{-4}
pKAC50	pBR322 Ω(F *ssb*$^+$) Ap^r	0.19
RP4	Ap^r Km^r Tc^r	9.9×10^{-7}
pLG273, pLG286		0.56
pLG288, pLG284		0.29
pLG272, pLG284		0.65
pLG274.2, pLG284		4.3×10^{-2}
pLG276.3, pLG284		0.52

[a] Colony formation at 44°C relative to that at 30°C. NT, Not tested.

RESULTS

Mapping of the ColIb *ssb* gene. A tetracycline resistance derivative of ColIb*drd-1*, pLG273, suppressed both the temperature sensitivity of the *ssb-1* strain KL450 and the UV sensitivity of this mutant at 30°C (Table 1 and Fig. 1). The location of the *ssb-1*-suppressing gene on ColIb*drd-1* was investigated first by screening a library of cosmids containing approximately 45 kilobase pairs (kb) of the plasmid. Suppressing cosmids, detected by qualitative streak tests, each carried the largest *Eco*RI fragment (E1, 20.3 kb) of ColIb. The E1 fragment overlaps three *Sal*I fragments (Fig. 2), and screening of appropriate pBR328-based recombinant plasmids showed that *ssb-1*-suppressing activity is specified by the middle fragment, S4 (10.1 kb). A recombinant plasmid carrying the complete S4 fragment was maintained unstably in a variety of strains, but a stable derivative (pLG283; Fig. 2) was isolated. This had suffered a spontaneous deletion of 2.35 kb but continued to suppress the temperature sensitivity of KL450 at 44°C (Table 1).

The putative ColIb *ssb* gene was located more precisely by Southern hybridization with the F *ssb* gene present on the 0.7-kb *Ava*II fragment in pKAC50 (8). This probe hybridized

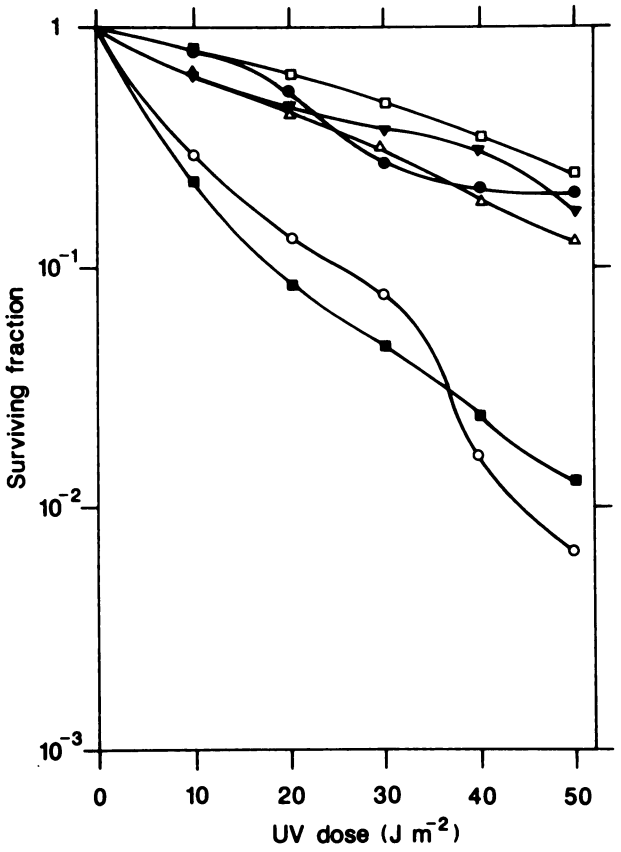

FIG. 1. Survival of *ssb-1* strains after UV irradiation. Strains were KL450 (○) and derivatives harboring plasmids pLG272 (△), pLG273 (□), pLG276.3 (▼), pLG288 (■), and both pLG288 and pLG284 (●). Plasmid pLG274.2 (not shown) conferred slightly greater UV resistance than did pLG276.3. Values are the means from at least two replicate experiments.

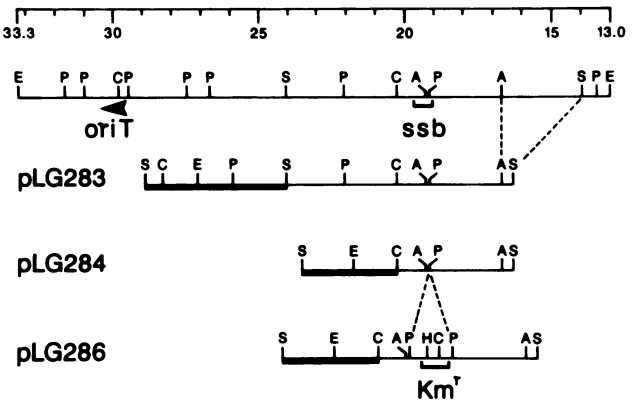

FIG. 2. Restriction maps of plasmids carrying the ColIb *ssb* locus. Line 1 (top) indicates kilobase coordinates on the ColIb map (34). Line 2 shows restriction sites in the E1 *Eco*RI fragment of Col*Idrd-1* and the location of the *ssb* gene. The defined *tra* genes extend to the left of the origin of transfer (*oriT*; arrowhead) and are transmitted late to the recipient cell (22, 34). The S4 *Sal*I fragment is between coordinates 13.9 and 24. Plasmids on lines 3 to 5 have been linearized at one end of the vector (——; pBR328 for pLG283 and pACYC184 for pLG284 and pLG286). - - - -, Deletion found in pLG283 and the insertion in pLG286 of the 1.2-kb *Pst*I fragment from pUC4.K, carrying the kanamycin resistance (Km^r) gene. The intact inverted repeat flanking the Km^r gene is at the left end of this insertion. Transcription of *ssb* and the Km^r gene is from right to left. Restriction sites are *Acc*I (A), *Cla*I (C), *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), and *Sal*I (S).

to the E1 and S4 fragments of ColIb (Fig. 3) and to a 3.95-kb *Cla*I-*Sal*I fragment of pLG283. When inserted into the Tc^r gene of pACYC184, this 3.95-kb fragment caused strong suppression of the temperature sensitivity of KL450 (plasmid pLG284; Table 1). pLG284 also enhanced the UV resistance of KL450 to almost the same extent as pLG273 (data not shown).

The probe containing the F *ssb* gene also hybridized to two *Pst*I fragments (5.9 and 2.9 kb) and two *Pst*I-*Cla*I fragments (3.5 and 1.1 kb) of pLG283, indicating that the putative ColIb *ssb* gene contains a *Pst*I site, identified as that at coordinate 19.2 on ColIb (Fig. 2). Confirmation that this *Pst*I site lies within the gene specifying the *ssb-1*-suppressing activity was obtained by inserting the kanamycin resistance determinant of Tn*903*, carried on a 1.2-kb *Pst*I restriction fragment of pUC4.K (32, 38, 39), into the unique *Pst*I site of pLG284. The resulting plasmid, pLG286 (Fig. 2), was unable to suppress the temperature sensitivity of KL450 (Table 1), and it enhanced the UV sensitivity of this strain (data not shown). Together, these findings indicate that the ColIb sequence homologous to F *ssb* is responsible for suppressing the *ssb-1* mutation of *E. coli*.

Study of RP4. In parallel studies of RP4, we found no evidence that this plasmid suppresses the temperature sensitivity of KL450 (Table 1). Furthermore, the 0.7-kb *Ava*II fragment of pKAC50 carrying F *ssb* failed to hybridize with restriction fragments of RP4 (Fig. 3), although the vector component of pKAC50 hybridized with the *bla* determinant on RP4 (data not shown).

Nucleotide sequence of ColIb *ssb*. A primer (5′ AGCAAC ACCTTCTTCACG) complementary to a sequence near the ends of the inverted repeats of Tn*903* initiated DNA synthesis on only one of the two *Eco*RI-*Hin*dIII fragments of pLG286. The lack of primer extension in one direction is explained by the recent finding that a deletion was sustained near one end of the Tn*903* fragment used to construct pUC4.K (38). Consequently, the primer could be used with intact pLG286 to determine the sequence of one strand of ColIb *ssb* to the left of the inserted *Pst*I fragment (Fig. 2). This sequence allowed the synthesis of further primers, which were used to complete the sequence determination of the *ssb* gene from both strands of pLG284. The nucleotide and predicted amino acid sequences are given in Fig. 4. The SSBs of F and *E. coli* lack the initiating methionine (8, 35), and in view of the sequence similarities, we have assumed that the same holds for ColIb SSB.

Detection of ColIb SSB in minicells. Extracts of minicells carrying pLG284 and pLG286 were compared by SDS-polyacrylamide gel electrophoresis to identify the product of ColIb *ssb* (Fig. 5). Plasmid pLG284 specified a product of 22,000 apparent molecular weight, which is the same size as that detected for F SSB in such a system (26). This polypeptide is assumed to be the ColIb SSB, since it was absent from minicells harboring the insertion mutant pLG286. The latter specifies a novel polypeptide of ~5,000 molecular weight, which is about the size of the 52-amino-acid fusion product predicted from the nucleotide sequence data for ColIb *ssb* carrying the Tn*903*-based insertion at the *Pst*I site.

Isolation of a ColIb*drd-1 ssb* mutant. As a prerequisite to studying the function of the *ssb* gene on ColIb, we constructed a mutant by recombining the Km^r insertion mutation in the cloned *ssb* gene on pLG286 into the ColIb*drd-1*

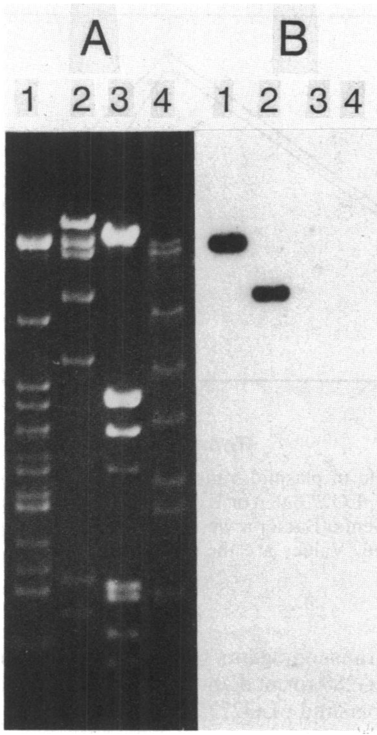

FIG. 3. Hybridization of a 0.7-kb fragment carrying F *ssb* to restriction fragments of ColIb but not of RP4. (A) Agarose gel electrophoresis of ColIb*drd-1* cleaved with *Eco*RI (lane 1) and *Sal*I (lane 2), λ*cI857 Sam7* digested with *Eco*RI and *Hin*dIII to provide size markers (lane 3), and RP4 cut with *Pst*I and *Sst*II (lane 4). (B) Southern blot analysis.

plasmid pLG273. The procedure (45) involved transformation of a *recBC sbcB* strain (JC7623) harboring pLG273 to kanamycin resistance, using pLG286 DNA linearized by cleavage with *Eco*RI and *Sal*I. Km^r pLG273 recombinants were then introduced into KL450 by transformation. Plasmid pLG288 was representative of eight such recombinants in being unable to suppress the *ssb-1* mutation (Table 1 and Fig. 1). As predicted, the S4 fragment of pLG288 was found to be ~1.2 kb larger than that of pLG273 and to contain a sequence that hybridized to the 1.2-kb *Pst*I Km^r fragment of pUC4.K (data not shown). Moreover, pLG288 was complemented by pLG284, which carries the cloned ColIb *ssb*$^+$ gene, showing the mutation to be recessive (Table 1).

Expression of ColIb *ssb*. Table 1 shows that the temperature sensitivity of *ssb-1* cells at 44°C was suppressed with high efficiency by plasmid pLG273, which carries a *drd* mutation derepressing the *tra* genes. In contrast, significantly lower suppression was detected with a wild-type *drd*$^+$ plasmid, pLG272, suggesting that the *ssb* locus on ColIb is regulated coordinately with the genes for conjugation. Plasmids pLG274.2 and pLG276.3 each carry a transposon insertion that inactivates part of the positive regulatory system required for expression of the ColIb conjugation genes (34). Neither of these mutant plasmids caused *ssb-1* suppression at 44°C, again favoring the conclusion that *ssb* expression on ColIb is controlled with the *tra* genes. Plasmid pLG284 in KL450 strains that also harbor pLG272, pLG274.2, or pLG276.3 retained its ability to suppress *ssb-1* at 44°C, confirming that the suppression phenomenon involves the plasmid SSB and not some undefined product of the *tra* region.

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                                                            10                                      20
ColIb  ssb          ATG AGT GCA CGT GGT ATC AAC AAG GTC ATC CTC GTC GGG CGT CTG GGC AAT GAT CCG GAG GTC CGT TAC ATC CCC
ColIb  SSB              Ser Ala Arg Gly Ile Asn Lys Val Ile Leu Val Gly Arg Leu Gly Asn Asp Pro Glu Val Arg Tyr Ile Pro
F      SSB              Ala Val                                                     Lys
E.coli SSB                  Ser         Val                             Asn         Gln                             Met

                    30      PstI                            40                                      50
AAC GGT GGC GCA GTG GCA AAC CTG CAG GTG GCC ACG TCA GAA AGC TGG CGC GAC AAA CAG ACG GGG GAG ATG CGG GAG CAG ACG GAA TGG
Asn Gly Gly Ala Val Ala Asn Leu Gln Val Ala Thr Ser Glu Ser Trp Arg Asp Lys Gln Thr Gly Glu Met Arg Glu Gln Thr Glu Trp

                            Ile Thr Leu                                     Ala                 Lys

                    60                                      70                          AccI        80
CAC CGT GTT GTG CTG TTC GGC AAG CTC GCG GAA GTG GCA GGT GAA TAT CTG CGC AAG GGC GCG CAG GTC TAC ATC GAG GGG CAA CTC CGT
His Arg Val Val Leu Phe Gly Lys Leu Ala Glu Val Ala Gly Glu Tyr Leu Arg Lys Gly Ala Gln Val Tyr Ile Glu Gly Gln Leu Arg
                                                            Cys
                                                    Ser                         Ser

                    90                                          100                                     110
ACC CGT AGC TGG GAC GAC     AAC GGC ATC ACC CGC TAC ATC ACT GAA ATT CTT GTT AAG ACC ACG GGC ACC ATG CAG ATG CTG GGG AGT
Thr Arg Ser Trp Asp Asp *** Asn Gly Ile Thr Arg Tyr Ile Thr Glu Ile Leu Val Lys Thr Thr Gly Thr Met Gln Met Leu Gly Ser
                    Glu                                 Val                                                     Val Arg
        Lys         Thr Gln Ser     Gln Asp             Thr         Val Val     Asn Val Gly                             Gly

                        120                                     130
GCA CCA CAG CAG AAC GCT CAG GCG CAA CCG AAG CCT CAG CAG AAT GGG CAG CCA CAG                 AGT GCT GAC GCG ACG AAA AAA
Ala Pro Gln Gln Asn Ala Gln Ala Gln Pro Lys Pro Gln Gln Asn Gly Gln Pro Gln *** *** *** *** Ser Ala Asp Ala Thr Lys Lys
    Ala Gly Ala Gln Thr     Pro Glu Glu Gly Gln     Phe Ser                 Pro Glu Pro Gln Ala Glu Ala Gly
Arg Gln Gly Gly Gly     *** Pro Ala Gly Gly Asn Ile Gly Gly                 Ser Gly Trp Gly Gln Pro Gln Gln Pro Gln Gly

140                                     150                                     160
GGT GGC GCG AAA ACG AAA GGC CGT GGA CGT AAG GCC GCG CAG CCC GAG CCT CAG CCG CAA ACG CCG GAG GGG GAG GAT TAC GGG TTT TCA
Gly Gly Ala Lys Thr Lys Gly Arg Gly Arg Lys Ala Ala Gln Pro Glu Pro Gln Pro Gln Thr Pro Glu Gly Glu Asp Tyr Gly Phe Ser
                                                                                Pro             Asp
    Asn Gln Phe Ser Gly     Ala Gln Ser Arg Pro Gln     Ser Ala     Ala Ala Pro Ser Asn     Pro Pro Met Asp ***     Asp

170             174
GAC GAC ATC CCG TTC TGA
Asp Asp Ile Pro Phe
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FIG. 4. Nucleotide and predicted amino acid sequence of the ColIb *ssb* gene and protein. It is assumed that the protein lacks an N-terminal methionine. The amino acid sequence of the F and *E. coli* SSBs (8, 35) are shown only where differences from ColIb SSB occur. Gaps introduced to maximize homology are indicated by asterisks. The *Pst*I site is underlined. Numbers correspond to the residues of the ColIb protein.

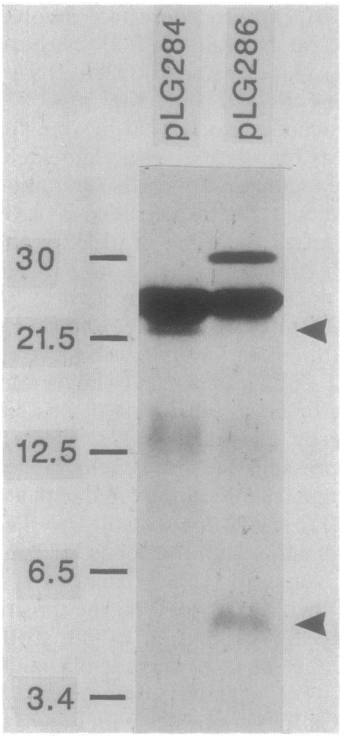

FIG. 5. Analysis of polypeptides made in minicells containing pLG284 and pLG286. The locations of molecular size markers (in kilodaltons) are shown on the left. Arrowheads indicate the presumptive products of the normal and mutant *ssb* genes. Other identifiable polypeptides are chloramphenicol aceyltransferase (25.7), specified by the vectors, and aminoglycoside phosphotransferase (30.7), encoded by the insertion in pLG286.

In contrast to the suppression of temperature sensitivity, the four *ssb*$^+$ ColIb plasmids enhanced the UV resistance of the *ssb-1* strain at 30°C to about the same extent, irrespective of the level of expression of the *tra* genes (Fig. 1). The ColIb *ssb* gene presumably contributes to this enhancement, because pLG284 complemented pLG288 in KL450 to restore a UV-resistant phenotype.

The difference between the abilities of the ColIb *ssb*$^+$ plasmids to suppress the temperature sensitivity and UV sensitivity of KL450 would be reconciled if the *ssb* gene in ColIb is expressed at two levels: constant low-level expression might be sufficient for suppression of UV sensitivity, whereas higher-level expression might be required for suppression of temperature sensitivity, with this state being obtained when the ColIb transfer system is fully expressed or when the plasmid *ssb* gene is cloned in a multicopy vector. To test this, the 3.95-kb *Cla*I-*Sal*I fragment containing ColIb *ssb* was inserted into the *tet* gene of pLG339, which is a pSC101 derivative with a copy number of six to eight per chromosome (37). Consistent with our hypothesis, the resulting plasmid (pLG289; Table 1) suppressed the temperature sensitivity of *ssb-1* bacteria less efficiently than did the pACYC184-based plasmid pLG284, which has an expected copy number of about 20 per chromosome (7). However, both recombinant plasmids enhanced the UV resistance of *ssb-1* cells to about the same extent (data not shown).

Effect of ColIb *ssb* mutation on conjugation. The conjugative ability of pLG288 was assessed in matings of *ssb-1* strains of *E. coli* at temperatures ranging from 30 to 44°C.

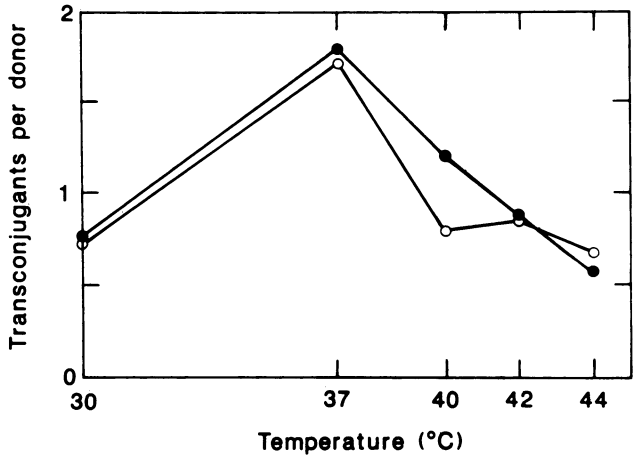

FIG. 6. Yield of plasmid transconjugants per donor cell in matings of KL450(pLG273) (○) or KL450(pLG288) (●) donor cells with KL450R recipients. Bacteria were mated for 30 min at the temperatures indicated. Values are the means from two replicate experiments.

The yield of transconjugants was highest when mating was at 37°C, and pLG288 formed about as many transconjugants as the parental plasmid pLG273 at all temperatures tested (Fig. 6). Similarly, pLG288 formed transconjugants with about the same efficiency as pLG273 in 37°C mating of standard *ssb*$^+$ laboratory strains (data not shown). These results indicate that the ColIb *ssb* gene has no major role in either the transfer of plasmid DNA or its processing in the recipient cell. While they also suggest that *E. coli* SSB has no essential function in DNA transfer itself, the temperature-sensitive bacterial protein might retain sufficient activity at 44°C to carry out conjugation-related functions. The possibility that the *E. coli* protein functions in the conjugative processing of the transferred strand remains, since any impediment to this process in *ssb-1* recipient cells might be removed once the mutants are plated out at low temperature, when the SSB-1 protein is able to renature (30).

Conjugative transfer of single-stranded ColIb DNA is normally associated with synthesis of a replacement strand in the donor cell. To examine whether ColIb SSB is essential for this aspect of conjugative processing of DNA, we measured the stability of pLG288 in the donor strain. The basis of the test is that IncI1 plasmids have a low copy number of one or two per chromosome (25) and, if ColIb SSB is required for replacement strand synthesis, some loss of pLG288 from donor cells might occur following strand transfer. This hypothesis was investigated by mating KL450R donors of pLG288 and pLG273 with rifampin-sensitive recipients for 30 min at 37°C. The yield of transconjugants in both matings was approximately 1.3 per donor cell, indicating efficient conjugation. There was no apparent loss of donor cell viability caused by mating (data not shown). Rif^r colonies were selected following mating, and when 600 from each mating were tested, all showed the Tc^r phenotype characteristic of the ColIb plasmids. Thus, no defect in the conjugative processing of the mutant plasmid in the donor cell was apparent.

Maintenance stability of ColIb *ssb* mutant. The stability of pLG288 was assessed in vegetatively growing cells. To limit conjugative reinfection of any plasmid-free segregants, recombinant plasmid pLG252 specifying entry exclusion was introduced into the test strain (BW97) harboring pLG221 (a

TABLE 2. Effects of plasmids on *sfiA::lacZ* expression in *recA441*, *recA730*, and UV-irradiated *rec*$^+$ cells

Plasmid(s) present	β-Galactosidase in strain[a]:		
	GC4597 (*recA441*)	GY7221 (*recA730*)	GC4415 (*rec*$^+$) + UV
None	1,630	2,429	553
pLG273	1,800	2,565	344
pLG288	313	148	328
pLG284	1,789	2,361	
pLG286	1,713	2,106	
pLG288, pLG284	433	156	

[a] Values are units mg of protein^{-1} measured in GC4597 after 1 h at 42°C in the presence of 0.5 mM adenine, in GY7221 after overnight growth at 30°C, and in GC4415 1 h after irradiation with 10 J m^{-2}. Values for GC4597 and GY7221 are the means of at least three independent experiments. Those for GC4415 are derived from two experiments.

Tn*5*-containing derivative of ColIb*drd-1*) or pLG288, and the medium was supplemented with 0.01% SDS, which inhibits conjugation by the depolymerization of pilin subunits (4). After growth for 13 generations at densities lower than 2×10^8 cells per ml, bacteria were plated on nutrient agar, and single colonies were tested for the Km^r phenotype determined by the ColIb plasmids. Irrespective of whether the strain carried pLG221 or pLG288, all 200 colonies tested retained the Km^r marker.

pLG288 confers a Psi$^+$ phenotype. Plasmids having sequence homology with F *ssb* also carry homologous *psi* determinants. Expression of *psi* interferes with induction of genes constituting the SOS regulon, which include *sfiA*, and the Psi function is monitored conveniently by measuring the β-galactosidase activity specified by *sfiA::lacZ*$^+$ fusions in a *recA441* or *recA730* background (2). Induction of the SOS response occurs in the former strain at high temperature, but it is constitutive in *recA730* strains. When tested in these strains (Table 2), pLG288 conferred a marked Psi$^+$ phenotype in contrast to the parental *ssb*$^+$ plasmid pLG273. A second independently isolated *ssb* mutant of pLG273 was identical to pLG288 in this respect. No Psi activity was detected when the cells carried pLG286, which contains the *ssb* mutation in the cloned ColIb fragment. Conversely, the Psi$^+$ phenotype of cells harboring pLG288 was unaffected by the presence of pLG284, carrying the cloned ColIb *ssb* gene. We have ruled out the trivial interpretation that the parental pLG273 plasmid, carried by the JC7623 strain in which pLG288 was constructed, had previously acquired the Psi$^+$ phenotype.

In contrast to the results obtained with *recA* cells, pLG288 was indistinguishable from pLG273 in its effect on SOS induction in UV-irradiated *rec*$^+$ cells (Table 2). This is consistent with the finding that R100.1 (IncFII) conferred a Psi$^+$ phenotype on *recA441* mutants at 40°C but it did not inhibit induction of SOS functions following UV irradiation (1).

DISCUSSION

ColIb carries an *ssb* gene homologous with the *ssb* determinant of plasmid F. There is 84% sequence homology between the two genes at the nucleotide level and 83% at the predicted amino acid level. The ColIb protein has a calculated molecular weight of 19,110 compared with 19,505 for F SSB (8). ColIb SSB also shows an overall 60% amino acid sequence identity with *E. coli* SSB, but this value masks extensive regional homologies. Hydropathy profiles are virtually identical for the three proteins. The number of residues in ColIb, F, and *E. coli* SSB differ slightly, being 174, 178, and 177, respectively. Both plasmid proteins differ from the bacterial protein by the addition of two common residues (positions 120 and 167 in the alignment shown in Fig. 4) and by the absence of a third (between residues 90 and 91). ColIb SSB also lacks four residues in the region (residues 130 to 140) that is highly variable among all three proteins.

The first two-thirds of *E. coli* SSB (residues 1 to 105) contain the DNA-binding domain and may be important for monomer-monomer interactions (44). All three SSBs have a very similar sequence in this region, which is predicted by Chou and Fasman analysis (11) to be highly ordered, containing α-helix and β-pleated sheet structures. The carboxy-terminal third of *E. coli* SSB may allow interactions with other proteins involved in DNA metabolism and control the ability of the protein to denature DNA (9, 44). The equivalent portions of all three SSBs are predicted to form predominantly random coil structures, but they vary extensively in sequence. The sequences of the last 38 residues of the two plasmid proteins are very similar, and the last five amino acids are identical in all three proteins.

Not only are the *ssb* genes of F and ColIb closely related but they are located at similar positions on their respective plasmids, mapping on the opposite side of *oriT* to the defined *tra* genes (26; Fig. 2). We have recently shown that relative to the *tra* genes, the direction of transfer of ColIb from the *oriT* site is the same as that established for F (22). Thus, both genes are located in the region that is transferred early during bacterial conjugation.

Golub and Low (19) reported that plasmid *ssb* genes are expressed coordinately with conjugation genes. The same applies to ColIb *ssb*, since efficient expression requires derepression of the *tra* system and active positive regulators controlling the *tra* genes. Thus, plasmid *ssb* genes appear to have been acquired as components of conjugation systems. The similarities between the sequences and locations of the ColIb and F *ssb* genes are remarkable considering that the plasmids belong to incompatibility groups having little DNA homology (13, 20). Moreover, the ColIb conjugation system is quite distinct from that of F, as judged by the organization of the *tra* genes, their specificities, and conferred phenotypes (34). We have speculated that the ColIb conjugation system, which specifies two types of conjugative pilus and two separate positive regulators, is hybrid and contains components of two different ancestral conjugation systems. Possibly the ColIb *ssb* gene originated from a system related to F-like plasmids.

The hypothesis that ColIb *ssb* functions in conjugation was examined using a mutant plasmid carrying a 1.2-kb insertion close to the 5′ end of the gene. The mutant showed no obvious defect in genetic tests of conjugative efficiency or of maintenance stability in growing bacteria. Golub and Low (19) likewise found no change of phenotype associated with *ssb* mutants of some other conjugative plasmids. It seems unlikely that ColIb SSB synthesized de novo in the recipient cell has a role in the processing of the transferred DNA strand, since any requirement would presumably be immediate and before the genes for high-level expression of the *ssb* locus are transferred. We have examined the possibility that plasmid SSB synthesized in the donor cell is transported to the recipient during the transfer process. Using a method allowing detection of transmitted polypeptides (29), no transfer of a protein of the size predicted for ColIb SSB was observed (manuscript in preparation). These considerations favor a role in the donor cell, possibly to promote synthesis

of DNA to replace the transferred strand. While this process was sufficiently normal to allow continued inheritance of the plasmid by donor cells following conjugation, plasmid loss would be masked if ColIb encodes a system analogous to the *ccd* or *hok/sok* mechanisms described for F-like plasmids (15, 31). These systems limit the appearance of plasmid-free segregants. However, no loss of donor cell viability was detected after pLG288-directed conjugations.

Clearly, there is no absolute requirement for ColIb SSB, and the gene may be present to augment the concentration of cellular SSB. Another possibility is that its function can be substituted by some other plasmid product. IncM, N, P, U, and W plasmids have phenotypically similar conjugation systems (5), and all lack F *ssb* homology (18; this paper). These plasmids may carry an *ssb* analog, as might ColIb, since the latter carries a hybrid conjugation system showing some resemblances to the systems specified by IncM and IncP plasmids (34).

We have sequence data (not shown) extending to about 150 nucleotides upstream and 130 nucleotides downstream of the ColIb *ssb* gene shown in Fig. 4. These flanking regions do not appear to contain a recognizable promoter or transcription terminator. On the contrary, there are open reading frames on either side of ColIb *ssb*, which read in the same direction as it does. The downstream sequence matches very closely that reported for the region downstream of F *ssb* (8), suggesting that this may be part of a conserved cistron. The *ssb* gene on ColIb may therefore be part of an operon with other unknown functions, and the Tn*903*-based cassette, used to produce the *ssb* insertion mutation described in this paper, may be giving polar effects on this operon.

It is significant that two independently isolated ColIb *ssb* mutants acquired the ability to confer a marked Psi^+ phenotype on the host cell. This result is probably not due to the mutant ColIb SSB protein per se but is likely the consequence of altered regulation of a downstream *psi* gene. The Psi function is thought to have evolved to permit transfer of single-stranded DNA without generating an SOS signal (2). There may therefore be a functional relationship between SSB and Psi proteins.

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