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The Single-Stranded-DNA-Binding Protein Encoded by the *Escherichia coli* F Factor Can Complement a Deletion of the Chromosomal *ssb* Gene

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Genes encoding single-stranded-DNA-binding proteins (SSBs) are carried by a variety of large self-transmissible plasmids, and it previously has been shown that these plasmid-borne genes can complement conditional lethal alleles of the *ssb* gene on the *Escherichia coli* chromosome for cellular viability. We have tested one of the plasmid-borne *ssb* genes, the *ssf* gene from the *E. coli* F factor, for its ability to complement total deletion of the chromosomal *ssb* gene for viability. We have found that *ssf* can complement the *ssb* deletion, but only when it is present on a high-copy-number plasmid. Cells that are totally dependent on the F-factor-encoded SSB for viability manifest growth properties indicative of problems in DNA replication.

The *Escherichia coli ssb* gene encodes a single-stranded-DNA-binding protein (SSB) that plays a crucial role in cellular DNA metabolism. Studies with temperature-sensitive *ssb* mutations have shown that *ssb* is an essential gene whose product is required for DNA replication (11, 23). *E. coli* SSB also plays an important role in genetic recombination (11, 12) and in the SOS response to DNA damage (17, 19, 33). Although only a handful of SSBs have been studied in detail, it is generally assumed that proteins of similar function, and possibly structure, are present in all prokaryotic and eukaryotic cells (8).

The existence of plasmid-encoded SSBs was initially discovered when it was observed that the presence of an *E. coli* F factor in an *ssb-1* strain partially reverses the temperature-sensitive growth phenotype (18). Further analysis demonstrated that the F factor carries its own *ssb* gene; this gene is called *ssf* and encodes a protein which is referred to as F-SSB (18). It has since been shown that many, but not all, conjugative plasmids from a number of different incompatibity groups carry their own *ssb* gene (13). These plasmid *ssb* genes appear to be coordinately regulated with the *tra* regulon (conjugal transfer) genes, but their presence does not appear to be necessary for conjugal transfer of the plasmids involved (14, 16). The ability of these plasmid-encoded SSBs to alleviate the temperature sensitivity of *ssb-1* seems to be dose dependent, as plasmids derepressed for fertility are more effective than their fertility-inhibited counterparts (13, 14).

As *ssf* appeared to have comparable effects on replication, recombination, and repair in an *ssb-1* strain at elevated temperatures, it was considered likely that the F-SSB from *ssf* could effectively replace *E. coli* SSB from the chromosomal *ssb* gene for functional purposes (14). However, the possibility that protein-protein interactions between monomers of F-SSB and *E. coli* SSB-1 were required for viability could not be ruled out. In order to eliminate this element of ambiguity, we have examined the ability of *ssf* to support the growth of a Δ*ssb* strain.

E. coli K-12 RDP268 is a derivative of the common laboratory strain AB1157 (F⁻ *thr-1 leuB6 proA2 his-4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL31 tsx-33 supE44* λ⁻) and genetically identical, except that the region of the *E. coli* chromosome containing the *ssb* gene has been replaced by a DNA fragment carrying the *aphA* gene for kanamycin-neomycin resistance (28). In initial studies, this strain showed an absolute dependence on the presence of a helper plasmid carrying a functional copy of *ssb* (28). Starting with a version of RDP268 that contained pRPZ146, a pBR322 derivative that carries the *ssb* gene from the *E. coli* chromosome (Fig. 1), we carried out a plasmid bumping or replacement protocol (28) using an *ssf*-containing pBR322 derivative called pKAC50 (18) (Fig. 1). As pRPZ146 is Tc^r and Ap^s while pKAC50 is Tc^s and Ap^r, Ap^r transformants were screened for Tc^s by replica plating as a means of identifying isolates in which pRPZ146 had been replaced by pKAC50. Restriction enzyme analysis of plasmid DNA from these isolates provided preliminary confirmation that only pKAC50 was present in these RDP268 transformants.

Total DNA was made from *E. coli* RDP268/pKAC50 and a number of control strains so that the absence of the *E. coli ssb* gene in this derivative could be confirmed by DNA hybridization. These total DNA preparations and control plasmid DNA preparations were digested with restriction enzymes *Nsi*I and *Pst*I (New England BioLabs and Bethesda Research Laboratories, respectively), and the nitrocellulose filter prepared from the subsequent 0.6% agarose gel was probed with an oligonucleotide 20-mer complementary to a region of the sequence of *ssb*. The results of this hybridization are shown in Fig. 2. The 2.4-kb *Nsi*I *ssb*-containing fragment from the *E. coli* chromosome of strain AB1157 that can be seen in lanes 3, 5, and 7 is clearly missing from both of the plasmid-containing RDP268 strains that were tested (lanes 4 and 6). pRPZ146 yields a 5.6-kb *Nsi*I-*Pst*I *ssb*-containing fragment (lanes 1, 3, and 4) that is not present in the pKAC50-containing version of RDP268 (lane 6). The *ssf* gene from pKAC50 is contained on a 1.9-kb *Pst*I fragment that shows a weak cross-hybridization signal with the *ssb* oligonucleotide probe (lanes 6, 7, and 9). The only signal seen in the lane containing total DNA from the RDP268/pKAC50 isolate (lane 6) is the *ssf* cross-hybridization signal.

Although it is clear from the data shown in Fig. 2 that *ssf* can support the growth requirement of a Δ*ssb* strain of *E. coli* when it is present on a high-copy-number plasmid, we

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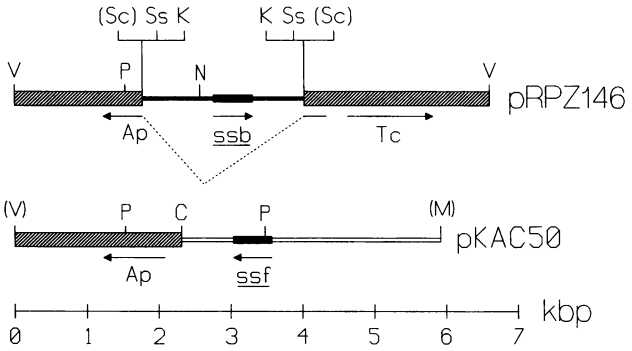

FIG. 1. Plasmid maps for pRPZ146 and pKAC50. pRPZ146 was constructed by inserting the 2.25-kbp *ssb*-containing *Kpn*I fragment from the *E. coli* chromosome into a derivative of pBR322 that contains a polylinker region inserted at the *Sca*I site within the *bla* (Ap) gene. The construction of the pBR322-derived pKAC50 plasmid has been previously described (18). pBR322 DNA (▨), *E. coli* chromosomal DNA (■), and F factor DNA (□) are shown. Gene position and transcriptional orientation are indicated by arrows below the maps. At the bottom is a size scale in kilobase pairs. The restriction enzyme site abbreviations: C, *Cla*I; K, *Kpn*I; M, *Sma*I; N, *Nsi*I; P, *Pst*I; Sc, *Sca*I; Ss, *Sst*I; V, *Pvu*II. Sites that have been inactivated are shown in parentheses.

also wanted to test for complementation with *ssf* present on a low-copy-number plasmid. To do this, F42*lac* was introduced into RDP269, a Δ*ssb* strain of *E. coli* that differs from RDP268 only in that it is Thr^+ and $\Delta(lac\text{-}pro)_{XIII}$. RDP269 that initially contained pACYC*ssb* (28) was mated with RDP186 [F42*lac*/Δ(*lac-pro*) *rpsE*], and Lac^+ transconjugants of RDP269/pACYC*ssb* were selected. These transconjugants were streaked on minimal lactose medium, and the resulting colonies were placed on a grid and tested for the loss of pACYC*ssb* by replica plating on LB plates containing chloramphenicol. Despite extensive screening of colonies derived from these transconjugants, we have thus far been unable to obtain an isolate in which pACYC*ssb* has been lost by random segregation in the presence of F42*lac*. Although Δ*ssb* strains can be supported by an *ssb* gene carried by a low-copy-number mini-F vector (data not shown), it appears that *ssf* is capable of complementing Δ*ssb* for viability only when present on the higher-copy-number pBR322 derivative.

The requirement that *ssf*, but not *ssb*, be present on a high-copy-number plasmid in order to complement Δ*ssb* could be the result of a difference in either expression level or protein function. To compare expression levels for *ssb*

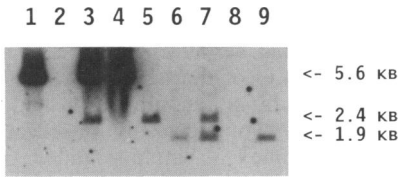

FIG. 2. Hybridization for the presence of *ssf* versus *ssb*. DNA samples (either purified plasmid DNA or total DNA prepared from the indicated strains) were cut with *Nsi*I and *Pst*I, and the resulting blot was probed with a radiolabeled oligonucleotide targeted to nucleotides 382 to 401 of the *ssb* coding sequence. Lanes: 1, pRPZ146; 2, blank; 3, AB1157/pRPZ146; 4, RDP268/pRPZ146; 5, AB1157; 6, RDP268/pKAC50; 7, AB1157/pKAC50; 8, blank; 9, pKAC50.

SSB-LACZ: (M)ASRGVNKVILVGNLGQDPDPVVLQRRDWEN...

SSF-LACZ: (M)AVRGINKVILVGRLGKDPVVLQRRDWEN...

FIG. 3. N-terminal amino acid sequence for the translational fusions. For each of the translational fusions, the N-terminal portion of *E. coli* SSB or F-SSB is shown in larger letters while the amino acids from β-galactosidase are shown in smaller letters. The extra amino acids in the *ssb-lacZ* fusion created by the insertion of the *Ppu*MI linker are underlined.

and *ssf*, we constructed and tested *ssb-lacZ* and *ssf-lacZ* translational fusions. Plasmid pBC26 (9) contains the *lacZ* gene without transcription or translation start signals and is designed to accept *Eco*RI-*Bam*HI fragments in a directional fashion for the purpose of making translational fusions. The naturally occurring *Bam*HI site in *ssf* (18) is in the correct reading frame (6), and a *Bam*HI linker was introduced at the unique *Ppu*MI site in *ssb* to allow the generation of a comparable fusion. The amino acid sequences of the amino-terminal portions of these two translational fusions are shown in Fig. 3.

The two pBC26 derivatives were introduced into a *recA1* derivative of KL791 (27; F^- $\Delta(lac\text{-}pro)_{XIII}$ *met his trp*), and β-galactosidase enzyme unit (EU) assays (27, 29) were run on samples from cultures grown to cell densities between 10^7 and 10^8 CFU/ml. CFU were determined by platings on LB plates containing 50 μg of ampicillin per ml. At least six independent determinations of β-galactosidase EU/CFU were made for each fusion. The *ssb-lacZ* fusion gave $(5.0 \pm 1.1) \times 10^{-5}$ EU/CFU, while the *ssf-lacZ* fusion gave $(1.7 \pm 1.3) \times 10^{-5}$ EU/CFU. In the same host strain, a fully induced *lac* operon from a pUC7 derivative called pUC7L (5) gave a value of 3.8×10^{-4} EU/CFU. One EU for β-galactosidase is the activity required to hydrolyze 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) in 1 min at 28°C (1).

As the β-galactosidase EU/CFU are about threefold lower for the *ssf-lacZ* fusion than for the *ssb-lacZ* fusion, it is possible that the inability of *ssf* to support the viability of a Δ*ssb* strain when present on a low-copy-number plasmid is the result of insufficient F-SSB production. If both *E. coli* SSB and F-SSB are of comparable functionality, this result would imply that *E. coli* SSB concentrations are normally less than threefold more than is absolutely required for cell viability.

As an additional means of comparing the functionality of F-SSB and *E. coli* SSB, we determined the relative exponential phase growth rates for both the RDP268 strain and the ssb^+ AB1157 parental strain containing either *ssf* or *ssb* on a pBR322 derivative. These generation times are shown in Table 1. In the ssb^+ AB1157 strain, the generation times were not substantially different with either the *ssb*-containing pRPZ146 plasmid or the *ssf*-containing pKAC50 plasmid present. With the Δ*ssb* RDP268 strain, however, the generation time is about 28% longer when the cell is dependent on the *ssf*-containing pKAC50 plasmid. It thus seems that F-SSB is not as functionally effective as *E. coli* SSB in supporting the growth of the cell.

While working with these strains, it was repeatedly noted that the CFU per optical density unit (optical density at 650 nm measured with a Bausch & Lomb Spectronic 20 spectrophotometer) were approximately fourfold lower for the Δ*ssb* strain containing pKAC50 than for any of the other strains in this series. Phase-contrast microscopy revealed that some of

TABLE 1. Generation times for various strains[a]

Host strain	Plasmid	Generation time (min)
AB1157	pRPZ146	21.3
	pKAC50	21.9
RDP268	pRPZ146	21.4
	pKAC50	27.5

[a] For each determination, 50 μl of an overnight culture was used to inoculate 20 ml of LB in a 125-ml flask. The cultures were maintained at 37°C and shaken at 300 rpm in a New Brunswick G24 environmental incubator shaker. Sample collection was started 1 h later, and samples were taken every 30 min for 4 h. Platings were done with a model D plating device from Spiral Systems, Inc., on LB plates containing 50 μg of ampicillin per ml for the pKAC50-containing strains and 10 μg of tetracycline per ml for the pRPZ146-containing strains. The data plots used to calculate the generation times all had R values of 0.992 or better.

the cells in the RDP268/pKAC50 culture exhibited filamentation to various degrees (Fig. 4), another indication of the growth problems experienced when the cell must utilize only F-SSB. Although filamentation has previously been observed with cells overexpressing *E. coli* SSB when the *ssb* gene was present on a pACYC184 derivative (24), we saw no evidence for filamentation when pRPZ146 was present in either strain RDP268 or AB1157. As it has also been reported (7) that a strain with pKAC4 (a pACYC184 derivative with *ssb*) produces approximately twice as much *E. coli* SSB as a strain with pDR1996 (a pBR322 derivative with *ssb*), such a twofold difference in actual *E. coli* SSB levels might explain our failure to observe filamentation in the pRPZ146-containing strains. We have not determined whether the filamentation shown by a Δ*ssb* strain containing only *ssf* is *lexA* dependent, but the observation of filamentation and the reduction of growth rate indicate that replication is perturbed when F-SSB must fully substitute for *E. coli* SSB.

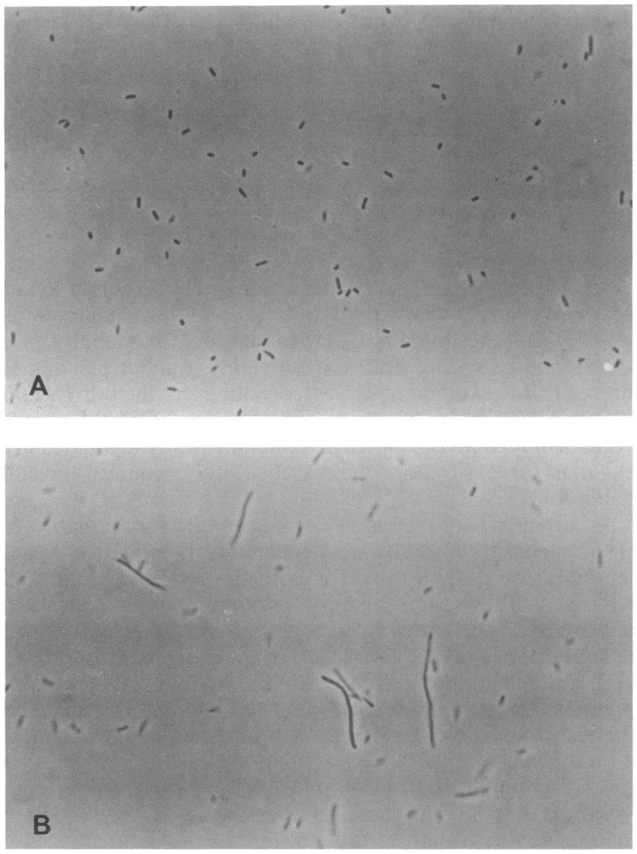

FIG. 4. Phase-contrast photomicrographs of exponentially grown RDP268 cells containing pRPZ146 (A) or pKAC50 (B). The photographs were taken at ×1,000 magnification with oil immersion and a Nikon Labophot microscope fitted with a Microflex AFX II camera attachment; Kodacolor Gold 200 film was used prior to black and white printing.

In addition to the *E. coli* chromosomal *ssb* gene (31) and the *ssf* gene from the F factor (6), the *ssb* genes from ColIb-P9 (16) and from pIP71a, pIP231a, and R64 (30) have been sequenced. The monomeric proteins made from all of these *ssb* genes are very similar in size, ranging from 174 to 178 amino acids. All of these *ssb* genes are also highly homologous for the amino-terminal 112 or 113 amino acids and have the identical Asp-Asp-Ile-Pro-Phe sequence at the carboxy terminus. The plasmid-derived *ssb* genes do diverge, however, considerably in both DNA and predicted amino acid sequence from the *E. coli* chromosomal *ssb* gene for the approximately 65 amino acids in the intervening region. The region of divergence between the plasmid-encoded SSBs is much more limited, however, as all of these proteins are also highly homologous in a region containing about 30 amino acids that is immediately adjacent to the carboxy terminus. A comparison of the remaining portion that differs from *E. coli* SSB indicates that there are at least two distinct families of plasmid-encoded SSB. Although there is yet no data indicating any functional differences for the plasmid-encoded SSBs, the amino acid sequence comparison for all of these SSBs clearly indicates four regions of possible functional significance that may provide a useful framework for dealing with structure and function questions concerning SSBs.

Because of the multiple roles which *E. coli* SSB must play in cellular DNA metabolism, analysis of the mechanistic basis of its in vivo action is necessarily complex. *E. coli* SSB exists in solution as a homotetramer (26, 32, 34), and these tetramers show several distinct binding modes that vary in site size from about 35 to 65 nucleotides per tetramer (2, 4, 21). The larger site size binding mode involves a very limited cooperativity between adjacent tetramers that is limited to octamer formation (3), and a structure with a beads-on-a-string morphology results (10). A greater degree of cooperativity between adjacent tetramers may occur in the smaller site size mode (22), and it is this mode that is generally favored at high protein/DNA ratios and low monovalent cation concentrations in vitro. It has been suggested that the various roles of *E. coli* SSB in vivo might well be affected by the protein's effective binding site mode (20). It is generally assumed that increased cooperativity shown in the small binding site mode would be favorable for replication, while the limited cooperativity of the larger site size and beaded structure mode might favor recombination and SOS induction. These ideas are supported by observations that a beaded structure complex of single-stranded DNA and *E. coli* SSB promotes the binding of RecA protein (15, 25).

The previously available data had not shown whether plasmid-encoded SSBs could completely replace the function of *E. coli* SSB. Herein we have shown that F-SSB can support the growth of an *ssb* deletion strain, but only when the *ssf* gene is present on a high-copy-number plasmid. Cells totally dependent on F-SSB for viability, however, show some differences in their growth properties that suggest

alterations in DNA replication. Further studies involving the plasmid-encoded SSBs may well prove very useful in understanding the assorted roles that *E. coli* SSB normally carries out in the cell.

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