Molecular Dissection of a Protein SopB Essential for Escherichia coli F Plasmid Partition*

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Biochemical and genetic experiments were carried out to deduce the structural and functional domains of SopB protein involved in the equi-partition of F plasmid. The protein is dimeric. Proteolytic and chemical footprinting studies support earlier genetic analyses that the binding of SopB to specific sites within the F plasmid sopC locus involves mainly the C-terminal region. In vivo, the expression of a high level of SopB protein is known to repress sopC-linked genes. This silencing activity is shown to be unaffected by the deletion of 35 N-terminal residues, but abolished when 71 or more were removed from the N terminus. An excess of SopB protein does not extend its in vitro binding outside sopC, implicating participation of a host factor(s) in SopB-mediated gene silencing. A database search identified a number of SopB homologues, including both chromosomally encoded bacterial proteins and plasmid-encoded proteins known to be involved in partition. Sequence homology is limited to the N-terminal half, suggesting that the N-terminal regions of these proteins are conserved to interact with a conserved cellular structure(s), whereas the C-terminal regions have diverged to bind different nucleotide sequences.

Low copy number bacterial episomes are known to encode systems which ensure their equi-partition into host cells undergoing division (reviewed in Hiraga (1992) and Williams and Thomas, 1992)). The partition system of the going division (reviewed in Hiraga (1992) and Williams and Thomas, 1992). It is uncertain what has been termed the IncG phenotype of E. coli cells expressing a high level of SopB protein, the linking numbers of plasmids which include or lack a sopC element are different (Lynch and Wang, 1994; Biek and Shi, 1994; Biek and Strings, 1995). The insertional inactivation of a single 43-bp repeat unit of the sopC element into a plasmid is sufficient in eliciting a substantial increment in the linking number (Lynch and Wang, 1994; Biek and Shi, 1994). Furthermore, a high intracellular concentration of SopB protein was found to repress plasmid-borne or chromosomally located genes if a sopC element is present in cis (Lynch and Wang, 1995). In a region extending by a minimum of 10 kb from a chromosomally located sopC element, accessibility of the DNA to gyrase and DNA adenosine methylase is severely restricted (Lynch and Wang, 1995). These observations suggest that the binding of SopB protein to one or more copies of the 43-bp motifs in sopC may initiate the sequestration of neighboring genes, for example by the formation of a nucleoprotein filament along the sopC-bearing DNA (Lynch and Wang, 1995). The silencing of sopC-linked genes by a high cellular concentration of SopB protein is believed to be the underpinning cause of what has been termed the IncG phenotype of sopB (Kusukawa et al., 1986), in which a sopC-bearing plasmid is incompatible with a multicycopy plasmid bearing sopB (Lynch and Wang, 1995).

To characterize further the nucleoprotein complex at the sopC locus, we have carried out detailed biochemical analysis of...
the SopB protein-sopC complex. Analysis of available nucleotide sequences also suggests a wide distribution of SopB homologues in diverse prokaryotic species. These homologues include episomal proteins that are known to be involved in partition, as well as putative products of open reading frames found in genomes of several distantly related bacteria. These results and their implications are presented below.

**EXPERIMENTAL PROCEDURES**

Cloning and Overexpression of sopB and Its Deletion Derivatives—Two plasmids were constructed to overexpress full-length SopB protein. In ptaconB (Lynch and Wang, 1995) and pET11b-sopB, the 3N-terminal coding sequence of sopB was copied from a miniprep of F-plasmid and placed between an NdeI and a BamHI site of the parent vectors by the polymerase chain reaction. Overexpression of sopB was from an inducible trp-lac hybrid or tac promoter in ptaconB, and from a phase T7 promoter in pET11b (Studier et al., 1990). Nucleotide sequencing of the sopB regions of the plasmids revealed a silent mutation at the 42nd codon (GCC → CGA) in pET11b-sopB, which was presumably introduced during the polymerase chain reaction. No difference was found between the sopB sequence in ptaconB and the published sopB sequence (Mori et al., 1986). Plasmids were also constructed for the overexpression of full-length SopB with either an N-terminal immunotag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either an N-terminal immuno- tag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either a N-terminal immunotag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either an N-terminal immuno- tag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either an N-terminal immuno- tag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either an N-terminal immuno- tag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either an N-terminal immuno- tag, a decapeptide Met-Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-De- overexpression of full-length SopB with either an N-terminal immuno-

**Protein Footprinting—Fifty μg of purified protein with or without the extraneous protein kinase site were phosphorylated with [γ-32P]ATP and the catalytic subunit of CAMP-dependent protein kinase (New England Biolabs). Phosphorylation was carried out in a buffer containing 10 mM TrisHCl, pH 7.5, 50 mM NaCl, 5 mM 2-mercaptoethanol, and 10 mM MgCl₂, for 10 min at room temperature. The reaction was terminated by addition of EDTA to a final concentration of 10 mM. The protein solution (160 μl) was diluted with 290 μl of the same buffer without MgCl₂, and divided into aliquots of 45 μl each. Where DNA was present, 1 nmol of a duplex oligonucleic DNA

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5' −TCCGAGCACACCCTGTCACCTC 3'
3' −GAGACCTTGGTGTCACGGTTGA 5'
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was used as a standard, and the purities of the partially purified proteins were estimated by densitometric tracing of electrophoretically resolved bands in a Coomassie-stained SDS-polyacrylamide gel.

The duplex 21-bp DNA oligomer as described in Hanai and Wang (1994), with minor modifications. Immunotagged g10sopB protein was dialyzed against 50 mM sodium phosphate, pH 8.3. Each reaction mixture (24 μl) contained 10 μg of the protein and 0.8 nmol of the duplex 21-bp DNA. To each mixture, 5 μl of 0.1 M citraconic anhydride, prepared by diluting immediately before use the anhydride (Aldrich) into 50 mM sodium phosphate (pH 8.6), were added. After 30 min at room temperature, 60 μl of 8.4 mM guanidine chloride in 50 mM sodium phosphate, pH 8.5, were added to denature the protein. Ten μl of 3% (w/v) N-hydroxysuccinimide acetate (Sigma) in dioxane were added and the mixture was kept at room temperature for 30 min. Five μl of 1 μM TrisHCl, pH 8.5, were added to stop lysine acetylation, and the mixture was dialyzed successively against 30% acetic acid (20 h), 0.1 M TrisHCl, pH 8.5 and 0.2% SDS (5 h), and 0.1 M TrisHCl, pH 8.5 (5 h). Each of the dialyzed samples, containing approximately 2 μg of protein, was digested with 60 ng of Lys-C protease (Sigma) for 16 h at 37°C. The peptides thus generated were separated on an SDS-15% polyacrylamide gel, electrophoresed on the chromed fragments were enzymatically transferred to a poly(vinylidene fluoride) membrane (Millipore), and visualized by alkaline phosphatase-mediated chemiluminescence. A monoclonal anti-T7 gene 10 peptide antibody conjugated to alkaline phosphatase (Novagen) and the Luminiphos 530 reagent (Boehringer Mannheim) were used in the immunochromatographic detection of the tagged polypeptides.

DNA Binding Assay of SopB Protein and Its Truncation Derivatives—The sopC-containing plasmid pASL54 (Lynch and Wang, 1994) was linearized at a unique BamHI site. DNA (0.2 μg) in 2.5 μl of 10 mM TrisHCl, pH 7.9, 10 mM MgCl₂, 50 mM NaCl, 1 mM dithiothreitol was mixed with different quantities of purified SopB protein or its derivative in 20 μl of 50 mM sodium phosphate, pH 8.0. Nco restriction enzyme (5 units) was then added to each mixture, and the reaction was terminated after 2 min at room temperature by phenol extraction.

**Gel Electrophoretic Mobility-Shift Assay of SopB Protein-DNA Interaction**—The duplex 21-bp DNA containing a SopB recognition sequence was “P-labeled at the 5’ termini. Fifty picomoles of the labeled DNA in 10 mM TrisHCl, 50 mM NaCl, 2-mercaptoethanol, 1 mM EDTA, and 10% glycerol were mixed with 25 pmol of SopB protein, the SopB(139–322) truncation derivative, or mixtures of the proteins in the same buffer. The protein-DNA mixtures were analyzed in a 6% polyacrylamide nondenaturing gel (acrylamide:bis = 30:0.8) which had been run in 50 mM Trisborate, pH 8.3, 1 mM EDTA prior to loading the samples.
The specific binding of purified SopB protein to the 43-bp repeats in sopC is further supported by experiments in which the DNA-protein complex was probed with Ncol restriction endonuclease. The sopC-containing pASLS4 DNA possesses two Ncol sites separated by 0.8 kb, one of which is within a 43-bp sopC motif. At ratios as high as 2,000 SopB monomer equivalents per sopC element, protection against cleavage by Ncol was observed only at the site within the 43-bp motif and not at the other site; cleavage at a HindIII site located 20 bp outside the sopC region was similarly found to be unaffected by the presence of excess SopB protein (data not shown).

Protein Footprinting Indicates That the DNA Binding Domain of SopB Protein Is Located at Its Carboxyl End—Several protein footprinting experiments were carried out to identify the DNA binding regions in SopB. Footprinting of SopB protein by various proteases was carried out using 32P-labeled SopB-hmk, which is SopB with a C-terminal nonapeptide tag containing a heart muscle kinase phosphorylation site. Heart muscle kinase was found to phosphorylate untagged SopB protein with an efficiency of approximately 15% of that of the C-terminally tagged derivative (data not shown). The N-terminal truncation derivative SopB(139–323) was not radiolabeled by the kinase (data not shown), indicating that SopB protein possesses at least one heart muscle kinase phosphorylation site in the N-terminal region before residue 139, and none after.

The patterns of proteolytic cleavages of the tagged SopB-hmk and untagged SopB protein by various proteases, in the absence and presence of a 21-bp DNA containing the sopC recognition site, are shown in Fig. 2, a and b, respectively. In Fig. 2a, unproteolysed SopB-hmk protein was run in lane 1, and size calibration markers generated by chemical cleavage of the radiolabeled protein at a cysteine or methionine were run in lanes 2 and 3, respectively. The next five pairs of lanes contained samples digested with chymotrypsin (lanes 4 and 5), Lys-C endoprotease (lanes 6 and 7), protease SV8 (lanes 8 and 9), subtilisin (lanes 10 and 11), and trypsin (lanes 12 and 13); in each pair, no DNA was present in the left lane sample, and DNA in molar excess over the protein was present in the right lane sample. In Fig. 2b, lane 1 contained unproteolysed SopB protein without the C-terminal tag, and lanes 2–9 contained samples corresponding to those run in lanes 4–11 of the Fig. 2a gel, the difference being that untagged SopB radiolabeled at its intrinsic site(s) rather than radiolabeled SopB-hmk protein was used.

The proteolytic sites can be deduced from a comparison of the corresponding samples in Fig. 2, a and b. Cleavage by chymotrypsin in the absence of DNA, for example, is most prominent around residue 275, yielding band 1 in the sample analyzed in lane 4 of Fig. 2a. Cleavage at this site is strongly protected by sopC binding, as band 1 is barely visible in lane 5 of Fig. 2a. Cleavage also occurs around residue 60, yielding band 2 in the sample analyzed in lane 4 of Fig. 2a. The assignments of these cleavage sites are confirmed by the patterns shown in lanes 2 and 3 of Fig. 2b. A single cleavage around residues 275 or 60 in untagged SopB protein radiolabeled within its N-terminal region would be expected to yield N-terminal fragments of these lengths and their C-terminal complements of around 49 and 264 residues in lengths, as the full-length untagged SopB protein is 323 residues long. Among the four expected cleavage products, the 60-residue N-terminal fragment and the 49-residue C-terminal fragment were not seen, presumably because they contained no site for radiolabeling by the kinase. Based on
the mobilities of the radiolabeled bands 1' and 2' in lane 2 of Fig. 2b, they were assigned as the 275-residue N-terminal fragment and the 264-residue C-terminal fragment, respectively. In support of these assignments, cleavage at residue at 275 to form band 1' was again found to be strongly dependent on the presence of sopC DNA (compare the intensity of band 1' in lanes 2 and 3 of Fig. 2b). Similar assignments were made for the strong cleavage sites by other proteases, and these results are summarized in Fig. 2c.

In the experiment shown in Fig. 3, the two-step lysine modification method of protein footprinting (Hanai and Wang, 1994) was applied. A derivative of SopB protein g10SopB, which has a phage T7 gene 10 decapeptide fused to the N terminus of SopB to serve as an immunotag, was first treated lightly with citraconic anhydride in the absence or presence of excess of a 21-bp DNA containing the SopB recognition sequence. The protein was then unfolded and treated with N-hydroxysuccinimide acetate to acetylate all reactive lysines that had not been citraconylated. The modified protein was exposed to dilute acetic acid to reverse citraconylation, and lysine-specific endoproteinase Lys-C was then used to cleave at the positions of the deacetylated lysines. In this procedure, lysines that are protected from citraconylation by DNA binding become irreversibly acetylated and are therefore resistant to cleavage by the Lys-C endoproteinase after deacetylation. The patterns of immunotagged Lys-C cleavage products resolved by SDS-polyacrylamide gel electrophoresis and detected by immunoblotting are shown in lanes 6 and 7 of Fig. 3, and various control samples and size calibration markers derived from chemical fragmentation of the immunotagged SopB were analyzed in the other lanes. From a comparison of these patterns, citraconylation at Lys-124, -246, -266, and -290, and one or both lysines in each of the closely spaced pairs Lys-201/204 and Lys-226/231, was clearly reduced by the presence of DNA (numbers of all amino acid residues refer to their positions in untaged SopB protein).

Studies of N- and C-terminal Truncations of SopB Protein for Binding to sopC and Repression of a sopC-linked Gene—Several sopB deletions were constructed and overexpressed by a phage T7 expression system to give polypeptides with a g10 decapeptide tag at their N termini (Studier et al., 1990). Binding of these tagged SopB truncations to sopC was monitored by the NcoI protection assay described earlier. Similar to the

Fig. 2. Proteolytic footprinting of SopB protein in the absence and presence of excess cognizant DNA. a, SopB protein with a C-terminal nonapeptide tag containing a site of phosphorylation by cAMP-dependent kinase was 32P-labeled at the site and subjected to partial proteolysis in the absence (lanes 4, 6, 8, 10, and 12) or presence (lanes 5, 7, 9, 11, and 13) of a 21-mer duplex DNA containing a SopB protein binding site. Lane 1 contained the protein not subjected to proteolysis, and lanes 2 and 3 markers generated by partial proteolysis of the labeled SopB protein at a cysteine (lane 2) or methionine (lane 3). The proteases used in the other samples were chymotrypsin (lanes 4 and 5), Lys-C endoproteinase (lanes 6 and 7), endoproteinase SV8 (lanes 8 and 9), subtilisin (lanes 10 and 11), and trypsin (lanes 12 and 13). No DNA was present during proteolysis of samples analyzed in the even-numbered lanes and excess DNA was present during proteolysis of samples analyzed in the odd-numbered lanes, b, SopB protein radiolabeled at its intrinsic phosphorylation sites by heart muscle kinase was subjected to proteolysis as for the C-terminally tagged SopB protein. Samples analyzed in lanes 2-11 corresponded to those in lanes 4-13 of a. Lane 1 contained unproteolyzed material. c, a summary of major proteolytic cleavage sites. C, cymotrypsin; L, Lys-C endoproteinase; V, endoproteinase SV; S, subtilisin; T, trypsin. Arrows with asterisks indicate sites that are protected by DNA binding.
SopB abolishes binding to hand, the deletion of 49 amino acids from the C terminus of duplex oligonucleotide and shift its electrophoretic mobility in consensus SopB-binding site. Either protein can bind to the ty-shift assays with a radiolabeled 21-bp DNA containing a truncation derivative SopB(139–323) were used in gel mobility assays, shown in Fig. 5. Full-length SopB protein and an N-terminal kinase tag in cells bearing pASLS4, which contains a tetracycline resistance marker, led to the repression of the sopC locus as well as a tetracycline-resistance marker, and a second plasmid carrying an ampicillin-resistance marker. In control experiments, expression of intact SopB, SopB with a C-terminal nonapeptide kinase site, or SopB with an N-terminal decapeptide immunotag in cells bearing pASLS4, which contains a tetracycline resistance marker and a tac promoter linked wild-type or mutant sopB gene, was assayed by colony formation on Luria broth agar supplemented with the appropriate combinations of ampicillin, tetracycline, and IPTG.

The results described for the full-length SopB protein, N-terminal truncation mutants lacking as many as the first 138 residues can block Ncol cleavage within the sopC locus. On the other hand, the deletion of 49 amino acids from the C terminus of SopB abolishes binding to sopC (data not shown).

The same set of sopB truncations were used to test the ability of the proteins to repress sopC-linked genes in vivo (Lynch and Wang, 1995). The SopB protein or its truncation derivative was expressed from a trp-lac (tac) hybrid promoter on a plasmid, which also carries an ampicillin-resistance marker. In control experiments, expression of intact SopB, SopB with a C-terminal nonapeptide kinase site, or SopB with an N-terminal decapeptide immunotag in cells bearing pASLS4, which contains the sopC locus as well as a tetracycline-resistance marker, led to the repression of the sopC-linked tetracycline-resistance marker. Among the SopB truncation proteins, only the mutant lacking the N-terminal 35 residues of SopB protein represses sopC-linked drug-resistance markers when expressed in trans. These results and the in vitro DNA binding data are summarized in Fig. 4.

The repression of sopC-linked genes by the mutant lacking the N-terminal 35 residues of SopB protein was unexpected in view of the IncG phenotype of the sopB P28L mutant (Kusukawa et al., 1987). A difference between wild-type and the P28L mutant sopB was observed, however, when pASLS4 was replaced by pASLS6, which contains a single 43-bp SopB protein binding site instead of the full sopC element in pASLS4 (Lynch and Wang, 1994). On tetracycline plates containing 1 mM IPTG, cellsdoublytransformedwithptacsopBandpASLS6 formed small colonies (results not shown). SopB Protein Is Dimeric—The specific binding of SopB protein to the 16-bp palindromic within each direct repeat of the sopC locus suggests a dimeric structure of the protein (Mori et al., 1989). This conjecture was confirmed by the experiment shown in Fig. 5. Full-length SopB protein and an N-terminal truncation derivative SopB(139–323) were used in gel mobility-shift assays with a radiolabeled 21-bp DNA containing a consensus SopB-binding site. Either protein can bind to the duplex oligonucleotide and shift its electrophoretic mobility in a polyacrylamide gel, as shown in lanes 1 and 2 of Fig. 5. The difference in the magnitudes of the shifts is in accordance with the expected size difference between the proteins. When the two proteins were first mixed and then stored at −20 °C for 60 h before incubation with the 21-bp DNA, a new band with a mobility intermediate of those of the bands in lanes 1 and 2 was observed (lane 3). This new band was not formed when the two proteins were mixed and immediately added to the DNA (lane 4), or when the two proteins were mixed after DNA binding (lane 5). The observation of a single extra band upon mixing and storage of the two proteins before DNA binding strongly suggests that SopB protein binds to the 21-bp DNA as a dimer. If it binds as a trimer or tetramer, protomer exchange would result in the generation of multiple bands with intermediate mobilities. In solution, the full-length SopB and the SopB(139–323) mutant protein eluted in gel filtration columns with apparent molecular masses of 95 and 50 kDa, respectively, relative to marker proteins of known sizes (data not shown; see also Fig. 3 in Watanabe et al. (1989)). The values calculated for dimeric SopB protein and SopB(139–323) are 71 and 41 kDa, respectively.

Both Chromosomal and Episomal Genes Encoding SopB Homologues Are Found in Diverse Bacterial Species.—A search of the amino acid sequence data base indicates that in addition to episome-encoded SopB homologues including previously identified ones (Williams and Thomas, 1992), open reading frames which translate to SopB homologues were found in the genomes of Pseudomonas putida, Bacillus subtilis, and Mycobacterium leprae (Ogasawara and Yoshikawa, 1992; De Rossi et al., 1995). Fig. 6 shows an alignment of the deduced amino acid sequences. Homology is found in the N-terminal but not the C-terminal half of the polypeptides.

**DISCUSSION**

The results described above indicate that SopB protein is a dimer when bound to DNA, and is probably a dimer as well in the unbound state. The positions of the proteolytic sites suggest that in each monomer there are at least two and probably more domains. Many of the prominent proteolytic sites were found in two regions in the N-terminal half of the protein, between residues 30 and 70, and around residue 130. Near the C terminus, strong cleavage sites by several proteases were found in the region between residues 265 and 295. Proteolytic cleavage within the N-terminal half of the protein is largely unaffected by DNA binding, with perhaps the exception of a Lys-C endopeptidase cleavage site at Lys-124. Cleavage by chymotrypsin and Lys-C near the C terminus, on the other hand, is strongly protected by DNA binding. These results support a model where the binding of dimeric SopB to the palindromic motifs in sopC involves mainly interactions between the C-terminal domain of the protein and the DNA.
The involvement of the C-terminal region in DNA binding was first hinted by genetic screens which resulted in the identification of incG mutants (Kusukawa et al., 1986). With the exception of a sopB point mutant P28L in which a leucine replaces Pro-28, most of the mutants identified were C-terminal deletions, the shortest deletion being 77 residues. The proteolytic footprinting results, and the observations that SopB(1–274) protein missing the last 49 amino acids does not bind to sopC but N-terminal truncations missing as many as 138 of the N-terminal residues of SopB protein do, provide strong evidence that the C-terminal half of SopB contains the sequence-specific DNA binding domain.

The model that the C-terminal half of SopB constitutes the DNA-binding region is further supported by the lysine-modification results. Other than Lys-124, all DNA-protected lysines were found between Lys-201 and the C terminus. Lys-124 probably also participates in DNA binding, as indicated by its protection from citraconylation and from Lys-C cleavage by DNA binding. Sequence-specific binding of the N-terminal truncation SopB(139–323) shows, however, that Lys-124 is not essential for DNA binding.

The enzymatic and chemical protein footprinting methods complement each other in the dissection of the structural features of a protein. The proteolytic method is advantageous in terms of its simplicity and in defining protease-resistant cores for further structural characterization by methods such as x-ray crystallography and nuclear magnetic resonance. The strong sequence and structural specificities of proteases, however, impose limitations on their usefulness as general protein footprinting reagents. For example, although chymotrypsin, subtilisin, and trypsin all cleave at about the same position in the C-terminal region of SopB protein (Fig. 2), only the chymotrypsin cleavage site showed a strong dependence on DNA binding (compare the proteolysis patterns shown in the pairs of lanes 4 and 10, and 12). Sites that are inaccessible to proteases are often accessible to small chemical reagents. Interaction between DNA and the region between residues 200 and 250 of SopB protein, for example, is not revealed by proteolytic footprinting but is suggested by the DNA-mediated protection of three or more lysines in this region from citraconylation.

Whereas the N-terminal half of SopB appears to be unim-

**Fig. 6. Homology alignment of SopB homologues.** SOPB_ECOLI, E. coli F plasmid SopB protein (Mori et al., 1986); VIRB_SHIFL, VirB of Shigella flexneri virulence plasmid PMYS5H6000 (Adler et al., 1989); S06100, ParB of phage P7 (Ludtke et al., 1989); PP1REP_3, ParB of phage P1 (Abeles et al., 1985); REPB_AGRRA, RepB of Agrobacterium rhizogenes plasmid pRIAB (Nishiguchi et al., 1987); TIPREPABC_2, RepB of Agrobacterium tumefaciens plasmid pTiB53 (Tabata et al., 1989); RLREPABC_2, RepB of Rhizobium leguminosarum cryptic plasmid PRBj (Turner and Young, 1985); QPHG_SOP_2, ParB of phage P1 (Kornacki et al., 1987; Theophilus and Thomas, 1987); CBOPH1_5, QsopB of Coxiella burnetii plasmid QpH1 (Thiele et al., 1993; Lin and Mallavia, 1994); MSGDNAB_17, RepB of M. leprae (De Rossi et al., 1995); YGI2_PSESEPU and OPHG_SOP_2 are open reading frames found in P. putida (Ogasawara and Yoshikawa, 1992) and a C. burnetii plasmid QpH1 (Thiele et al., 1993), respectively; YAA_BACSU and SPOJ_BACSU are open reading frames found in B. subtilis (Ogasawara and Yoshikawa, 1992). Conserved and semiconserved residues are indicated by shaded boxes.
portant for DNA binding, deletions past Arg-72 eliminate in vivo silencing of sopC-linked genes by the protein. Thus the protein appears to be bipartite structurally and functionally: the C-terminal half constitutes the DNA-binding part and the N-terminal half constitutes the part important for the silencing function of the protein, and probably its function in partition as well. As mentioned earlier, the point mutant P28L was found to be IncG− and defective in partition (Kusukawa et al., 1987), implicating the involvement of the N-terminal portion of SopB protein in eliciting the IncG phenotype and in F plasmid partition. The interpretation that the silencing of sopC-linked genes by SopB is responsible for the IncG phenotype (Lynch and Wang, 1994) is not necessarily contradicted by the IncG− phenotype of P28L mutant (Kusukawa et al., 1987) and the present findings that the mutant protein retains at least partial activity in silencing sopC-linked genes, and that deletion of the N-terminal 35 residues does not abolish the silencing capability of SopB protein (see Fig. 4). The apparent difference between the present results and those of Kusukawa et al. (1987) can be attributed to differences in the physiological assays employed, and in the intracellular levels of the SopB proteins.

The essentiality of the N-terminal region of SopB protein in F plasmid partition and in the silencing of sopC-linked genes suggests that this region may be involved in interaction with protein(s) encoded by chromosomal gene(s). Two types of mechanisms have been suggested for the partition systems represented by that of the F plasmid: one invokes specific protein-mediated association between sopC and a chromosomal site, and the other invokes distinct or related systems for episomal and chromosomal partition. The former mechanism is not supported by the finding that F plasmid is stably inherited in E. coli mukB mutants, which have a deficiency in chromosome partition (Ezaki et al., 1991). The latter mechanism would most likely involve chromosomally encoded proteins in F plasmid partition. The presence of open reading frames in a diverse collection of bacteria that code for proteins with their N-terminal regions homologous to that of SopB suggests that these chromosomally encoded proteins may participate in some chromosome partition, and that these proteins and SopB may interact with a common target protein in the chromosomal partition apparatus. Genetic and/or biochemical identification of protein(s) involved in both partition systems may hold the key to the elucidation of partition mechanisms.

As reported previously, SopB protein has the remarkable ability of shielding a long segment of sopC-containing intracellular DNA from cellular proteins such as gyrase and DNA adenine methylase (Lynch and Wang, 1995). Several plausible mechanisms were raised for this phenomenon, including SopB protein-mediated sequestration of DNA to a cellular compartment inaccessible to many cellular proteins, and nucleoprotein filament formation or microcondensation initiated by sopC-bound SopB protein (Lynch and Wang, 1995). The present study provides no new information to distinguish between these possibilities. The electron microscopy and NcoI restriction endonuclease protection results indicate, however, that SopB protein by itself is incapable of nucleoprotein filament formation or microcondensation of sopC-containing DNA. Even in the presence of a vast molar excess of purified SopB protein, binding of the protein outside the sopC element appears insignificant.

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REFERENCES

Abeles, A. L., Friedman, S. A., and Austin, S. J. (1985) Mol. Biol. 185, 261–272
Adler, B., Sasakawa, C., Tobe, T., Makino, S., Komatsu, K., and Yoshikawa, M. (1989) Mol. Microbiol. 3, 627–635
Biek, D. P., Shi, J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8027–8031
Biek, D. P., Strings, J. (1995) J. Mol. Biol. 246, 388–400
De Rossi, E., Cantoni, R., Labo’, M., and Riccardi, G. (1995) EMBO/GenBank™/DDBJ accession no. L39923
Ezaki, B., Ogura, T., Niki, H., and Hiraga, S. (1991) J. Bacteriol. 173, 6643–6646
Gross, E. (1997) Methods Enzymol. 11, 238–255
Hanai, R., and Wang, J. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11904–11908
Hiraga, S. (1992) Annu. Rev. Biochem. 61, 283–306
Jackson, G. R., Schaffer, M. H., Stark, G. R., and Vanaman, T. C. (1973) J. Biol. Chem. 248, 6583–6591
Kendrilli, P. J., and Krebs, E. G. (1991) J. Biol. Chem. 266, 15555–15558
Kornacki, J. A., Balderes, P. J., and Figurski, D. H. (1987) J. Mol. Biol. 198, 211–222
Kusukawa, N., Mori, H., Kondo, A., and Hiraga, S. (1987) Mol. & Gen. Genet. 208, 365–372
Lin, Z., and Mallavia, L. P. (1994) Mol. Microbiol. 13, 513–523
Looman, A. C., Bodlaender, J., Comstock, L. J., Eaton, D., Hurani, P., de Boer, H. A., and van Knippenberg, P. H. (1987) EMBO J. 6, 2489–2492
Ludtke, D. N., Eichorn, B. G., and Austin, S. J. (1989) J. Mol. Biol. 209, 393–406
Lynch, A. S., and Wang, J. C. (1994) J. Mol. Biol. 236, 679–684
Lynch, A. S., and Wang, J. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1896–1900
Mori, H., Kondo, A., Ohshima, A., Ogura, T., and Hiraga, S. (1986) J. Mol. Biol. 192, 1–15
Mori, H., Mori, Y., Ichinose, C., Niki, H., Ogura, T., Kato, A., and Hiraga, S. (1989) J. Biol. Chem. 264, 15335–15341
Nishiguchi, R., Takanami, M., and Oka, A. (1987) Mol. & Gen. Genet. 206, 1–8
Ogasawara, N., and Yoshikawa, H. (1992) Mol. Microbiol. 6, 629–634
Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
Tabata, S., Hoorykaa, P. J., and Oka, A. (1989) J. Bacteriol. 171, 1665–1672
Theophilius, B. D. M., and Thomas, C. M. (1987) Nucleic Acids Res. 15, 7443–7450
Thielen, S., Willems, H., Haas, M., and Krauss, H. (1993) EMBO/GenBank™/DDBJ accession no. X75356
Turner, S. L., and Young, J. P. W. (1995) EMBO/GenBank™/DDBJ accession no. X89447
Watanabe, E., Inamato, S., Lee, M.-H., Kim, S. U., Ogura, T., Mori, H., Hiraga, S., Yamashita, M., and Nagai, K. (1989) Mol. & Gen. Genet. 218, 431–436
Williams, D. R., and Thomas, C. M. (1992) J. Gen. Microbiol. 138, 1–16