Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1896–1900, March 1995
Biochemistry

SopB protein-mediated silencing of genes linked to the *sopC* locus of *Escherichia coli* F plasmid

(position-dependent gene repression/plasmid incompatibility/nucleoprotein structure)

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ABSTRACT **Expression of a high level of F-plasmid-encoded SopB protein in *Escherichia coli* is found to repress genes linked to *sopC*, a sequence element of F consisting of 12 tandemly joined imperfect repeats of a 43-bp motif. Repression of a gene can occur over a distance of at least 10 kb from the *sopC* element and is not affected by the relative orientation of *sopC*. In the repressed state, accessibility of intracellular DNA to cellular proteins is greatly reduced in the region containing *sopC*, as monitored by the trapping of the covalent intermediate between DNA and DNA gyrase and by Dam methylase-catalyzed DNA methylation. These results signify the formation of a nucleoprotein structure emanating from *sopC* and are discussed in terms of position-dependent silencing of genes in general and the IncG type of plasmid incompatibility in particular.**

Stably maintained low-copy-number episomes usually encode systems for equipartition of their replication products during cell division. The partition system of the single-copy F plasmid of *Escherichia coli* has been extensively studied by Hiraga and coworkers (1, 2). This system consists of two proteins, SopA and SopB, and a DNA sequence element, *sopC*. The *sopC* locus contains 12 tandemly joined imperfect repeats of a 43-bp motif. *In vitro*, purified SopB protein protects a pair of 7-bp inverted repeats, centrally located within each of the 43-bp motifs, from digestion by DNase I; thus *sopC* is thought to serve a centromere-like function by the binding of SopB protein and perhaps chromosomally encoded proteins yet to be identified. Binding of purified SopB protein to *sopC* is unaffected by SopA protein. SopA protein appears to bind by itself, or more likely as a complex with SopB protein, to the promoter–operator region of the *sopA* gene. This result supports genetic evidence that the SopA protein has a major role in the regulation of the *sopAB* operon. It is less clear, however, whether the SopA protein is directly involved in the partition process.

Stable maintenance of F plasmid or its shortened mini-F derivatives appears to be strongly dependent on the stoichiometry of the SopB protein and its binding sites in *sopC*. In *E. coli* cells harboring both a multicopy plasmid bearing the *sopC* region and a low-copy mini-F or *oriC*-based plasmid carrying the *sopABC* DNA segment, the low-copy plasmid is destabilized by an apparent failure in equipartition. This phenomenon is known as the IncD type of incompatibility. Presumably, the binding of SopB protein to multiple copies of the *sopC* elements reduces the amount of available SopB protein to a level insufficient for the formation of the centromere-like structure at the *sopC* locus of the low-copy-number plasmid.

Inheritance of mini-F plasmids or *oriC*-based replicons carrying the *sopABC* region is also known to be destabilized in *E. coli* cells harboring plasmids expressing a high level of SopB protein, a phenomenon termed the IncG type of incompatibility (1, 2). A molecular interpretation of the IncG-type incompatibility has been lacking. It is known, however, that the rate of loss of a mini-F plasmid under IncG incompatibility conditions is faster than that of partition defective mini-F plasmids, suggesting that the IncG phenotype is associated with an inhibitory principle (3).

Recent work in our laboratory utilized the induced expression of a tightly regulated site-specific recombinase to efficiently form intracellular DNA rings of well-defined nucleotide sequences in yeast or *E. coli* (4, 5). It was observed that in *E. coli* cells expressing a high level of the SopB protein, the linking numbers of plasmids bearing either the complete *sopC* element or a single 43-bp *sopC* motif were much higher than those of control plasmids containing no *sopC* motif (5). A similar conclusion has been reported by Biek and Shi (6). Because the observed linking-number change is too large to be attributable to structural changes within the *sopC* element, it was proposed that the *sopC* element or even a single 43-bp *sopC* motif may serve as a nucleation site, from which the SopB protein can initiate the formation of a nucleoprotein structure that can extend into adjacent DNA sequences (5). In agreement with this interpretation, we have observed that in cells overexpressing a high level of SopB protein, a region of plasmid or chromosomal DNA containing the *sopC* element becomes genetically silent. The silenced region can be at least 10 kb in size, and within this silenced region general accessibility of the DNA to cellular proteins appears to be greatly reduced. These findings are reported here and are discussed in terms of IncG-type plasmid incompatibility and the general mechanisms of position-dependent silencing of genes.

MATERIALS AND METHODS

***E. coli* Strains and Plasmids.** *E. coli* K-12 strains HB101 (F−, λ−, *hsdR*, *hsdM*, *supE44*, *ara-14*, *galK2*, *lacY1*, *proA2*, *rpsL20*, *xyl-5*, *recA13*, *mcrB*) and DH5α [F−, λ−, φ80d*lacZ*ΔM15, Δ(*lacZYA–argF*)*U169*, *deoR*, *recA1*, *endA1*, *hsdR17*, *supE44*, *thi-1*, *gyrA96*, *relA1*] were obtained from commercial sources. Strain AS19 (*argH*, *leu*, *actD*^{S}), an *E. coli* B derivative permeable to actinomycin D and a variety of other antibiotic agents (7), was obtained from David Morris (University of Washington, Seattle). *E. coli* DH5α *pir*::amp^R, which carries an integrated copy of the R6K plasmid *pir* gene encoding the π function required for replication of plasmids containing the R6K γ origin of replication, was kindly provided by M. Koob and W. Szybalski (University of Wisconsin, Madison). The construction of strains ASL1270, -1276, -1358, and -1356 from strain HB101 is described in *Results*.

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Abbreviations: CAT, chloramphenicol acetyltransferase; IPTG, isopropyl β-D-thiogalactopyranoside.

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In the construction of ptacsopB, a 1016-bp fragment containing the *sopB* gene was amplified from the mini-F plasmid DF41 (8) by DNA polymerase chain reaction (PCR) using the oligonucleotides 5′-GGGGATCCTAGGAATTC*catatg*AAG-CGTGCGCCTGTTATTC-3′ and 5′-GGGGATCCGCTA-G*ctgcag*GTCGCATCAGGGTGCTGGC-3′ as primers. The underlined sequences in the pair of primers correspond to nt 1887–1908 and 2865–2846, respectively, in the *sopABC* sequence (9), and the locations of the *Nde* I and *Pst* I sites in the oligonucleotide primers are shown in lowercase italics. After digestion with *Nde* I and *Pst* I restriction enzymes, the 979-bp *Nde* I–*Pst* I segment from the PCR was cloned in between the corresponding sites in pASLR2 (5), placing the *sopB* open reading frame under transcriptional regulation of a modified *E. coli tac* promoter that is tightly controlled by the *lac* repressor.

A derivative ptacsopBX was constructed from ptacsopB by cutting the plasmid at a unique *Age* I site in the beginning part of the *sopB* coding region; the 3′ recessed ends were then repaired and the resulting DNA was religated to introduce a termination codon near the site of ligation. The construction of pASLS3, pASLS4, and pASLS5 has been described (5). The latter two plasmids contain the *sopC* element of the F plasmid and a fragment of pACYC184 containing a chloramphenicol-resistance determinant and the p15A origin of replication; pASLS3 is identical to pASLS4 except for the absence of the *sopC* element. Integration of DNA sequences into the λ*attB* site of the *E. coli* chromosome was achieved with a plasmid-based system kindly provided by M. Koob and W. Szybalski.

Methods. The relative amounts of mRNAs in *E. coli* ASL1270 and ASL1276 cells harboring ptacsopB were analyzed as follows. Cultures were grown at 37°C in Luria broth containing ampicillin at 100 μg/ml. At a cell density of about 10^8/ml, 1 ml of each culture was removed and immediately combined with an equal volume of 75% (vol/vol) ethanol/and 2% (vol/vol) phenol/20 mM sodium acetate, pH 5.3/2 mM Na_2EDTA) to quench metabolic processes (10). The remaining culture was split into three equal portions: isopropyl β-D-thiogalactopyranoside (IPTG, 1 mM) was added to one, rifampicin (250 μg/ml) was added to the second, and no addition was made to the third. Aliquots were removed at various times from each culture and quenched as described. The quenched cultures were briefly centrifuged at 4°C, and cell pellets were kept frozen on dry ice. RNA was prepared from the frozen cell paste (11). The resulting RNA samples were treated exhaustively with RNase-free DNase (Promega) to remove any contaminating DNA; the integrity of the RNA in the resulting preparations was confirmed by visualization of ethidium-stained rRNA after agarose gel electrophoresis; the concentration of RNA was estimated from absorbance at 260 nm.

Semiquantitative analysis of the RNA samples by reverse transcription and PCR was done by the procedure of Aatsinki *et al.* (12), with the following four oligonucleotide primers in each sample: CAT1, 5′-GCCCGCCTGATGAATGCTC-3′; CAT2, 5′-CGCCCCGCCCTGCCACTC-3′; PYRG1, 5′-TGCCGCAGCCTCCCTCGC-3′; and PYRG2, 5′-CGT-TCGCCGGAACAGCGCGATC-3′. The pair of CAT primers were designed for amplification of a 467-bp region of the Tn*9*-derived gene encoding chloramphenicol acetyltransferase (CAT). The PYRG primers were designed for amplification of a 598-bp region of the *pyrG* gene of *E. coli* K-12, encoding CTP synthetase (13).

Oxolinic acid-mediated gyrase cleavage sites were mapped essentially as described (14). To isolate genomic DNA for analysis of methylation by Dam methylase, *E. coli* ASL1270 and ASL1276 cells harboring ptacsopB were grown to about 10^7 cells per ml in Luria broth containing ampicillin at 100 μg/ml, each culture was split into two and IPTG (1 mM) was added to one to induce the *tac* promoter-linked *sopB* gene. After 2–2.5 hr, both IPTG-induced and uninduced cultures were quenched with the phenol/ethanol mixture described earlier, and genomic DNA was prepared from each culture following lysis with SDS, digestion with proteinase K, and multiple phenol/chloroform (1:1 by volume) extractions.

RESULTS

Repression of Genes Adjacent to *SopC* by a High Cellular Level of SopB Protein. Table 1 summarizes the growth characteristics of *E. coli* cells harboring various plasmids. HB101 cells transformed with pASLS4, which contains the *sopC* element and the determinants of tetracycline and chloramphenicol resistance (5), and ptacsopBX, which carries the

Table 1. Colony sizes of various *E. coli* strains bearing the indicated plasmid or plasmids on Luria broth/agar plates containing antibiotics

		Colony size					
		Ampicillin		Ampicillin + chloramphenicol		Ampicillin + tetracycline	
Strain	Plasmid(s)	− IPTG	+ IPTG	− IPTG	+ IPTG	− IPTG	+ IPTG
HB101	pASL4 + ptacsopBX	+++	+++	+++	+++	+++	+++
	pASLS4 + ptacsopB	+++	++	+++	−	+++	−
	pASLS3 + ptacsopB	+++	++	+++	++	+++	++
ASL1270	ptacsopBX	+++	+++	+++	+++	NR	NR
	ptacsopB	+++	++	+++	−	NR	NR
ASL1276	ptacsopBX	+++	+++	+++	+++	NR	NR
	ptacsopB	+++	++	+++	−	NR	NR
ASL1358	ptacsopBX	+++	+++	+++	+++	NR	NR
	ptacsopB	+++	++	+++	−	NR	NR
ASL1356	ptacsopBX	+++	+++	+++	+++	NR	NR
	ptacsopB	+++	++	+++	−	NR	NR

Ampicillin and tetracycline were at 100 and 12.5 μg/ml, respectively, when present, and the concentration of IPTG was 1 mM when present. The concentration of chloramphenicol was 30 μg/ml for transformants of HB101 and 17 μg/ml for the others. Colony size was assessed after 18 hr at 37°C; +++ indicates that the size was the same as that of untransformed HB101 cells plated on Luria broth plates, and ++ indicates a slight reduction in size; − indicates that no colonies were observable. NR, not relevant.

ampicillin-resistance marker and a defective *sopB* gene linked to the IPTG-inducible *tac* promoter, grew normally on nutrient plates containing ampicillin plus tetracycline or chloramphenicol, in either the absence or the presence of IPTG. However, when the cells harbored pASL4 and ptacsopB, which is identical to ptacsopBX except that it expresses functional SopB protein from the *tac* promoter, colony formation on plates containing ampicillin plus tetracycline or chloramphenicol was observed in the absence but not in the presence of IPTG. On plates containing ampicillin but no tetracycline or chloramphenicol, the effect of IPTG was much less severe on pASLS4/ptacsopB double transformants: the presence of IPTG resulted in only a slight reduction of colony size. Similarly, HB101 cells transformed with ptacsopB and pASLS3, the latter of which does not carry the *sopC* locus but is otherwise identical to pASLS4, formed colonies on plates containing ampicillin, chloramphenicol, or tetracycline and IPTG; the presence of IPTG had only a minor effect on colony size, as noted earlier for colony formation of cells harboring both pASLS4 and ptacsopB on plates without chloramphenicol or tetracycline. These results indicate that a high cellular level of the SopB protein leads to either the elimination of a plasmid containing the *sopC* element or the repression of the antibiotic-resistance genes that reside on the *sopC*-containing plasmid.

To test whether plasmid loss or gene repression was responsible for the inactivation of the *sopC*-linked tetracycline and chloramphenicol markers on pASLS4, a DNA segment containing a *sopC* element and a chloramphenicol-resistance marker ≈500 bp apart was inserted into the chromosomal λ*attB* site of *E. coli* HB101 to give strain ASL1270. Whereas ASL1270 cells bearing ptacsopBX formed normal-size colonies on nutrient plates containing IPTG and chloramphenicol, growth of ASL1270 cells bearing the functional SopB-overexpression plasmid ptacsopB was severely retarded on the same plates (see Table 1). Thus, it appears that overexpression of SopB represses the expression of the *sopC*-linked chloramphenicol-resistance marker encoding CAT.

That a high level of *sopB* represses the expression of the *sopC*-linked CAT gene was further supported by measurements of the effect of IPTG on the steady-state level of CAT mRNA in ASL1270 cells harboring ptacsopB. The relative amounts of CAT mRNA and mRNA of a control gene, *pyrG*, which encodes CTP synthetase (13), were assessed after the simultaneous amplification of the mRNAs by the use of reverse transcription and PCR. In this experiment, *pyrG* was chosen for comparison because of its remote chromosomal location from the integrated CAT marker, being at 59.7 min of the *E. coli* map and separated from the λ*attB* site by some 1.9 Mbp. IPTG induction of SopB synthesis in strain ASL1270 cells harboring ptacsopB resulted in a significant decrease in the steady-state level of the CAT mRNA but showed no detectable effect on the level of the pyrG mRNA (Fig. 1, compare lanes 5–8 with lanes 1–4). When rifampicin, an inhibitor of *E. coli* RNA polymerase, was added to the growth medium instead of IPTG, the levels of both mRNAs were much reduced (lanes 9–12). Quantification of these effects by performing assays in the presence of [α-[^{35}S]thio]dATP showed that in *E. coli* ASL1270 cells harboring ptacsopB, there was a 5-fold decrease in the level of the CAT mRNA 20 min after the addition of either IPTG or rifampicin. Between 20 and 60 min, this level of repression of CAT mRNA remained essentially unchanged in cells treated with IPTG; in cells treated with rifampicin, the steady-state level of the same mRNA decreased 10-fold after 60 min. Quantitation of the relative amounts of the ^{35}S-labeled PCR products of *pyrG* mRNA confirmed that its steady-state level was unaffected by IPTG.

Effects of the Orientation and Position of the *SopC* Element on SopB-Mediated Repression of *SopC*-Linked Genes. Several additional *E. coli* strains were constructed to test the effects of inverting the orientation of the *sopC* element and varying the spacing between the *sopC* element and a marker gene on the SopB protein-mediated repression of the gene. Strain ASL1276 differs from strain ASL1270 only in the orientation of the *sopC* element relative to the other sequences, and strains ASL1356 and ASL1358 are identical to strains ASL1270 and ASL1276, respectively, except that in the former strains a 9.79-kb *Sph* I fragment of phage λ DNA (corresponding to nt 12,002 to 2212) is present between the *sopC* element and the CAT gene. These strains were similarly transformed with ptacsopB or ptacsopBX and the transformants were again tested for their ability to grow on nutrient agar plates supplemented with various antibiotics, in the presence or absence of IPTG. Neither the inversion of the *sopC* element nor the insertion of a 9.79-kb DNA segment between the *sopC* element and the CAT gene abolished the SopB protein-mediated repression of the chloramphenicol-resistance marker (Table 1).

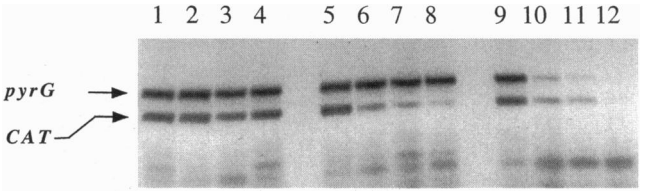

FIG. 1. Analysis of the relative amounts of CAT and *pyrG* mRNA in untreated *E. coli* ASL1270 cells harboring ptacsopB (lanes 1–4), the same cells following the addition of IPTG to induce the expression of SopB protein (lanes 5–8), and the same cells following the addition of rifampicin to inhibit RNA synthesis (lanes 9–12). For the last two sets of samples, the leftmost lane of each set contained the sample removed at the time of addition of IPTG or rifampicin, and the next three lanes from left to right contained, respectively, samples taken 20, 40, and 60 min afterwards; the untreated samples were taken at the same times as the corresponding treated ones. See text for further details.

Effect of a High Level of SopB Protein on the Accessibility of Intracellular DNA Containing the *sopC* Element to Cellular Proteins. Results of two experiments indicate that the SopB protein-mediated silencing of genes linked to *sopC* involves the formation of an extended nucleoprotein structure in which accessibility of the DNA to other cellular proteins is greatly reduced. In one, the distribution of DNA gyrase in the region of intracellular DNA containing the *sopC* element was examined. *E. coli* AS19 was used in these experiments because of the known permeability of the strain to numerous antibiotics, including oxolinic acid (7, 15). Cells harboring ptacsopB and pASLS5 were grown in medium containing ampicillin and chloramphenicol to ensure the presence of both plasmids. The culture was split into two equal portions and IPTG was added to one to induce the *tac* promoter-linked *sopB* gene. One hour after induction, oxolinic acid was added to both cultures to trap the covalent intermediate between gyrase and intracellular DNA (16, 17). After the addition of SDS to the oxolinic acid-treated cultures to denature the covalently bound gyrase and to reveal the double-stranded breaks in the DNA, extensive deproteination by proteinase K treatment and phenol extraction was carried out, and the purified DNA was used for mapping the locations of the oxolinic acid-induced gyrase cleavage sites (15). In the autoradiogram shown in Fig. 2*a*, the DNA was digested with *Sac* II restriction endonuclease, and blot hybridization of the gel-resolved fragments was done with a ^{32}P-labeled probe that hybridizes to one end of the 2989-bp *Sac* II fragment containing *sopC* (see Fig. 2*b*). Four identical pairs of samples were run in lanes 1–8 of Fig. 2*a*; the odd-numbered lanes contained samples obtained from cells exposed to IPTG and the even-numbered lanes contained samples obtained from cells unexposed to IPTG. Whereas oxolinic acid-induced gyrase cleavage sites were readily detectable in the *sopC*-containing fragment from cells unexposed to IPTG (see the distinct pattern of multiple bands below the

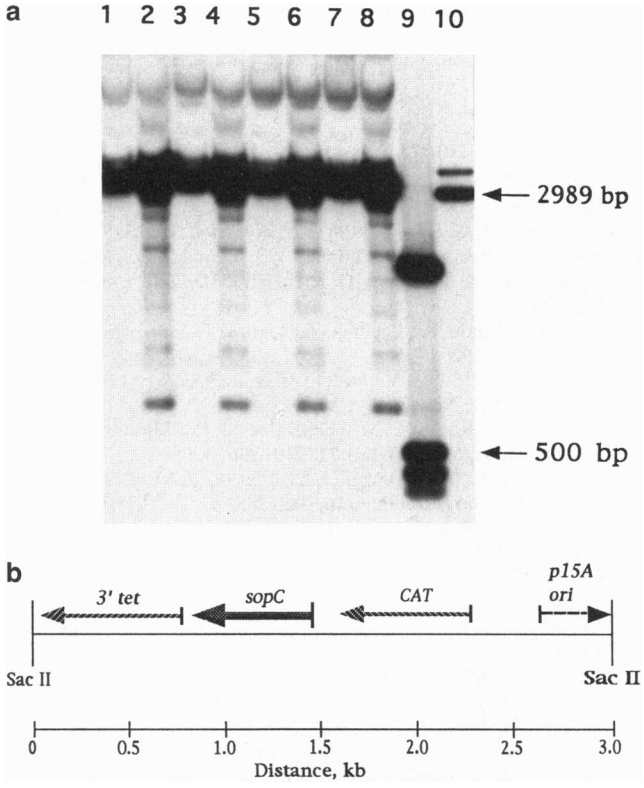

FIG. 2. Mapping of oxolinic acid-induced gyrase cleavage sites in the *sopC*-containing pASLS5 DNA in cells harboring also ptacsopB, which express a high level of SopB protein upon induction by IPTG. (*a*) Four identical pairs of samples were analyzed in lanes 1–8, with the IPTG-induced sample of each pair run in the left lane and the uninduced sample run in the adjacent right lane. Lanes 9 and 10 contained restriction fragments used as size markers, with the sizes of some of these indicated at right in the figure. (*b*) A diagram of the 2989-bp *Sac* II fragment of pASLS5 used in the experiment shown in *a*. The locations of the *sopC* element and the *tet* gene fragment are indicated; the radiolabeled probe used hybridizes specifically to the *tet* region.

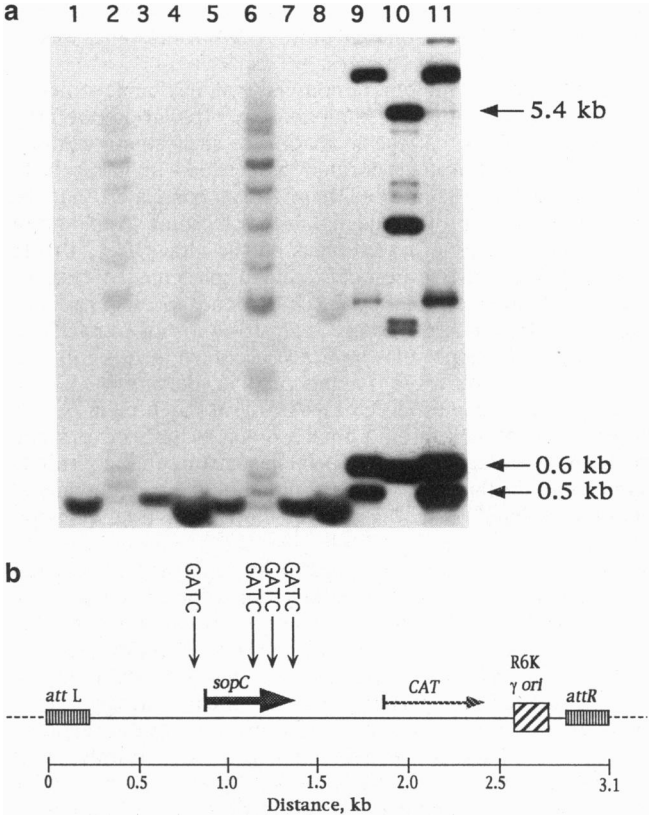

FIG. 3. Dependence of Dam methylase-mediated modification of intracellular DNA containing *sopC* before and after induction of SopB protein. (*a*) Overnight cultures were diluted by a factor of 10^3, and the diluted cultures were grown in Luria broth containing ampicillin at 100 μg/ml and 0 or 1 mM IPTG. Total cellular DNA was harvested at a cell density of about 3×10^8/ml and digested exhaustively with either *Sau*3AI or *Dpn* I. Lanes 1–4 contained DNA samples from ASL1276 cells bearing ptacsopB. Lane 1, *Sau*3AI-digested, IPTG-induced; lane 2, *Dpn* I-digested, IPTG-induced; lane 3, *Sau*3AI-digested, uninduced; lane 4, *Dpn* I-digested, uninduced. Samples run in lanes 5–8 were DNA from ASL1270 cells bearing ptacsopB; these samples were treated as described for the other set run in lanes 1–4. Lanes 9–11 contained length markers, the sizes of three of which are indicated at right margin in the figure. A 600-bp fragment of the integrated *sopC* region was used in the preparation of radiolabeled probe for blot hybridization. (*b*) Schematic drawing of the region of strain ASL1270 DNA containing *sopC*; four GATC sites spanning 632 bp in the immediate vicinity of the *sopC* region are indicated by the vertical arrows. The corresponding region in strain ASL1276 is the same except that the orientation of the 632-bp *Bam*HI segment containing *sopC* is inverted.

2989-bp band in lanes 1, 3, 5, and 7 of Fig. 2*a*), no such sites were detectable in the same DNA isolated from IPTG-induced cells (see lanes 2, 4, 6, and 8 of Fig. 2*a*). On the other hand, gyrase cleavage sites in ptacsopB obtained from the same pair of cultures were found to be the same, independent of the addition of IPTG (results not shown). Similar results were obtained with several additional pairs of restriction fragments.

In a second series of experiments, modification of chromosomal DNA by the endogenous DNA adenine methylase, the product of the *E. coli dam* gene, was examined. *E. coli* strains ASL1270 and ASL1276 cells were transformed with ptacsopB and grown in the absence or presence of IPTG. As described earlier, both strains were derived from strain HB101 by the integration of a *sopC*-containing DNA segment into the chromosomal λ*attB* site, and the two strains differ only in the orientation of the *sopC* element. Chromosomal DNA preparations of the cells were then digested with either *Sau*3AI restriction endonuclease, which cuts DNA at all 5′-GATC-3′ sites irrespective of their state of adenine methylation, or *Dpn* I restriction endonuclease, which cuts DNA at unmodified but not at Dam-modified 5′-GATC-3′ sites. Analysis of the digestion products showed that the Dam-methylation pattern in the *sopC* region was strongly dependent on the induction of *sopB* by IPTG (Fig. 3). Protection from Dam methylase due to the expression of a high level of SopB protein extended over a region of ≥5 kbp from the location of the *sopC* element.

DISCUSSION

The results support strongly the notion that in *E. coli* cells expressing a high level of SopB protein, a nucleoprotein structure can form by initiating at the *sopC* element and then spreading outward to adjacent DNA sequences. Whereas the *sopC* element normally contains 12 tandemly arranged imperfect repeats of a 43-bp sequence, the presence of even a single copy of the 43-bp motif appears to be sufficient for the formation of this nucleoprotein structure (5). Accessibility of the DNA within this nucleoprotein structure to other cellular proteins seems to be much reduced, as evidenced by measurements of DNA methylation by Dam methylase and oxolinic acid-induced cleavage of DNA by gyrase. Sequestration of DNA segments of ≥10 kb by alternative mechanisms that do not involve the formation of an extended nucleoprotein structure, such as microcondensation of DNA triggered by the binding of a limited number of protein molecules, or localization of the DNA to a cellular compartment inaccessible to DNA gyrase and Dam methylase, seems less likely, as the *sopC*-dependent silencing of genes requires the presence of a high level of SopB protein. Further, because the linking numbers of *sopC*-containing intracellular plasmids show large

changes upon induction of SopB protein (5), these plasmids must be accessible to one or more DNA topoisomerases to permit the topological changes.

The formation of multimeric complexes along double-stranded DNA has been reported in a number of cases (reviewed in ref. 18). It seems plausible that the formation of such complexes may displace a class of proteins normally associated with intracellular DNA. In adenovirus-infected cells, it was postulated that the binding of a viral protein may inhibit nucleosome formation or even displace existing nucleosomes on the viral DNA (19). In phage ϕ29-infected *Bacillus subtilis* cells, displacement of histone-like DNA-binding proteins by the phage gene *6* protein has been suggested (18, 20–22). Because the association of intracellular DNA with various proteins in *E. coli* accounts for a significant fraction of the observed linking number deficits of *E. coli* nucleoids or covalently closed DNA rings isolated from *E. coli*, displacement of such chromosomal proteins by the SopB protein-mediated formation of a nucleoprotein structure may contribute significantly to the observed net linking-number increments of plasmids isolated from cells expressing a high level of SopB protein (5).

The silencing of *sopC*-linked genes by SopB protein provides a paradigm for position-dependent repression of genes by the formation of a nucleoprotein structure. It is striking that the structure can extend by at least 10 kb in one direction; because the orientation of the *sopC* element has no effect on the repression of genes in its neighborhood, the propagation of the nucleoprotein structure from *sopC* is presumably bidirectional and thus the silenced region is likely to be significantly larger than 10 kb. It is plausible, however, that certain existing macromolecular complexes on the DNA may set boundaries for the spread of the silenced region.

A large number of "DNA silencing" phenomena have been described in various organisms. In bacteria, these range from genetic inactivation of whole chromosomes (23–26) to more localized effects (27–31). Formation of multimeric complexes between intracellular *E. coli* DNA and H-NS protein, for example, has been implicated in the repression of a number of genes (29–31); the *B. subtilis* phage ϕ29 gene *6* protein described above has also been shown to inactivate two early promoters at the left end of the viral DNA (20–22). In eukaryotes, some of the most extensively studied cases of gene silencing are X chromosome inactivation in XX diploids, the repression of genes close to centromeres and telomeres, and the silencing of genes at the yeast mating-type loci (for recent reviews, see refs. 32 and 33; see also refs. 34–37). Silencing of a large segment of eukaryotic DNA through the formation of a nucleoprotein structure emanating from an inactivation center is thought to be a common mechanism in many if not all cases, but the complexity of these systems makes their genetic and biochemical dissection a formidable task. In the SopB–*sopC* system reported here, at least the nucleation or initiation step appears to be relatively simple and readily amenable to detailed molecular characterization. We are uncertain about whether chromosomally encoded proteins might be involved. Biochemical experiments have previously identified two proteins of 75 and 33 kDa that appear to be associated with *sopC* in a SopB-dependent manner (38).

The SopB protein-mediated silencing of a long segment of intracellular DNA containing *sopC* provides a mechanistic basis of the IncG-type incompatibility. In the 100-kb F plasmid, the *sopC* element is separated by some 3.5 kb from *oriS*, the primary origin of replication (39). It is likely that the spreading of the nucleoprotein structure from the *sopC* region interferes with the replication of F plasmid or *oriC* plasmids containing the *sopC* locus.

We wish to thank Drs. Michael Koob and Waclaw Szybalski for gifts of plasmids and strains. This work was supported by a grant from the U.S. Public Health Service (GM24544).

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