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Genetics

A single 43-bp *sopC* repeat of plasmid mini-F is sufficient to allow assembly of a functional nucleoprotein partition complex

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ABSTRACT **Stable maintenance of the low-copy-number mini-F plasmid in *Escherichia coli* is dependent on a functional partition system. The *sop* partition region encodes proteins SopA and SopB and a cis-acting element *sopC*, which contains multiple sites to which SopB binds. We have found that SopB protein acting at *sopC in vivo* is associated with a marked effect on plasmid DNA supercoiling, which may reflect the formation of a wrapped nucleoprotein complex. In this study, we demonstrate that a functional partition complex can form with a single 43-bp SopB binding site. Our experiments suggest that SopB bound at a single site nucleates the binding of additional SopB protein and wrapping of adjacent DNA sequences, such that approximately equal numbers of supercoils are restrained regardless of the number of tandem *sopC* repeats present. It is likely that this unusual nucleoprotein complex allows interaction of the plasmid with the partition apparatus.**

The F plasmid is stably maintained in *Escherichia coli* at a low copy number of one or two per chromosome (1). Numerous plasmid-encoded functions contribute to low loss rates, but of primary importance is the effective segregation of plasmid molecules accomplished by the partition system (2). The F *sop* (stability of plasmid) partition genes have been extensively characterized (3, 4). A 2.5-kb segment encodes proteins SopA and SopB and an adjacent cis-acting region, *sopC*, which includes 12 tandem repeats of a 43-bp segment to which SopB binds (5, 6). Partitioning of an unstable *oriC* plasmid containing the *sopC* segment can occur if the Sop proteins are supplied in trans, suggesting that the *sopC* region may function analogously to a chromosomal centromere to allow interaction of the plasmid DNA with the partition apparatus (3).

Mini-F plasmids derived from the 9.6-kb *f5 Eco*RI fragment of F are replicated and stably maintained with properties similar to the intact F plasmid (7); for general reviews of F, refer to Kline (8) and Willetts and Skurray (9). Plasmid pZC209 used in our studies (shown in Fig. 1) is stably maintained at low copy number and consists of a 4.8-kb mini-F fragment that includes only the *oriS* (*ori2*) replication region (8, 11) and the *sopABC* partition genes. The mechanistic aspects of the partition process are not well understood, and knowledge of host functions and their roles remains incomplete. It has been found that DNA supercoiling is an important factor in partitioning of plasmid pSC101 (12). We have also found that the partition functions of mini-F influence superhelicity of mini-F plasmid DNA (unpublished results cited in ref. 13; D.P.B. and J. Strings, unpublished data). Mini-F plasmids lacking the *sop* genes were more highly supercoiled than *sop*$^+$ plasmids, consistent with the idea that the *sop* system contributes to a relaxation of negative superhelical turns *in vivo*. The relaxation of negative supercoils by the *sop* system was somewhat unexpected in light of the stabilizing effect of *topA* mutations on mini-F and other partition-defective plasmids (12). In this report, we demonstrate that a single SopB binding site (one of 12 present in the wild-type *sopC* region) is sufficient for both the relaxation of multiple negative superhelical turns as well as for assembly of a functional partition complex. Lane *et al.* (14) have previously demonstrated that a mini-F plasmid carrying only a small portion of the *sopC* locus was partitioned properly. Our studies confirm and extend these findings. Moreover, our results provide strong support for the hypothesis that the binding of SopB protein to *sopC* is associated with altered DNA conformation, which is likely to reflect wrapping of the DNA about a protein core with the restraint of positive superhelical turns.

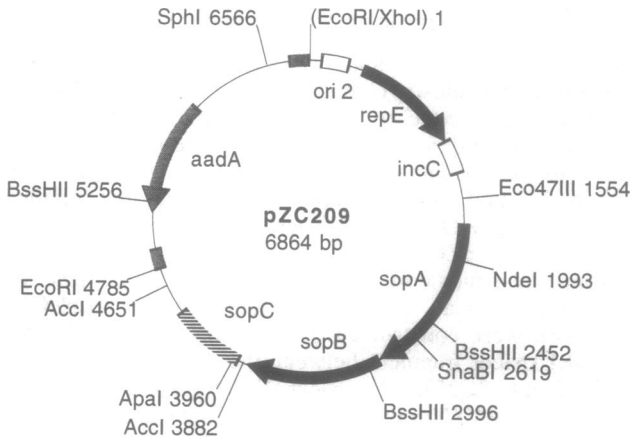

FIG. 1. Mini-F plasmid pZC209. Relevant restriction endonuclease sites are indicated. Numbering begins at the *Eco*RI/*Xho* I Ω/mini-F junction (corresponds to bp 4781 in the 9.57-kb *f5 Eco*RI fragment of F; GenBank data base, accession no. M12987). The *aadA* gene encodes resistance to streptomycin and spectinomycin (10). Partition functions are encoded by the *sopABC* genes.

MATERIALS AND METHODS

Strains, Media, and Growth Conditions. *Escherichia coli* K-12 strain MG1655 and LB growth medium have been described (15). When selecting for inheritance of plasmid pZC209 or its derivatives after transformation or when scoring for the presence of plasmid by replica plating, the concentrations of streptomycin sulfate and spectinomycin dihydrochloride were 20 μg/ml and 40 μg/ml, respectively; selection under other conditions was in medium containing antibiotics at one-half these concentrations, which allowed better growth of the cells. Plasmid stability studies were performed as described (15). Loss rates per cell per generation were calculated from curves fitted to the data.

Plasmid Constructions. The plasmids used in supercoiling studies are listed in Table 1. Mini-F plasmids used in these studies were derived from plasmid pZC209 (Fig. 1), a 6.86-kb

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plasmid that contains the 4.79-kb *Xho* I/*Eco*RI fragment from pMF21 (16) and the 2.08-kb *Eco*RI Ω fragment from pHP45-Ω, which carries the *aadA* gene encoding resistance to streptomycin and spectinomycin flanked by transcription terminators (10). Plasmid pZC230 contains a 10-bp *Hin*dIII linker at the *Bss*HII site in the *sopB* gene. Deletion of the *sopC* region of pZC209 by digestion with restriction endonuclease *Acc* I and ligation produced plasmid pZC252.

Plasmid pZC209 was digested with *Acc* I and ligated with oligonucleotides encoding a synthetic consensus *sopC* repeat (4)

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5'-CTGGTCTGATTATTAGTCTGGGACCACGGTCCCACTCGTATCGT-3'
  3'-CCAGACTAATAATCAGACCCTGGTGCCAGGGTGAGCATAGCAGC-5'
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(referred to here as a single-*sopC* repeat). Plasmid pZC253 contains one single-*sopC* repeat, while plasmid pZC254 contains two single-*sopC* repeats at the *Acc* I site; the spacing between the tandem repeats differs by 2 additional bp compared to the spacing of the repeats in the wild-type *sopC* region. Plasmid pZC260 contains a *lac* operator fragment derived from pAO-SLO (17) inserted between the *Acc* I and *Eco*RI sites of pZC252.

Mini-F plasmids containing no (pZC264) or one (pZC265) single-*sopC* repeat, which were similar in size to pZC209 (6.86 kb), were constructed by insertion of an 825-bp *Acc* I/*Eco*RI spacer fragment between the *Acc* I/*Eco*RI sites of pZC252 and pZC253. (The spacer DNA fragment was derived from the *Eco*RV/*Nru* I tetracycline-resistance gene fragment of pBR322.) A synthetic 24-bp oligonucleotide

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5'-ACTCGAGGCGAATTCTAGAGCTCG-3'
3'-TGAGCTCCGCTTAAGATCTCGAGC-5'
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that contained *Xho* I and *Sac* I sites near its ends was inserted at the *Eco*47III or *Sph* I sites of mini-F plasmid pZC252. A 113-bp *Xho* I/*Sac* I fragment, which contained the synthetic single-*sopC* repeat (located between the *Hin*dIII and *Acc* I sites of pBluescriptII SK$^-$; Stratagene), was then inserted at these sites to produce plasmids pZC269 and pZC282. Mini-F plasmid pZC270 that contained a single-*sopC* repeat at both *Acc* I and *Eco*47III sites was made by replacing the 2.4-kb *Nde* I/*Sph* I *repE*-containing fragment of pZC253 with the corresponding fragment from pZC269. Plasmid pZC193 is a derivative of pACYC184 that contains the *sopA*$^+$*B*$^+$ genes, and plasmid pZC194 lacks these genes (D.P.B. and J. Strings, unpublished data).

Plasmid DNA Supercoiling. Exponential cultures in LB medium were grown to $A_{600} \approx 0.4$ and then chilled on ice, and plasmid DNA was isolated from 5–10 ml of cell culture by an alkaline SDS lysis procedure (18). Plasmid topoisomers were separated in 20-cm-long 0.7% agarose gels in TBE buffer (100 mM Tris borate, pH 8.3/2 mM EDTA) containing 0.5–2 μg of chloroquine diphosphate per ml (Sigma). Electrophoresis at 3 V/cm was for 25–30 hr with buffer circulation. At least two different chloroquine concentrations were used in order to assess whether the plasmid DNA was negatively or positively supercoiled. To enhance visualization of plasmid topoisomer bands, the DNA was transferred from agarose gels to nitrocellulose filter paper and hybridized with ^{32}P-labeled mini-F DNA probes (18). Autoradiographs were scanned and quantitated by using a Visage 2000 imaging system.

Table 1. Mini-F plasmids used in supercoiling studies

Plasmid	Size, kb	Relevant marker(s) or derivation
pZC209	6.86	*sop*$^+$
pZC230	6.87	*sopB*$^-$
pZC252	6.09	*sopA*$^+$B$^+$Δ*C* (*Acc* I deletion)
pZC253	6.13	*sopA*$^+$B$^+$ + 43-bp *sopC* (at *Acc* I)
pZC254	6.18	*sopA*$^+$*B*$^+$ + 2 tandem 43-bp *sopC* (at *Acc* I)
pZC260	6.04	*sopA*$^+$*B*$^+$Δ*C* + *lacO*
pZC264	6.78	*sopA*$^+$*B*$^+$Δ*C*
pZC265	6.83	*sopA*$^+$*B*$^+$ + 43-bp *sopC* (at *Acc* I)
pZC269	6.22	*sopA*$^+$*B*$^+$ + 43-bp *sopC* (at *Eco*47III)
pZC270	6.26	*sopA*$^+$*B*$^+$ + 2 separated 43-bp *sopC* (at *Acc* I + *Eco*47III)
pZC269	6.22	*sopA*$^+$*B*$^+$ + 43-bp *sopC* (at *Sph* I)

All plasmids confer streptomycin/spectinomycin resistance.

RESULTS

A Single Repeat from the *sopC* Region Allows Stable Maintenance of Mini-F. The *sopC* region of the F plasmid includes 12 nearly identical 43-bp SopB binding segments arranged in direct tandem array (4, 14). We were interested in knowing whether a single SopB binding site from the *sopC* locus (i.e., what we have referred to as a single-*sopC* repeat) would be sufficient to allow proper partitioning of mini-F. We replaced the 769-bp *Acc* I *sopC* fragment of pZC209 with a synthetic 43-bp consensus single-*sopC* repeat and measured plasmid stability. Plasmid pZC209, which contained the wild-type *sopC* segment, was stably maintained in MG1655 grown in the absence of selection (≤0.01% loss per cell per generation) as were plasmids containing a single-*sopC* repeat (pZC253; ≤0.02% loss rate) or two tandem single-*sopC* repeats (pZC254; ≤0.03% loss rate). Plasmid pZC252, which lacked the entire *sopC* segment, was not stably maintained (1.5% loss rate), and was similar to the *sopB* mutant plasmid pZC230 (2.1% per cell per generation). As measured by this assay, a single 43-bp *sopC* repeat was both necessary and sufficient to allow stable maintenance of mini-F.

Superhelicity of Mini-F Plasmids as a Function of the Number of 43-bp *sopC* Repeats. We have previously found that the partition genes of plasmid mini-F affect plasmid DNA superhelicity as measured by electrophoresis of isolated DNA in agarose gels containing chloroquine (unpublished results quoted in ref. 13; J. Strings and D.P.B., unpublished data). Activity of the *sop* system was reflected in a relaxation of negative supercoils; *sop* mutant plasmids presumably became more highly negatively supercoiled by the cellular topoisomerase DNA gyrase. We were interested in determining what effect the presence of a single-*sopC* repeat would have on supercoiling of the mini-F plasmid inasmuch as our prior findings had suggested that binding of SopB to multiple sites within *sopC* resulted in the restraint of multiple positive superhelical turns.

Topoisomer distributions of DNA isolated from strains containing these plasmids are shown in Fig. 2. The results of the supercoiling study were striking. Plasmid pZC253, which contained a single-*sopC* repeat, gave a topoisomer distribution that was relaxed by ≈9 superhelical turns (which was more clearly visible in a gel containing 2 μg of chloroquine per ml; data not shown) relative to the highly negatively supercoiled *sopC*-deletion plasmid pZC252 [compare pZC253 (single-*sopC* repeat) in lane 2 with pZC252 (deletion of entire *sopC* region) in lane 1]. In addition, plasmid pZC254 containing two single-*sopC* repeats in tandem (lane 3) exhibited supercoiling that was very similar to the single-*sopC* repeat plasmid pZC253 (lane 2). At the chloroquine concentrations used in all of these studies, DNA molecules that were more highly negatively supercoiled migrated more rapidly than species that were more relaxed.

To directly compare the effects of single and multiple *sopC* repeats on supercoiling of mini-F, we added spacer fragments to make the plasmids similar in size to the parent plasmid pZC209. Plasmid pZC265 (Fig. 2, lane 6) containing a single-*sopC* repeat was more relaxed than *sopC*-deletion pZC264 (lane 5) by ≈9 turns (which was more clearly quantitated in a gel with 1.5 μg of chloroquine per ml; data not shown), while pZC209 with the wild-type 12-repeat *sopC* region (lane

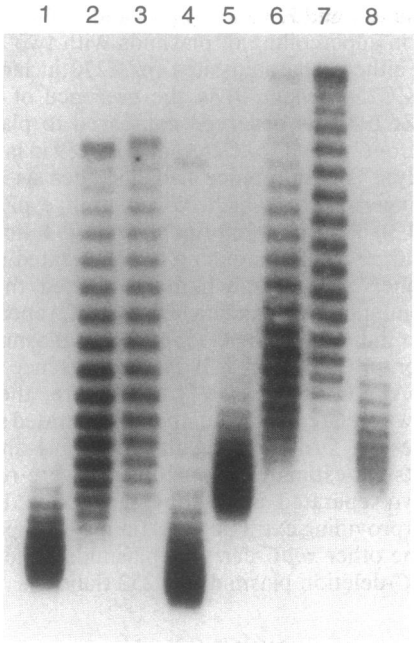

FIG. 2. Supercoiling of mini-F plasmids with altered *sopC* regions. Plasmid DNA from exponential MG1655 cultures was isolated and topoisomers were separated in an agarose gel containing 1 μg of chloroquine per ml. DNA was transferred to nitrocellulose, hybridized with ^{32}P-labeled mini-F pZC178 (D.P.B. and J. Strings, unpublished data), and detected by autoradiography. Nicked and fully relaxed species at this chloroquine concentration migrate most slowly and correspond to the uppermost band visible in some of the lanes (e.g., lanes 2 and 6). More negatively supercoiled species migrated more rapidly, and all covalently closed species are negatively supercoiled in this gel. Lanes 1–4 contained plasmids of ≈6.1 kb. Lane 1, pZC252 (*sopC* deletion); lane 2, pZC253 (single-*sopC* repeat); lane 3, pZC254 (two tandem single-*sopC* repeats); lane 4, pZC260 (*sopC* region replaced by *lacO*). Lanes 5–8 contained plasmids of ≈6.9 kb. Lane 5, pZC264 (*sopC* deletion); lane 6, pZC265 (one single-*sopC* repeat); lane 7, pZC209 (wild-type *sopC*); lane 8, pZC230 (*sopB* mutant of pZC209).

7) was more relaxed than *sopC*-deletion pZC264 (lane 4) by 12 or 13 turns.

As a control to ensure that the stability and supercoiling effects promoted by a single SopB binding site were *sop* specific, we introduced a binding site for the unrelated *lac* repressor protein in place of *sopC*. Plasmid pZC260, which contained the *lac* operator segment (*lacO*) was as unstable as *sop*-deletion plasmid pZC252 in *lacI*$^+$ strain MG1655 (1.9% loss rate for pZC260 compared to 1.5% loss rate for pZC252) and exhibited high negative supercoiling characteristic of *sop*-mutant mini-F plasmids (compare pZC260 in lane 4 with pZC252 in lane 1, Fig. 2), which was unlike plasmids that carried 1 or 2 single-*sopC* repeats (pZC253 in lane 2 and pZC254 in lane 3, Fig. 2). This indicated that an unrelated DNA binding protein could not substitute for the *sop* system with respect to stability or supercoiling of mini-F.

Superhelicity of Mini-F Plasmids Carrying *sopC* Repeats at Separated Sites. The effect of the *sop* system on supercoiling has been suggested to reflect the formation of a higher-order wrapped nucleoprotein complex rather than a topoisomerase activity acting at *sopC* (D.P.B. and J. Strings, unpublished data). The finding that even a single SopB binding site was associated with large changes in supercoiling (≈9 turns) was difficult to reconcile with the most simple version of the DNA wrapping model. For such a model to be correct, we must hypothesize that the binding of SopB protein to a single site allows additional binding of SopB protein (perhaps through cooperative protein–protein interactions) with associated wrapping of adjacent non-*sopC* DNA. Similar numbers of supercoils would be restrained by plasmids with single or multiple tandem *sopC* repeats, since most tandem sites would presumably be part of one wrapped complex.

A prediction of this model was that multiple separated-*sopC* repeats in a plasmid would contribute in an additive fashion to supercoiling compared to plasmids with only one single-*sopC* repeat and that multiple tandem-*sopC* repeats would not. Plasmid pZC270 contains a single-*sopC* repeat at both the *Acc* I and *Eco*47III sites, which are located 2.3 kb apart. Topoisomer distributions of plasmid pZC270 (Fig. 3, lane 5) and plasmid pZC254 (two tandem single-*sopC* repeats) (lane 4) appeared very similar, a result that seemed at odds with the wrapping model. We know that a single-*sopC* repeat was active when placed at the *Eco*47III site as evidenced by the finding that plasmid pZC269 (containing one single-*sopC* repeat at *Eco*47III) was both stably maintained (≤0.01% loss rate) and exhibited relaxed supercoiling associated with a functional *sop* system (compare pZC269 in lane 3 to pZC253 in lane 2; Fig. 3). Plasmid pZC269 DNA was not relaxed to quite as great an extent as plasmid pZC253 with a single-*sopC* repeat at the *Acc* I site (or pZC282, which contains a single-*sopC* repeat at the *Sph* I site; data not shown). Plasmid pZC282, which contained a single-*sopC* repeat only at the *Sph* I site, was stably maintained (≤0.02% loss rate) and exhibited relaxed supercoiling that was virtually identical to pZC253 (data not shown).

While not supportive of the wrapping model, these findings did not disprove it. It was possible that SopB protein was limiting, which precluded the efficient formation of separate wrapped complexes, or that some other physical property limited the maximal extent of complex formation on a single DNA molecule.

Providing Excess SopAB Proteins Reveals Differences in Supercoiling of Mini-F Containing Separated or Tandem *sopC* Repeats. A derivative of plasmid pACYC184 carrying the *sopA*$^+$*B*$^+$ genes (plasmid pZC193) was introduced into strains carrying mini-F. Plasmid DNA was isolated and mini-F

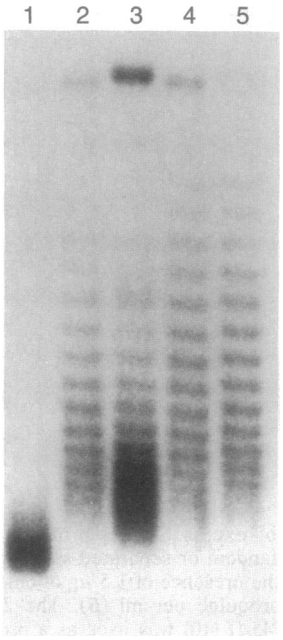

FIG. 3. Comparison of supercoiling of mini-F plasmids containing two single-*sopC* repeats at separated or tandem sites. Electrophoresis was in the presence of 2 μg of chloroquine per ml (see Fig. 2 legend for other details). All plasmids were ≈6.1 kb. Lane 1, pZC252 (*sopC* deletion); lane 2, pZC253 (one single-*sopC* repeat at *Acc* I site); lane 3, pZC269 (one single-*sopC* repeat at *Eco*47III site); lane 4, pZC254 (two tandem single-*sopC* repeats at *Acc* I site); lane 5, pZC270 (single-*sopC* repeats at *Acc* I and *Eco*47III sites).

topoisomer distributions were examined. Plasmid pZC193 (pACYC184::*sopA*$^+$*B*$^+$) affected supercoiling of all mini-F plasmids that carried *sopC* relative to the pACYC184-derived control plasmid lacking the *sopA*$^+$*B*$^+$ genes (pZC194). The results shown in Fig. 4 indicate that mini-F plasmids pZC253 (lane 2, single-*sopC* repeat at *Acc* I), pZC269 (lane 3, single-*sopC* repeat at *Eco*47III), and pZC254 (lane 4, two tandem single-*sopC* repeats at *Acc* I) were relaxed to a similar degree (4 or 5 turns) in the presence of pZC193 compared to pZC194

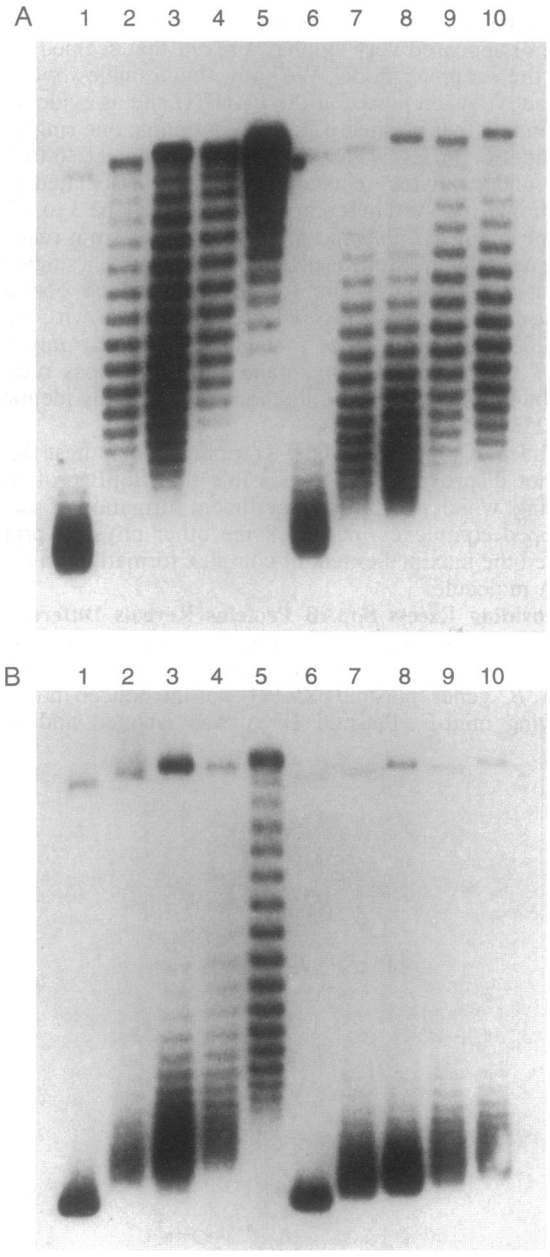

FIG. 4. Effect of excess SopAB proteins on supercoiling of plasmids with two tandem or separated single-*sopC* repeats. Electrophoresis was in the presence of 1.5 μg of chloroquine per ml (*A*) and 0.5 μg of chloroquine per ml (*B*). The 2.1-kb *Eco*RI *aadA* fragment from pHP45-Ω (10) was used as a probe specific for the mini-F plasmids (see Fig. 2 legend for other details). All plasmids were ≈6.1 kb. Lanes 1–5, plasmid DNA was from strains that carried pZC193 (pACYC184::*sopA*$^+$*B*$^+$), while the corresponding plasmids in lanes 6–10 were from strains that contained pZC194 (pACYC184 without *sopAB*). Lanes 1 and 6, pZC252 (*sopC* deletion); lanes 2 and 7, pZC253 (single-*sopC* repeat at *Acc* I site); lanes 3 and 8, pZC269 (single-*sopC* repeat at *Eco*47III site); lanes 4 and 9, pZC254 (two tandem single-*sopC* repeats at *Acc* I); lanes 5 and 10, pZC270 (single-*sopC* repeats at both *Acc* I and *Eco*47III sites).

(compare lanes 2 and 7, 3 and 8, and 4 and 9). In Fig. 4*A*, some difference in supercoiling of plasmids with two single-*sopC* repeats at either separated sites (pZC270 in lane 10) or in tandem (pZC254 in lane 9) in the presence of the control plasmid pZC194 was observed compared to plasmids with one single-*sopC* repeat (pZC253 and pZC269 in lanes 7 and 8, respectively). This difference had not been as apparent in previous experiments, which did not utilize pZC194 (e.g., Figs. 2 and 3). The important finding in Fig. 4, however, was that plasmid pZC270, containing two separated single-*sopC* repeats (lanes 5), was much more relaxed than plasmid pZC254 containing two tandem single-*sopC* repeats (lanes 4) or than the individual single-*sopC* repeat plasmids pZC253 (lanes 2) or pZC269 (lanes 3) in the presence of plasmid pZC193 (pACYC184::*sopA*$^+$*B*$^+$). Therefore, the degree of relaxation was related to the number of separated single-*sopC* repeats present. From the results in Fig. 4 and previous experiments we estimate that ≈20 turns were restrained in pZC270 (two separated single-*sopC* repeats) in the presence of pZC193 (providing extra SopAB) (lane 5) compared to ≈12 turns for the other *sopC*-carrying plasmids (lanes 2–4) relative to *sopC*-deletion plasmid pZC252 (lane 1).

DISCUSSION

The *sopABC* segment of mini-F directs plasmid segregation and ensures that each daughter cell receives at least one plasmid copy at the time of cell division (3). The wild-type *sopC* region contains multiple binding sites for SopB protein (6). Previous studies have suggested that not all of these repeats are required for effective partition (4, 14). Here we demonstrate that a functional partition complex can be assembled on a single 43-bp SopB binding site with architecture that is apparently similar to that formed with the wild-type *sopC* region. A single-*sopC* repeat was able to function at all three mini-F sites where it was tested, indicating that a functional partition complex was able to assemble largely independently of the surrounding sequence context. Previous studies by Lane *et al.* (14) suggested that the unique segment located adjacent to the *sopC* region was important for partition. Our present findings indicate that this region is not essential for partition of mini-F plasmids that contain a shortened *sopC* segment. Results to be described elsewhere indicate that transcription into the wild-type *sopC* locus has an effect on function of the partition system (M. Zgoda and D.P.B., unpublished results) and may account for the behavior observed by Lane *et al.* (14) in their studies of plasmids containing Tn*5* insertions near or in *sopC*.

The partition region of the mini-F plasmid encodes two essential proteins, SopA and SopB. The SopA protein has multiple activities. It binds to sites in its promoter region where it is thought to repress *sopAB* gene expression (6). It may also participate more directly in the partition process (ref. 3; unpublished results), although the mechanism has not been elucidated. The activities of the SopB protein may be similarly complex in that SopB appears to act in repression of *sopAB* gene expression by promoting SopA protein binding (6), and it binds to sites in the cis-acting centromere-like *sopC* region where it may contribute to formation of a partition complex (5, 6).

Our previous studies have demonstrated that the mini-F *sop*-encoded proteins produced a large effect on supercoiling of plasmids carrying the cis-acting *sopC* segment (unpublished results quoted in ref. 13; D.P.B. and J. Strings, unpublished data). We believe that the differences in linking number reflect the action of cellular topoisomerases acting on the DNA *in vivo*, which compensate for altered superhelicity associated with the formation of a wrapped nucleoprotein complex. Our present findings provide support for the idea that Sop protein interaction with *sopC* results in formation of

a complex in which DNA structure is altered. Lynch and Wang (19) have recently published very similar findings concerning the nature of the complex formed between SopB protein and *sopC* based on a different topological assay. The suggestion that the partition complex is a wrapped nucleoprotein structure is consistent with our data; however, conformation and physical characterization await direct biochemical studies.

Restraint of positive superhelical turns by DNA wrapping has been observed previously for *Methanothermus fervidus* Hmf protein, which forms nucleosome-like structures on DNA (20), and for the p6 replication-activator protein of *Bacillus subtilis* phage ϕ29, which organizes the phage DNA in a solenoidally wrapped complex (21, 22). Presumably many (about 8–10) positive superhelical turns would normally be restrained in each SopB–*sopC* complex. This might be accomplished through multiple right-handed turns about a protein core as for the p6 protein with ϕ29 DNA or, alternatively, as a string of wrapped complexes resembling closely spaced nucleosomes. That a single 43-bp *sopC* repeat was able to allow formation of such a structure suggests that some of the protein–DNA interactions in the wrapped complex occur largely independently of DNA sequence (once SopB protein is localized to a single-*sopC* repeat) and suggest that protein–protein interactions may play an important role in formation of the partition complex. Similarly, a *par*-mutant P1 plasmid that contained an altered ParB binding site located adjacent to the IHF binding site within the P1 centromere-like *incB* segment abolished ParB binding to this site *in vitro*; however, in the presence of both ParB and IHF a DNase I footprint was observed that was very similar to the wild type, suggesting that ParB could be localized to the normal site by protein–protein interactions in the absence of some of the normal protein–DNA contacts (23).

Our results indicate that the amount of SopB protein is likely to limit the extent of complex formation *in vivo*, since increased amounts of SopAB were associated with increased numbers of restrained turns (Fig. 4). Function of the partition system is greatly influenced by the amount of Sop proteins present, and overexpression of *sopB* results in plasmid instability (3, 28). The biological significance of the multiple 43-bp repeats in the wild-type *sopC* region is unknown, since even a single repeat appears to be sufficient for partition. One possibility is that SopB binding is enhanced by multiple tandem repeats and that this is important under certain growth conditions or in particular genetic backgrounds.

The partition functions (*par*) of the low-copy P1 plasmid have similarities with the F *sop* genes. In addition to limited homologies between the partition proteins (4, 24), each plasmid has a cis-acting centromere-like region to which its cognate B protein binds (3, 5, 6, 23, 25, 26), and both the P1 *parAB* and F *sopAB* operon are autoregulated (6, 27). The cis-acting elements located downstream from the partition protein-coding regions appear quite distinct, but they are sites at which specific nucleoprotein structures are assembled (6, 23, 26). The P1 ParB–*incB* complex appears to form a DNA loop in which ParB protein interacts cooperatively at separated sites aided by DNA bending promoted by IHF (23, 26). It seems likely that such nucleoprotein complexes are important in allowing low-copy-number plasmids F and P1 to interact precisely with the partition apparatus.

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