

## The pSC101 *par* Locus Alters Protein-DNA Interactions In Vivo at the Plasmid Replication Origin

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**We report here direct evidence that mutations in the *par* locus affect protein-DNA interactions in vivo at the replication origin of plasmid pSC101. Concomitant with *par*-mediated plasmid stabilization, two sites in the origin region show an altered methylation pattern as detected by in vivo footprinting with dimethyl sulfate. One site is located near an integration host factor-binding sequence adjacent to the first of three direct repeats known to be involved in the initiation of pSC101 replication; the second site is within the third direct repeat.**

pSC101, a medium-copy-number nonconjugative plasmid (8), has been studied extensively as a model for the replication of chromosomal DNA in *Escherichia coli* (11, 22) and the distribution of extrachromosomal replicons to daughter cells at division (10, 12-14, 20). Stable inheritance of this plasmid in logarithmically growing cell populations ordinarily depends on the presence of the *par* locus, which is contained on a 375-bp DNA segment adjacent to the region essential for plasmid DNA replication (Fig. 1); while pSC101 plasmids containing *par* are maintained stably in the population without selection, derivatives lacking *par* are lost in fewer than 40 generations. Deletion analysis has shown that three partition-related (PR) DNA segments, two direct repeats and an intervening inverted repeat, are important for *par* function (20). Plasmids having one PR segment deleted are stably maintained in the absence of a wild-type pSC101 replicon but are rapidly and preferentially lost when introduced into the same cell as pSC101-derived plasmids containing an intact *par* locus; this phenomenon defines the Cmp phenotype. Deletion of two PR elements results in an unstable plasmid (the Par<sup>-</sup> phenotype), whereas deletion of all three PR segments leads to extreme plasmid instability (the Superpar<sup>-</sup> phenotype [20]).

Recent work has suggested that the *par* locus accom-

plishes stability by affecting interactions taking place at the replication origin of pSC101 rather than being directly involved in plasmid distribution per se (1, 4, 14). We sought to obtain direct evidence for such interactions in vivo by using a modification of the combined in vivo footprinting and primer extension technique described by Sasse-Dwight and Gralla (15), in which methylated DNA products that have been subsequently cleaved by piperidine are amplified linearly by polymerase chain reaction. The procedure, which has been used previously for in vivo footprinting of chromosomal DNA (16), allows satisfactory footprinting data to be obtained on relatively low-copy-number plasmids such as pSC101. Dimethyl sulfate methylates primarily the N-7

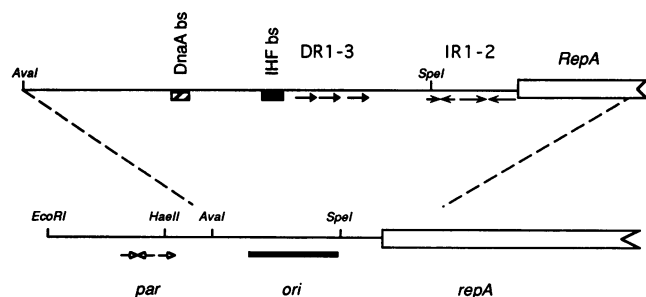


FIG. 1. The pSC101 replication origin and *par* region. The minimal origin comprises binding sites (bs) for IHF and DnaA proteins as well as three direct repeats (DR) to which the pSC101-encoded RepA protein binds (7, 9, 19, 21). The *repA* gene is autoregulated by binding of RepA to the inverted repeats (IR) in the promoter region. *par* is located approximately 200 bp from the replication origin and contains two direct repeats and an intervening inverted repeat (20).

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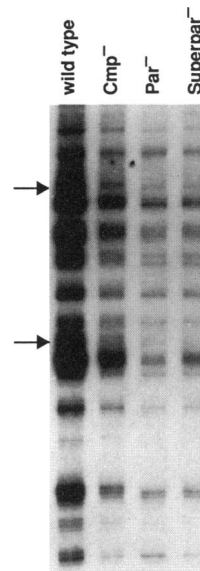


FIG. 2. In vivo footprinting of *par*<sup>+</sup> and *par* mutant plasmids. Cells containing either wild-type, Cmp<sup>-</sup>, Par<sup>-</sup>, or Superpar<sup>-</sup> pSC101 plasmids were grown exponentially in rich medium to an optical density at 600 nm of 0.3 and treated with dimethyl sulfate (10 mM; 5 min at 37°C). The piperidine-treated plasmid DNA was analyzed by primer extension analysis using a 5'-end-labelled 20-mer primer (5'-GCTTGGTTTCATGCTCCGTT-3') located within the *repA* gene 50 bp from the 5' end. Differences in methylation between the wild-type and *par*-mutated plasmids are indicated (arrows).

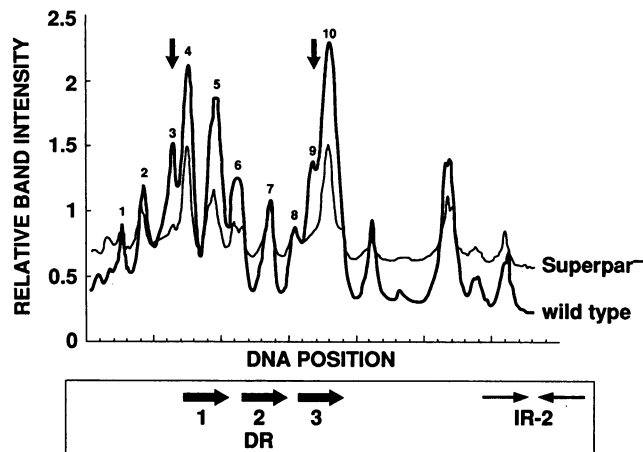


FIG. 3. Densitometric tracing of *par*<sup>+</sup> and Superpar<sup>-</sup> DNAs. The lanes containing DNAs from the wild-type and Superpar<sup>-</sup> plasmids shown in Fig. 2 were traced and displayed in relation to landmarks within the origin and promoter region (see the legend to Fig. 1). The numbers correspond to positions in Table 1. Regions that show differences between the wild-type and *par*-mutated plasmids are indicated (vertical arrows).

position of guanosine and the N-3 position of adenosine, and the dimethyl sulfate-induced modification of DNA in vivo can be either prevented or enhanced at specific locations as a result of interaction of DNA with protein(s) (5, 6).

The result of an in vivo footprinting experiment with *E. coli* K-12 strain DPB635 (3), using the wild-type plasmid pZC20 (2) and pSC101 derivatives containing deletions of various lengths in the *par* locus (pZC119 [Cmp<sup>-</sup>] [4], pZC127 [Par<sup>-</sup>; *EcoRI-HaeIII*Δ*par*] [constructed by D. P. Biek], and pZC132 [Superpar<sup>-</sup>] [4]) as substrates, is shown in Fig. 2. In a 300-bp region covering the pSC101 replication origin, two specific segments showed reproducible differences in methylation pattern in the wild-type versus *par*-mutated plasmids. In both segments, more-intense bands (marked by arrows) were observed for plasmid DNA containing the *par* locus than for pSC101 plasmids defective in *par*. The results of densitometric tracings obtained in such footprinting experiments are shown in Fig. 3 and are summarized in Table 1.

The relevant DNA segments are diagrammed in Fig. 4 in relation to certain landmarks within the pSC101 origin region. One region showing differential methylation of the wild-type and *par*-deleted plasmids (positions 3 and 4) is located near a previously identified integration host factor

TABLE 1. Densitometric tracing results from in vivo footprinting experiments

Position <sup>a</sup>	Relative band intensity <sup>b</sup>			
	Wild type	Cmp <sup>-</sup>	Par <sup>-</sup>	Superpar <sup>-</sup>
1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.2
2	1.6 ± 0.1	1.6 ± 0.1	2.0 ± 0.2	1.8 ± 0.2
3	2.1 ± 0.2	1.7 ± 0.2	1.5 ± 0.3	1.1 ± 0.1
4	3.0 ± 0.2	4.3 ± 0.3	4.6 ± 0.5	3.6 ± 0.3
5	2.6 ± 0.2	2.7 ± 0.3	2.6 ± 0.5	1.9 ± 0.3
6	1.6 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
7	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
8	1	1	1	1
9	2.0 ± 0.2	1.4 ± 0.3	1.0 ± 0.1	1.0 ± 0.1
10	3.4 ± 0.2	4.7 ± 1.0	5.0 ± 1.0	3.7 ± 0.2

<sup>a</sup> See Fig. 3 and 4.

<sup>b</sup> Relative to the value obtained for position 8, which was judged to be unaffected by the *par* locus. The data are averages with standard deviations for at least three determinations. The data for the wild-type plasmid were obtained from autoradiographs that had been exposed for shorter periods of time than those required for the *par*-deleted derivatives.

(IHF)-binding site (9) adjacent to the first of three direct repeated sequences shown to bind RepA protein (19, 21). In this region, an adenosine residue shows increased sensitivity to methylation for the wild-type plasmid compared with the *par*-deleted plasmid (position 3), whereas the adjacent site appears to be partially protected in the presence of the *par* locus (position 4). The importance of IHF protein for pSC101 replication has been shown previously (2, 3, 9, 18), and a functional interaction between plasmid stability and IHF has been suggested by the finding that mutations and conditions that allow a pSC101 plasmid to replicate in the absence of IHF stabilize the inheritance of *par*-deleted plasmids when IHF is present (2-4, 14).

A second region showing differences between the wild-type and *par*-defective pSC101 plasmids is located in the origin region direct repeat closest to the *repA* gene. The site affected is also an adenosine residue that is more sensitive to methylation in the wild-type plasmid than in the *par*-deleted plasmid (position 9); this adenosine is followed by an adenosine that is slightly protected from cleavage by piperidine in the *par*<sup>+</sup> plasmid (position 10). The increased sensitivity to dimethyl sulfate at one position accompanied by an adjacent protected locus has been observed previously in vivo as a consequence of protein binding (6). Since in vitro footprinting has shown that both RepA and DnaA proteins can bind to the origin region direct repeats (17, 19, 21), the observed *par*-induced changes in the in vivo footprint may reflect differential abilities of the wild-type and *par*-deleted



FIG. 4. In vivo footprint positions within the pSC101 origin. The nucleotide sequence of the pSC101 origin and promoter region is numbered as reported by Churchward et al. (7), and positions 1 through 10 (Table 1 and Fig. 3) are indicated. For abbreviations, see the legend to Fig. 1.

plasmids to bind either of these proteins. However, the footprints determined *in vitro* have little similarity to the footprints we have observed on actively replicating circular DNA plasmids *in vivo*, suggesting that DNA superhelicity, which has been implicated in the function of the *par* locus (1, 14), has a role in the binding of these proteins to the origin.

Our data show that the pSC101 *par* region affects the formation of protein-DNA complexes at the pSC101 replication origin, which is situated more than 200 bp from the *par* locus. While the mechanism of the observed effect has not been elucidated, we postulate that it may result from the previously demonstrated *par*-induced changes in DNA supercoiling (14). Evidence suggesting that transcriptionally induced changes in pSC101 DNA superhelicity (1) are associated with *in vivo* footprint patterns on *par*-deleted plasmids resembling those on wild-type replicons (unpublished data) supports this notion.

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