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Effects of the pSC101 partition (*par*) locus on *in vivo* DNA supercoiling near the plasmid replication origin

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ABSTRACT

Previous work has shown that deletion of the partition (*par*) locus of plasmid pSC101 results in decreased overall superhelical density of plasmid DNA and concommitant inability of the plasmid to be stably inherited in populations of dividing cells. We report here that the biological effects of *par* correlate specifically with its ability to generate supercoils *in vivo* near the origin of pSC101 DNA replication. Using OsO_4 reactivity of nucleotides adjoining 20 bp (G–C) tracts introduced into pSC101 DNA to measure local DNA supercoiling, we found that the wild type *par* locus generates supercoiling near the plasmid's replication origin adequate to convert a (G–C) tract in the region to Z form DNA. A 4 bp deletion that decreases *par* function, but produces no change in the overall superhelicity of pSC101 DNA as determined by chloroquine/agarose gel analysis, nevertheless reduced (G–C) tract supercoiling sufficiently to eliminate OsO_4 reactivity. Mutation of the bacterial *topA* gene, which results in stabilized inheritance of *par*-deleted plasmids, restored supercoiling of (G–C) tracts in these plasmids and increased OsO_4 reactivity in *par*$^+$ replicons. Removal of *par* to a site more distant from the origin decreased supercoiling in a (G–C) tract adjacent to the orgin and diminished *par* function. Collectively, these findings indicate that *par* activity is dependent on its ability to produce supercoiling at the replication origin rather than on the overall superhelical density of the plasmid DNA.

INTRODUCTION

Stabilization of plasmid inheritance by the partition (*par*) locus of plasmid pSC101 is mediated at least in part by the ability of *par* to generate negative supercoils in plasmid DNA (1). Deletion of *par* is associated with both plasmid instability and a decrease in overall DNA superhelicity, and host mutations that increase DNA superhelicity enable the partitioning of *par*-deleted pSC101 plasmids and a variety of other partition-defective extrachromsomal replicons (1–3). Conversely, mutations in *E.coli* genes encoding DNA gyrase subunits reduce plasmid DNA superhelicity, and concurrently accentuate defects in *par* (1). While a strong DNA gyrase binding site in *par* (4) is congruent with the sequences that mediate plasmid stabilization (5), another DNA sequence that contains a comparably strong gyrase binding site (6) does not stabilize plasmid inheritance (7; Miller and Cohen, unpublished).

par locus partial deletions that decrease gyrase binding do not affect the overall superhelical density of the plasmid DNA as monitored by chloroquine/agarose gels (1,4). Moreover, despite the observed ability of superhelical density to affect plasmid stability, increased *overall* supercoiling is not invariably associated with stabilized inheritance; for example, active transcription, which leads to the transient build up of negative supercoils behind the transcription complex and a consequent increase in the overall superhelical density of the plasmid DNA, may or may not affect stability depending on the location and orientation of the transcriptional unit relative to the plasmid's replication origin (8). This observation has suggested that local supercoiling may be more relevant to partitioning than overall superhelical density.

Domains of local supercoiling in plasmid DNA have been identified *in vivo* by exploiting the observation that tracts of alternating (G–C) base pairs undergo a conformational change from right handed B form DNA to the left handed Z form when present in a region that contains a high concentration of negative supercoils (9–11). We used such (G–C) tracts to assess the effect of mutations in *par*, and of the location of *par* on the plasmid, on the supercoiling of a region of pSC101 DNA near the replication origin. Our results show that *par* in its native position induces supercoils locally in the origin region, and that mutations that reduce or eliminate *par* function dramatically affect the extent of such supercoiling. Additionally, displacement of *par* to a site distant from the origin reduces supercoiling of origin region (G–C) tracts and concurrently partially impairs *par* function. Collectively, these findings support the notion that stabilization of plasmid inheritance by the pSC101 *par* locus is dependent on the ability of *par* to increase the concentration of supercoils at the plasmid's replication origin.

MATERIALS AND METHODS

Bacterial strains

All strains used are derivatives of *E.coli* K-12: DPB635 (*zch*-2250::mini-kan) and DPB636 (*zch*-2250::mini-kan *top*A66)

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(12), and PM191 (*dra*, *drm*, *thy*$^+$, *thr*, *leu*3, *thi*, *lac*Y, *sup*E, *rec*A56) (13).

Enzymes and chemicals

Restriction endonucleases were purchased from New England Biolabs, Beverly, MA; Life Technologies, Inc., Bethesda, MD; or Boehringer Mannheim, Indianapolis, IN. T4 DNA ligase and kinase were purchased from Life Technologies, Inc. The Klenow fragment of DNA polymerase I was purchased from Promega, Madison, WI. Calf intestinal alkaline phosphatase was purchased from Pharmacia, Piscataway, NJ. All enzymes were used according to supplier's instructions.

Ampicillin (Ap), kanamycin monosulfate (Km) and tetracycline (Tc) were purchased from Sigma, St Louis, MO, as were osmium tetroxide and 2,2′-bipyridine. Deoxynucleotides were purchased from Pharmacia. γ-ATP was purchased from Amersham, Arlington Heights, IL.

General procedures

LB medium (14) was used for growth of liquid cultures. Antibiotics were used at the following concentrations in both solid and liquid media: Ap, 20 μg/ml; Km, 30 μg/ml; Tc, 10 μg/ml.

DNA fragments produced by digestion with restriction endonucleases were purified on agarose gels and isolated by a freeze and squeeze method (15). Low melting temperature agarose (Seaplaque) was purchased from FMC, Rockland, ME.

Plasmids were isolated from logarithmically growing cells by the method of Biek and Cohen (16). Plasmid stability was determined as described by Meacock (13). The constructs used to compare stability of plasmids with and without the (G–C) tract inserted near the origin and their phenotypes are: pPM20 (Par$^+$, Km^r); pPM24 (Par$^-$, Km^r) (13); pCM461 (Cmp$^-$, Km^r, C. Miller, unpublished); pZC20 (Par$^+$) (16); pDLC18 [Par$^+$, (G–C)]; pDLC827+20 [Cmp$^-$, identical to pZC119 (7)]; pDLC93 [Cmp$^-$, (G–C)]; pDLC829+20 (Par, identical to pZC127); and pDLC94 [Par$^-$, (G–C)]. These plasmids were introduced into strain PM191 by transformation using competent cells prepared by the method of Hanahan (17). To determine the relative transformation frequency, a compatible Ap^{r}Tc^s pBR322 derivative, pZC9 (18), was also used to transform PM191. Transformants were selected on LB medium containing Km and Ap, grown in non-selective liquid medium for 20 generations and scored for Ap^r or Km^r cells.

The competition phenotype was determined as described by Tucker *et al.* (5), with the following modifications. Incoming plasmids were introduced into PM191 cells harboring pPM20 (Par$^+$Cmp$^+$) or pCM461 (Cmp$^-$). Following initial selection on solid medium, cells were grown overnight in liquid medium with selection for both the incoming and the resident plasmids. Cells were inoculated into liquid LB medium without drug selection and grown for 8–9 h (approximately 20 generations). Cells were diluted and plated on LB for subsequent spotting of individual colonies on selective solid medium to determine the percentage of cells containing each plasmid. Cmp$^+$ refers to the ability of the incoming plasmid to compete equally with pPM20 and to exclude pCM461. Cmp$^-$ plasmids competed equally with pCM461 but were preferentially lost in the presence of pPM20. Par$^-$ plasmids are also Cmp$^-$. Cmp$^\pm$ refers to plasmids that were preferentially lost from cells carrying pPM20 (Par$^+$) but were in turn able to displace the Cmp$^-$ plasmid pCM461. Thus, Cmp$^\pm$ refers to an intermediate competition phenotype that is observable only after growth following initial selection for both resident and incoming plasmids.

Plasmid constructions

The chimeric plasmids pDLC11, pDLC87 and pDLC92 used in this study contain the following relevant DNA fragments: the *Nde*I–*Pst*I fragment of a pSC101 derivative containing an intact or mutated *par* locus, the pSC101 origin of replication, the *rep*A gene, and the upstream portion of the *bla* gene. pDLC11 contains an wild type *par* locus from pZC20 (Par$^+$) (16); pDLC87 contains a *par* locus having a 4 bp deletion at *Hae*II of the third PR segment (5) from pZC119 (Cmp$^-$) (7); and pDLC92 contains a *par* locus from pZC127 (Par$^-$) (7) having a deletion from *Eco*RI to *Hae*II. Each pSC101 derivative fragment was ligated to the *Nde*I–*Pst*I fragment of pBR322 containing the ColE1 replication origin and the downstream portion of the *bla* gene. pDLC11 and pDLC87 contain a single copy of the 20 bp (G–C) sequence inserted into the *Ava*I site. pDLC92 contains a single copy of the 20 bp (G–C) sequence inserted into the *Eco*RI site. The (G–C) sequence was obtained by digesting an annealed double-stranded oligomer having the sequence 5′-CTAGTC CCGAGAATTCGCGCGCGCGCGCGCGAATTCCCG AGG-3′ (purchased from the PAN facility, Stanford University, Stanford, CA). A diagram of the chimeric plasmids is shown in Figure 2.

Plasmids pDLC18, pDLC93 and pDLC94 were constructed from the chimeric plasmids by deleting the ColE1 origin-containing *Pst*I–*Afl*III fragment of each plasmid and replacing it with the *Pst*I–*Afl*III of the pSC101 derivative, pZC20. The resultant constructs contain only the pSC101 replicon. Plasmid pDLC103 contains the intact *par* locus on an *Eco*RI fragment [derived from pDLC11 and containing no (G–C) tract] introduced into the *Hae*II site downstream of *rep*A by blunt end ligation. The entire *par* locus near the origin has been deleted from the *Eco*RI site to the *Ava*I site and the *Eco*RI site restored (Biek, D., unpublished). The (G–C) tract was introduced at the restored *Eco*RI site.

Assay of OsO_4 reactivity

The OsO_4 reaction (11), the sample preparation and primer extension assays (19) were performed as described for the higher copy number chimeric plasmids. For experiments involving the lower copy number pSC101-derived plasmids (10 copies per cell at division), the OsO_4 reaction and sample preparation were increased in scale to obtain sufficient DNA for primer extension reactions. In this case, 30 ml of cells grown to $OD_{600} = 0.8$ were concentrated to 2 ml and treated as described. Alternatively, 200 ml of cells were concentrated to 13 ml and reacted with OsO_4 as described, followed by an alkaline lysis procedure and CsCl gradient purification (20).

The primer (designated Ava3) used for the extension reaction has the sequence 5′-CAAAAGGATGTCGCAAACGC-3′ (purchased from Operon, Inc., Alameda, CA) and hybridizes to a region between the *Ava*I site and the pSC101 origin at bp 4956–4975 (21). The primer was ^{32}P end-labelled using γ-ATP and T4 kinase. 7-Deaza-dGTP (Boehringer Mannheim) was substituted for dGTP in the primer extension reactions. The bottom strand of the plasmid sequence was extended using this primer.

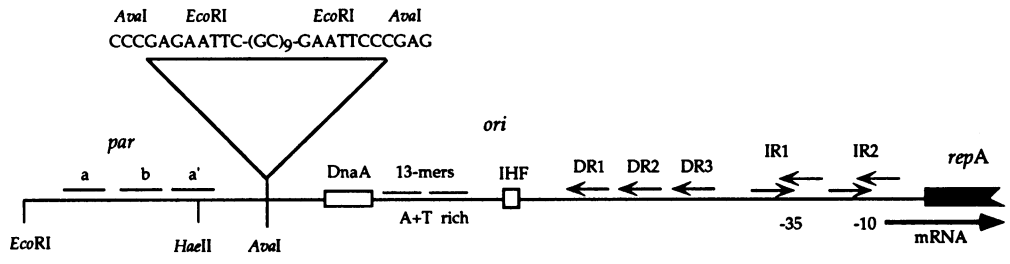

Figure 1. pSC101 origin of replication and *par* locus showing the location of the inserted (G–C) tract. The origin region contains consensus sequences for the binding of DnaA, integration host factor (IHF) and three direct repeats (DR1, DR2, DR3) that bind the RepA protein (32). Two pairs of inverted repeats (IR1 and 2) that bind RepA overlap the *repA* promoter and act in the autoregulation of RepA synthesis (33–35). Relevant restriction sites are indicated. The *AvaI* site is ~115 bp from the DnaA binding site in the origin and defines the end of a DNA segment containing *par* locus (5,13). The region to which the Ava3 primer (see Materials and Methods) hybridizes is indicated. Primer extension of the bottom strand proceeds upstream away from the origin and through the (G–C) tract inserted at *AvaI*.

Gel electrophoresis was performed as described (11) except that 8% acrylamide:bis-acrylamide gels (Long Ranger, J. T. Baker, Phillipsburg, NJ) and 40% formamide (Ultrapure, J. T. Baker) were used. Fixed and dried gels were exposed to film (Amersham, Arlington Heights, IL) according to manufacturer's instructions.

RESULTS

Effects of *par* on supercoiling in the pSC101 origin region *in vivo*

To assess the ability of *par* in its native location to induce superhelicity in the origin region, a 20 bp tract containing alternating dG and dC nucleotides was inserted at the *AvaI* site between *par* and the origin of replication (Figs 1 and 2), creating *Eco*RI sites at the junctions of the insert and the plasmid. In regions of sufficiently high superhelicity, this (G–C) tract converts from B form to Z form DNA, distorting and dissociating A–T pairs at the interface between the Z form (G–C) tract and the adjacent B form plasmid sequences (9–11). This distortion can be detected by treatment of cells containing the construct with OsO_4, which oxidizes thymidine residues at the dissociated base pairs; the resulting single strand breaks are seen as sites of chain termination during primer extension analysis (11). Thymidine residues located in *Eco*RI sites adjacent to (G–C) tracts that have a superhelical density too low to produce Z form DNA show no detectable OsO_4 reactivity.

We analyzed supercoiling of (G–C) tracts in pSC101-derived replicons that have three different phenotypes related to *par* locus function (Table 1). pDLC 18 (par^{wt}) carries an intact *par* locus and consequently is retained by 100% of viable cells after 100 generations of growth in non-selective medium (13). pDLC93 lacks 4 bp of *par* sequence at the *Hae*II cleavage site (par^{H}). This Cmp$^-$ plasmid (5) ordinarily is stably inherited but is lost preferentially in the presence of a pSC101 replicon that carries an intact *par* locus. pDLC92 contains an *EcoRI–AvaI* deletion (par^{EA}) that removes the entire *par* locus and results in rapid plasmid loss in the absence of selection (5). Introduction of (G–C) tracts into the corresponding parental plasmids to generate these constructs had no detectable affect on the Cmp or Par phenotype (data not shown).

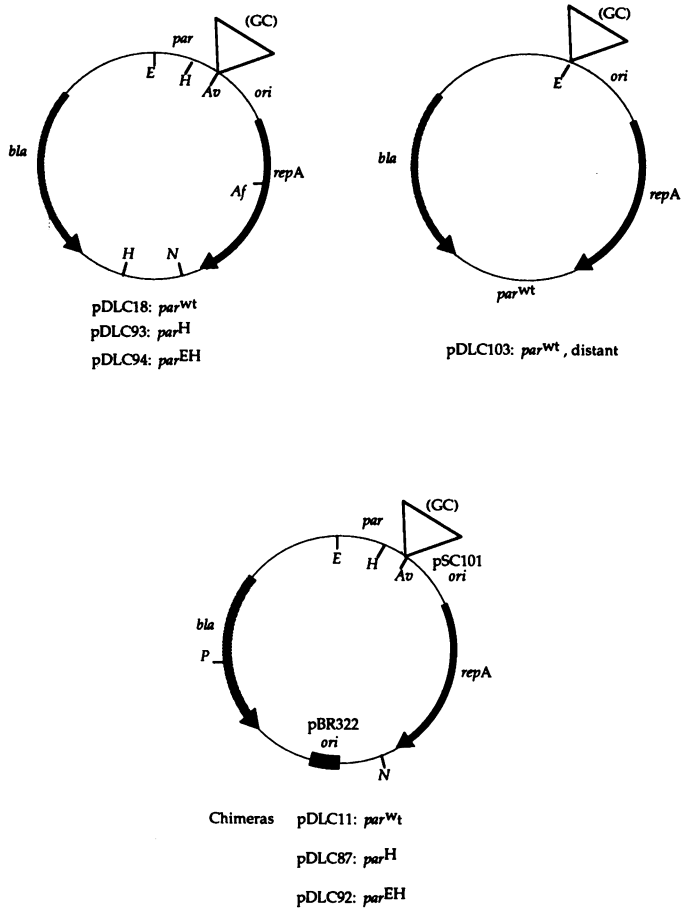

Figure 2. Diagrams of constructs containing the 20 bp (G–C) tract used for analysis of supercoiling *in vivo*. Plasmids pDLC18, 93 and 94 replicate from the pSC101 origin and contain respectively the intact *par* locus (par^{wt}), a 4 bp deletion at the *Hae*II site of *par* (par^{H}), and a deletion in *par* from *Eco*RI to *Hae*II (par^{EH}). Plasmid pDLC103 contains an intact *par* locus inserted at the *Hae*II site downstream of the *repA* gene; in this plasmid the *par* locus has been deleted from its native site near the origin. Plasmids pDLC11, 87 and 92 are par^{wt}, par^{H} and par^{EH} chimeras of pBR322 and pSC101 as described in Materials and Methods. These plasmids replicate only from the ColE1 origin under the experimental conditions used (24).

Table 1. Relevant features of plasmid constructs used in the analysis of supercoiling *in vivo*

Plasmid	Replicon	Insertion site of (G–C) tract[a]	Status of *par* locus[b]	Phenotype[c]
pDLC18	pSC101	*Ava*I	intact	Cmp$^+$
pDLC93	pSC101	*Ava*I	Δ4 bp *Hae*II	Cmp$^-$
pDLC94	pSC101	*Ava*I	Δ*Eco*RI–*Hae*II	Par$^-$
pDLC103	pSC101	*Hae*II	intact, distant from origin	Cmp$^\pm$
pDLC11	ColE1/pSC101	*Ava*I	intact	Cmp$^+$
pDLC87	ColE1/pSC101	*Ava*I	Δ4 bp *Hae*II	Cmp$^-$
pDLC92	ColE1/pSC101	*Ava*I	Δ*Eco*RI–*Hae*II	Par$^-$

[a]All (G–C) insertions are 20 bp tracts of alternating dGdC, plus the flanking *Eco*RI sites and *Ava*I sites where applicable.
[b]Deletions in *par* locus unless *par* is intact. For pDLC103, an intact *par* locus was inserted at the *Hae*II site downstream of *rep*A. The orientation of the *par* locus, which functions in either orientation (13) is unknown.
[c]The Cmp phenotypes of pDLC18, pDLC93, pDLC94, pDLC95 and pDLC103 were determined experimentally in this study. The Cmp phenotype listed for chimeric plasmids refers to the phenotype of the parent pSC101 componant (1,8).

While earlier work has shown that deletion of the entire *par* locus can decrease supercoiling of plasmid DNA as assayed by electrophoresis in chloroquine/agarose gels, small deletions that alter the DNA gyrase binding site within *par* and produce the Cmp$^-$ phenotype have no detectable effect on overall superhelical density (1,4). However, as seen in Figure 3, pDLC 93, which contains a 4 bp *par* locus deletion that yields the Cmp$^-$ phenotype (5) and affects other *par*-dependent functions such as the formation of the origin region protein/DNA complex (22) and pSC101-related incompatibility (23) showed reduced local supercoiling in a (G–C) tract adjacent to the pSC101 replication origin when assayed by the OsO_4/primer extension assay. While sites of potential reactivity of pSC101 DNA with OsO_4 are present at both junctions of the (G–C) tract, only one junction showed significant cleavage; analogous differential reactivity at junctions of (G–C) tracts has been observed previously on other plasmids (11).

Whereas Rhamouni and Wells observed that supercoiling of plasmid DNA induced by transcription from strong promoters can lead to OsO_4 reactivity at interfaces between (G–C) tracts and plasmid DNA (11), we were unable to detect effects of transcription on OsO_4 reactivity at the junctions of the 20 bp (G–C) tracts we inserted into pSC101 derivatives that were stabilized by transcription initiated near the origin region of the plasmid (data not shown). Our finding that transcription sufficient to stabilize plasmid inheritance (8) fails to produce detectable supercoiling in 20 bp (G–C) tracts is consistent with results showing that such transcriptionally stabilized plasmids cannot compete with Cmp$^+$ (PDLC18) plasmids, for which supercoiling was observed (Fig. 3).

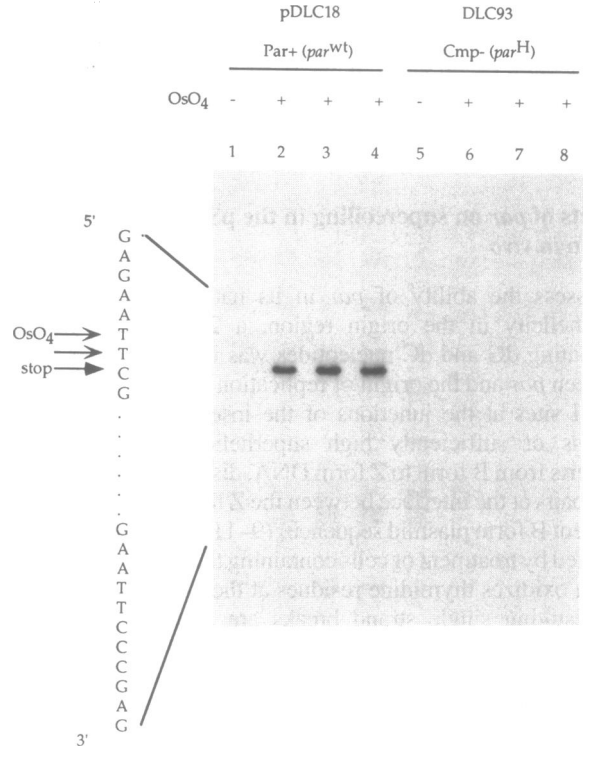

Figure 3. Primer extension analysis of OsO_4-treated *par*^{wt} and *par* mutant pSC101 plasmids containing a 20 bp (G–C) tract at the *Ava*I site between *par* and *ori*. The DNA sequence of the region is indicated at the left of the figure. The (G–C) tract sequences in the primer-extended samples were aligned with the DNA sequence by the parallel primer extension and analysis of pDLC18 reacted with DMS and piperidine (19), which cleaves the DNA at guanine residues. Lanes 1 and 5 are control samples that have not been treated with OsO_4. Lanes 2–4 are triplicates of OsO_4 reactions with the *par*^{wt} derivative. Lanes 6–8 are triplicate OsO_4 reactions of the *par*^{H} derivative. The arrows point to the reactive thymine residues at the 5′ end of the tract and the primer extension stop site.

Effects of *par* on supercoiling near the pSC101 replication origin are independent of origin function and are accentuated by *topA* mutation

We wished to know whether the observed *par*-induced effects on pSC101 DNA supercoiling in the vicinity of the pSC101 replication origin require functioning of this origin. Previous work has shown that the pSC101 replication origin is not used on chimeric plasmids comprised of pSC101 and ColE1 when the ColE1 origin, which maintains the plasmid at an elevated copy number, can function (24). As seen in Figure 4A, OsO_4/primer

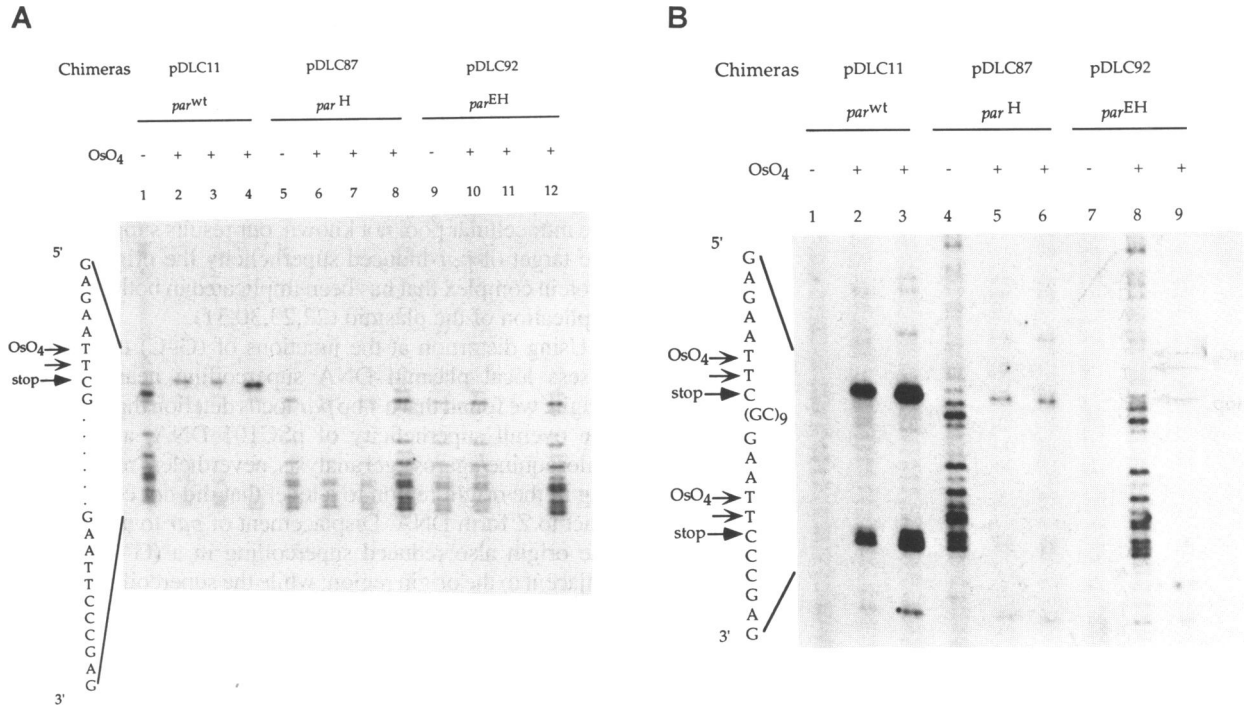

Figure 4. (**A**) *In vivo* OsO_4 reaction and primer extension of the chimeric plasmids containing the *par*^{wt} and mutant *par* loci in a *top*A$^+$ strain. Lanes 1, 5 and 9 are untreated control sample. Lanes 2–4, 6–8 and 10–12 show OsO_4-treated plasmids containing *par* loci as indicated. The sequence of the region is shown at left and the reactive thymine residues are indicated by arrows. The stop site associated with OsO_4 cleavage immediately precedes the reactive thymines. (**B**) *In vivo* OsO_4 reaction in *top*A66 mutant strain DPB636 and primer extension of the chimeric plasmids as described in Figure 3. Lanes 1, 4 and 7 are untreated control samples. Lanes 2, 3, 5, 6, 8 and 9 contain OsO_4-treated plasmids carrying *par* loci as indicated. The sequence of the region is shown at left and the reactive thymine residues at the 5′ and 3′ ends of the (G–C) tract are indicated by arrows. An arrow at the left of the lanes indicates the location of the primer extension stop site. In the *top*A mutant, all of the plasmids contain Z DNA.

extension analysis of (G–C) tracts introduced into chimeric replicons comprised of pSC101 and the ColE1-related plasmid pBR322 indicated that the topological effects of *par* on a (G–C) tract inserted near the pSC101 replication origin is independent of whether that origin is being used to initiate DNA synthesis. Additional bands representing DNA breaks at various other locations in the chimeric plasmids were observed in these experiments. The cause of these bands is not known; however, they cannot be due to OsO_4 reactivity as they were observed also in untreated controls (Fig. 4A).

Whereas supercoiling near the pSC101 origin of replication was not seen for *par*-mutated plasmids in wild type hosts, it was observed (Fig. 4B, lanes 5 and 6) in bacteria defective in topoisomerase I, which antagonizes the negative DNA supercoiling produced by DNA gyrase (25) and leads to stabilization of a variety of partition defective plasmid replicons (1). While OsO_4 reactivity at the (G–C) tract was observed in the *topA66* mutant for plasmids that contain full or partial deletions of *par*, it was much greater in the construct containing only the 4 bp deletion. Additionally, the *topA66* mutation dramatically increased OsO_4 reactivity at (G–C) tract junctions on plasmids containing an intact *par* locus, enabling the detection of cleavages at the junction furthest from *par* as well as at the *par*-proximate junction (pDLC11, Fig. 4B, lanes 2 and 3). Based on the relationship between insert length and supercoil density elucidated by Zacharias *et al.* (10), we estimate the negative supercoil density at the midpoint of the B to Z transition for a 20 bp (G–C) tract to be 0.029. Thus the wild type pSC101 plasmid has a local superhelical density of at least $-\sigma = 0.029$ near the origin in a wild type *E.coli* host strain. This is an increase above the effective superhelix density of $-\sigma = 0.025$ for plasmids in *E.coli* (10). Increased OsO_4 reactiviy of *par*-mutant plasmids in a *top*A66 strain indicates that this host mutation increases the percentage of mutant plasmids having a superhelical density of at least $-\sigma = 0.029$ near the origin.

The *par* locus generates negative supercoils in the origin region when present at an alternative location

Is the proximity of *par* to the pSC101 replication origin important to its ability to generate supercoils in the origin region? Earlier work has shown that displacement of *par* to a site distant from the origin does not eliminate its ability to stabilize inheritance of the plasmid (13; C. A. Miller, unpublished). We confirmed that pSC101 stability did not decrease when *par* was displaced to site ~2.2 kb from the origin in the counterclockwise direction and 1 kb from the origin in the clockwise direction (plasmid pDLC103, Fig. 2). The retention of OsO_4-reactivity at the (G–C) tract located near the origin (Fig. 5) is consistent with the observed ability of *par* to act at a distance; however, the primer extension band representing OsO_4 cleavage at this site was only 15–20% of the intensity, by densitometry, of the band seen at the same position when *par* was present at its native location (Fig. 5).

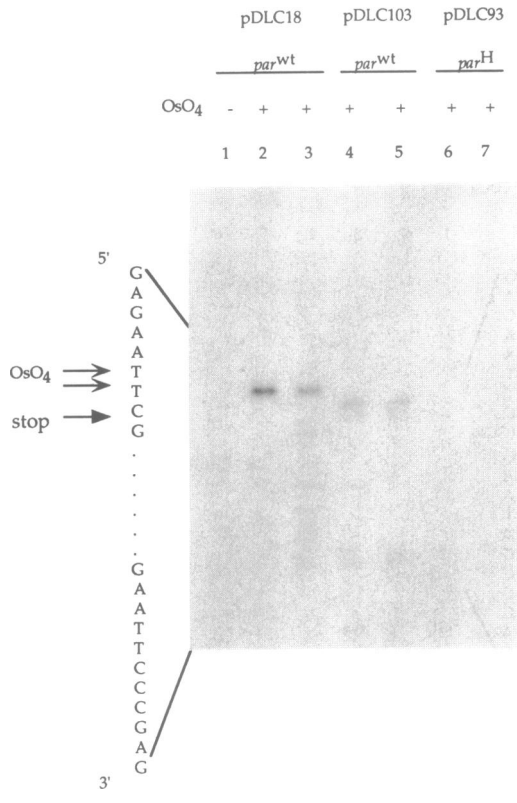

Figure 5. *In vivo* supercoiling of pSC101 derivatives containing par^{wt} at its native location near the origin of replication (pDLC18) compared to a plasmid having the par^{wt} locus at a site distant from the origin and the Cmp^- pSC101 plasmid (pDLC93). Lane 1 is a control that received no treatment with OsO_4. Lanes 2 and 3, pDLC18. Lanes 4 and 5, pDLC103. Lanes 6 and 7, pDLC93. As a result of the cloning procedure for pDLC103, the distance between the primer and the end of the (G–C) tract insert is two nucleotides shorter than in pDLC18 and pDLC93 resulting in slightly faster migration of the band. The sequence of the region is shown at left and the reactive thymine residues are indicated by arrows. The stop site associated with OsO_4 reactivity immediately precedes the reactive thymines.

The reduced supercoiling observed at the (G–C) tract of plasmid pDLC103 was associated with impairment of *par* function; although the plasmid was still stably inherited, it was lost preferentially when present in the same cell as a pSC101 replicon containing the *par* locus at its native location. However, pDLC103 was preferentially *retained* when introduced concurrently with pDLC93, a Cmp^- plasmid containing a *par* locus deletion (par^H) that eliminates detectable supercoiling of the (G–C) tract in wild type cells (Fig. 3). These observations, which define a new phenotype that we designate as $Cmp^{\pm}$, indicate the existance of a hierarchy of competition phenotypes that correlate with the extent of *par*-induced supercoiling in the origin region: pDLC18 (Par^+ Cmp^+) > pDLC103 ($Cmp^{\pm}$) > pDLC93 (Cmp^-).

DISCUSSION

Loci that promote partitioning have been identified on a variety of bacterial plasmids, including pSC101, F, R1, NR1 and P1 (13,26–29). For all of these replicons, the pSC101 *par* locus and/or other conditions that generate negative supercoils in plasmid DNA have been found to enable the partitioning of derivatives that ordinarily are not stably inherited. It has been proposed that the stabilizing effects of *par* on the inheritance of pSC101 result from its ability to allow the multiple copies of the plasmid in the intracellular pool to be recognized as individual molecules (5). The findings reported here suggest that supercoiling induced by *par* at the pSC101 replication origin is at least in part responsible for the effects of *par* on plasmid stability. While the mechanism by which topological changes in plasmid DNA might affect the physical or functional separation of plasmids in the intracellular pool not known, our results support the view that the target of *par*-induced superhelicity the origin region DNA/protein complex that has been implicated in both partitioning and replication of the plasmid (22,23,30,31).

Using distortion at the junctions of (G–C) tract insertions to assess local plasmid DNA supercoiling near the replication origin, we found that a 4 bp *par* locus deletion that does not reduce the overall superhelicity of pSC101 DNA, as determined by chloroquine/agarose gel analysis, nevertheless reduced supercoiling in the origin region to a level that did not convert the (G–C) tract to Z form DNA. Displacement of *par* to a site distant from the origin also reduced supercoiling in a (G–C) tract inserted adjacent to the origin region; while the supercoiling that did occur was sufficient to allow stable inheritance of the plasmid, *par* function was impaired, as indicated by the inability of construct pDLC103 to compete equally with a pSC101 replicon containing *par* at its native location. As pDCL103 was preferentially retained in the presence of a Cmp^- pSC101 plasmid, its phenotypic expression of the *par*-determined Cmp function is intermediate between Cmp^+ and Cmp^- plasmids, and therefore the plasmid was designated a $Cmp^{\pm}$ replicon. The extent of origin region OsO_4 reactivity observed for the pDLC103 plasmid was intermediate to Cmp^+ and Cmp^- replicons.

Origin region supercoiling induced by the intact *par* locus was increased greatly in a host mutated in the *top*A gene (Fig. 4B). In *top*A mutant bacteria the OsO_4/primer extension assay we used also detected (G–C) tract supercoiling in *par*-defective plasmids defective in *par*, consistent with the known ability of *top*A mutations to stabilize the inheritance of even plasmids that lack the entire *par* locus (1). The extent of OsO_4 reactivity was substantially greater for plasmids that carry a small *par* deletion than for a plasmid deleted for the entire locus.

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