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Boundaries of the pSC101 Minimal Replicon Are Conditional

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The DNA segment essential for plasmid replication commonly is referred to as the core or minimal replicon. We report here that host and plasmid genes and sites external to the core replicon of plasmid pSC101 determine the boundaries and competence of the replicon and also the efficiency of partitioning. Missense mutations in the plasmid-encoded RepA protein or mutation of the *Escherichia coli* topoisomerase I gene enable autonomous replication of a 310-bp pSC101 DNA fragment that contains only the actual replication origin plus binding sites for RepA and the host-encoded DnaA protein. However, in the absence of a *repA* or *topA* mutation, the DNA-bending protein integration host factor (IHF) and either of two *cis*-acting elements are required. One of these, the partitioning (*par*) locus, is known to promote negative DNA supercoiling; our data suggest that the effects of the other element, the inverted repeat (IR) sequences that overlap the *repA* promoter, are mediated through the IR's ability to bind RepA. The concentrations of RepA and DnaA, which interact with each other and with plasmid DNA in the origin region (T. T. Stenzel, T. MacAllister, and D. Bastia, Genes Dev. 5:1453–1463, 1991), also affect both replication and partitioning. Our results, which indicate that the sequence requirements for replication of pSC101 are conditional rather than absolute, compel reassessment of the definition of a core replicon. Additionally, they provide further evidence that the origin region RepA-DnaA-DNA complex initiating replication of pSC101 also mediates the partitioning of pSC101 plasmids at cell division.

pSC101 is a medium-copy-number plasmid that has been studied extensively as a model system for DNA replication (for a review, see reference 23). The region that encodes its essential replication functions (see Fig. 1) shows multiple similarities to *oriC*, a DNA segment that contains the origin of replication of the *Escherichia coli* chromosome. These similarities include a binding site for the DnaA protein (14, 16), 13-mer repeats in an A+T-rich region (which in *oriC* have been shown to allow the entry of DnaB-DnaC [7]), and a binding site for the DNA-bending protein integration host factor (IHF) (31, 33). However, like a variety of other plasmids (e.g., F [36], P1 [1], and R6K [13]), pSC101 also contains in its essential replication region three direct repeat sequences (DRs or "iterons"), which bind the plasmid-encoded replication protein RepA and have a central role in regulating pSC101 incompatibility (28, 42) and copy number (2). Nearby in the native pSC101 plasmid are the *repA* gene, which is autoregulated by interaction of the RepA protein product with inverted repeat (IR) sequences that overlap the *repA* promoter (22, 38, 41), and the partition (*par*) locus, which increases negative DNA supercoiling (11, 27), alters nucleoprotein interactions in the origin region (17), and facilitates stable inheritance of the plasmid in actively growing cell populations (26).

Minimal or core replicons, which contain the site of initiation of DNA synthesis plus binding sites for proteins that act on the replication origin, have been identified and cloned for many bacterial plasmids, including pSC101 (25, 30, 34). The property commonly used to define such minimal replicons is the ability to exist autonomously when essential replication proteins are provided in *trans*. We report here that for pSC101, the boundaries and requirements of the minimal replicon are conditional, rather than absolute. A 310-bp DNA fragment is sufficient for replication when accompanied by a mutation of *repA* or by bacterial mutations that affect DNA conformation (i.e., *topA*); however, under wild-type conditions the boundaries of the region required must expand to include nearby plasmid loci (i.e., the *par* locus or additional RepA binding sites). Moreover, the conditions that determine the sequences required for the pSC101 minimal replicon to function are the same ones shown previously to affect the stable inheritance of *par*-deleted plasmids and pSC101 DNA replication in the absence of IHF (4–6, 27). The data we present provide further support for the view that pSC101 replication and partitioning, which can occur independently of each other (10, 26, 37), are mediated by the same origin region DNA-protein complex, which we designate the replication-partition (R/P) complex.

MATERIALS AND METHODS

Bacterial strains, media, and general methods. *E. coli* K-12 strains PM191 (a *recA* derivative of C600 [26]) and DPB636 (a *topA* mutant strain [5]) were used. Growth of bacteria was in LB (29). Antibiotics (Sigma) were used at the following concentrations: ampicillin, 20 μg/ml; kanamycin, 30 μg/ml; chloramphenicol, 20 μg/ml. Transformation (20) and stability assays (26) were done as described previously. Plasmid DNA was isolated by the Triton X lysis procedure and purified on CsCl-ethidium bromide gradients (9).

Miniplasmid construction. Restriction enzymes were purchased from Pharmacia and New England Biolabs; T4 polymerase and T4 DNA ligase were purchased from Life Technologies Inc. Enzymes were used as recommended by the manufacturers. Cleaved plasmid DNA (Table 1 and Fig. 1) was made blunt ended with T4 polymerase, gel purified from 1% low-melting-point agarose (SeaPlaque; FMC) gels, and then ligated in the gel (28). Miniplasmids were purified by electrophoresis on 1% agarose (Life Technologies Inc.) gels to separate the small miniplasmid from the helper plasmid providing RepA in *trans*; DNA was electroeluted from gels, extracted with phenol, and precipitated with ethanol prior to transformation.

Copy number assays. Relative plasmid copy number was determined as described previously (6), except that plasmid DNA isolated from 200-ml cultures grown to mid-log phase with selection for plasmids was purified on CsCl-ethidium bromide gradients. Films were scanned on a PDI densitometer.

Protein concentrations. Proteins isolated from approximately equal amounts of log-phase (optical density, 0.6) cultures containing plasmids encoding RepA were separated on a sodium dodecyl sulfate–10% polyacrylamide gel, reacted with antibody against RepA, visualized by using the ECL system (Amersham), and scanned on a PDI densitometer (18).

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TABLE 1. Plasmids used in this study

Plasmid	Description	Source or reference
pFHC871	*dnaA* gene cloned with its own promoter onto pBR322; Cm^r	3
pHI1061	Like pPM30 with mutation in IR (21)	This study
pHI1158	*repA* cloned onto ColD replicon expressed from a fortuitious promoter; Cm^r, twice normal amount of RepA	This study
pHI1183	*repA7* under control of T7 gene 10 promoter on RSF1010; Km^r, fourfold normal amount of RepA	18
pPM30	Wild-type pSC101 plasmid; Ap^r	26
pSC101*oriP-IR*	Origin (par^+ ori^+ IR^+) and Ap^r gene of pPM30, *Afl*III to *Nde*I (Fig. 1)	This study
pSC101*oriM*	Origin (*par*-deleted ori^+ IR^-) and Ap^r gene of pWTT315, *Spe*I to *Nde*I (Fig. 1)	This study
pSC101*oriIR*	Origin (*par*-deleted ori^+ IR^+) and Ap^r gene of pWTT315, *Afl*III to *Nde*I (Fig. 1)	This study
pSC101*oriP*	Origin (par^+ ori^+ IR^-) and Ap^r gene of pPM30, *Spe*I to *Nde*I (Fig. 1)	This study
pSC101$oriP_M$-*IR*	Origin (Cmp^- ori^+ IR^+) and Ap^r gene of pWTT316, *Afl*III to *Nde*I	This study
pSC101*oriP-IR*$_M$	Origin (par^+ ori^+ IR^M) and Ap^r gene of pHI1061, *Afl*III to *Nde*I	This study
pWTT315	*par*-deleted pPM30	37
pWTT316	Cmp^-, *Hae*II site-deleted pPM30	37
pZC84	*repA* with its own promoter and IRs cloned on pBR322; Km^r, fourfold normal amount of RepA	4
pZC109	*repA* cloned under *lacUV5* control on mini-F; Km^r, 20% normal amount of RepA	4

RESULTS

Identification of a conditionally replicating pSC101 DNA segment. Earlier work has shown that a pSC101 DNA fragment containing the *par* locus, binding sites for IHF and DnaA, DR iterons and A+T-rich 13-mers, and the IRs of the *repA* gene promoter can replicate in *E. coli* as a plasmid when wild-type RepA protein is provided in *trans* from a gene on a coexisting pBR322 plasmid (8, 21, 35, 41). While investigating pSC101 partitioning, we found that a much smaller pSC101 DNA segment containing only sequences bracketing the site believed to be the actual origin of replication can be maintained as an autonomous replicon in *E. coli topA* mutants or in wild-type bacteria synthesizing a mutant RepA protein (RepA7), both of which conditions have been shown also to circumvent the need for IHF and to stabilize the inheritance of plasmids lacking the *par* locus (4–6, 10, 27) (Table 2 and Fig. 2). We designate this DNA segment the pSC101 minimal origin (pSC101*oriM*). However, under normal conditions (i.e., in the absence of a *repA* or *topA* mutation), either of two additional pSC101 DNA regions, the *par* locus or a DNA segment containing two sets of IR sequences, was required in *cis* for plasmid propagation (Table 2) (see also reference 30). The resulting *cis*-enhanced minireplicons containing *par* or the IR segment are designated pSC101*oriP* and pSC101*oriIR*, respectively. The minireplicon containing both elements is pSC101 *oriP-IR*.

Replication and stability properties of minimal and enhanced pSC101 replicons. Previous work has shown that pSC101 plasmids lacking *par* but expressing RepA7 and other mutant RepA proteins in *cis* are stably inherited (6, 10); *par*-defective plasmids are also stably inherited in *topA* mutant bacteria that express the wild-type RepA protein (27). However, during studies of pSC101 partitioning we observed that pSC101 minireplicons lacking *par* (i.e., the pSC101*oriIR* plasmid) or carrying a mutated *par* locus that has a 4-bp deletion affecting *par* function (37) (pSC101$oriP_M$-*IR*) were not stabilized by *topA* mutations or by RepA7 provided in *trans* (Table 3). Moreover, pSC101*oriP*, which contains an intact *par* locus but lacks the IR sequences, was stable in wild-type bacteria when *trans*-complemented by the RepA7 protein and in *topA* mutant cells when *trans*-complemented by wild-type RepA but otherwise was unstable (Table 3). Notwithstanding the observed differences in stability, the copy numbers of pSC101 *oriM*, pSC101*oriP*, pSC101*oriIR*, and pSC101*oriP-IR* plasmids *trans*-complemented by RepA7 were similar (Table 4). Analogous differences in stability were observed in *topA* mutants; however, in these *topA* cells the copy number of pSC101*oriM* was greatly reduced relative to that of the other miniplasmids (Fig. 2 and Tables 3 and 4). The small reduction in copy number produced by deletion of *par* or the IR sequence in cells containing wild-type RepA (Table 4) (see also references 25 and 30) is associated with pronounced plasmid instability. These results provide further evidence that partitioning and replication of pSC101 are independent functions (cf. references 10, 18, and 37).

Effects of RepA protein concentration. Recent work has shown that the intracellular concentration of RepA is critically important for the propagation of pSC101 (18). Excessive amounts of RepA prevent propagation of both *par*-deleted and wild-type pSC101 replicons (18), apparently by altering the intracellular ratio of RepA monomers to dimers (19). However, our finding that both the stability and replication properties of pSC101 are affected when RepA is provided in *trans* on pBR322 (see above) raised the possibility that tight regulation of the RepA concentration relative to its DR target may be necessary for normal replication and/or partitioning. We therefore determined whether the observed differences in the phenotypic properties of *cis*- versus *trans*-complemented pSC101 derivatives would be eliminated by supplying a *trans*-complementing RepA concentration that more closely approximates the native *repA*/DR ratio. A strain that synthesizes RepA protein under control of the *lacUV5* promoter from the miniF-derived pZC109 (5) plasmid produces only 20% of the wild-type RepA concentration (Table 5). Another plasmid, pHI1158 (a ColD derivative carrying *repA* transcribed from a fortuitous promoter), provides RepA at twice the normal levels (Table 5). pZC84 supplies RepA transcribed from its own promoter, which is known to be autoregulated by binding of its product to IR sequences, at about four times the normal level (Table 5) (18).

We found that the pBR322-derived pZC84 plasmid carrying *repA* expressed from its own promoter provided the best RepA concentration we tested for miniplasmid replication, allowing normal stability and copy number of a *trans*-complemented miniplasmid containing both *par* and the IR region (i.e., pSC101*oriP-IR*). Complementation of pSC101*oriP-IR* by pZC109 resulted in loss of the miniplasmid from half of the population in 40 generations (Table 6) and reduction of the miniplasmid copy number to about one-fifth of the normal level (data not shown). Increasing the RepA concentration to twice the normal level was still insufficient to stabilize inheritance of pSC101*oriP-IR*. Only with pZC84 providing RepA in *trans* from its own promoter at four times the normal level was the complete miniplasmid, pSC101*oriP-IR*, stable (Table 6).

While the *par* locus alters binding of proteins within the

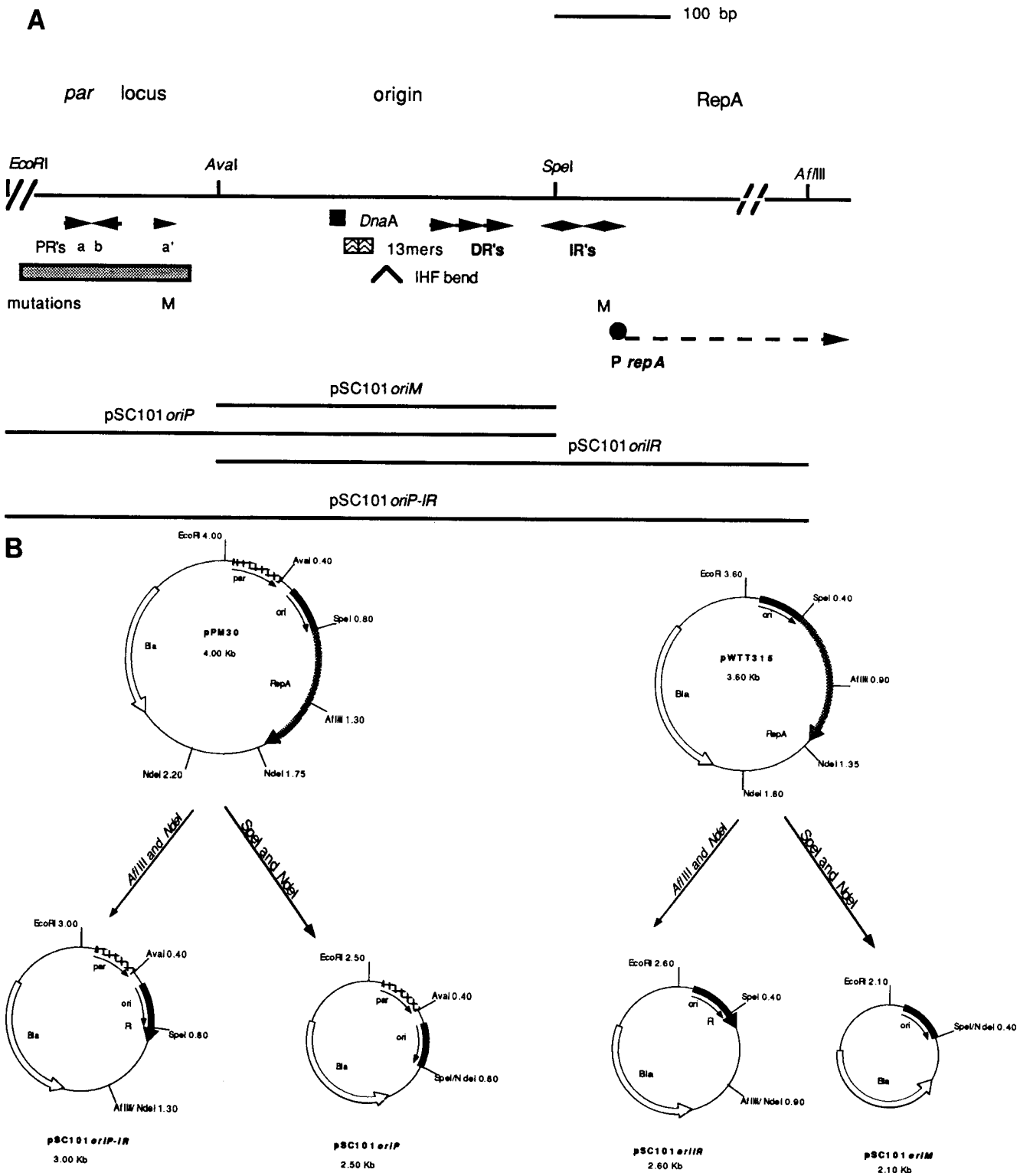

FIG. 1. pSC101 plasmid maps. (A) The features of the replication region of pSC101 and the restriction sites used for cloning the minimal origin regions are shown. The DRs (arrowheads) and the IRs (diamonds) are where the RepA protein binds. The IR sequences overlap the promoter of the RepA gene. The DnaA boxes (black squares), the 13-mers (squares with wavy lines), and the IHF binding site (inverted V) are shown. The PRs (partition-related sequences [37]; marked as a, b, and a′ arrowheads) of the *par* locus are shown, as is the DNA gyrase binding footprint (shaded box) that overlaps the *par* locus (39). The M's mark the sites of mutations in the *par* locus and the IRs. The origin regions contained in the miniplasmids are as follows: pSC101*oriM*, *Ava*I to *Spe*I; pSC101*oriP*, *Eco*RI to *Spe*I; pSC101*oriIR*, *Ava*I to *Afl*III; pSC101*oriP-IR*, *Eco*RI to *Afl*III. (B) Maps of the pSC101 miniplasmids used and their derivations from the *par*$^+$ pPM30 plasmid and the *par*-deleted pTWW315 plasmid. The *par* locus is marked as a hatched area, the *ori* region is designated with a thick black line, and the IR sequences are marked as an arrowhead. The relevant restriction sites are shown; slashed lines indicate where two unmatched ends of restriction sites were ligated and the ends were made blunt with T4 polymerase, thus destroying the sites. The sizes of the DNA fragments are indicated from the *Eco*RI site of each plasmid.

TABLE 2. Effect of wild-type RepA, RepA7, and *topA* mutations on transformation of miniplasmids

Miniplasmid	Relative transformation frequency[a]		
	PM191(pZC84)[b]	PM191(pHI1183)[c]	DPB636(pHI1158)[d]
pSC101*oriP-IR*	0.75	1.0	2.0
pSC101*oriM*	ND[e]	1.0	1.6
pSC101*oriIR*	0.09	1.0	1.0
pSC101*oriP*	0.01	1.0	1.1

[a] Frequencies are expressed relative to those obtained for PM191(pHI1183) and have been corrected for slight differences in efficiency of transformation as measured by using the unrelated pBR322 plasmid. Platings are on media containing 20 μg of ampicillin per ml. Results are averages for at least three experiments.
[b] PM191(pZC84) is a wild-type strain that supplies wild-type RepA at fourfold the normal concentration from its own promoter with the IRs (Table 5) (18).
[c] PM191(pHI1183) is a wild-type strain that supplies mutant RepA7 at fourfold the normal concentration (Table 5) (18).
[d] DPB636(pHI1158) is a *topA* mutant strain that supplies wild-type RepA at twice the normal concentration (Table 5).
[e] ND, no colonies detected.

TABLE 3. Effect of wild-type RepA, RepA7, and *topA* mutations on stabilities of miniplasmids

Miniplasmid	Stability[a] (% cells containing plasmid/no. of generations of growth in absence of selection for plasmid) in:		
	PM191(pZC84)[b]	PM191(pHI1183)[c]	DPB636(pHI1158)[d]
pSC101*oriP-IR*	100/40	100/40	100/40
pSC101*oriM*	—[e]	74/40	68/40
pSC101*oriIR*	18/20	74/40	88/40
pSC101*oriP*$_M$*-IR*	50/20	92/40	98/40
pSC101*oriP*	10/20	100/40	100/40

[a] Results are averages for four experiments.
[b] PM191(pZC84) is a wild-type strain that supplies wild-type RepA at fourfold the normal concentrations from its own promoter with the IRs (Table 5) (18).
[c] PM191(pHI1183) is a wild-type strain that supplies mutant RepA7 at fourfold the normal concentrations (Table 5) (18).
[d] DPB636(pHI1158) is a *topA* mutant strain that supplies wild-type RepA at twice the normal concentrations (Table 5).
[e] —, not done.

origin region (17) and consequently enhances replication (24, 28, 30, 37) and promotes partitioning (26) by altering negative supercoiling of plasmid DNA (11, 27), the mechanism by which the IR sequences can enhance both replication and stability is not known. However, the IR sequences have been shown to bind the RepA protein 10 times more strongly than the DR region does in vitro (35, 38, 41), and we speculated, as have others (25, 30), that the IRs may direct RepA to the adjacent origin region binding sites. To test this notion, we introduced into the IR of pSC101*oriP-IR* a point mutation that has been reported to reduce RepA protein binding in vitro by 16-fold (22). As shown in Table 6, this miniplasmid (i.e., pSC101*oriP-IR*$_M$) showed much greater instability than did a miniplasmid carrying the wild-type IR when the supply of RepA was limited; however, reduced binding of RepA to the IR had no detectable effect when the amount of RepA protein was increased to the level that allowed stable replication of a miniplasmid carrying the wild-type IR sequence (see results for pZC84 in Table 6).

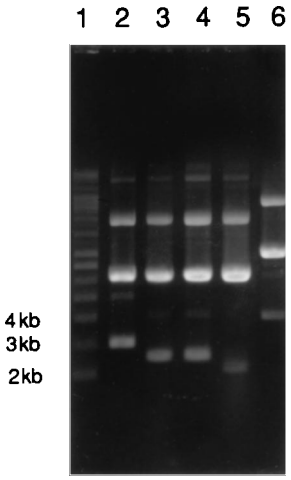

FIG. 2. Miniplasmid DNA in a *topA* mutant host. The *topA* mutant strain DPB636 enables replication of all miniplasmids when RepA is provided in *trans* from a ColD derivative (pHI1158, which provides twofold the normal concentration of RepA [Table 1 and Fig. 3]). Plasmid DNA was taken from DPB636 (pHI1158) cells in mid-log phase (optical density of about 0.4). Lanes: 1, supercoiled ladder (the sizes of the three smallest bands are 2, 3, and 4 kb); 2, pSC101*oriP-IR*, miniplasmid of 3 kb; 3, pSC101*oriP*, miniplasmid of 2.5 kb; 4, pSC101*oriIR*, miniplasmid of 2.6 kb; 5, pSC101*oriM*, miniplasmid of 2.1 kb; 6, wild-type pPM30 (4 kb) and a plasmid similar to pHI1158 but lacking the *repA* gene.

Effects of DnaA protein overexpression on plasmid replication and stability. Overexpression of the *E. coli* DnaA protein both stabilizes the inheritance of *par*-deleted pSC101 plasmids and suppresses the inhibitory effects of RepA excess on pSC101 DNA replication (18). Additionally, we observed that the transformation frequency of pSC101 miniplasmids was increased in cells containing suboptimal amounts of wild-type RepA when excess DnaA was provided from a gene carried by the pBR322 replicon (data not shown). Collectively, these results imply that an increased intracellular concentration of DnaA can compensate for a variety of events that otherwise might adversely affect the formation or function of the origin region R/P complex. Consistent with this interpretation was the ability of excess DnaA to stabilize the inheritance of pSC101*oriP-IR* miniplasmids when RepA was supplied by pZC109 at 20% of the normal concentration (Table 6). The stability of pSC101*oriIR* and pSC101*oriP* was also increased when excess DnaA protein was provided along with wild-type RepA protein (Fig. 3). However, the effect of DnaA excess was

TABLE 4. Effect of wild-type RepA, RepA7, and *topA* mutations on copy number of miniplasmids

Miniplasmid	Copy number[a] in:		
	PM191(pZC84)[b]	PM191(pHI1183)[c]	DPB636(pHI1158)[d]
pSC101*oriP-IR*	1.1 ± 0.1	1.0 ± 0.1	1.5 ± 0.2
pSC101*oriM*	—[e]	1.2 ± 0.1	0.4 ± 0.1
pSC101*oriIR*	0.7 ± 0.1	0.9 ± 0.1	1.3 ± 0.2
pSC101*oriP*	0.5 ± 0.1	0.9 ± 0.1	1.5 ± 0.2

[a] The copy number of the miniplasmids was determined from cells in mid-log phase (optical density, 0.4) (see Materials and Methods). The values are expressed relative to those for the wild-type pPM30 plasmid and normalized against an internal control. Measurements from gels such as the one shown in Fig. 2 were made with a PDI densitometer; data are averages for four experiments.
[b] PM191(pZC84) is a wild-type strain that supplies wild-type RepA at fourfold the normal concentrations from its own promoter with the IRs (Table 5) (18).
[c] PM191(pHI1183) is a wild-type strain that supplies mutant RepA7 at fourfold the normal concentrations (Table 5) (18).
[d] DPB636(pHI1158) is a *topA* mutant strain that supplies wild-type RepA at twice the normal concentrations (Table 5).
[e] —, not done.

TABLE 5. Relative RepA production

Plasmid	Replicon	Promoter	Relative amt of RepA protein[a]
pPM30	pSC101	*repA*	1
pZC109	mini-F	*lacUV5*	0.2 ± 0.1
pHI1158	ColD	Fortuitous	2 ± 0.4
pZC84	pBR322	*repA*	4 ± 0.4
pHI1183[b]	RSF1010	T7 gene 10	4 ± 0.4

[a] The amount of RepA protein was determined by Western blot (immunoblot) analysis (see Materials and Methods) and, after densitometric tracing, normalized to the optical cell density measured at 600 nm. The results are normalized to the value obtained for RepA encoded by the wild-type pSC101 plasmid, pPM30, and are averages for at least six experiments.
[b] pHI1183 produces mutant RepA7 protein.

insufficient to allow propagation of pSC101*oriM* mediated by wild-type RepA protein in a *topA*$^+$ host.

DISCUSSION

Our results show that the sequences required for replication of the pSC101 plasmid are determined by factors external to the origin itself. Mutation of the host *topA* gene (in cells expressing wild-type RepA protein) or of the pSC101-encoded *repA* gene (i.e., *repA7*) allows replication of a miniplasmid (pSC101*oriM*) consisting of the DnaA binding site, A+T-rich 13-mers, and the three directly repeated iterons (DRs) that bind the RepA protein. However, pSC101*oriM* cannot be propagated in wild-type bacteria by wild-type RepA at any protein level that we have been able to supply. In contrast, pSC101 *oriP-IR*, which contains the core replicon plus both the *par* locus (26, 37) and the IR region (25, 28, 34, 38) can be maintained stably when wild-type RepA is provided in *trans* at appropriate levels. Plasmids that include either *par* or the IR segment (i.e., pSC101*oriP* and pSC101*oriIR*) can be propagated under wild-type conditions; however, these plasmids are unstable and have a slightly lower than normal copy number (Tables 3 and 4).

The pSC101 minimal replicon we identified contains *cis*-acting sites for the binding of two proteins essential for replication, DnaA and RepA, and these sites appear to be sufficient for formation of the R/P complex, and hence replication of the plasmid, when RepA, DnaA, and certain other host replication proteins are provided in *trans*. The formation of the R/P complex at the origin region is influenced by the negative supercoiling associated with the *par* locus (11, 27) or *topA* mutation (5). The *repA7* mutation (4) and other mutations in *repA* (10) produce proteins that appear not to require an optimal DNA conformation to form an R/P complex that functions effectively for both replication and partitioning; this conclusion is supported by evidence obtained in this study that the *par* locus is dispensable for replication or partitioning of miniplasmids in the presence of the RepA7 mutation and by previous evidence that IHF also is not required under these conditions (5, 10). The IRs may enhance formation of the R/P complex by loading RepA onto its DR binding sites, as recently has been speculated by others (25, 30) and as shown in this work by the effects on plasmid stability under conditions of RepA deficiency of a mutation that lowers RepA binding to the IRs. The ability of sequences external to core replicons to increase binding of a replication initiator protein to the origin (15) or to allow replication under suboptimal conditions (40) has also been observed for other plasmids.

The construction of pSC101 miniplasmids lacking the *repA* gene enabled us to show that RepA deficiency reduces plasmid copy number and stability (Table 6). Thus, the intracellular concentration of RepA must be maintained within a narrow range for optimal plasmid replication, as RepA excess, which increases the ratio of RepA dimers to monomers (19), is also detrimental to replication and partitioning (18). However, a higher RepA concentration was required for stable maintenance of pSC101*oriP-IR* when the protein was provided in *trans*. Moreover, conditions that stabilize *par*-deleted plasmids when *repA* is in *cis* (i.e., the *repA7* mutation or a mutation in the host *topA* gene) do not stabilize the *par*-deleted *trans*-complemented miniplasmids. The replication protein of the plasmid NR1 (12) also has been observed to be more effective in *cis* than in *trans*. In contrast to excess RepA, which has detrimental effects, excess DnaA, which is known to bind cooperatively with RepA to the pSC101 origin region in vitro (32), increases the stability of pSC101 miniplasmids (Table 6 and Fig. 3). Excess DnaA protein can stabilize *par*-deleted pSC101 plasmids and allow their replication in the presence of excess RepA protein (18).

Our current findings, as well as the results of earlier work from our laboratory, strongly support the view that the R/P complex consisting of DnaA, RepA, and DNA sites within the origin mediates pSC101 partitioning (6, 10, 18), as well as replication (18, 28, 32) and replication-related incompatibility (28); these functions are facilitated by loci (*par* and the IR sequences) that bracket the minimal replicon. Consistent with this view is evidence that multiple factors that positively or negatively affect plasmid stability have analogous effects on replication: the host *topA* mutation (5, 27), an excess of the DnaA protein (18), suboptimal amounts of the RepA protein (this study and reference 18), the RepA7 mutation (4, 6), and the *par* locus (24, 26, 37). However, the role of the R/P complex in partitioning appears to be separate from its role in DNA replication: the effects of *par* and the IR sequence on copy number and stability are not congruent (data presented in this report), equipartitioning can occur in the absence of plasmid DNA replication (18, 37), fivefold elevation of plasmid copy number does not ensure stability of *par*-deleted plasmids (37), and there is no correlation between the effects of different *repA* mutations on plasmid stability and their effects on copy number (10). Together, these observations argue strongly that the stabilization of plasmid inheritance by factors that also enhance plasmid DNA replication does not result from enhanced replication per se.

TABLE 6. Stabilities of miniplasmids at limiting RepA concentrations

Miniplasmid	Stability[a] in:			
	PM191 (pZC109)[b]	PM191(pZC109, pFHC871)[c]	PM191 (pHI1158)[d]	PM191 (pZC84)[e]
pSC101*oriP-IR*	56/40	100/40	68/40	100/40
pSC101*oriP-IR*$_M$	8/20	—[f]	20/40	100/40

[a] Stabilities are indicated as in Table 3.
[b] PM191(pZC109) is a wild-type strain that supplies wild-type RepA at 20% of the normal concentration (Table 5).
[c] PM191(pZC109, pFHC871) is a wild-type strain that supplies wild-type RepA at 20% of the normal concentration and excess DnaA.
[d] PM191(pHI1158) is a wild-type strain that supplies wild-type RepA at twice the normal concentration (Table 5).
[e] PM191(pZC84) is a wild-type strain that supplies wild-type RepA at fourfold the normal concentration from its own promoter with the IRs (Table 5).
[f] —, not done.

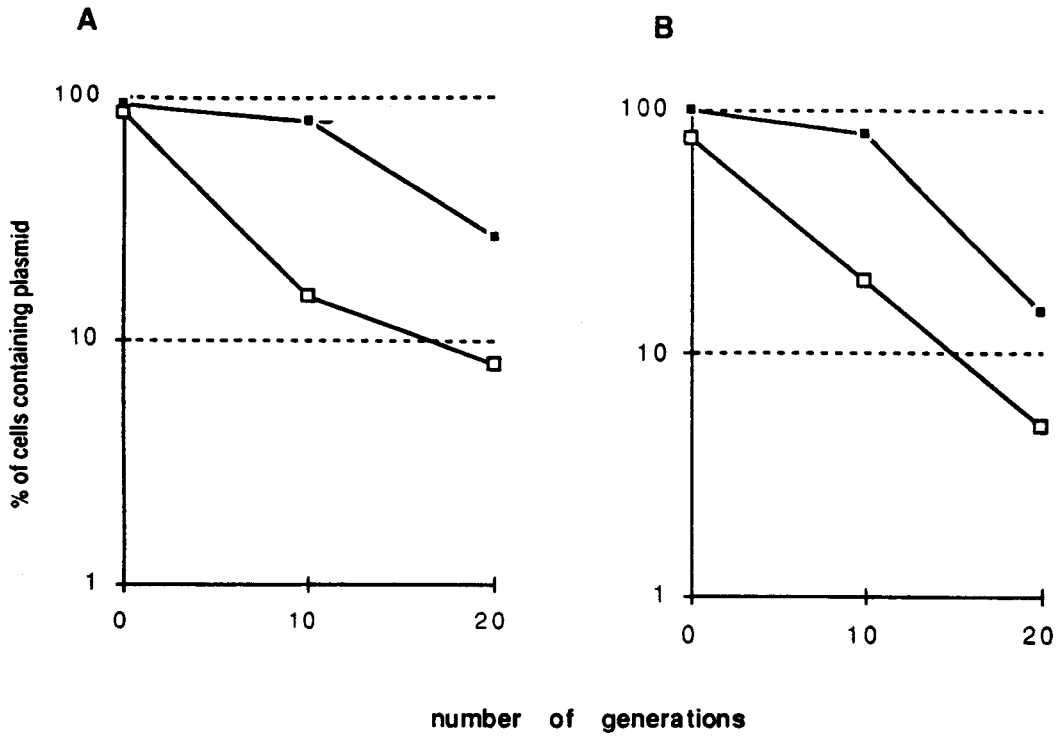

FIG. 3. Effect of excess DnaA protein on stability of miniplasmids lacking one *cis*-acting enhancer element. The rates of loss of the plasmids with PM191(pZC84) (a wild-type cell providing fourfold the wild-type amount of RepA in *trans* from its own promoter with IRs), with and without pFHC871 (a pBR322 plasmid providing excess DnaA from its own promoter [3]), are shown. (A) pSC101*oriIR*; (B) pSC101*oriP*. Samples were taken at 0′ (from a colony grown with selection for all the plasmids) and following 10 and 20 generations of growth without selection for the miniplasmid; cells were plated on LB plates with selection for the other two plasmids (chloramphenicol and kanamycin used), and colonies were then picked onto plates and grown under ampicillin selection for the miniplasmid. The open squares represent the cells containing the miniplasmid in the presence of RepA and the chromosomally provided DnaA, and the closed squares represent the cells that also contain pFHC871, which expresses excess DnaA.

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