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Genetics

# Host participation in plasmid maintenance: Dependence upon *dnaA* of replicons derived from P1 and F

(replication origin/autoregulation/pSC101/plasmid evolution)

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**ABSTRACT** **Nonparticipation of the bacterial *dnaA* gene in plasmid replication has been assumed to be the general rule. In conditional *dnaA* mutants of *Escherichia coli*, only plasmid pSC101 has been shown to have a *dnaA* requirement. Experiments with *dnaA* null mutants of *E. coli*, presented here, show that *dnaA* plays a critical and direct role in the replication of miniplasmids derived from P1 and F as it does in the initiation of bacterial replication. Evidence is also presented for the existence of a *dnaA*-independent secondary replicon of P1 that is able to drive bacterial chromosome replication but is inadequate to support the maintenance of P1 as a plasmid in *E. coli*.**

Initiation of DNA synthesis at the chromosomal origin, *oriC*, in *Escherichia coli* is dependent upon the product of the *dnaA* gene (1, 2), a protein that binds specifically to *oriC* at four or five nearly identical 9-base-pair dnaA boxes (3, 4). The DnaA protein also binds to dnaA boxes elsewhere, including the promoter region of the autoregulated *dnaA* gene (3). dnaA boxes occur in the origin regions of the diverse bacteria (5, 6) and in the origin regions of several bacterial plasmids (listed in ref. 3).

The evidence from structure for a widespread role of *dnaA* in replication appears to be contradicted by genetic evidence that most plasmids and phages tested are *dnaA* independent. The only known exceptions are pSC101 among plasmids (7–9) and 186 among phages (8, 10). The paradox rests on the interpretation of experiments with *E. coli* bearing thermosensitive (ts) mutations in *dnaA*. Any of several suitably integrated replicons can suppress the conditional lethality of *dnaA*ts mutations by providing an alternative, apparently *dnaA*-independent, replication origin. This phenomenon of integrative suppression has been demonstrated with F (11), P1 (12) and miniP1 (13), R1 and miniR1 (14), and a variety of other plasmids (12, 15–20) as well as with prophage P2*sig* (21). The capacity for integrative suppression of *dnaA*ts lethality has been widely accepted as evidence of *dnaA* independence. There are reasons for doubting this interpretation. We expect *dnaA*ts mutants at elevated temperature to be partially active (leaky), because an autoregulatory circuit (22–24) assures that the decreasing affinity of the denaturing protein for a dnaA box is accompanied by a compensatory overproduction of that protein. Leakiness is consistent with observations of phenotypic suppression of certain *dnaA*ts mutations attributable to increased dosage of the mutant gene (25) or altered superhelicity of the genome (26). Integrative suppression of *dnaA*ts lethality may indicate only that a relatively low DnaA protein activity satisfies the *dnaA* requirement.

Reliable evidence for independence of *dnaA* can be obtained only from null mutants. An *rnh* mutation [which prevents the destruction of potential primer RNAs by RNase H (27–29)] or an appropriately integrated miniR1 plasmid (Kaspar von Meyenburg and Tokio Kogoma, personal communication) allows bacterial replication to proceed from an unusual origin that is totally *dnaA* independent. In such bacteria other replicons can be tested for a possible absolute *dnaA* requirement.

We have chosen to reexamine the *dnaA* dependence of miniplasmids that are derived from P1 and F because of a striking similarity to pSC101 in the organization of the basic replicons (30, 31). As seen in Fig. 1, all three plasmids have a similarly positioned dnaA box or pair of dnaA boxes in the minimal origin, despite the near absence of homology in the remainder of their DNA sequences. Each plasmid bears a unique *rep* gene that determines a required replication protein. Upstream of each *rep* gene are repeated sequences that specifically bind the Rep protein and are implicated in replication and *rep* autoregulation. If overproduced, the Rep protein of P1 is known to interfere with the plasmid replication (39), a characteristic that we exploit in an experiment reported here. Plasmid replication in both P1 (13) and F (40) is normally limited by sets of repeated sequences [designated *incA* in P1 (38)] that lie downstream of the *rep* gene and sequester Rep protein. Plasmid destabilization by a cloned incompatibility element of this kind is indicative of dependence upon the Rep protein for which the repeats have specific affinity.

P1 is a composite of more than one replicon (41, 42). In addition to reassessing the role of *dnaA* function in the miniplasmids, we have retested the parental P1 for *dnaA* independence under the same stringent conditions.

## MATERIALS AND METHODS

**Bacteria and Phages.** Strains of *E. coli* K-12 used are listed in Table 1. The following phages were used: P1Cm (48); a wild-type λ (this laboratory); λ-miniP1, λΔ*att*-P1:5R-3 (41); λ-miniF, λΔ*att*-F:585 (*ori*-2) (49); λ-miniColE1, a λ*imm*434 Q21 derivative of the λ*col106A* (50); λ*tna*7, a *dnaA*$^+$*tnaA*$^+$ λ*c*I857 transducing phage (51); λDKC231 and λDKC236, described in the text, were constructed by Dhruba Chattoraj and have not been described elsewhere.

**Media and Microbiological Methods.** Standard media and methods were as described (46, 52). The minimal medium M56 was a phosphate-buffered medium supplemented with glucose and Casamino acids (53). Antibiotics were used in the following concentrations: ampicillin, 25 μg/ml; chloramphenicol, 12 μg/ml; kanamycin, 25 μg/ml; tetracycline, 7 μg/ml.

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Abbreviations: ts, thermosensitive; Cm, chloramphenicol; Tc, tetracycline; $^R$, resistant; $^S$, sensitive.

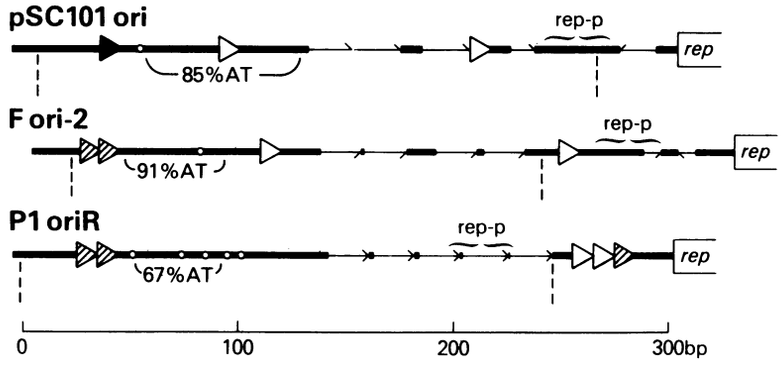


FIG. 1. dnaA boxes at similarly organized origins of replication from plasmids that exhibit minimal DNA sequence homology. Minimal functional origins lie between the vertical dashed lines. Solid, shaded, and open triangles: dnaA boxes that conform to the consensus sequence TTAT(C/A)CACA for DnaA protein binding sites (3) perfectly, deviating by one base, or deviating by two bases, respectively. Each origin requires a specific plasmid-encoded *rep* gene product transcribed from the indicated promoters. Thin arrows, repeated sequences to which the different Rep proteins bind. Circles, GATC sequences. Distance is in base pairs (bp). All three origins have similarly positioned AT-rich regions and, at least in the case of pSC101 *ori* and F *ori*-2, replication is known to be leftward (32, 33). Data are from refs. 31, 34, and 35 for pSC101; refs. 36 and 37 for F; refs. 38 and 39 for P1.

## RESULTS

**Characterization of *dnaA* Null Strains.** Strains carrying the miniR1 plasmid pKN500 integrated into the *E. coli* chromosome (near 80 min) have been found not to require the *dnaA* gene (Kaspar von Meyenburg and Tokio Kogoma, personal communication). From a strain carrying this integrated miniR1 plasmid, (CM1793), *dnaA*::Tn*10* derivatives (like EH3787) were constructed. All isolates tested were found to be sensitive to rich medium (LB broth) at all temperatures and sensitive to temperatures below 40°C in minimal medium (Kaspar von Meyenburg and Tokio Kogoma, personal communication), which considerably diminishes their usefulness.

Cold-resistant mutants, readily selected on minimal plates, simultaneously acquired the ability to grow on rich medium at 30°C and 37°C, but still showed rich-medium sensitivity at 42°C. We attribute cold resistance to a mutation (tentatively named *mad*) that impairs the expression of a gene or genes responsible for misery in the absence of *dnaA*. Studies of rich-medium resistant mutants similarly selected suggest that the *mad* mutation may be a large chromosomal inversion (54). The mutant allele in one isolate (EH3791) is called *mad*-1. For this work the following results are important: the *mad*-1 mutation is unlinked to the integrated miniR1 in P1 transductions and is unlinked to the *dnaA*::Tn*10* allele. Replication of the *mad*-1 mutant is still dependent on the integrated miniR1 plasmid (as determined by its *copA* sensitivity, see legend of Table 1).

A Δ*dnaA* derivative of the *dnaA*::Tn*10* *mad*-1 strain EH3791 was isolated as described in Table 1. In this strain (EH3827) the deletion removes part of Tn*10* along with an internal part of the *dnaA* gene, as determined by Southern blotting. Loss of the *tet* (tetracycline) marker permitted its

Table 1. *E. coli* K-12 strains

| Strain | Genotype | Source |
|---|---|---|
| CM1565 | *asnB*32 *relA*1 *spoT*1 *thi*-1 *fuc*-1 *lysA ilv*-192 *dnaA*$^+$ | Ref. 43 |
| CM1793 | CM1565 *zia*::pKN500 (=miniR1) *dnaA*$^+$ | Ref. 43 |
| EH3787 | CM1565 *zia*::pKN500 *dnaA*::Tn*10* | This work |
| EH3791 | CM1565 *zia*::pKN500 *dnaA*::Tn*10* *mad*-1 | This work |
| EH3827 | CM1565 *zia*::pKN500 Δ*dnaA* *mad*-1 | This work |
| EH3894 | CM1565 *zia*::pKN500 Δ*dnaA* *mad*-1 *tnaA*::Tn*10* | This work |
| EH3896 | CM1565 *zia*::pKN500 *dnaA*$^+$ *mad*-1 *tnaA*::Tn*10* | This work |
| EH3868 | CM1565 *loxB*::P1Cm *dnaA*::Tn*10* | This work |
| EH3872 | CM1565 *loxB*::P1Cm *dnaA*$^+$ Δ1071(*oriC-asnA*)::Tn*10* | This work |
| EH4032 | CM1565 *loxB*::λ-[miniP1 *loxP*(=*Bam*HI-9)] *dnaA*$^+$ Δ1071(*oriC-asnA*)::Tn*10* | This work |

Each of the *dnaA* alleles was verified by Southern blotting using the DNA of a λ *dnaA*-transducing phage as a probe. Constructions were as follows: EM3787 from CM1793 × λ*tna*2420 (relevant alleles: *c*I857 *dnaA*::Tn*10*) (44), selecting Tc$^R$ at 42°C on minimal medium. Growth of EH3787 is stimulated by tetracycline (7 μg/ml) but not dependent upon it. There is possibly a disruption of the *dnaA-dnaN* operon by the Tn*10* insertion that places *dnaN* at least partially under the control of a tetracycline-inducible promoter within the transposon. EH3791 was derived from EH3787 as spontaneous survivor at 30°C on minimal medium. Replication of EH3791, EH3827, and EH3894 is dependent upon the integrated miniR1 plasmid (pKN500) as shown by cold sensitivity of these strains following transformation to ampicillin resistance with the *copA*ts plasmid pOU420 (45). EH3827 was derived from EH3791 as spontaneous Tc$^S$ deletion selected on Bochner plates (46). EH3894 was derived from EH3827 × λ*tna*2123 (relevant alleles: *c*I857 *tnaA*::Tn*10* *dnaA*$^+$) (44), selecting Tc$^R$ at 42°C on minimal medium, screened for Δ*dnaA*. EH3896 was derived from EH3827, as EH3895, but screened for *dnaA*$^+$ by P1-mediated transduction of *dnaA*ts strain CM734 (44) to heat resistance. EH3868 was derived from CM1565, P1Cm introduced by λ-P1:*Eco*RI-7 prophage displacement (47); *dnaA*::Tn*10* introduced as above. The *loxB* location of the integrated prophages in EH3868, EH3872, and EH4032 was verified by Southern blotting using the DNA of a plasmid carrying *loxB*, supplied by Nat Sternberg, as a probe. EH3872 was derived from CM1565, P1Cm integrated as above; Δ*oriC* introduced via λ*asn*1071 [relevant alleles: *c*I857, Δ1071(*oriC-asnA*)::Tn*10*] (43). EH4032 was derived from CM1565; the λ-miniP1 *loxP* (supplied by Dhruba Chattoraj) was introduced by prophage displacement and Δ*oriC* by P1 transduction. R, resistant; Tc, tetracycline; S, sensitive; CM, chloramphenicol.

reuse in subsequent strain construction. We have found no other phenotypic differences between the deletion strain and its parent.

**Failure to Establish Particular Replicons in *dnaA* Null Strains.** MiniP1 and miniF cloned into a Δ*att*λ vector are unable to lysogenize by site-specific recombination but are able to lysogenize in the plasmid mode. They fail to become established in *dnaA* null strains, whereas carriers of λ-miniColE1 and pBR322 are obtained with a frequency only slightly reduced by the *dnaA* defect (Table 2). The lysogenization failure of λ-miniP1 and λ-miniF is not due to a defective expression of λ immunity in the *dnaA* null strains as seen by comparing lines 1 and 3 with lines 4 and 5, nor is it due to the secondary mutation *mad*-1. The *mad*-1 mutation did not affect the lysogenization frequency by λ-miniP1 in a *dnaA*$^+$ strain.

Surprisingly, the frequency of P1Cm lysogenization was only moderately reduced by the *dnaA* defect that completely blocks establishment of the miniP1 plasmid (Table 2). Lysogens obtained with P1Cm were analyzed further (Fig. 2). The Southern blots show a cleavage pattern consistent with the published P1 map (57) when the lysogen is *dnaA*$^+$. Lysogens of *dnaA* null strains were found to harbor prophages missing *Bgl* II fragment 2 in each of the 13 cases tested and generally show a new band of higher molecular weight. As the IS*1*-flanked transposon Tn*9* of P1Cm is located in the *Bgl* II fragment 2, the disappearance of this fragment in the lysogens suggests that the prophage has been integrated into the bacterial chromosome. Integration could occur via homologous recombination with bacterial IS*1* elements (58) or via a transposon-mediated cointegration event. The different sizes of the junction fragments in different lysogens shows that the integration did not happen at a single preferred site in the bacterial chromosome. The preferred plasmid site of integration noted here has been remarked previously under conditions where its selection appeared less exclusive (12).

These results allow us to conclude that neither P1 nor miniP1 can establish itself stably as a plasmid in a *dnaA* null strain.

**Loss of MiniP1 After the Removal of the *dnaA* Gene.** The failure of λ-miniP1 to lysogenize *dnaA*::Tn*10* strain EH3791 might be attributed to a polar effect of the transposon on expression of the downstream *dnaN* gene (59). The failure might also be specifically associated with the establishment phase of lysogeny rather than with plasmid maintenance. These alternatives are excluded by the following experiment.

Table 2. Replicon establishment in *dnaA* null strains

| Line | Replicon | Frequency of establishment, *dnaA* null/*dnaA*$^+$ |
|---|---|---|
| 1 | λ-miniP1 (*oriR*) | <0.001 |
| 2 | P1Cm | 0.4 ± 0.3 |
| 3 | λ-miniF (*ori*-2) | <0.001 |
| 4 | λ | 0.2 |
| 5 | λ-miniColE1 | 0.5 ± 0.1 |
| 6 | pBR322 | 0.6 ± 0.1 |

In each case, EH3791 and CM1793 were infected or transduced [using T4 GT7 (55)], and the frequencies of λ-immune or antibiotic-resistant colonies were compared. The results of lines 1, 2, and 6 were also obtained when the Δ*dnaA* strain EH3827 was substituted for EH3791. Similarly the results of lines 1 and 3 were also obtained when the isogenic pair EH3894 and EH3896 were used, indicating that the lysogenization failures are not due to the *mad*-1 mutation. Transductants obtained with pBR322 were found to contain pBR322 as free supercoiled plasmids in both *dnaA*$^+$ and *dnaA* null strains, whereas P1Cm was found to have integrated into the host chromosome in *dnaA* null strains. We have also observed that λ-miniP1 and λ-miniF fail to lysogenize an *rnh*::*cat* strain [M1C1020 (Mitsuhiro Itaya and Robert Crouch, personal communication)] into which *dnaA*::Tn*10* was introduced by transduction (data not shown).

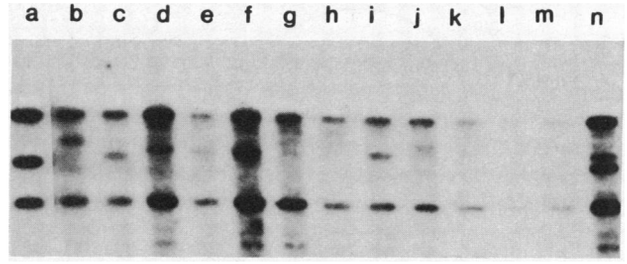


FIG. 2. Integrated P1 prophage DNA in a *dnaA* null *E. coli*. The total DNA of Cm$^R$ colonies resulting from P1Cm infection of CM1793 (lane a), of EH3791 (lanes b–f) and of EH3827 (lanes g–n) was digested with *Bgl* II and probed with $^{32}$P-labeled P1Cm DNA. Only the three largest *Bgl* II fragments are shown. Note that in lanes b–m, no band of the mobility of the middle band of lane a is found. The pattern in lane n might be explained by the presence of a pair of integrated P1Cm prophages, yielding (in nonequimolar amounts) a typical *Bgl* II-2 fragment and a "novel junction" fragment. Minor bands, due to bacterial sequences homologous to P1, are visible in the most heavily loaded lanes.

First, a Cm$^R$ miniP1 plasmid [pSP102 (60)] is introduced by transformation at 30°C into a bacterial strain in which a chromosomal *dnaA*::Tn*10* defect is complemented by a *dnaA*-transducing λ prophage (λ*tna*7). The feasibility of this transformation implies that the maintenance of the plasmid is not impaired by a possible polar effect of the Tn*10* in *dnaA* on the *dnaN* gene. λ*tna*7 does not carry the *dnaN* gene.

The lysogen was then cured of the *dnaA*$^+$ λ prophage by transient thermal induction (5 min at 42°C), segregation at low temperature, and subsequent incubation at 42°C. The heat-resistant colonies were screened for miniP1 (Cm$^R$), Tn*10* (Tc$^R$), and absence of *dnaA* function (small size and cold sensitivity). Of the colonies that appeared at 42°C, 99% were small, cold sensitive, and Tc$^R$. Of these *dnaA*::Tn*10* isolates, all tested (45/45) had lost the miniP1 plasmid. One percent of the heat-resistant colonies were large, cold-resistant, and Tc$^S$ *dnaA*$^+$ recombinants. Of these *dnaA*$^+$ isolates, all tested (24/24) had retained the plasmid. The existence of the latter class shows that, were it not for the *dnaA* defect, the plasmid would survive the heat pulse and nonselective growth at 42°C. Control experiments with pBR322 showed no plasmid loss in either class of cured cells.

The miniP1 plasmid used in this experiment was a high copy deletion mutant (lacking the control element *incA*). Loss of this plasmid cannot be accounted for by a slight reduction in copy number. We conclude that replication from *oriR* of an established miniP1 plasmid is severely or totally impaired by the loss of *dnaA* function.

***dnaA* Function Is a Direct Participant in Replication from the P1 Origin *oriR*.** In the experiments described above, the failure of *oriR* to function in *dnaA* null strains was revealed by the absence of colonies containing miniP1 plasmids. A test allowing viable colonies to be scored only when *oriR* is nonfunctional has been described by Chattoraj *et al.* (60). The test depends upon the lethality caused by replication from *oriR* when it is located at *att*λ. Strains lysogenic for a recombinant λ phage carrying *oriR* (but neither *repA* nor *incA*) could be transformed with *repA*-carrying plasmids only if the expression of RepA was insufficient (pALA178) or excessive (pALA162) for replication. *repA*-carrying plasmids known to support *oriR* replication in *trans* (pALA177, pALA176) could not be introduced.

We used this test to obtain direct evidence for the absence of *oriR* function in a Δ*dnaA* strain. The Δ*dnaA* strain (EH3827) and its *dnaA*$^+$ parent (CM1793) were lysogenized separately with *oriR*$^+$ and *oriR*$^-$ phages (λDKC231 and λDKC236) as diagrammed in Fig. 3. The relative efficiency with which these four lysogens could be transformed by four

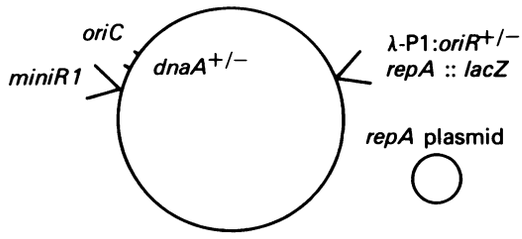


FIG. 3. Map of constructs of Table 3.

plasmids supplying the RepA protein in different amounts is shown in Table 3. The immunologically determined relative amounts of RepA protein are taken from ref. 60. In contrast to the situation in the *dnaA*$^+$ strain, the transformation frequencies in the Δ*dnaA* strain were not affected by whether the λ prophage was *oriR*$^+$ or *oriR*$^-$. None of the four plasmids induced lethal replication in the Δ*dnaA* strain.

The absence of lethal replication cannot be attributed to a lowered *repA* gene expression in the absence of *dnaA*. The presence of RepA protein activity was shown in this experiment by determination of the repression of the RepA regulated *repA* promoter on the prophage. Both the λ-P1:*oriR*$^+$ and λ-P1:*oriR*$^-$ prophages have the *repA* promoter fused to the *lacZ* gene, allowing the *repA* promoter activity to be determined as β-galactosidase expression (Fig. 3 and Table 3). We conclude that replication from *oriR* of P1 is *dnaA* dependent even when the essential replication protein RepA is provided from a source that is *dnaA* independent.

**A *dnaA*-Independent Replicon in P1.** *E. coli* carrying a P1Cm or λ-miniP1 prophage chromosomally integrated at *loxB* (66 min) were analyzed for the dispensability of *oriC* and *dnaA*. We were able to construct *oriC* deletion derivatives of both lysogens (EH3872 and EH4032, Table 1), suggesting that the *oriR* replicon that is present in both λ-miniP1 and P1 can drive replication of the bacterial chromosome. The P1Cm lysogen but not the λ-miniP1 lysogen tolerated loss of *dnaA* gene function (EH3868), indicating that P1 possesses a *dnaA*-independent replicon in addition to the *dnaA*-dependent *oriR*. That this secondary replicon is *repA* independent is revealed by its insensitivity to an *incA* element cloned on pBR322 (30). Two lines of evidence show that it is unable to maintain P1 as a stable plasmid in *E. coli*. The P1 plasmid prophage is *incA* sensitive (56), and P1 does not lysogenize as a plasmid in *dnaA* null strains (Fig. 2). If the secondary replicon of P1 is active upon infection (e.g., prior to the establishment of immunity), it would promote chromosomal integration by homologous recombination between IS*1*s and thus account for the observed efficiency with which P1Cm lysogenizes a *dnaA* null strain (Table 2). The function of the *dnaA*- and *repA*-independent replicon in P1 remains to be determined. It is possibly a replicon used in vegetative replication of P1 since neither a *dnaA* null mutation nor the presence of a high-copy plasmid bearing *incA* significantly affects growth of P1 phage in the lytic cycle.

## DISCUSSION

Our reexamination of the *dnaA* dependence of P1 and F replicons that are organized similarly to the basic replicon of pSC101 shows that all three resemble bacterial *oriC* plasmids in having a clear *dnaA* requirement. Each of these replicons carries one or more presumptive binding sites for the DnaA protein. However, the possession of such sequences in an origin region does not correlate well with *dnaA* dependence. Both of the *dnaA* independent plasmids used in the experiments reported here, miniR1 and pBR322, possess a consensus dnaA box within 100 base pairs of the minimal replicon (61–63).

Table 3. Prevention of lethal replication from *oriR* of P1 by a *dnaA* null mutation

| repA plasmid | Relative yield of RepA protein | Frequencies with which *repA* plasmids transform λ-P1:*oriR*$^+$ *E. coli* | |
|---|---|---|---|
| | | *dnaA*$^+$ | Δ*dnaA* |
| pALA178Cm | 0.5 | 0.1 | 1 |
| pALA177Cm | 3.5 | <0.01 | 1 |
| pALA176Cm | 10 | <0.01 | 1 |
| pALA162Cm | 50 | 0.2–1.0 | 1 |

The frequencies of Cm$^R$ transformants obtained with a λ-P1:*oriR*$^+$ lysogen are normalized with respect to the frequencies obtained with the otherwise isogenic λ-P1:*oriR*$^-$ lysogen. The sources of RepA protein are Cm$^R$ derivatives of described plasmids (39). The yields of RepA protein generated from the parental (Cm$^S$) plasmids are normalized with respect to the yield generated from the intact basic replicon of λ-miniP1; data are from ref. 60. The plasmids were used to transform the two λ lysogens of CM1793 (*dnaA*$^+$) and the two λ lysogens of EH3827 (Δ*dnaA*). Both λ prophages carry a *repA::lacZ* protein fusion expressed from the autoregulated *repA* promoter. This fusion allows RepA activity in the transformants to be assayed as inhibition of β-galactosidase production. In *dnaA*$^-$, as well as *dnaA*$^+$ cells, the *repA* promoter was repressed to the same degree by each plasmid (pALA178Cm caused 80% repression, the other three caused >98% repression).

Dependence on *dnaA* is seen in miniRts1, a plasmid that is largely homologous to the miniP1 of Fig. 1 (64). We find that miniRts1 is highly unstable in a *dnaA* null strain, but, unlike miniP1 and miniF, is not totally replication defective (data not presented). Deletion of the tandem pair of dnaA boxes from the miniRts1 origin region lowers plasmid stability but does not eliminate the capacity for plasmid replication (Y. Itoh, Y. Kamio, and Y. Terawaki, personal communication). We speculate that plasmids bearing dnaA boxes have evolved from *oriC* plasmids and, in some cases, have lost dependence upon *dnaA*. MiniRts1 may represent a replicon in transition.

Evidence adduced here for a secondary replicon in P1 that is independent of *dnaA* suggests that P1, like F (65), has evolved by the union of disparate replicons.

**Note Added in Proof.** Since this manuscript was submitted, we have learned that B. C. Kline, T. Kogoma, J. E. Tam, and M. S. Shields (personal communication) have independent evidence that plasmid F maintenance requires the *E. coli dnaA* gene product.

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