JOURNAL OF BACTERIOLOGY, Aug. 1990, p. 4386–4391
0021-9193/90/084386-06$02.00/0

Vol. 172, No. 8

A Single DnaA Box Is Sufficient for Initiation from the P1 Plasmid Origin

ANN L. ABELES,* LUCRETIA D. REAVES, AND STUART J. AUSTIN

Laboratory of Chromosome Biology, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21701

Received 12 January 1990/Accepted 3 May 1990

The P1 plasmid replication origin requires the host DnaA protein for function. Two DnaA-binding boxes lie in tandem within the previously defined minimal origin, constituting its left boundary. Three more boxes lie 200 base pairs to the right of these, in the leader region for the P1 *repA* gene. We show that either set alone is active for origin function. One of the two origin boxes is relatively inactive. Constructs with just one of the five boxes are active for specific origin function as long as the box conforms exactly to the published consensus. This single consensus box is functional when placed either to the left or right of the core origin sequences. The flexibility shown by this system suggests that the boxes play a role different from those in the host *oriC* origin, where the number and position of boxes are critical.

The phage P1 lysogenizes its *Escherichia coli* host as an autonomous plasmid. Its copy number is as low as that of the bacterial chromosome (34). The plasmid is stable, showing that the control of replication is highly precise. Relatively few replicons are capable of such precise control. P1 and the host chromosome driven by the *oriC* replication system are prime examples. The mechanisms by which such replication systems are controlled are not well understood.

The minimal P1 replicon responsible for stringently controlled replication (the P1-R replicon) has been defined and studied in isolation (2). It consists of an origin of replication followed by the gene for the essential RepA protein and a copy-control locus, *incA*. A minimal P1 plasmid replication origin has been defined and consists of a maximum of some 254 base pairs (bp) (13) (Fig. 1). A central core region of the origin is distinguished by a group of five 7-bp direct repeats, including four with nested GATC sequences that are substrates for the *E. coli* DNA deoxyadenine methylase (19, 21). To its right is a group of five 19-bp repeats (2) that are specifically bound by the essential P1 replication initiator protein, RepA (5). The leftmost portion of the origin contains a tandem direct repeat of the putative DnaA recognition sequence (the DnaA box) (17). An additional region contains three closely apposed repeats that match or resemble DnaA boxes. It is present outside and to the right of the minimal functional origin. It lies in the untranslated leader region of the *repA* gene (20) (Fig. 1). Despite earlier indications to the contrary (14), DnaA protein is required for P1 plasmid replication, both in vivo and in vitro (20, 38). Thus P1, like several other plasmids (10, 20), requires DnaA, the *oriC* initiator protein, and its P1-specific initiator protein, RepA.

Several features of the P1 origin have counterparts in *oriC*, which also has repeated sequences, multiple sites for DNA-deoxyadenine methylation, and multiple DnaA boxes (17, 42). In *oriC*, the number and spacing of the four DnaA boxes are critical for function (9). Here, we construct mutant P1 origins with differing numbers and arrangements of boxes and test their effectiveness, both in vivo and in an in vitro replication system.

* Corresponding author.

MATERIALS AND METHODS

Buffers and other reagents. TE buffer and other buffers used for DNA preparation and electrophoresis were as described by Maniatis et al. (28) or Abeles et al. (5). Cells were grown in L broth (1) or L agar with 50 μg ampicillin per ml or 25 μg of spectinomycin per ml if needed. Restriction enzymes and ligase were obtained from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.), New England Bio-Labs, Inc. (Beverly, Mass.), or Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and used according to the instructions of the suppliers. Other materials were as previously described (1, 3, 5).

Bacterial strains. *E. coli* N100 (*recA13*) (16) was used for initial cloning and DNA preparation. BR825 (*polA*) (27) and BR827 [the *polA12*(Ts) of Monk and Kinross (31)], were used for the experiments testing the P1 *ori* function in *polA* hosts. JJ119 (26) and JM103 (29) were used to prepare the f1- and M13-based DNA, respectively, for in vitro replication.

Plasmids and cloning vectors. Plasmids pUC19 (39) and pSP102 (33) and vectors M13mp10 (30), M13mp11 (30), and $f1h_0$ (26) were as described previously. Plasmid pSP102 contains the P1 replicon, deleted for the *incA* region, ligated to the chloramphenicol resistance gene from Tn*9* (6). Plasmid pALA619 was derived from the pSC101-based vector pGB2 (15) as follows. The *Ssp*I-to-*Nru*I fragment of pALA69 (12), which encompasses the P1 *repA* open reading frame and an uncharacterized constitutive promoter in the pBR322 sequences, was inserted into the *Hin*cII site of pGB2 oriented such that the *Ssp*I site lay near the pGB2 *Hin*dIII site. Like its parent, pALA69 (8, 35), this plasmid was able to complement mini-P1 plasmids that were deleted for *repA* or carried the *repA103* amber mutation (data not shown).

The P1 *ori* clones shown in Table 2 were constructed as follows. The *Hin*dIII-to-*Eco*RI fragment of M13-P1 *ori* 49 (3) encompassing P1 *ori* was cloned into pUC19 to form pALA618. (In M13-P1 *ori* 49, the P1 sequence had been cut at the *Hin*dIII site at bp 606, filled in with DNA polymerase thus reproducing the P1 sequence through bp 610, and ligated to *Bam*HI linkers.) A double-stranded synthetic oligonucleotide encompassing the P1 *ori* sequence from bp 386 to 502 (with a *Hin*dIII site at the end at bp 386) was used to replace the *Hin*dIII-to-*Nru*I (P1 bp 502) region, thus forming pALA631. The oligonucleotide had a T-to-A muta-

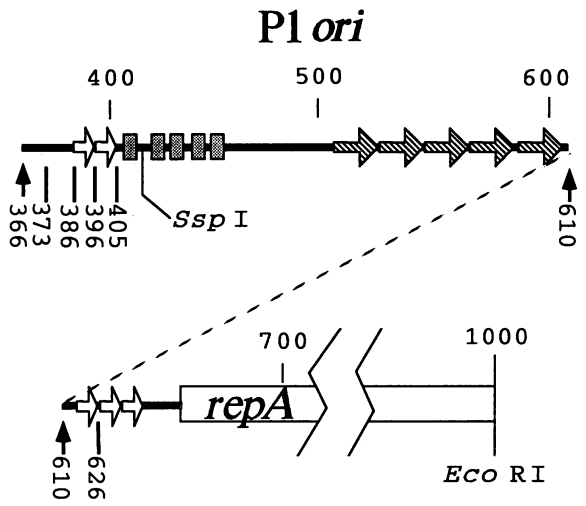

FIG. 1. Structure of the P1 R-origin region and adjacent portion of the *repA* gene. Base pair coordinates are conventional (2). Putative DnaA boxes are shown as open arrows. Five 7-bp repeats, four of which include GATC *dam* methylase sites, are shown as shaded boxes. The 19-bp repeats bound by RepA are shown by striped arrows. Part of the *repA* open reading frame is shown (open box).

tion at bp 419, creating an *Ssp*I site. This mutation was essentially silent for origin function (data not shown) and was present in all of the clones except pSP102. The other plasmids were made by cutting at the *Hin*dIII and *Ssp*I sites of pALA631 and ligating to suitable double-stranded synthetic oligonucleotides. The oligonucleotide used to construct pALA654 had a consensus 5′ TTATCCACA box, whereas the one for pALA645 had the wild-type sequence with its defective DnaA box, 5′ TTATCCACt. Plasmids pALA657 and pALA658 were made by replacing the *Nru*I-to-*Eco*RI fragments of pALA646 and pALA631, respectively, with the *Nru*I-to-*Eco*RI fragment (P1 bp 503 to 1000) of pALA33 (2) to bring in the downstream DnaA boxes. Plasmid pALA662 was made by ligating a double-stranded synthetic oligonucleotide between the *Hin*cII site (P1 bp 599) and the *Eco*RI site of pALA646. This oligonucleotide reproduced the natural P1 sequences from bp 599 through bp 626, except that the C and A at positions 623 and 624 were reversed to give a consensus box, 5′ TTATCCACA. The oligonucleotide terminated with the *Eco*RI sequence to facilitate cloning and produced a clone similar to pALA657 but deleted for all *repA* sequences downstream of the box. The P1 origin sequences of each pUC19 derivative were confirmed by DNA sequencing with Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) as recommended by the supplier.

For the in vitro assays (see Table 3), the relevant P1 *ori* regions were recloned into the M13 or f1 vectors as follows. The *Hin*dIII-*Eco*RI fragments of the pUC19-P1 clones (see Table 2) were excised and introduced into the homologous sites of M13mp10 (30) for the m-series templates. For the f series, the *Hin*dIII site of each clone was converted to *Eco*RI with a synthetic adapter, and the resulting *Eco*RI P1 *ori* fragment was introduced into the *Eco*RI site of the $f1h_0$ vector (26). Clone numbers relate to the parent pUC19-P1 clones; i.e., m655 is the M13mp10 clone from pALA655, etc. The double-stranded replicative forms were used as templates. The choice of vector had no measurable effect on the efficiency or specificity of the system (data not shown).

DNA preparation. Plasmid DNA and replicative-form DNA for the M13 and f1 clones were prepared as described (3, 28).

In vivo assay for P1 *ori* function. The pUC19 derivatives were introduced by transformation into a strain in which DNA polymerase I is temperature sensitive [*polA*(Ts12) (31)]. The strain also contained a compatible plasmid supplying the P1 RepA protein (pALA619) or a control plasmid (pGB2). Transformants were isolated and purified at 30°C, at which temperature the origin of pUC19 is functional (7). After a period of growth at 30°C in antibiotic medium selective for the retention of the test plasmid (ampicillin), the culture was diluted into antibiotic-free medium and shifted to 42°C, blocking pUC19 origin function (7, 25). Samples were withdrawn periodically during growth and tested for the proportion of cells retaining the plasmid by counting the number of cells able to form colonies at 30°C on agar with and without an antibiotic.

In vitro replication. In vitro replication was performed as described by Abeles and Austin (3), except that the reactions were incubated at 37°C for 20 min and purified DnaA protein was added where indicated. The reactions contained about 0.04 pmol of template DNA and, when present, 3 pmol of DnaA protein (gift from Jon Kaguni) and 6 pmol of purified RepA protein (1).

RESULTS

Assay for origin function. We have developed a convenient system to assay the function of the P1 plasmid origin in vivo. It allows us to manipulate and sequence the origin region in a vector that uses an alternative origin capable of driving the construct to high copy number. The activity of the P1 origin is then tested under conditions where the high-copy-number replicon is inactive.

The P1 origin or its mutant derivatives were cloned into the vector pUC19 (39). Using synthetic oligonucleotides, we constructed clones containing the previously defined minimal P1 origin with its two DnaA boxes and variants with deletions of different amounts of sequence. The pUC19 derivatives were introduced by transformation into a strain in which DNA polymerase I is temperature sensitive [*polA*(Ts12)]. The strain also contains a compatible plasmid supplying the P1 RepA protein (pALA619) or a control plasmid (pGB2). After growth under antibiotic selection at 30°C, the culture was diluted into antibiotic-free medium and shifted to 42°C. Under these conditions, pUC19 origin function is blocked (T. Brendler, manuscript in preparation), as this origin is dependent upon DNA polymerase I (25). Samples were withdrawn during growth and tested for the proportion of cells retaining the plasmid. Under these conditions, pUC19 was rapidly lost from the population (Fig. 2). Its loss was not influenced by the presence of either pGB2 or pALA619, which supplies RepA. Note that the starting population already contained cured cells. This reflects the low copy number of plasmids driven by the pUC origin in the *polA*(Ts12) strain at 30°C coupled with the random distribution of the copies to daughter cells (8). At 42°C, where pUC origin replication is blocked (7), the loss rate presumably reflects the dilution of the initial plasmid copies by growth. In contrast, the starting population containing a pUC19 clone with the minimal P1 origin (pALA655) had no plasmid-free cells and continued to maintain the plasmid for some considerable time at 42°C without selection (Fig. 2). Under selection, the cells grew well and maintained the plasmid indefinitely at 42°C (data not shown). The plasmid was not

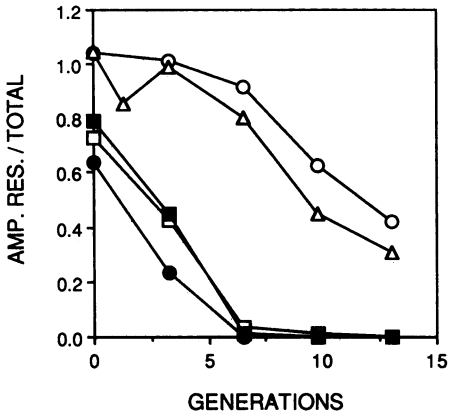

FIG. 2. Maintenance of P1 *ori* derivatives in a *polA*(Ts) host at 42°C. The vector pUC19 and various P1 *ori* plasmids derived from it (Table 2) were tested (see Materials and Methods). The ratios of viable colonies formed on plates with and without ampicillin are plotted against the number of generations of unselected growth at 42°C. Plasmids were relatively stable only when the P1 origin was present (pALA655, pALA631) and RepA was supplied. Removing the 38 bp that lie to the left of the first DnaA box had little or no effect (pALA631). Symbols: □, pUC19; ○, pALA655; △, pALA631 in BR827(pALA619); ■, pUC19; ●, pALA655 in BR827(pGB2); □, ○, and △, RepA supplied; ■ and ●, no RepA.

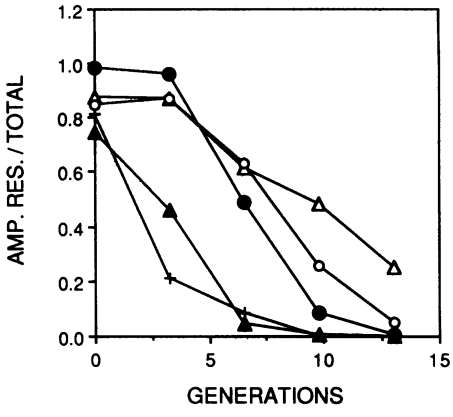

FIG. 3. Maintenance of P1 *ori* derivatives in a *polA*(Ts) host at 42°C. Deletions of the minimal origin removing one (pALA645) or both (pALA646) DnaA boxes blocked replication. Symbols: △, pALA631; ▲, pALA646; +, pALA645 (each with RepA supplied). If a single box matching the consensus was substituted for that in pALA645 (pALA654), some replication was restored. A single consensus box downstream of the origin (pALA662) was also active. Symbols: ●, pALA654; ○, pALA662 (each with RepA supplied).

maintained unless the RepA protein was supplied (Fig. 2), showing that the improved maintenance is due to P1 origin function. The gradual loss of the P1-driven plasmid without selection is presumably due to the random distribution of the plasmid at cell division. These clones have no plasmid partition system (4). After an initial dilution of the copies present at zero time, the rate of loss should reflect the average number of copies produced by the P1 origin per dividing cell (32). The data in Fig. 2 do not allow an accurate estimation of the copy number of the plasmid, but analysis of data from several repeat determinations suggests that P1 *ori* generates an average of five to nine copies per dividing cell under these conditions (data not shown).

Activity of deletion and single-box constructs. The test was repeated with the clones with deletion derivatives of the P1 origin. Deletion of sequences to the left of the DnaA boxes had only a minimal effect (pALA655 and pALA631) (Fig. 2). Deletion of the leftmost DnaA box had a very deleterious effect on the origin function (pALA645) (Fig. 3). Further deletion through the remaining box had little or no additional effect, suggesting that two boxes are required for origin function (pALA646). However, there is an alternative hypothesis. The leftmost box, TTATCCACA, matches the published consensus sequence, $TTAT^{C}/_{A}CA^{C}/_{A}A$ (17), but the second repeat differs by a single base (T) at the last position (Table 1). Thus, the right box could theoretically be defective and unable to support replication when the left box is deleted. In this case, the left box could be both essential and sufficient for activity. To test this, we constructed a pUC-P1 *ori* plasmid in which only a right-hand box was present but the sequence of the box was altered to that of the missing left box (pALA654). The construction with one good box functioned considerably better than its defective counterpart (pALA645), which differed by only a single base pair (Fig. 3). We conclude that, although perhaps not as effective as the wild-type arrangement of tandem boxes, a single box is sufficient for origin function as long as it conforms to the consensus.

The relative efficiencies of the mutant origins were confirmed independently by measuring the transformation frequencies in an unconditional *polA repA*$^{+}$ strain and its *repA* counterpart (Table 2). Mutants leaving the DnaA boxes intact transformed efficiently. The mutant with a single good box transformed nearly as well, but the mutant with one bad box gave only a few slow-growing colonies. The mutant with no boxes gave no transformants. All transformation was dependent upon the presence of RepA (Table 2).

In vitro activity of deletion and single-box constructs. The hierarchy of effectiveness of the mutant origins seen in vivo was also maintained when constructs containing them were used as templates for DNA synthesis in vitro (Table 3). The activity was always dependent upon the presence of exogenously added P1 RepA, as expected for authentic P1 origin function. The DnaA protein present in the cell extract was limiting, as shown by a general stimulation of activity, when purified DnaA protein was added (Table 3). However, the differences in template efficiency of the mutants was maintained under these conditions, showing that the effectiveness with which the limiting DnaA protein is utilized depends on the number and sequence of the boxes. Again, the construct with a single consensus DnaA box was clearly active, whereas those with only a bad box or none at all were not, confirming that a single box is both necessary and sufficient for initiation.

Role of a second group of DnaA boxes. Three potential

TABLE 1. DnaA boxes in the P1 *ori* region[a]

Location of boxes	5′ nucleotide	Sequence	3′ nucleotide
Upstream	387	TTATCCACA	395
	396	TTATCCACt	404
Downstream	618	TTATCacCA	626
	628	TTATAaAAA	636
	637	CTATCCACA	645

[a] The consensus sequence is $^{T}/_{C}TAT^{C}/_{A}CA^{C}/_{A}A$. Nonconsensus bases are in smaller type. This sequence is as published previously (17), except that C is permitted in first position (C. Schaefer and W. Messer, personal communication).

TABLE 2. Transformation efficiency in a *polA* host

Clone	P1 sequences present[a] (bp)	Transformants[b]	
		BR825(pGB2) (no RepA)	BR825(pALA619) (supplies RepA)
pUC19		0	0
pALA658	386–1000	0	2×10^6
pALA655	366–610	0	2×10^6
pALA631	386–610	0	4×10^6
pALA657	405–1000	0	4×10^6
pALA654	396–610[c]	0	1×10^6
pALA662	405–626[d]	0	1.5×10^6
pALA645	396–610[c]	0	50
pALA646	405–610	0	0
pSP102[e]	−228–1529	0.1×10^6	0.4×10^6

[a] See schematic diagram of origin (Fig. 1).
[b] Number of ampicillin-resistant transformants (at 30°C) per microgram of DNA of the *polA* strain BR825 (27) containing plasmid pALA619 (supplies P1 RepA) or the control plasmid, pGB2 (15).
[c] pALA654 has a single consensus DnaA box, 5′ TTATCCACA, whereas pALA645 has the wild-type sequence 5′ TTATCCACt at the same position.
[d] pALA662 has a single consensus DnaA box to the right of the core origin sequences (downstream position).
[e] pSP102 (33) contains a complete P1 replicon, including *repA* but deleted for *incA*.

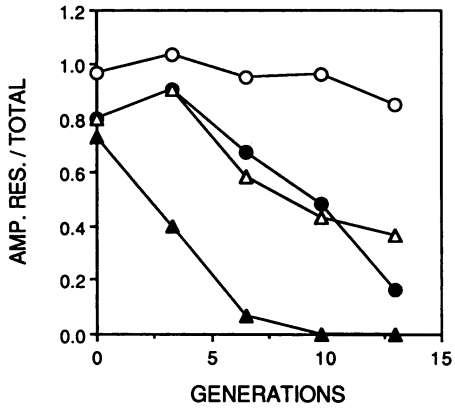

FIG. 4. Maintenance of P1 *ori*-containing plasmids in a *polA*(Ts) host at 42°C. Plasmids with upstream (pALA631) or downstream (pALA657) sets of boxes were equally effective, but one with both sets (pALA658) was better. Symbols: △, pALA631; ▲, pALA646; ○, pALA658; ●, pALA657 (each with RepA supplied).

DnaA boxes lie closely apposed in the RepA leader sequence outside the minimal origin. We will refer to these as the downstream boxes, as they lie downstream of the initiation point of the *repA* gene. One is a perfect match to a revised consensus, and the others differ by one and two base pairs, respectively (Table 1). Two explanations for the presence of these boxes seem reasonable. First, they could act as regulatory sequences, either modulating origin function or, more likely, modulating the expression of the *repA* gene of which they are a part. The DnaA box acts as a regulatory element in other systems, notably in the autoregulation of the *dnaA* gene (11, 37). Second, they could contribute to origin function directly. The latter would imply that this set of boxes and the origin set are at least partially redundant. To test this hypothesis, we constructed a pair of pUC19-P1 *ori* clones. One, pALA658 (Table 2), contained the downstream cluster of boxes plus the intact origin including its upstream pair of boxes. The other, pALA657, had the same sequences but lacked the upstream pair of boxes. The results of quantitative in vivo assays are given in Fig. 4, and transformation assays are given in Table 2. They show that the construct containing only the downstream cluster of boxes (pALA657) was as effective as the classical minimal origin containing only the upstream boxes (pALA631). Thus, although a regulatory role for the downstream cluster is not ruled out, it must be in addition to a direct role in origin function. Since the two sets of boxes showed redundancy, a second version of the minimal origin can be defined that excludes the upstream boxes and extends through the downstream sequences. This new minimal origin was fully RepA dependent (Table 2). Sequences within the original origin (bp 366 to 610) were still required; clones containing only the downstream region (bases 606 through 1856) but no origin failed to replicate (data not shown). As confirmed below, the new minimal origin makes use of the same core origin sequences for initiation as the original one, simply utilizing DnaA boxes that are differently placed. The data in Fig. 3 also allow us to redefine the original minimal origin to the 225-bp sequence from bp 386 to 610.

The construct with both clusters of boxes (pALA658) is more effective than either of the minimal origins (Fig. 4). Its low rate of loss suggests that a higher average copy number is maintained. Thus, the full wild-type complement of five boxes appears to make initiation more efficient.

Origin with a single consensus box to its right. Using oligonucleotide-directed sequence manipulation, we constructed a second origin with a single consensus box. In this construct (pALA662), the box 5′ TTATCCACA was placed to the right of the origin in the region normally occupied by the downstream box cluster. This box matched the good box of the upstream cluster, but had no exact counterpart in the wild-type downstream cluster (Table 1). There were no additional P1 sequences beyond the boundary of the box. The construct transformed the *polA* strain efficiently (Table 2), showing that the origin is active. The data in Fig. 3 confirm that pALA662 has origin activity and shows that, if anything, it is more active than pALA654, which has the same consensus box placed to the left of the origin. We conclude that a single consensus box is sufficient to allow initiation when placed either to the left or right of the core origin sequences and that no additional P1 sequences are necessary in either case.

TABLE 3. Effect of deletion mutations in vitro

Clone	P1 sequences present[a]	pmol of [^{3}H]dTTP[b]			
		Without DnaA[c]		With DnaA	
		RepA$^-$	RepA$^+$	RepA$^-$	RepA$^+$
M13mp10		4	5	6	6
f1h$_0$		4	3	6	7
m655	355–610	4	40	3	76
f631	386–610	4	38	4	61
m654	396–610[d]	4	12	4	37
f645	396–610[d]	4	6	4	10
f646	405–610	5	6	4	6

[a] The f and m series constructs are derived from f1h$_0$ and M13mp10 vectors, respectively (see Materials and Methods).
[b] In vitro replication was determined in a crude *E. coli* system (18) by using purified P1 RepA protein (1) and template DNA (see Materials and Methods).
[c] The crude extract contained a limiting amount of DnaA protein before any addition.
[d] f645 has the wild-type sequence with its defective DnaA box; m654 has a 5′ TTATCCACA consensus box in the same position.

DISCUSSION

Our results show that, although the DnaA box motif is essential for P1 origin function, such boxes can be placed either to the right or to the left of the core origin sequences. This result has a parallel in observations on the plasmid mini-Rts1 (22, 23). Mini-Rts1, although a member of a different incompatibility group than P1, has a similar replicon structure (24). Its origin is similarly organized with tandem DnaA boxes in roughly the same position as in the P1 origin (22, 24). Mini-Rts1 will replicate when both of these DnaA boxes are deleted, giving a plasmid with a somewhat lower copy number (23). The deleted plasmid still requires DnaA protein for replication. Itoh and co-workers point out that a sequence that lies just outside the origin in the control region for RepA initiator synthesis resembles a single DnaA box and might be the target for DnaA in the deletion mutant.

Our results show that a single DnaA box, as long as it conforms to the consensus, is necessary and sufficient to allow initiation at the P1 origin. Again, the placement of the box does not seem to be critical. With single-box constructs, the efficiency of initiation may be lower than that obtained when multiple boxes are present. However, the average copy number appears to be at least as high as that of the P1 wild type. Wild-type P1, unlike our origin constructs, has a strict negative feedback involving a distal locus (*incA*) that limits the copy number to one or two per unit cell (12, 33). Thus it is possible, although unproven, that a single box could support replication of an otherwise wild-type P1 plasmid with little detriment.

The observation that the position of boxes relative to the origin is not critical is an intriguing one. Constructions with only downstream boxes replicate as well as those with only upstream boxes, and the consensus upstream box can function in its original position, in a downstream position, or in the position normally occupied by its defective upstream neighbor. These observations contrast strongly with those on *oriC*, where deletion of just one of the four boxes inactivates the origin and where the precise positioning of the boxes is critical (9).

Bramhill and Kornberg (9) show that interaction of DnaA protein with the four boxes in *oriC* induces strand opening within the origin. They proposed that winding of the repeated DnaA boxes around a core of DnaA protein distorts the DNA, causing the strands to open nearby in a region that features three 13-bp tandem repeats. Evidence has also been presented for a direct role for DnaA in recognizing this 13-bp repeat unit (41). The DnaA protein may also play a role in directing the DnaB protein into the melted region to initiate primosome assembly and subsequent primer formation (10). The presence of multiple DnaA boxes in P1 *ori* originally suggested to us that they might play roles similar to those in *oriC*. However, Bramhill and Kornberg (10) have pointed out that the five direct repeats that bind the P1 RepA protein might play the role of a strand-opening system for the P1 origin. In this case, the requirement for DnaA for P1 origin function might reflect its participation in only a subset of the DnaA activities required for initiation at *oriC*. We believe that our present results provide strong support for this hypothesis for P1. Clearly, the DnaA box is a critical sequence for P1 origin function, but a single box can work and different positions of the DnaA boxes are effective. These facts argue against the obligate involvement of the P1 DnaA boxes in a complex, coiled structure that initiates the precise strand opening event as proposed for *oriC*. Rather, it is probable that the P1 boxes play a subsidiary role such as local capture of the protein for its subsequent binding to an origin primosome assembly region, probably the origin core region containing the short repeats and nested GATC sequences. In this case, the efficiency of delivery of DnaA to the origin might improve with increasing numbers of boxes, but the lack of requirement for a precise number or position of boxes could be explained.

An interesting parallel is exhibited by the *inc*FII plasmids. Here DnaA protein is not required but does facilitate replication (36). The origin of these plasmids contains a single DnaA box, and unlike in the *oriC* case, the ADP form of the protein is sufficient (40). Perhaps the action of DnaA at the P1 and *inc*FII origins is similar, except that in the P1 case the requirement is far more stringent. It would be interesting to determine whether the ADP form of DnaA is sufficient to promote initiation from the P1 origin or whether the ATP form is required, as it is for *oriC*.

ACKNOWLEDGMENTS

We thank Christof Schaefer for the suggestion that the second DnaA box with the final T might be inactive and Jon Kaguni for the gift of the DnaA protein. We also thank Julie Ratliff for preparation of the manuscript.

This research was sponsored by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with ABL.

ADDENDUM IN PROOF

S. Wickner, J. Hoskins, D. Chattoraj, and K. McKenney (J. Biol. Chem., in press) showed that P1 *ori* replicates in vitro with the ADP form of DnaA.

LITERATURE CITED

1. **Abeles, A.** 1986. P1 plasmid replication: Purification and DNA-binding activity of the replication protein RepA. J. Biol. Chem. **261**:3548–3555.
2. **Abeles, A., K. Snyder, and D. Chattoraj.** 1984. P1 plasmid replication: replicon structure. J. Mol. Biol. **173**:307–324.
3. **Abeles, A. L., and S. J. Austin.** 1987. P1 plasmid replication requires methylated DNA. EMBO J. **6**:3185–3189.
4. **Abeles, A. L., S. A. Friedman, and S. J. Austin.** 1985. Partition of unit-copy miniplasmids to daughter cells. III. The DNA sequence and functional organization of the P1 partition region. J. Mol. Biol. **185**:261–272.
5. **Abeles, A. L., L. D. Reaves, and S. J. Austin.** 1989. Protein-DNA interactions in the regulation of P1 plasmid replication. J. Bacteriol. **171**:43–52.
6. **Alton, N. K., and D. Vapnek.** 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. Nature (London) **282**:846–869.
7. **Austin, S., S. Friedman, and D. Ludtke.** 1986. Partition functions of unit-copy plasmids can stabilize the maintenance of plasmid pBR322 at low copy number. J. Bacteriol. **168**:1010–1013.
8. **Austin, S. J., R. J. Mural, D. K. Chattoraj, and A. L. Abeles.** 1985. *trans*- and *cis*-acting elements for the replication of P1 miniplasmids. J. Mol. Biol. **183**:195–202.
9. **Bramhill, D., and A. Kornberg.** 1988. Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. Cell **52**:743–755.
10. **Bramhill, D., and A. Kornberg.** 1988. A model for initiation at origin of DNA replication. Cell **54**:915–918.
11. **Braun, R. E., K. O'Day, and A. Wright.** 1985. Autoregulation of the DNA replication gene *dnaA* in *E. coli* K12. Cell **40**:159–169.
12. **Chattoraj, D., K. Cordes, and A. Abeles.** 1984. Plasmid replication: negative control by repeated DNA sequences. Proc. Natl. Acad. Sci. USA **81**:6456–6460.
13. **Chattoraj, D. K., K. M. Snyder, and A. Abeles.** 1985. P1 plasmid replication: multiple functions of RepA protein at the origin. Proc. Natl. Acad. Sci. USA **82**:2588–2592.

14. **Chesney, P. H., J. R. Scott, and D. Vapnek.** 1979. Integration of the plasmid prophages P1 and P7 into the chromosome of *Escherichia coli*. J. Mol. Biol. **130**:161–173.
15. **Churchward, G., D. Belin, and Y. Nagamine.** 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene **31**:165–171.
16. **Das, A., D. Court, and S. Adhya.** 1976. Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in transcription termination factor rho. Proc. Natl. Acad. Sci. USA **73**:1959–1963.
17. **Fuller, R. S., B. Funnell, and A. Kornberg.** 1984. The dnaA protein complex with the *E. coli* chromosome replication origin (*oriC*) and other DNA sites. Cell **38**:889–900.
18. **Fuller, R. S., J. M. Kaguni, and A. Kornberg.** 1981. Enzymatic replication of the origin of *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. USA **78**:7370–7374.
19. **Geiger, G. E., and P. Modrich.** 1979. Recognition sequence of the *dam* methylase of *Escherichia coli* K12 and mode of cleavage of *Dpn*I endonuclease. J. Biol. Chem. **254**:1408–1413.
20. **Hansen, E. B., and M. Yarmolinsky.** 1986. Host participation in plasmid maintenance: dependence upon dnaA of replicons derived from P1 and F. Proc. Natl. Acad. Sci. USA **83**:4423–4427.
21. **Hattman, S., J. E. Brooks, and M. Masurekar.** 1978. Sequence specificity of the P1 modification methylase (M. Eco PI and the DNA methylase M. Eco dam) controlled by the *Escherichia coli dam* gene. J. Mol. Biol. **126**:367–380.
22. **Itoh, Y., Y. Kamio, and Y. Terawaki.** 1987. Essential DNA sequence for the replication of Rts1. J. Bacteriol. **169**:1153–1160.
23. **Itoh, Y., and Y. Terawaki.** 1989. Replication properties of mini-Rts1 derivatives deleted for dnaA boxes in the replication origin. Plasmid **21**:242–246.
24. **Kamio, Y., A. Tabuchi, Y. Itoh, H. Katagiri, and Y. Terawaki.** 1984. Complete nucleotide sequence of a mini-Rts1 and its copy mutant. J. Bacteriol. **158**:307–312.
25. **Kingsbury, D., and D. Helinski.** 1970. DNA polymerase as a requirement for maintenance of bacterial plasmid colicinogenic factor-E1. Biochem. Biophys. Res. Commun. **41**:1538–1544.
26. **Lahue, R. S., S.-S. Su, and P. Modrich.** 1987. Requirements for d(GATC) sequences in *Escherichia coli MutHLS* mismatch correction. Proc. Natl. Acad. Sci. USA **84**:1482–1486.
27. **Ludtke, D. N., B. G. Eichorn, and S. J. Austin.** 1989. Plasmid-partition functions of the P7 prophage. J. Mol. Biol. **209**:393–406.
28. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
30. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest fragments. Gene **19**:269–276.
31. **Monk, M., and J. Kinross.** 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **109**:971–978.
32. **Nordstrom, K., and S. J. Austin.** 1989. Mechanisms that contribute to the stable segregation of plasmids. Annu. Rev. Genet. **23**:37–69.
33. **Pal, S., R. J. Mason, and D. K. Chattoraj.** 1986. Role of initiator titration in copy number control. J. Mol. Biol. **192**:275–285.
34. **Prentki, P., M. Chandler, and L. Caro.** 1977. Replication of the prophage P1 during the cell cycle of *Escherichia coli*. Mol. Gen. Genet. **152**:71–76.
35. **Swack, J. A., S. K. Pal, R. J. Mason, A. L. Abeles, and D. K. Chattoraj.** 1987. P1 plasmid replication: measurement of initiator protein concentration in vivo. J. Bacteriol. **169**:3737–3742.
36. **Tang, X. B., D. D. Womble, and R. H. Rownd.** 1989. DnaA protein is not essential for replication of IncFII plasmid NR1. J. Bacteriol. **171**:5290–5295.
37. **Wang, Q., and J. M. Kaguni.** 1987. Transcriptional repression of the *dnaA* gene of *Escherichia coli* by dnaA protein. Mol. Gen. Genet. **209**:518–525.
38. **Wickner, S. H., and D. K. Chattoraj.** 1987. Replication of mini-P1 plasmid DNA in vitro requires two initiation proteins encoded by the *repA* gene of phage P1 and the *dnaA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:3668–3672.
39. **Yanish-Peron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
40. **Yung, B. Y.-M., E. Crooke, and A. Kornberg.** 1990. Fate of the initiator protein in replication at the origin of the *Escherichia coli* chromosome in vitro. J. Biol. Chem. **265**:1282–1285.
41. **Yung, B. Y.-M., and A. Kornberg.** 1989. The dnaA initiator protein binds separate domains in the replication origin of *Escherichia coli*. J. Biol. Chem. **264**:6146–6150.
42. **Zyskind, J. W., J. M. Cleary, W. S. A. Bruilow, N. E. Harding, and D. W. Smith.** 1983. Chromosomal replication origin from the marine bacterium *Vibrio harveyi* functions in *Escherichia coli*: *oriC* consensus sequence. Proc. Natl. Acad. Sci. USA **80**:1164–1168.