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Partition of P1 Plasmids in *Escherichia coli mukB* Chromosomal Partition Mutants

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The partition system of the low-copy-number plasmid/prophage of bacteriophage P1 encodes two proteins, ParA and ParB, and contains a DNA site called *parS*. ParB and the *Escherichia coli* protein IHF bind to *parS* to form the partition complex, in which *parS* is wrapped around ParB and IHF in a precise three-dimensional conformation. Partition can be thought of as a positioning reaction; the plasmid-encoded components ensure that at least one copy of the plasmid is positioned within each new daughter cell. We have used an *E. coli* chromosomal partition mutant to test whether this positioning is mediated by direct plasmid-chromosomal attachment, for example, by pairing of the partition complex that forms at *parS* with a bacterial attachment site. The *E. coli* MukB protein is required for proper chromosomal positioning, so that *mukB* mutants generate some cells without chromosomes (anucleate cells) at each cell division. We analyzed the plasmid distribution in nucleate and anucleate *mukB* cells. We found that P1 plasmids are stable in *mukB* mutants and that they partition into both nucleate and anucleate cells. This indicates that the P1 partition complex is not used to pair plasmids with the host chromosome and that P1 plasmids must be responsible for their own proper cellular localization, presumably through host-plasmid protein-protein interactions.

The plasmid/prophage of bacteriophage P1 is lost from its bacterial host less than once per 10^4 generations (38). This exceptional stability is due to several maintenance systems (reviewed in reference 46). They include a stringently controlled replication system and the *lox*-Cre site-specific recombination system, which ensure that at least two copies are present at cell division; the partition system, which properly positions these copies in dividing cells; and the *phd-doc* addiction system (26), which impairs the growth of the occasional plasmid-less daughter cells that arise.

The P1 partition system consists of two plasmid-encoded genes, *parA* and *parB*, and a *cis*-acting site, *parS* (1). The only known host component of P1 plasmid partition is the *Escherichia coli* integration host factor (IHF), which assists ParB in forming a specific protein complex at *parS* (9, 16, 17). The assembly of the ParB-IHF partition complex at *parS* is thought to be an early step in partition. The P1 ParA protein is not required at this stage, and its direct role in partition is not understood.

The partition system is essential for plasmid stability. However, two different plasmids partitioned by the same *par* system are unable to coexist stably in the same cell, a phenomenon called incompatibility. Models for plasmid partition must explain both the stability and incompatibility properties of the *par* systems (reviewed in references 3, 22, and 45). One model proposes that plasmids pair with each other, and the paired complex is then positioned so that the cell division septum forms between them. Incompatibility results from formation of heterologous plasmid pairs. In a second model, plasmids attach directly to specific locations or sites within a dividing cell. These sites must be limited, that is, fewer than or equal to the number of plasmid copies, and must also flank the position of the new septum. Incompatibility would result from competition for these cellular sites. Both models predict that plasmid attachment is directed by the protein complex at their partition sites.

The organization of protein recognition sequences in *parS* and the biochemical properties of the P1 partition complex bear striking similarities to the bacteriophage λ *attP* site and to the intasome (*attP*-Int-IHF complex), respectively. At *parS*, IHF binds to and bends a sequence between two sets of ParB recognition sequences (10, 16, 17). At *attP*, IHF binds to and bends several sequences that are flanked by λ Int protein recognition sequences (37, 42). It is likely that the IHF-directed bends promote P1 ParB and λ Int to contact their multiple recognition sequences in specific three-dimensional conformations (18, 19, 28, 29, 35). These structures are substrates for subsequent steps in partition and λ integrative recombination, respectively.

We found it intriguing that the P1 partition complex biochemically and organizationally resembles the λ *attP* intasome, and we wondered whether this represents a functional similarity. During λ site-specific recombination, the protein complex at *attP* captures and pairs with a naked bacterial DNA site, *attB* (36). A similar pairing function of the P1 partition complex would advocate the second partition model described above: in this case, the specific intracellular location to which a plasmid attaches prior to cell division would be a DNA site on the bacterial chromosome. No recombination need occur, as long as pairing persists during cell division. (In fact, early experiments by Ikeda and Tomizawa (24) show that recombination into the host chromosome is an extremely rare event.) This model predicts that P1 can actively partition only into daughter cells that receive a bacterial chromosome.

We have used an *E. coli* partition mutant to examine the role of the bacterial chromosome in P1 partition. The *E. coli mukB* mutation makes cell growth very temperature sensitive and disrupts normal chromosomal partition at all temperatures. At 22°C (permissive for growth), about 5% of *mukB* mutant cells contain no bacterial chromosomes (are anucleate), whereas <0.3% of $mukB^+$ cells are anucleate (14, 23). It has been proposed that MukB is a motor protein that propels the chro-

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mosome to specific intracellular locations prior to cell division (22, 32, 33). We have tested whether P1 partition requires the MukB protein directly and whether P1 plasmids can partition properly into daughter cells that do not receive a bacterial chromosome at cell division.

E. coli mukB mutants have been used to show that partition of F plasmids does not depend on partition of the bacterial chromosome (14). The F plasmid partition system encodes two proteins, SopA and SopB, and a site, *sopC* (34). SopB binds to *sopC* to form the F partition complex. Short regions of similarity have been found between the sequences of SopA and ParA and between the sequences of SopB and ParB; however, the overall homology is poor (31, 45). The P1 and F systems are functionally distinct; their components can neither substitute for nor interfere with each other. In addition, the organization of SopB recognition sequences in *sopC* is very different from the organization of ParB recognition sequences in *parS* (1, 21) and IHF is not involved in F partition complex assembly. Although we suspect that P1 partition and F partition may be similar, these differences compelled us to test whether P1 partition complexes functionally resemble λ Int-IHF-*attP* complexes rather than F partition complexes. Our results show that P1, like F, does not partition by chromosomal attachment and argue that the P1 partition complex is used to promote pairing between P1 plasmids or to attach to other intracellular (protein and/or membrane, for example) sites.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strain DH5 (*endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1*) was used to construct and maintain all plasmids. *E. coli* SH7718 (*mukB*::Km^r λ^r λ*c*I857) and SH7714 (λ^r λ*c*I857) were kindly provided by S. Hiraga (14).

Media. LB medium and M9 minimal medium were prepared as previously described (39). M9/CT is M9 containing 0.2% Casamino Acids (Difco) and 50 μg of tryptophan per ml. Antibiotics, when used, were present at the following concentrations: chloramphenicol, 25 μg/ml; piperacillin, 20 μg/ml.

Construction of P1 miniplasmids. pLG44 (Fig. 1) was constructed as follows. The 7-kb *Kpn*I fragment from λ-P1:5R-3 (41) was cloned into the *Kpn*I site of pUC19. Next, a 1.9-kb *Pst*I fragment containing the *cat* gene from Tn*9* (from plasmid pST52 [40]) was cloned (by using synthetic linkers) in the *Sal*I site of the chimeric mini-P1–pUC19 plasmid. The mini-P1 and pUC19 replicons of the resulting plasmid were separated by digestion with *Sac*II and *Pst*I (sites in the pUC19 polylinker sequence), treatment with T4 DNA polymerase to create blunt ends, and circularization by ligation. Chloramphenicol-resistant plasmids (pLG44) were isolated following transformation of DH5 cells. The *par* mutant (pLG48) was created by cloning a 1.2-kb *Sal*I fragment containing a Tn*903* gene encoding kanamycin resistance (from pUC4-K; Pharmacia) into the *Xho*I site of the *parA* gene of pLG44. Finally, pLG49 resulted from the insertion of a 1.7-kb fragment containing *lacI* from pMC9 (7) as a *Bam*HI fragment into the pLG44 unique *Bam*HI site. λCm-P1:5R was constructed by cloning the above-described *cat* gene between the *Sal*I sites of λ-P1:5R-3 as described previously (15).

Isolation of total and anucleate cell samples. *E. coli* SH7718 cultures (200 ml) containing mini-P1 plasmids were grown in M9/CT medium at 22°C until the A_{600} was approximately 0.2. A 10-ml sample was taken (total cells) and processed as described below. The culture was shifted to a water bath at 42°C for 1 hour and then left at 37°C for 2 to 3 h. The cell debris and remaining cells were collected by centrifugation at 10,000 rpm in a Sorvall GS-A rotor for 20 min and resuspended in 30 ml of 0.84% NaCl. This mixture was filtered through coffee filters and Whatman no. 40 filters to remove most of the debris (filtered cells) and then processed as described below.

Analysis of DNA content. Cell samples were collected by centrifugation and resuspended in 1.1 ml of 0.84% NaCl. One milliliter of each cell sample was treated with DNase I and then lysed as described by Ezaki et al. (14). Genomic DNA was extracted once with phenol, twice with phenol-$CHCl_3$ (1:1), and once with $CHCl_3$ and then precipitated with ethanol. The precipitate was washed twice with 70% ethanol and then resuspended in 150 μl of 10 mM Tris HCl (pH 8)–1 mM Na_2EDTA containing 15 μg of RNase A and incubated for at least 24 h at 4°C.

DNA samples were cut with appropriate restriction endonucleases (see below), separated by agarose gel electrophoresis, transferred by capillary action and cross-linked to hybridization membranes (Amersham Hybond N), and hybridized to various ^{32}P-labeled DNA probes as previously described (Southern hybridization analysis [39]). The radioactive probes were purified DNA restriction fragments primed with random sequence dN_6 primers and labeled with [α-^{32}P]dATP and DNA polymerase I large fragment (39). The radioactive filters were washed at high stringency and exposed to film and to storage phosphor screens for quantification.

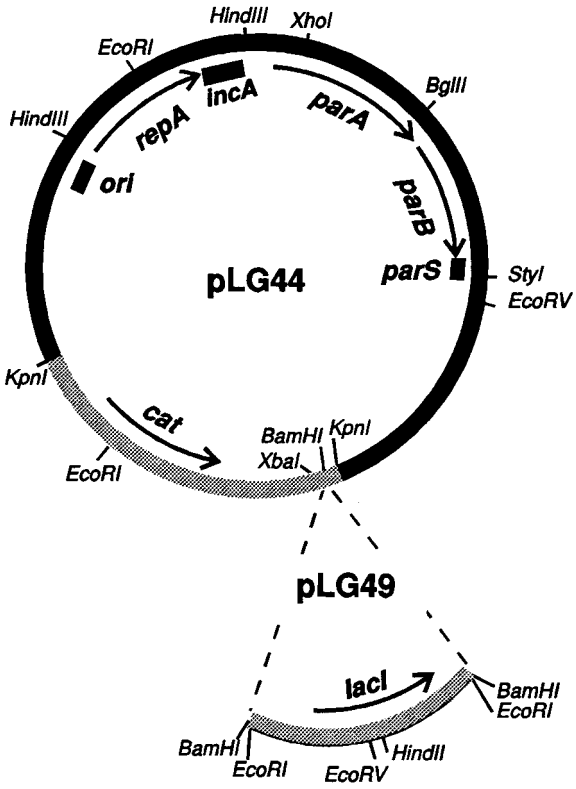


FIG. 1. Map of mini-P1 plasmid pLG44. The positions of the replication region (*ori*, *repA*, and *incA* [2]), the partition region (*parA*, *parB*, and *parS* [1]), and the chloramphenicol resistance gene (*cat*) are indicated. On the plasmid circle, black represents P1 DNA and grey is non-P1 DNA (Tn*903*, *E. coli*, and pUC19 polylinker sequences). The *lacI* fragment was inserted into the *Bam*HI site of pLG44 to create pLG49 as shown. The sizes of pLG44 and pLG49 are 8.9 and 10.6 kb, respectively.

The plasmids used as sources for chromosomal probes were pMC9 for *lacI* (7), pRPG18 for *trpR* (20), and pDX1 for *purB* (a gift from Donghong Xu). pBEF101 (15) was the source of P1 *par* DNA for the plasmid-specific probes.

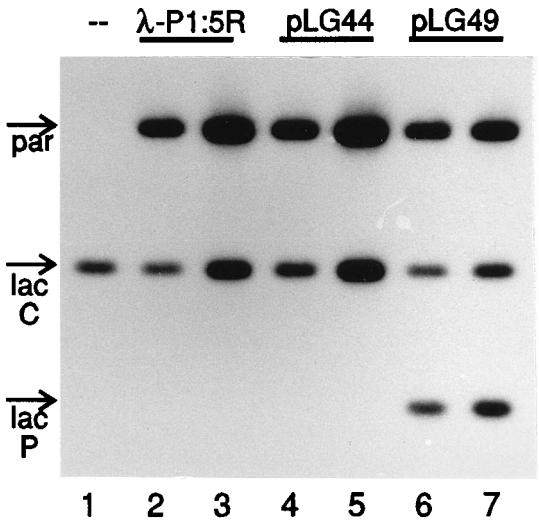
Copy number determinations. Cells containing the mini-P1 plasmids were grown in M9/CT until the A_{600} was between 0.2 and 0.4 and then chilled on ice. Three A_{600} equivalents were collected by centrifugation, the cells were lysed, and DNA was collected as previously described (39). DNA was analyzed by Southern hybridization as described above.

Quantification. The radioactivity from the DNA bands was measured with a Molecular Dynamics PhosphorImager. The P/C ratio, or the ratio of plasmid to chromosomal hybridizing sequences, was calculated as the radioactivity of the plasmid band divided by the radioactivity of the chromosomal band in each gel lane. This does not represent the absolute ratio of plasmids to chromosomes because the probes had different specific activities (except pLG49 probed for *lac*). The relative number of plasmids to chromosomes was calculated as the P/C ratio of one plasmid or culture divided by the P/C ratio of the reference plasmid or culture.

Plasmid stability assays (18). Cells with mini-P1 plasmids were grown overnight in M9/CT with chloramphenicol. The culture was diluted at least 1,000-fold in M9/CT (no drug) and grown for approximately 18 generations. Samples were plated onto LB plates before and after the growth period in nonselective medium. Subsequent colonies were transferred with toothpicks to LB plates containing chloramphenicol to test for the presence of the plasmid.

RESULTS

The *E. coli mukB* mutation does not affect the copy number or stable maintenance of P1 plasmids. *E. coli* SH7718 (14) contains a *mukB* null chromosomal partition mutation, which we used to determine whether the bacterial MukB protein



Plasmid	Copy number relative to λ-P1:5R	Copy number relative to *E. coli*
λ-P1:5R	(1.0)	–
pLG44	0.9 ± 0.1	–
pLG49	1.0 ± 0.1	1.2 ± 0.1

FIG. 2. Determination of pLG44, λCm-P1:5R, and pLG49 copy numbers in DH5. DNA was isolated from DH5 (lane 1), DH5(λCm-P1:5R) (lanes 2 and 3), DH5(pLG44) (lanes 4 and 5), and DH5(pLG49) (lanes 6 and 7); digested with *Eco*RI and *Eco*RV; and analyzed by Southern hybridization as described in Materials and Methods. The amount of DNA in lanes 3, 5, and 7 is twice that in lanes 2, 4, and 6. The plasmid *par* probe was the 1-kb P1 *Xho*I-*Bgl*II fragment (Fig. 1), and the chromosomal *lac* probe was a 789-bp *Hin*dII-*Eco*RI *lacI* fragment from pMC9 (7). On the autoradiogram, arrows indicate the positions of the plasmid bands hybridizing to the *par* and *lac* probes and are designated par and lac P, respectively. Their sizes are 3.5 and 0.85 kb, respectively. The chromosomal band (1.5 kb) that hybridizes to the *lac* probe is designated lac C. The P/C ratio, i.e., the ratio of plasmid to chromosomal hybridizing sequences, was calculated for each gel lane (see Materials and Methods) and averaged for each plasmid sample. The pLG44 or pLG49 copy number relative to that of λ-P1:5R is the respective P/C ratio divided by the λ-P1:5R P/C ratio calculated from the *par* band and the chromosomal *lac* band. The copy number of pLG49 relative to that of the *E. coli* chromosome is the pLG49 P/C ratio calculated from the plasmid and chromosomal *lac* bands.

plays a role directly or indirectly, via its effect on chromosomal partition, in P1 plasmid partition. To study plasmid maintenance, we routinely use the P1-derived plasmid λ-P1:5R, which contains essentially only the P1 replication (*rep*) and partition (*par*) functions in an integration-deficient λ vector (41). However, for these studies, we constructed mini-P1 plasmid pLG44 (Fig. 1) because the presence of λ sequences prohibits the use of λ-P1:5R in SH7718 (this strain is λ^r, and induction of a λ*c*I857 prophage was used to kill cells with chromosomes [14]; see below). As measured by Southern hybridization, the pLG44 copy number was the same as that of λ-P1:5R (Fig. 2). We then measured the copy number of pLG44 in *mukB* null mutant strain SH7718 relative to the copy number in isogenic *mukB*$^+$ strain SH7714. Southern blots of DNAs from these two strains showed no difference in the ratio of pLG44 to the chromosome (Fig. 3). We probed Southern blots with chromosomal probes from three different regions of the chromosome

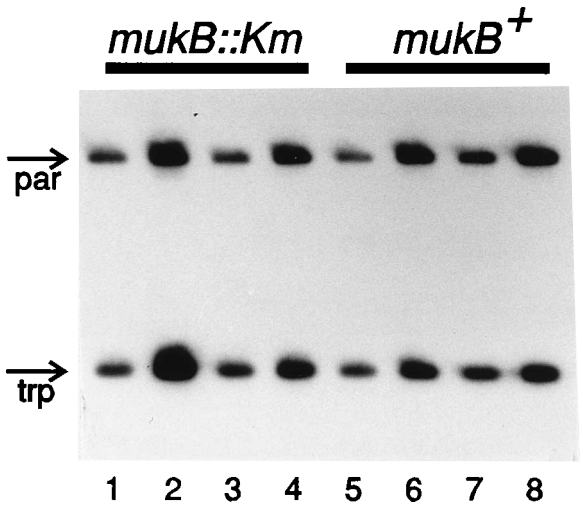


FIG. 3. Southern blot of genomic DNAs from *E. coli* SH7718 (*mukB*::Km^r) and SH7714 (*mukB*$^+$) cells containing pLG44 probed for chromosomal (*trp*) and plasmid (*par*) sequences. In this blot, DNAs from two cultures of each strain (from independent pLG44 transformants) were digested with *Bam*HI and *Hin*dIII. The chromosomal probe was a 1.2-kb *Bam*HI fragment from pRPG18 (20) containing the *E. coli trpR* gene, and the plasmid probe was a 1.5-kb *Eco*RI-*Sal*I fragment from pBEF101 (15) containing the P1 *parA* gene. On the autoradiogram, arrows indicate the positions of the plasmid band (4.3 kb) hybridizing to the *par* probe and the chromosomal band (1.2 kb) hybridizing to the *trp* probe. Lanes: 1 and 2, SH7718 culture 1; 3 and 4, SH7718 culture 2; 5 and 6, SH7714 culture 1; 7 and 8, SH7714 culture 2. The amount of DNA in lanes 2, 4, 6, and 8 was about twice that in lanes 1, 3, 5, and 7, respectively.

(*lacI* at 8 min, *purB* at 25 min [data not shown], and *trpR* [Fig. 3] at 100 min [5]). The average ratio of the pLG44 copy number in SH7718 (*mukB*::Km^r) to that in SH7714 (*mukB*$^+$) was 1.0 ± 0.1.

Next, we constructed a *parA* mutant version of pLG44, called pLG48 (see Materials and Methods). Measured in our standard Rec$^-$ host, DH5, pLG44 was very stable but *par* mutant pLG48 was unstable (Table 1). Therefore, pLG44 stability is dependent on *par*. We then tested plasmid stability in *E. coli* SH7718. Again, pLG44 was stable and this stability was dependent on *par* (Table 1). Therefore, P1 partition occurs in the absence of the MukB protein. There was a slight difference in plasmid stability when DH5 was compared with SH7718, probably because SH7718 is Rec$^+$. Mini-P1 plasmids are less stable in Rec$^+$ than in Rec$^-$ strains because the plasmids lack the *lox*-Cre site-specific recombination system that resolves plasmid dimers, which can arise from homologous recombination (4). Plasmid stability assays measured the plasmid content of viable cells, that is, cells that contained a host chromosome. The results demonstrated that P1 plasmids do not require the

TABLE 1. Stability of mini-P1 plasmids

E. coli strain	Mini-P1 plasmid	*par*	% Retention of mini-P1[a]
DH5	pLG44	+	>99
DH5	pLG48	–	20
SH7718	pLG44	+	98
SH7718	pLG48	–	<5

[a] Stabilities of pLG44 and pLG48 were measured after approximately 18 generations of growth in M9/CT medium. DH5 cultures were tested at 37°C, and SH7718 cultures were tested at 22°C. pLG48 contains a *parA* null mutation (see Materials and Methods).

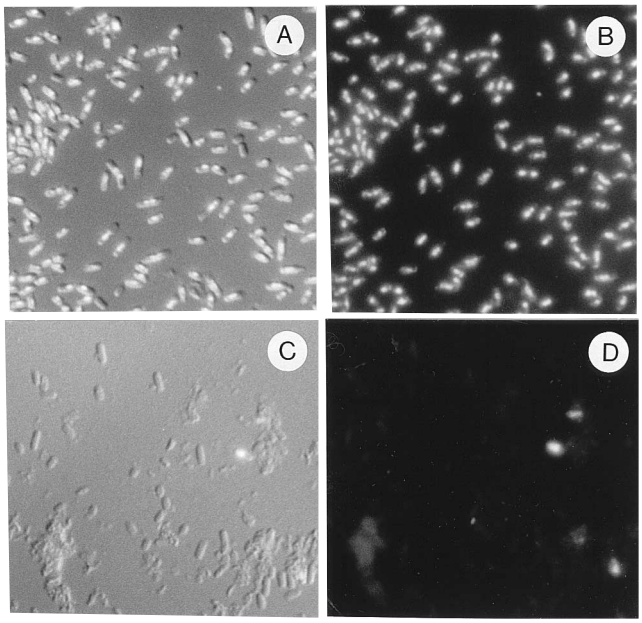


FIG. 4. Microscopy of total (A and B) and filtered (C and D) samples of SH7718(pLG49) cells. Cells were prepared for microscopy, stained with 4′,6-diamidino-2-phenylindole (DAPI, a fluorescent nucleoid stain) essentially as described by Hiraga et al. (23), and examined in a Nikon Microphot FX-A microscope by using a combination of Nomarski optics and fluorescence (A and C) or fluorescence only (B and D). SH7718 cultures (total cells [A and B]) contained about 5% anucleate cells. The presence of mini-P1 had no detectable effect on this frequency. This preparation of filtered cells (C and D) contained 95% anucleate cells. Bar, 5 μm.

MukB protein directly (independently of the host chromosome) for stability. We concluded that pLG44 was Rep^+ and Par^+ and that P1 stability was *par* dependent in *mukB* mutant cells. If pairing does occur between P1 and bacterial chromosomes, every bacterial attachment site must be occupied by a P1 plasmid.

P1 plasmid DNA segregates into anucleate cells. We measured the segregation of P1 plasmids into anucleate cells to determine whether P1 partition requires the MukB protein indirectly via MukB's role in chromosomal partition. The observation that P1 partitions only with the chromosome would support (although not prove) the idea that P1 specifically pairs with the bacterial chromosome. For unit copy plasmids, this model predicts that cells that do not inherit a bacterial chromosome also will not inherit a plasmid chromosome. Alternatively, partition into anucleate cells would show that plasmid-chromosome pairing or attachment cannot be required for plasmid maintenance.

We used *E. coli* SH7718 to isolate anucleate cells and examine their DNA content. This strain is λ^r and contains an integrated λ*c*I857 prophage. Heat induction of λ kills only cells with chromosomes, and released phage cannot reinfect any cells (14). SH7718 cells containing pLG44 were grown to mid-log phase and then heated to induce killing and lysis. The mixture of anucleate cells and cell debris was concentrated and filtered to remove most of the debris. Samples were taken before heat induction (total cells) and after filtration (filtered cells). They were examined by microscopy (Fig. 4) and lysed to extract DNA. The ratio of chromosomal to plasmid DNA was measured by Southern hybridization of radioactive DNA probes to total cellular DNA after digestion with restriction endonucleases (Fig. 5). The filtered sample contained a much higher ratio of plasmid to chromosomal sequences than did the total cell population (Fig. 5 and Table 2). We used various chromosomal probes from different *E. coli* map locations to measure this ratio and obtained similar results. In addition, the enrichment of plasmid sequences depended on the fraction of nucleate cells in the filtered sample, as expected since the nucleate cells supply the chromosomal sequences. In similar experiments with F plasmids, Ezaki et al. found a similar correspondence of plasmid enrichment and frequency of cells with nucleoids in the filtered cell sample, and our data fit the theoretical curve calculated for unit copy plasmid partition into anucleate cells (14). They also used a cell division inhibitor, furazlocillin, to produce long filamentous nucleate cells and normal-size anucleate cells prior to cell lysis. When we used piperacillin (whose action is similar to that of furazlocillin [6]) to inhibit cell division, a similar increase in the ratio of plasmid sequences to chromosomal sequences was observed (Table 2).

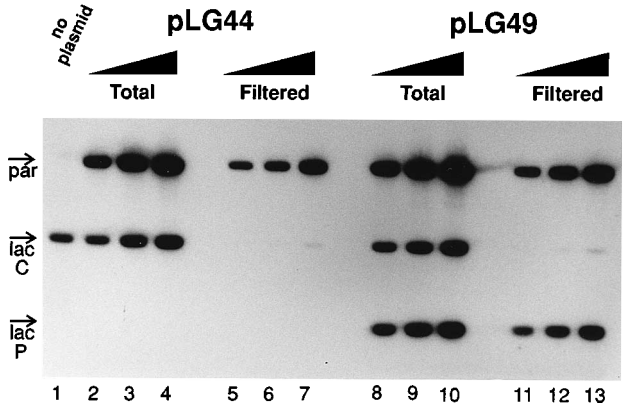


FIG. 5. Southern blot of cellular DNAs isolated from total and filtered cell populations. DNAs from SH7718 cells (no plasmid; lane 1), from SH7718(pLG44) cells before (total; lanes 2 to 4) and after (filtered; lanes 5 to 7) isolation of anucleate cells; and from SH7718(pLG49) cells before (total; lanes 8 to 10) and after (filtered; lanes 11 to 13) isolation of anucleate cells were digested with *Eco*RI and *Eco*RV and analyzed by Southern hybridization to the *par* and *lac* probes as described in the legend to Fig. 2. The ramp above the lanes represents increasing amounts of each DNA sample. The positions of the plasmid *par* (par) and *lacI* (lac P) fragments and of the chromosomal *lacI* (lac C) fragment are indicated on the left.

TABLE 2. Ratios of plasmid DNA contents in filtered and total cell populations

Expt no.	Mini-P1 plasmid	Piperacillin treatment[a]	Enrichment of plasmid DNA in filtered vs total samples[b]	% Nucleate cells in filtered sample
1	pLG44	+	8 ± 1	14
2	pLG44	−	15 ± 2	7
3[c]	pLG44	−	12 ± 2	6
3[c]	pLG49	−	14 ± 2	5

[a] Where indicated, cells were treated with piperacillin for 4 h prior to heat induction. The ratio of plasmid to chromosomal sequences was not changed by this treatment (data not shown).

[b] The DNAs were analyzed on Southern blots which had been probed with both plasmid- and chromosome-specific probes. The P/C ratio (see Materials and Methods) was averaged for each sample (total P/C and filtered P/C). Plasmid enrichment is the filtered P/C divided by the total P/C. Each value was derived from at least two different blots. The chromosomal probes used were from the *lacI* (8 min), *purB* (25 min), and *trpR* (100 min) regions of the chromosome (5). There was no significant change in the ratio when different probes were used.

[c] Quantification of the experiment shown in Fig. 5.

From our results, we concluded that P1 segregates into anucleate cells.

The data rule out the possibility that all copies of P1 plasmids segregate by attachment to the bacterial chromosome. However, it is important for this analysis that the number of copies of P1 is similar to the number of bacterial chromosomes. Extra copies could segregate randomly, and some of these should end up in anucleate cells. Previous studies have shown that the copy number of P1 prophage is about the same as that of the host chromosome (24, 41), implying that there are no other copies. We thought it essential to confirm this conclusion for pLG44. We cloned a chromosomal sequence, the *lacI* gene, into pLG44, creating pLG49 (Fig. 1). The same radioactive probe, a DNA restriction fragment that was internal to *lacI*, was used to measure the copy number of pLG49 with respect to that of the host chromosome (the ratio of plasmid *lacI* to chromosomal *lacI* sequences). We also probed for *par* sequences to compare the copy numbers of pLG49, λ-P1:5R, and pLG44. In DH5 cells, the copy numbers of all plasmids were similar to each other and to that of the host chromosome (Fig. 2).

We then repeated the isolation of anucleate cells from an *E. coli* SH7718 population containing pLG49. Again, P1 plasmid DNA was found to be enriched in the anucleate population (Fig. 5 and Table 2). The copy number of pLG49 in SH7718 relative to that of the host chromosome was 1.3 ± 0.2. We think it very unlikely that the small excess of plasmid copies accounts for the P1 DNA found in anucleate cells. In this experiment (Fig. 5), we estimated the amount of plasmid DNA in anucleate cells (compared to that in the total population) by assuming 100% recovery of anucleate cells (5% of total SH7718 cells are anucleate and thus resistant to the heat treatment). The copy number of pLG44 in the filtered sample with respect to the total sample was 0.8, and that of pLG49 was 1.0. If the recovery of anucleate cells were less than that of total cells, the values would be higher. Although we cannot be sure that recovery was quantitative, especially after the filtration steps, these numbers are consistent with similar plasmid copy numbers in anucleate and nucleate cells. Therefore, any small excess of plasmid to chromosomal copies most likely cannot account for the plasmid DNA recovered in the anucleate population. We conclude that P1 plasmids are actively partitioned into anucleate cells and do not depend on proper chromosomal segregation for their stability.

DISCUSSION

P1 plasmids encode two proteins, ParA and ParB, and contain a DNA site, *parS*, all of which are required for proper positioning prior to cell division (1). Our study addressed the question of whether P1 uses these components to associate with the bacterial chromosome or with the bacterial MukB partition protein. Our experiments indicate that neither association can account for plasmid stability. First, mini-P1 plasmids were Par^+ in the absence of the MukB protein. Mini-P1 plasmids were stable in populations of *E. coli mukB* null mutants as measured in cells that contained bacterial chromosomes (viable cells) (Table 1). Therefore, P1 partition does not require MukB directly or indirectly through possible MukB effects on the expression of other proteins.

Second, our results show that P1 plasmids partition into cells that do not receive a host chromosome (anucleate cells). Copy number measurements indicated that our mini-P1 plasmids are essentially unit copy (Fig. 2 and 5), which agrees well with previous measurements of P1 prophage and λ-P1:5R copy numbers (24, 41). Since (at least) one plasmid per chromosome must be actively partitioned into nucleate cells to account for P1 stability (Table 1), there are very few extra copies that could be randomly distributed into anucleate cells. Our data agree well with a theoretical curve calculated for partition of unit copy plasmids into anucleate cells (Fig. 4 in reference 14). We conclude, therefore, that P1 partition into anucleate cells was active, and consequently P1 plasmids do not partition via physical pairing or attachment to the host chromosome.

Our conclusions are supported by experiments with minicell-producing mutants of *E. coli* which argue against any significant random segregation of P1 Par^+ plasmids. *E. coli minB* mutants are defective in proper placement of the cell division septum (8, 12). These mutants produce small minicells without chromosomes because the septum forms close to the poles rather than at the center of the cell. Analyses of the plasmid DNA content of minicells have shown that F and P1 plasmids are depleted rather than enriched in minicells (13, 25). If the P1 plasmids in anucleate cells were only a result of random distribution, one would expect to see an enrichment of P1 DNA in minicells similar to that observed in anucleate cells (i.e., regardless of the position of the septum). In addition, these data suggest that the actively segregated P1 plasmids are normally positioned in the center half of the cell (closer to the normal septum site) rather than nearer to the poles.

Therefore, the similarities between the λ Int-IHF complex at *attP* and the partition complex at *parS* do not represent a common bacterial pairing function. Why have these similarities evolved? One possibility is that the important common element is IHF, for example, that both episomes (λ and P1) use IHF as some kind of physiological sensor. We suspect that the higher-order nucleoprotein complexes assembled at these sites share some type of functional similarity, but this has not been confirmed.

The results reported here are consistent with data from similar experiments that measured the partition of F plasmids into anucleate cells (14) and provide further evidence that the maintenance functions of F and P1 are similar. The two *par* systems are organized analogously. Both F SopB and P1 ParB bind to their respective *par* sites (9, 16, 30, 43). F SopA and P1 ParA are ATPases (11, 44). Although the regions of similarity between these proteins are limited, a variety of plasmid proteins that share these similarities have been identified (27, 31, 45). The result that two plasmids, F and P1, find their cellular location independently of the location of the bacterial chromosome suggests that this feature is common to the other plasmid partition systems.

The test of the partition model described here for P1 and previously for F (14) has important implications for the study of partition in P1, F, and similar plasmids. A discovery that P1 partitions by chromosomal attachment would have demanded efforts to identify the chromosomal *att* site. Since this is not the case, we deduce that plasmid positioning is mediated by protein-protein interactions between plasmid components and between plasmid and host factors. The definition of such protein-protein interactions is essential to our understanding of the mechanism of plasmid partition.

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