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Biochemistry

Participation of *Escherichia coli* integration host factor in the P1 plasmid partition system

(ParB protein/*parS*/segregation/centromere/replication)

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ABSTRACT **Stable maintenance of the plasmid prophage of bacteriophage P1 requires the P1 ParB protein, which acts on a DNA site termed *parS*. Fractionation of extracts from *Escherichia coli* cells overproducing ParB revealed that a host factor, in addition to ParB, is required to observe maximal binding to *parS*, as detected by a nitrocellulose filter retention assay. Two observations indicated that this factor is *E. coli* integration host factor (IHF): purified IHF substituted specifically for host factor from a crude lysate, and lysates prepared from cells deficient in the β subunit of IHF (*E. coli hip* mutants; also called *himD*) contained no host factor activity. Binding studies *in vitro* and competition experiments *in vivo* suggest that two types of ParB–*parS* DNA complexes can exist that differ in (*i*) the presence of IHF, (*ii*) the amount of *parS* sequence with which the proteins interact, and (*iii*) the specificity of their participation in partition. Under normal conditions, with the intact P1 partition region and wild-type bacteria, P1 plasmids apparently use IHF to assist ParB in the assembly of a functional partition complex at *parS*.**

The partition systems of low-copy-number plasmids, such as the P1 prophage, help to ensure that every newborn cell receives at least one plasmid copy at cell division. The P1 partition region, *par*, encodes two proteins, ParA and ParB, and contains a cis-acting site, *parS* (1). Another low-copy-number plasmid, the sex factor F, possesses a similarly organized *par* region (2). Although the two systems do not functionally interact, they probably utilize similar mechanisms to accomplish partition. It has been proposed that one or both plasmid proteins recognize and bind to the *par* site (a centromere analog), that this complex either alone or with other proteins results in plasmid pairing, and that the paired complex attaches to the host partition apparatus at the nascent septum of the dividing cell (2, 3).

Initial analysis of P1 plasmid segregation has focused on the plasmid-encoded functions contained within a 2.6-kilobase segment of DNA (1, 3–5). Recent *in vivo* experiments from this laboratory, using P1-derived plasmids, indicated that ParB recognizes *parS* (4). ParB, when produced in excess, specifically destabilizes both low- and high-copy-number plasmids containing *parS*. The destabilization does not require ParA and only affects plasmids with *parS*—evidence that ParB can recognize and bind to this site. The interpretation of the results is that excess ParB aggregates with several molecules of *parS* plasmid DNA, and random distribution of such structures explains the severity of the segregation defect (4).

The participation of host functions in the P1 partition process has been unexplored. In the course of studying the interaction of ParB with *parS*, it was discovered that a host factor, in addition to ParB, contributes to *parS*-specific DNA binding activity. The experiments described here demonstrate that the integration host factor (IHF) of *Escherichia coli* is a component of the P1 partition system.

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MATERIALS AND METHODS

Bacterial Strains. *E. coli* K-12 strains were M5219 [containing the defective prophage λ*bio*252 *c*I857 Δ*H*1 (6)], MC4100 (7), and DH5 (*recA1*) (Bethesda Research Laboratories). DH5 and MC4100 were transduced to *hip* (gene encoding β subunit of IHF; also called *himD*) by P1 transduction using a P1 *vir* lysate grown on the strain RW2014 {Δ3[*hip*] (8); a gift from R. Weisberg, National Institute of Child Health and Human Development}, which lacks part of the *hip* gene, replaced by a transposon chloramphenicol-resistance gene, *cat* (8). DH5 was first made *rec*$^+$ by lysogenization with λ*precA*, λ*c*I857 carrying the *recA* gene (9), followed by isolation of chloramphenicol- and heat-resistant transductants (cured of λ and the *recA* gene). Chloramphenicol-resistant transductants of DH5 and MC4100 were defective for phage λ integration and φ80 growth, confirming the presence of the Δ3[*hip*] *cat* allele (8).

Phage and Plasmids. All cloning procedures and reagents have been described (4). λkan-miniP1 is a kanamycin-resistant derivative of λ-P1:5R-3 (4, 10). The P1 *parB* gene, from the *Bgl* II to *Dra* I (changed to *Sal* I) sites of P1 *par* (1), was inserted into the *Bam*HI–*Sal* I sites of the vector pPLc2819 (11) to give the plasmid pBEF105. Plasmids containing *parS* were pALA207 (1) and pBEF127; plasmid pBEF127 was constructed by insertion of a 925-base-pair (bp) *Dra* I (changed to *Bam*HI)–*Bam*HI fragment from pALA270 (1) into pBR322. Finally, insertion of the kanamycin-resistance gene from Tn*903* into the *Bam*HI site of pALA318 (12) yielded pALA318kan.

Reagents and Buffers. Bio-Rex-70 resin (100–200 mesh) and Bradford concentrated dye were from Bio-Rad. *S*-Adenosyl-L-[*methyl*-^{3}H]methionine (80 Ci/mmol; 1 Ci = 37 GBq) was from Amersham. Buffer A is 50 mM Tris·HCl, pH 7.5/100 mM NaCl/0.1 mM EDTA/2 mM dithiothreitol/20% (vol/vol) glycerol/400 μg of bovine serum albumin per ml. Buffer B is 50 mM Hepes·KOH, pH 7.5/0.1 mM EDTA/2 mM dithiothreitol/25% glycerol. Buffer B′ is buffer B with 50 mM KCl. Buffer C is 50 mM Tris·HCl, pH 7.5/10% (wt/vol) sucrose. Binding buffer is 50 mM Hepes·KOH, pH 7.5/150 mM KCl/1 mM EDTA/2 mM dithiothreitol.

Proteins. *E. coli* IHF and HU proteins were generously provided by Howard Nash (National Institute of Mental Health) and Robert Craigie (National Institute of Diabetes and Digestive and Kidney Diseases), respectively. Partial purification of ParB is described in the text. Dialyzed control lysate was produced by dialysis against buffer A of the supernatant from the control lysate (see text for definition) after precipitation with 0.25 g of $(NH_4)_2SO_4$ per ml.

Abbreviation: IHF, integration host factor.

[^{3}H]DNA Labeling. Supercoiled plasmid was prepared by the alkaline lysis method and purified through two successive CsCl/ethidium bromide gradients as described (13). DNA was ^{3}H-labeled *in vitro* by methylation with *Hha* I methylase (New England Biolabs). The standard reaction mixture contained 50 mM Tris·HCl (pH 7.5), 10 mM EDTA, 5 mM 2-mercaptoethanol, 0.6 μM *S*-adenosyl-L-[*methyl*-^{3}H]methionine (50 μCi/ml), and 100–200 μg of DNA per ml. The mixture was incubated with 1 unit of *Hha* I methylase per μg of DNA at 37°C for 1 hr, and methylation was stopped by the addition of NaDodSO$_4$ to 0.2%. There are >30 *Hha* I sites in the pBR322-derived plasmids and none within the region defined as *incB* or *parS* (1). Typical final specific activities were between 50,000 and 100,000 cpm/μg of DNA. The DNA was extracted once with phenol/CHCl$_3$, 1:1 (vol/vol), and twice with CHCl$_3$, precipitated with ethanol, washed with 70% ethanol, and resuspended in 10 mM Tris·HCl, pH 7.5/1 mM EDTA. This purification removed >95% of unincorporated *S*-adenosylmethionine.

Preparation of Cell Extracts. Cells were grown in LB medium as described in the text, collected by centrifugation, washed, and resuspended in buffer C to an OD$_{600}$ of 300–400, frozen in liquid N$_2$, and stored at −80°C until use. The final lysis mixture contained in buffer C thawed cells at A_{600} of 200, $(NH_4)_2SO_4$ at 29.1 g/liter, egg white lysozyme at 200 μg/ml, 20 mM spermidine·HCl, 10 mM EDTA, and 2 mM dithiothreitol. An incubation at 0°C for 45 min was followed by another at 37°C for 15–60 sec with constant inversion. Occasionally, Brij 58 (to 0.1%) was added to assist lysis during this last step. After the mixture was chilled to 0°C, cell debris was removed by centrifugation in a Beckman JA-20 rotor at 15,000 rpm for 60 min for large (10–30 ml) lysates or in a Beckman TL100.2 rotor at 50,000 rpm for 30 min for small (0.5 ml) lysates.

DNA Binding Activity Assays. Binding reaction mixtures (20 μl) contained 2.5 μg of sonicated salmon sperm DNA and 200 ng of ^{3}H-labeled plasmid DNA in binding buffer. Nitrocellulose filters (25-mm Schleicher & Schuell BA85) were boiled in three changes of distilled water, stored in water at 4°C, and soaked in wash buffer (binding buffer without dithiothreitol) at room temperature for 30 min before use. The mixtures were assembled on ice, incubated with protein (diluted when necessary in buffer A) at 30°C for 20 min, and filtered with gentle suction through nitrocellulose. The filters were washed twice with 0.3 ml of wash buffer at 30°C and dried. Radioactivity was measured by liquid scintillation counting.

RESULTS

DNA Binding by ParB and a Host Factor. The behavior of various P1 miniplasmids *in vivo* suggested that ParB binds to *parS* (4) and that this activity might be used to follow purification of the protein. To overproduce ParB, the *parB* gene was cloned under the control of the phage λ P_L promoter in the vector pPLc2819 (11) to give the plasmid pBEF105. Crude lysates were prepared from heat-induced *E. coli* M5219 (cI857) cells containing either pBEF105, producing the ParB lysate, or pPLc2819, producing the control lysate. The *parS* DNA substrate was the plasmid pALA207, which contains a 794-bp P1 sequence covering *parS* inserted in pBR322; *parS* is the only site necessary for partition within this sequence (1, 5). Only the ParB lysate showed significant specific binding to *parS*-containing DNA, using a nitrocellulose filter retention assay (Fig. 1). There was very little binding of the ParB lysate to pBR322 and of the control lysate to either plasmid. Retention of [^{3}H]DNA was eliminated when NaDodSO$_4$ (to 0.1%) was added after the binding reactions (data not shown), indicating that the protein caused no irreversible change in DNA structure sufficient to allow it to stick to the filter. The ParB lysate-pALA207 binding reaction was linear up to a plateau corresponding to 75–80% of added DNA. Thus, this assay appeared to be an adequate measure of *parS*-binding activity, presumed to be due to ParB protein.

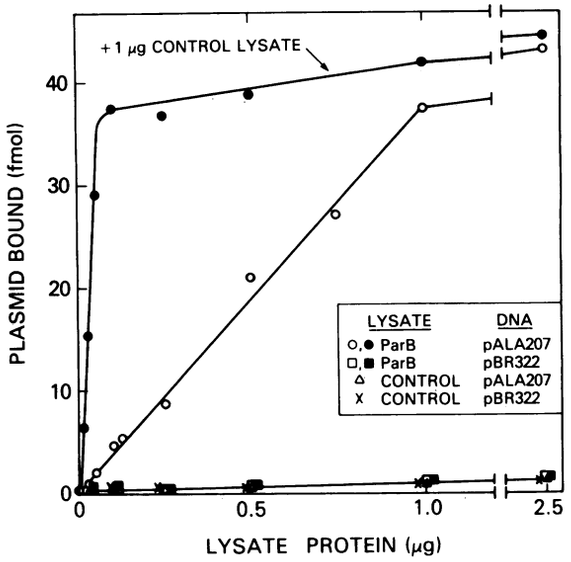

FIG. 1. Nitrocellulose filter retention assay for ParB. *E. coli* M5219 cultures containing pPLc2819 or pBEF105 were induced for 3 hr at 42°C and lysed as described to produce the control and ParB lysates, respectively. The [^{3}H]DNA substrates were pALA207 (*parS*) and the vector pBR322. Data points are: ○, ParB lysate with pALA207; ●, ParB lysate plus 1 μg of control lysate with pALA207; □, ParB lysate with pBR322; ■, ParB lysate plus 1 μg of control lysate with pBR322; △, control lysate with pALA207; ×, control lysate with pBR322.

Subsequent fractionation of the ParB lysate indicated that at least two components were involved in the reaction. After precipitation with ammonium sulfate (0.28 g/ml), <3% of binding activity remained in the supernatant, yet the recovery in the salt pellet was only 50%. Mixing the supernatant and pellet fraction together in their original proportions yielded 85% of the original activity (data not shown). The stimulatory activity of the supernatant was attributed to a host factor because it could be supplied by the control lysate. In fact, the control lysate specifically stimulated ParB lysate binding to pALA207 about 20-fold but could not increase binding to pBR322 (Fig. 1). Lysates made from cells containing no plasmid also showed stimulatory activity (see below), indicating that there was a host component to the binding reaction that was limiting in the ParB lysate.

Ion-exchange chromatography separated ParB from the host factor (Fig. 2*A*). Simple DNA binding activity (assayed with no additional protein) migrated as two small peaks. The first peak, which was eluted between 0.45 and 0.55 M KCl, corresponded to ParB. True ParB activity, measured with saturating host factor from the control lysate, was about 200-fold greater than simple binding (note the difference in the scale in Fig. 2*A*).* Denaturing gel electrophoresis of the peak fractions revealed a major protein band, estimated to be at least 80% pure, that migrated with an apparent molecular mass of 44–45 kDa (Fig. 2*B*), consistent with the gel mobility of ParB from maxicell extracts (1). The host factor activity, measured as stimulation of ParB (from the low-salt peak), was eluted between 0.65 and 0.85 M KCl, with a smaller amount that comigrated with the ParB peak itself. The latter is probably due to site-specific binding activity of ParB alone, rather than to host factor contamination in the ParB peak (see

*The lower stimulation (by a factor of ≈10) afforded by added host factor to the ParB lysate (Fig. 1) is due to the presence of host factor in this crude ParB fraction.

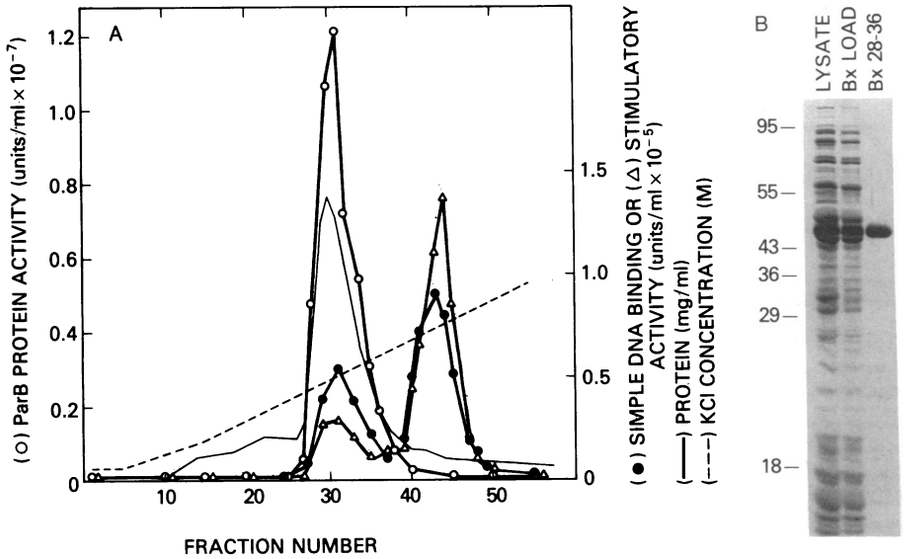

FIG. 2. Bio-Rex 70 purification of ParB. A 2-liter culture of M5219 (pBEF105) was induced for 2 hr at 42°C and lysed as described. The lysate (19 ml) was mixed with 4.0 g of $(NH_4)_2SO_4$, and the precipitate was removed by centrifugation at 15,000 rpm for 40 min in a Beckman JA-20 rotor. An additional 2.66 g of $(NH_4)_2SO_4$ was mixed with the supernatant. After centrifugation as above, the pellet was resuspended in 2 ml of buffer B′, dialyzed for 2 hr against 500 ml of buffer B′, and diluted with buffer B until the conductivity was equivalent to that of buffer B′ (final vol, 27 ml). This fraction (the Bio-Rex load) contained 194 mg of protein and 2.1×10^8 units of ParB activity (73% of lysate protein and 83% of lysate ParB activity). It was applied onto a 22-ml Bio-Rex 70 column equilibrated with buffer B′ by first mixing with 8 ml of the resin for 1 hr and then pouring the slurry over the remaining resin. After the column was washed with 2 column volumes of buffer B′, the protein was eluted with a 200-ml gradient of 0.05–1 M KCl in buffer B. Equal portions of fractions 28 through 36 were pooled to determine recovery; 89% of ParB activity from the load was recovered in 30 mg of protein. (*A*) pALA207 (*parS*) binding activity and protein assays. One unit of binding activity is defined as the amount of protein necessary to bind 1 fmol of plasmid. Simple DNA binding activity (●) refers to the binding capacity of each fraction measured with no additional protein. ParB activity (○) was assayed with 1 μg of protein from a dialyzed control lysate. Stimulatory activity (△) was measured with 50 ng of ParB from fraction 31. Protein concentrations were determined by the Bradford method (14). (*B*) $NaDodSO_4$/polyacrylamide gel electrophoresis of ParB. Samples of ParB lysate (LYSATE lane, 20 μg), Bio-Rex load (Bx LOAD lane, 15 μg), and the pool of Bio-Rex fractions 28–36 (Bx 28–36 lane, 3.6 μg) were analyzed on a 12% polyacrylamide gel and stained with Coomassie blue (15). The positions of molecular mass standards, run in the same gel, are indicated in kDa. Electrophoresis of individual fractions (not shown) revealed that the major 45-kDa band comigrated with ParB activity measured in *A*.

below). The profile of simple binding activity almost paralleled that of the stimulatory activity, because ParB from the trailing edge of the ParB peak contributed to the binding reaction. Nevertheless ParB was now essentially free of host factor, and each component could be measured separately by saturating the binding reaction with the other.

The Host Factor Is IHF. Several observations suggested that the host factor could be a small heat-stable protein. It did not pass through a dialysis membrane (molecular weight limit was 8000), was insensitive to RNase, and was precipitable by ammonium sulfate (0.3–0.5 g/ml). Eighty-two percent to 95% of the activity was recovered after an incubation at 100°C for 10 min (data not shown).

Two experiments showed that the factor was IHF, a small heat-stable protein in *E. coli* required for phage λ site-specific recombination (16, 17). First, purified IHF was able to specifically stimulate ParB binding to pALA207 (Fig. 3*A*). IHF consists of two subunits, α and β (18), encoded by the bacterial *himA* and *hip* (*himD*) genes, respectively (19, 20), and binds site-specifically to DNA (17). In the linear range of the binding curve, approximately two IHF dimers were added per plasmid retained on the filter (Fig. 3*A*). Second, lysates of *hip* mutant cultures were unable to provide host factor activity (Fig. 3*B*). Purified IHF was able to complement the *hip* deficiency (Fig. 3*A*). Lysates of the isogenic hip^+ strain contained about as much host factor as the original control lysate contained (Fig. 3*B*). It has been shown (18) that IHF–DNA complexes stick poorly to nitrocellulose filters, consistent with the behavior of host factor and IHF (alone) in these experiments (Figs. 1 and 3*A*).

Protein HU is another heat-stable DNA binding protein that is structurally related to IHF but apparently binds nonspecifically to DNA (see ref. 21 for review). HU stimulated ParB binding but less effectively than a crude lysate or IHF. For example, 140 ng of purified HU contained about as much stimulatory activity as 125 ng of control lysate or 0.25 ng of IHF. Therefore HU was not the host factor; either the protein preparation may be contaminated with IHF or HU may substitute weakly for IHF in this reaction.

IHF Functions *in Vivo* During Plasmid Partitioning. λ-miniP1 is a unit-copy-number plasmid partitioned by P1 *par* (3, 10). It was less stably maintained in *E. coli hip* cells than in wild-type cells (Table 1). However, the defect was not as severe as that caused by the loss of *par* function; >95% of cells lose λ-miniP1 *par* mutants after 20 generations of nonselective growth (3, 4). Thus, it appears that IHF contributes to stability but is not absolutely required. One simple explanation is that two types of ParB–*parS* complexes can form, one dependent and the other independent of IHF. This possibility appeared intriguing in the light of the observation by Martin *et al.* (5) that two types of *parB*-dependent partition sites are found within *parS*. A small or minimal *parS* site ("*parS*-small") includes a 34-bp palindrome between the *Dra* I and *Sty* I restriction sites in P1 *par*, and a larger *parS* site ("*parS*-large") contains the small site as well as the region between the *Dra* I and *Taq* I restriciton sites to its left (5) (Fig. 4). Both sites can accomplish partition (with ParA and ParB), but the large and small site partition complexes are distinct. Members of each type can compete only with themselves and not with the other. Each complex can be recognized *in vivo* by its ability to destabilize another plasmid dependent on a particular type of *parS* site for stability. λ-miniP1 contains the *parS*-large site (the natural context) and is destabilized only

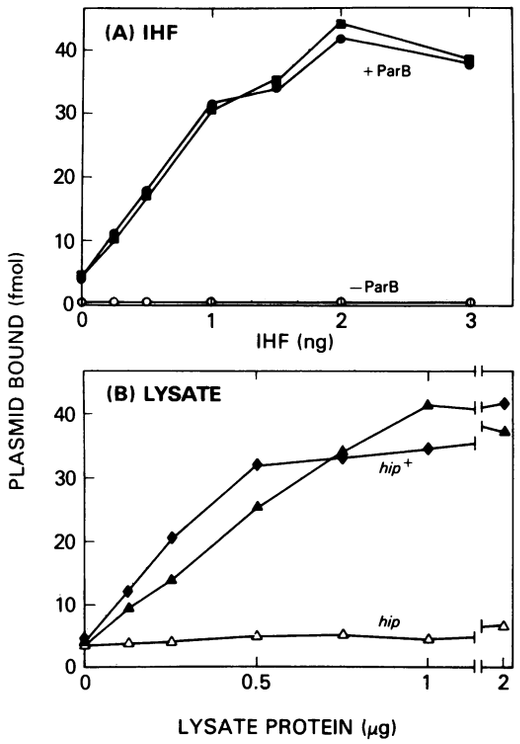

FIG. 3. Host factor analysis. (*A*) Binding activity of purified IHF alone (○), with 50 ng of ParB (●), and with 50 ng of ParB and 1 μg of MC4100 *hip* lysate (■). (*B*) Binding activity of MC4100 (▲), MC4100 *hip* (△), and M5219 (pPLc2819) (◆) lysates. The M5219 lysate is the control lysate described in Fig. 1. MC4100 derivatives were grown in LB at 37°C to an A_{600} of about 3 and lysed as described. All assays in *B* contained 50 ng of ParB.

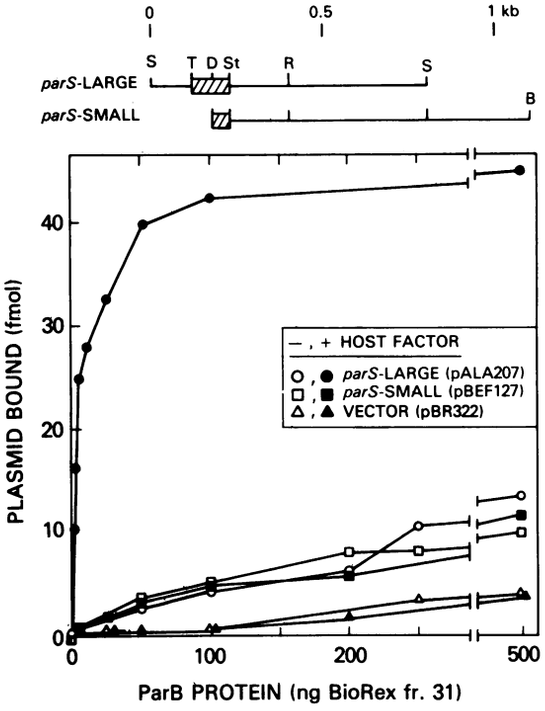

FIG. 4. Binding behavior of plasmids containing *parS*-large and *parS*-small. (*Upper*) Diagrams representing the P1 DNA from *par* (thin lines) inserted into the *Bam*HI site of pBR322 yielding pALA207 (*parS*-large) and pBEF127 (*parS*-small). The hatched boxes on the lines indicate the regions conferring *parS* activity determined by Martin *et al.* (5). Restriction sites: B, *Bam*HI; D, *Dra* I; R, *Eco*RV; S, *Sau*3AI; St, *Sty* I; and T, *Taq* I. Scale: kb, 1000 bp. (*Lower*) ParB binding activity to pALA207 (●, ○), pBEF127 (■, □), and pBR322 (▲, △) in the presence (filled symbols) and absence (empty symbols) of host factor, supplied by 1 μg of dialyzed control lysate. ParB (Bio-Rex fraction 31) was first concentrated 8-fold by centrifugation in Centricon-30 filters (Amicon) and then was diluted in buffer A to reduce salt inhibition at high levels of protein.

by plasmids containing *parS*-large and not by those containing *parS*-small (Table 2) (5).

It seemed reasonable that the two types of *parS* sites seen *in vivo* might represent two types of ParB–*parS* complexes, with and without IHF. This hypothesis led to two predictions. First, the *parS*-small site should be IHF independent, because *parS*-large (on pALA207) was the site stimulated by IHF (Figs. 1–3). Second, in the absence of IHF, a large site should behave as a small site. In *hip* mutants, both *parS*-large- and *parS*-small-containing plasmids destabilized λ-miniP1 (Table 2), indicating that without IHF, the partition system now recognized these sites as equivalent and competitive. Thus, IHF is required for site discrimination—further evidence that this protein functions in plasmid partitioning.

In vitro, IHF stimulated ParB binding only to pALA207 (*parS*-large) (Fig. 4). ParB binding to pBEF127 (*parS*-small) was unaffected by added IHF, yet was greater than to the vector pBR322. This argues that ParB can bind to *parS* in the absence of IHF. IHF was supplied from a crude lysate; purified IHF gave identical results (data not shown). The experiment also shows that no other lysate component could stimulate ParB binding to *parS*-small (Fig. 4). In the absence of IHF, the *parS*-large plasmid (pALA207) behaved as the *parS*-small plasmid (pBEF127), evidence that this unstimulated binding (in Fig. 4, and in Fig. 2 at the ParB peak) was due to ParB alone and not contaminating host factor. Therefore, both predictions were confirmed; only the *parS*-large

Table 1. Stability of miniP1 plasmids *in vivo*

Strain	Generations, no.	% retention of resident plasmid: λ-P1:5R (*rep*⁺*par*⁺)	% retention of resident plasmid: pALA318 (*rep*⁺)
DH5	20	97	99
	60	97	92
DH5 *hip*	20	84	98
	60	63	92

The resident plasmids (tested separately) were kanamycin-resistant derivatives of λ-P1:5R (λ-miniP1), which contains both the P1 replication (*rep*) and partition (*par*) regions (10), and pALA318, which contains only *rep* (12). The copy number of pALA318 is about 4-fold higher than that of λ-P1:5R (12) and was identical in both DH5 and DH5 *hip* (measured as described in ref. 4; data not shown). Plasmid retention was measured after the indicated number of generations (calculated from the viable cell count) in nonselective LB medium. Cells were plated onto nonselective plates, and at least 100 subsequent colonies were transferred with toothpicks to kanamycin plates (25 μg/ml) to detect the presence of the plasmid.

Table 2. Competition of λ-miniP1 by *parS* plasmids

Competing plasmid	% retention of λ-P1:5R: DH5	% retention of λ-P1:5R: DH5 *hip*
pALA207 (*parS*-large)	3	≦1
pBEF127 (*parS*-small)	99	≦1

Plasmid retention was measured after 18 generations in LB medium nonselective for the resident λ-miniP1 (without kanamycin), whereas selection was continuously maintained for the competing plasmid (100 μg of ampicillin per ml). The copy numbers of the competing plasmids were identical in DH5 and DH5 *hip* (data not shown). These values are for one experiment; variability in different experiments was <4%.

complex could use IHF, and *parS*-large and *parS*-small sites were indistinguishable in the absence of IHF.

Finally, the observation that in wild-type cells a high-copy plasmid containing *parS*-small cannot destabilize λ-miniP1 (5) (Table 2) argues strongly that the ParB–*parS* complex with IHF is the one predominantly used during the normal partition process; there were no complexes without IHF that would be blocked by competition with *parS*-small.

By using the miniP1 plasmid pALA318, which contains the P1 replication system but lacks *par* (12), it was apparent that the *hip* mutation had no measurable effect on replication. Because of partial deletion of the regulatory locus *incA*, the copy number of pALA318 is 4-fold higher than that of λ-miniP1, and pALA318 is moderately stable without a partition system (12). Its stability and copy number were identical in wild-type and *hip* mutant cells (Table 1). These results, as well as those of the competition experiments, argue that the role of IHF in plasmid stability is in plasmid partitioning and not in replication.

DISCUSSION

Proper partition of P1 plasmids at cell division requires two plasmid proteins, ParA and ParB, and a centromere-like site, *parS*. The experiments presented here identify the bacterial IHF as a component of the P1 partition system. IHF is a small, site-specific DNA binding protein (17) originally characterized as a host factor required for phage λ site-specific recombination (16). It binds to three sites within *attP*, and with phage λ Int protein assembles a structure competent to recombine with *attB*. The protein plays a role in a variety of other processes (see ref. 21 for a review), such as insertion sequence IS*1* and transposon Tn*10* transposition (22, 23), pSC101 replication (24, 25), and regulation of several phage and bacterial genes (26–31). Plasmid partitioning can now be added to this list.

In vitro, P1 ParB protein and *E. coli* IHF contribute to *parS*-specific binding activity as measured by retention on nitrocellulose filters. It appears that ParB can bind to *parS* without IHF and that IHF dramatically alters the nature of this binding (Fig. 4). These observations appear to be true *in vivo* as well (Table 2). The two types of ParB–*parS* complexes differ in three respects: (*i*) the dependence on IHF, (*ii*) the amount of DNA required from the *parS* region, and (*iii*) the ability to compete in the partitioning process. The IHF-dependent and -independent complexes correlate with the two types of *parS* sites examined *in vivo* by Martin *et al.* (5). The small site, *parS*-small, is IHF independent and presumably lacks information for IHF binding. When IHF is absent, a large site behaves as a small site. Where does IHF exert its effect? Searches through *parS* revealed no perfect matches to the IHF box consensus sequence 5′-$^{T}_{C}$AANNNNTTGAT$^{A}_{T}$ (17, 22, 32), although several degenerate boxes were observed. For example, a possible candidate is the sequence 5′-TAACTGACTGTTT, found immediately to the left of the *Dra* I site (Fig. 4) and thus only in *parS*-large. Similarly, one can deduce that the minimal *parS* site, a 34-bp palindrome (5), contains the ParB recognition site. This analysis is speculative; direct binding studies must be done to define the sequences with which both IHF and ParB interact. Nevertheless, it is clear that both proteins cooperate to act at or near *parS*.

The two types of *parS* complexes apparently cannot interact and pair with each other [pairing would lead to competition (1, 3)] and must, therefore, be significantly different. How can IHF cause such a difference between complexes that both contain ParB? It is presumably not simply due to an altered binding affinity of ParB; a low copy number miniF plasmid containing *parS*-small (the "low affinity" site) is not destabilized by a plasmid containing *parS*-large (5). *In vitro*, the affinity of ParB–*parS* complexes for nitrocellulose is altered by IHF (Fig. 4). Because some DNA–protein complexes do not stick well to nitrocellulose, this alteration does not necessarily represent a difference in ParB binding affinity for DNA. Perhaps the conformation of ParB is altered by the presence of IHF, changing how ParB recognizes itself or is recognized by another as-yet-undefined protein.

In a natural context, with IHF in *E. coli* and *parS*-large in P1, the competition experiments (Table 2) argue that the ParB–IHF–*parS* complex is the normal and predominant substrate for partition. Therefore, the experiments described here identify IHF as a host participant in P1 partition and define a preliminary step in the construction of an active partition complex prior to cell division.

This paper is dedicated to Arthur Kornberg on the occasion of his 70th birthday. I am extremely grateful for his support, encouragement, and advice, which have been invaluable contributions to my development as a scientist. I thank Michael Yarmolinsky for his help and support and Sue Wickner, Kit Tilly, Dhruba Chattoraj, and Howard Nash for the many discussions and suggestions that have improved this study and this manuscript.

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