Stoichiometry of P1 Plasmid Partition Complexes

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The P1 plasmid prophage is faithfully partitioned by a high affinity nucleoprotein complex assembled at the centromere-like parS site. This partition complex is composed of P1 ParB and Escherichia coli integration host factor (IHF), bound specifically to parS. We have investigated the assembly of ParB at parS and its stoichiometry of binding. Measured by gel mobility shift assays, ParB and IHF bind tightly to parS and form a specific complex, called I + B1. We observed that as ParB concentration was increased, a second, larger complex (I + B2) formed, followed by the formation of larger complexes, indicating that additional ParB molecules joined the initial complex. Shift Western blotting experiments indicated that the I + B2 complex contained twice as much ParB as the I + B1 complex. Using mixtures of ParB and a larger polyhistidine-tagged version of ParB (His-ParB) in DNA binding assays, we determined that the initial I + B1 complex contains one dimer of ParB. Therefore, one dimer of ParB binds to its recognition sequences that span an IHF-directed bend in parS. Once this complex forms, a second dimer can join the complex, but this assembly requires much higher ParB concentrations.

Low copy number plasmids in bacteria, such as the P1 plasmid/prophage, are faithfully maintained in growing cell populations. This stable inheritance is dependent on active partition systems that are responsible for proper intracellular localization of these plasmids (reviewed in Refs. 1 and 2). Fluorescently tagged plasmids and fluorescence in situ hybridization have been used to show that P1 and F plasmids are specifically located at the ¼ and ¾ positions in the cell for most of the cell cycle (3, 4). Localization is directed via a partition complex, which consists of a plasmid protein bound specifically to a cis-acting plasmid site.

In P1, the par operon contains three elements that are essential for proper segregation: the parA and parB genes and a centromere-like site, parS (5). ParB and the Escherichia coli integration host factor (IHF)3 assemble specifically at parS to form the partition complex (6, 7). A highly organized nucleoprotein structure has also been reported for F plasmid (8, 9), and a similar complex may form on the Bacillus subtilis chromosome at the binding sites of the SpoOJ protein, a ParB homolog (10, 11). In addition to their role as the substrate for localization, it is speculated that partition complexes serve to promote pairing between newly replicated replicons, which are subsequently separated by an as yet unidentified mechanism. Pairing between partition complexes has recently been described for the R1 plasmid, which has a similar but nonhomologous partition system (12).

The current picture of the P1 partition complex is derived from a variety of protein-DNA binding experiments in vitro and examination of mutant parS sites in vivo (13–17). ParB recognizes two distinct sets of repeated sequences, called box A and box B repeats, that flank an IHF binding site in parS (Fig. 1A) (6, 13). Binding of IHF to parS creates a large bend in the DNA, which greatly increases ParB’s affinity for parS (13). Proper phasing of the A and B boxes across this bend is functionally important for formation of the partition complex (15, 17). ParB affinity is also greatly increased by superhelicity in the DNA substrate. All of these data suggest a partition complex structure in which the DNA is wrapped around a core composed of IHF and ParB. IHF binds to its specific sites as one IHFα/IHFβ heterodimer (18, 19), but the stoichiometry of ParB assembled on the partition complex at parS is not known.

Another characteristic of the centromeric parS site is the ability to silence neighboring genes when ParB is present (20, 21). It has been proposed that silencing occurs when ParB molecules spread along and cover the DNA from a nucleation point, which is parS. The role of this larger structure in plasmid segregation is unknown, but this observation suggests that the partition complex can adopt different structures in vivo.

One important question that remains is the stoichiometry of ParB in the P1 partition complex. ParB is a dimer in solution (13). In this study, we have examined the nature and stoichiometry of the binding of ParB to parS by gel electrophoresis. We find that one dimer is sufficient to interact with the ParB recognition sequences that span the IHF-directed bend, resulting in the high affinity binding to parS that is observed for ParB and IHF. At higher concentrations of ParB, more dimers of ParB join this complex to create even higher order protein-DNA complexes.

EXPERIMENTAL PROCEDURES

Reagents and Enzymes—Sources for reagents were as follows: polyvinylidene fluoride (PVDF) membranes, Millipore; bovine serum albumin (fraction V), guanidine HCl, 1,10-phenanthroline-copper (OP-Cu), Sigma; [α-32P]dATP and [α-32P]dCTP, NEN Life Science Products; and dithiothreitol, Bio-Rad. Restriction enzymes and DNA polymerases were purchased from New England Biolabs or Roche Molecular Biochemicals.

DNA and Proteins—The plasmids pBEF165 and pBEF166 contain the P1 parS sequence between the P1 TaqI and Styl restriction sites.

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cloned in opposite orientations into a modified pBlueScript vector (13). For gel mobility shift assays, the DNA substrates were total restriction digests of pBEF166 and thus included both parS and vector DNA fragments. Digestion with different restriction enzymes yielded parS DNA fragments of different size. Digestion with XhoI or BamHI produced parS-132 or parS-252, respectively (the numbers correspond to the length of the DNA in bp). DNA fragments were labeled at their 3'-ends with [32P]dATP or [32P]dCTP and DNA polymerase I large fragment and purified by phenol-chloroform extraction and ethanol precipitation steps (22). For Op-Cu footprinting substrates, parS-211 DNA fragments were generated by digestion of pBEF165 or pBEF166 with BamHI and SmaI and were 32P-labeled at the 3'-end of the BamHI site. parS-211 DNA fragments labeled on the upper strand (as parS is shown in Fig. 1) were generated from pBEF165, and DNA fragments labeled on the lower strand were prepared from pBEF166.

His-ParB has 36 amino acids, including a 10X polyhistidine tag, fused to the N terminus of ParB (23). ParB (fraction V), His-ParB, and ParB were purified as described previously (23, 24).

**Gel Mobility Shift Assays**—The standard reaction mixture (10 μl) contained 0.5 nM 32P-labeled DNA in a buffer containing 50 mM Hepes KOH (pH 7.5), 50 mM NaCl, 10 mM MgCl2, 1% glycerol, 100 μg of bovine serum albumin/ml, 100 μg of sonicated salmon sperm DNA/ml, and 1 mM dithiothreitol. The mixtures were assembled on ice, incubated for 15 min at 30 °C, and analyzed by electrophoresis in 5% polyacrylamide gels in 90 mM Tris borate, 1 mM EDTA. Electrophoresis was performed at 150 V for 10 h at 4 °C. The gels were dried onto Whatman DE81 paper and exposed to a phosphor screen for imaging by a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA).

**In Situ OP-Cu Footprinting**—In situ footprinting assays were performed as described in Sigman et al. (25) as follows. Gel mobility shift assays were performed as described above. Protein-DNA complexes were transferred from gels to PVDF membranes in 39 mM glycine, 48 mM Tris, 0.0375% SDS, and 20% methanol with a semidy apparatus. Membranes were dried briefly and exposed to a phosphor screen to quantitate radiolabeled parS DNA. For protein detection, the membranes were then treated with anti-ParB antibodies, and ParB was visualized using an ECF Western blotting kit (Amersham Pharmacia Biotech) as recommended by the manufacturer, analyzed with a Molecular Dynamics Storm 860 imager, and quantified by ImageQuant software (Molecular Dynamics). Free ParB, determined as the ParB “background” that migrated adjacent to (just above) the protein-DNA complex in each gel lane, was subtracted from the ParB that was bound to parS DNA. In Situ OP-Cu Footprinting—In situ footprinting assays were performed as described in Sigman et al. (25) with the modifications reported previously (24). Briefly, protein-DNA complexes were separated by gel electrophoresis, and the entire gel was treated with a solution containing OP-Cu to cleave the DNA (24). Complexes were visualized by autoradiography and excised from the gel. DNA was extracted and analyzed by electrophoresis in sequencing gels. The cleavage patterns for each complex and for unbound DNA were scanned using a PhosphorImager and ImageQuant software (Molecular Dynamics). To correct for differences in loading, the scan of each protein-DNA complex was normalized in the area outside parS to the cleavage pattern of unbound parS DNA that was isolated from the same gel.

**RESULTS**

**Characterization of Two Forms of the Partition Complex**—ParB and IHF cooperate to form the partition complex at parS. We have been interested in the structure of this complex, since its assembly represents an early step in the partition process. Here, we have examined the nature and number of complexes of ParB and IHF that assemble at parS by gel mobility shift assays (Fig. 2). We were specifically interested in ParB-parS complexes with IHF. Even though IHF is not essential for plasmid stability, in vivo competition (or “incompatibility”) assays indicate that IHF is always a component of wild-type P1 partition complexes (6, 13). By gel mobility shift assays, we observed two distinct ParB-IHF-parS complexes (I + B1 and I + B2) in addition to IHF complexes alone (I complex) (Fig. 2). This is consistent with previous studies of these complexes by gel electrophoresis that revealed a single ParB-IHF-parS complex at low concentration and a second, more slowly migrating, complex at higher concentrations (7, 20). When ParB concentration was increased further, we also observed complex I + B3 (Fig. 2) and higher complexes (data not shown).

ParB formed complex I + B1 with much greater affinity than it formed complex I + B2. We measured the relative affinity of complexes formed on a 252-bp DNA fragment containing parS (parS-252; Fig. 3). The concentration of ParB required for 50% complex formation is a measure of the dissociation constant, assuming that free protein is approximately equal to total protein. This assumption is valid as long as the concentrations of substrate and thus of protein-DNA complexes are significantly lower than the total ParB concentration, and the binding stoichiometry is low. These Kd values are estimates, since the binding reaction is more complicated than a simple bimolecular reaction (each complex does not necessarily saturate before becoming the substrate for the next higher complex). Nevertheless, they demonstrate the large difference in affinity of ParB for naked parS DNA (apparent Kd of 4 nM ParB dimers to form complex I + B1) compared with its affinity for complex I + B1 (apparent Kd of 800 nM ParB dimers to form complex I + B2) (Fig. 3).

**Relative Stoichiometry between the Two Partition Complexes**—The difference between complexes I + B1 and I + B2 could represent (i) a difference in the shape of these complexes, (ii) a difference in the amount of ParB bound in each complex, or (iii) a pairing event between two parS DNA molecules. To discriminate among these possibilities, we first performed shift Western blotting analysis (25) to measure the relative stoichiometry of ParB on parS in the two complexes. ParB-IHF DNA complexes were assembled on parS-132 DNA fragments, separated by electrophoresis, and then transferred to PVDF membranes, which retained both the DNA and proteins (see “Experimental Procedures”). DNA and ParB present in the different complexes were detected successively on the same membrane by PhosphorImager scanning (Fig. 4A, left panel) and quantitative Western blot analyses (Fig. 4A, right panel), respectively. Quantification revealed that complex I + B2 contains, on average, 2.1-fold more ParB than complex I + B1 (Fig. 4B). A similar result was obtained with parS-211 (average I + B2/I + B1 = 1.90; data not shown). Therefore, if we consider that an I + B1 complex is composed of 1 unit of ParB, then an I + B2 complex contains 2 units of ParB per parS DNA molecule.

This result makes it unlikely that the I + B2 complex corresponds to a pairing event between two I + B1 complexes. If this
increasing ParB concentration. The positions of free DNA fragments and protein-DNA complexes are indicated on the left. Note that the large vector DNA fragment also contains an IHF binding site.

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**Fig. 2.** Multiple partition complexes form at parS with increasing ParB concentration. The positions of free DNA fragments and protein-DNA complexes are indicated above each lane. IHF, when present (+), was at 400 nM. The positions of free DNA fragments and protein-DNA complexes are indicated on the left. Note that the large vector DNA fragment also contains an IHF binding site.

**Fig. 3.** Quantification of ParB binding in complexes I + B1 and I + B2. Binding of ParB and IHF to a 252-bp DNA fragment containing parS was analyzed by gel mobility shift assay (as in Fig. 2) and quantified using a PhosphorImager. The DNA substrate was a mixture of three fragments (3040, 252, and 120 bp) resulting from BamHI digestion of pBEF166 (see “Experimental Procedures”), which were present at 0.5 nM. Filled circles (●) represent complex I + B1, and open circles (○) represent complex I + B2. The inset (same axes as main graph) shows the I + B1 curve at low ParB concentration on an expanded scale.

If the stoichiometry of ParB were true, then the protein:DNA ratio for both complexes should be the same; complex I + B2 would have twice as much ParB and twice as much DNA as complex I + B1. Similarly, if I + B2 represented an I + B1 complex that had captured and paired with a naked (or IHF-bound) parS DNA molecule, the ParB:DNA ratio would decrease by half in complex I + B2 (I + B2/I + B1 = 0.5). Nevertheless, we checked for pairing by including an unlabeled parS DNA fragment of different size in the binding mixture (Fig. 5). The expectation was that paired complexes would form between DNA fragments of different size, producing two distinct I + B2 complexes when only one of the fragments was radioactively labeled. Conversely, ParB bound to only one parS DNA fragment would simply be competed by the unlabeled parS DNA fragment. The latter result was observed (Fig. 5), indicating that the I + B2 complex does not represent a pairing event.

**Fig. 4.** ParB prototympAR DNA ratios in partition complexes. A, shift Western blotting analysis. The left (lanes a–g) and right (lanes a’–g’) panels represent detection of 32P-labeled DNA (by a PhosphorImager) and of protein (via immunoblots), respectively, from the same gel. DNA fragments (4 nM concentration of an XbaI digest of pBEF166) were incubated in the presence (+) or the absence (−) of IHF (200 nM) and ParB (lane d and d’, 250 nM; lanes a, e, a’, and e’, 500 nM; lanes f and f’, 750 nM; lanes g, and g’, 1000 nM). The protein-DNA complexes were separated by gel mobility shift assay and then transferred to a PVDF membrane for analysis (see “Experimental Procedures”). The positions of free and bound parS-132 are indicated on the left (same nomenclature as in Fig. 2). B, determination of the relative stoichiometry of ParB on parS-132. Ratios of the “counts” of parS (radiolabeled) versus ParB (fluorescence) were determined in the I + B2 shifts and in the I + B1 ParB shifts for each reaction in A (referred to by their corresponding gel lanes). The relative ratio of each I + B2 complex was then divided by the corresponding ratio of the I + B1 complex to determine the relative stoichiometry of ParB on parS.

**Fig. 5.** The I + B1 Complex Contains One ParB Dimer—ParB is a dimer in solution (13), but its stoichiometry at parS is unknown. Since ParB recognizes four box A and two box B sequences (14, 15), it seemed reasonable to predict that a tetramer would be required to occupy all sites. On the other hand, both genetic and chemical interference studies suggest that only two box A sequences are essential for parS activity (A2 and A3 in Fig. 1; Refs. 14–16). To examine the stoichiometry of
ParB in the I + B1 and I + B2 complexes, we designed a mixing experiment using ParB and a larger polyhistidine-tagged version of the protein, His-ParB (23) (Fig. 6). His-ParB binds to parS, but produces a larger, more slowly migrating complex (complex I + hB1; Fig. 6A, lane 4). When ParB and His-ParB were both added to a binding reaction mixture, we expected that complexes containing more than one dimer would produce hybrid bands in a gel mobility shift assay. At low concentration of both ParB proteins, only ParB (I + B1) and His-ParB (I + hB1) complexes were observed (Fig. 6A, lanes 5 and 6). At high protein concentrations, an intermediate complex was observed (I + B2/hB2) that migrated between the ParB I + B2 and His-ParB I + hB2 complexes (Fig. 6A, lanes 9 and 10). The simplest explanation for these results is that the I + B1 complexes contain only one dimer of ParB and that the I + B2 complexes contain two dimers of ParB.

Formally, the lack of an intermediate I + B1 complex could be interpreted as indicating that this complex contains only one monomer of ParB, since heterodimers of ParB/His-ParB would also be expected to produce hybrid complexes. However, we favored the idea that the ParB dimerization interaction is too strong to completely reassert during this experiment. Furthermore, it seemed unlikely that a single monomer would be able to interact with two box B sequences across the IHF bend or to protect all of the sites within parS that were detected by OP-Cu footprinting (see below). Nonetheless, to test whether the I + B1 complex contains one dimer or one monomer of ParB, we forced the protein to form heterodimers by denaturing and renaturing a mixture of both ParB proteins. ParB and His-ParB, alone or mixed together, were denatured by treatment with 6 M guanidine and then renatured by successive dialysis steps to remove the guanidine. When used in a DNA binding experiment at low ParB concentrations, the ParB/His-ParB mixture now produced an intermediate I + B1/hB1 complex (Fig. 6B, lane 5). This result is consistent with the formation of a complex containing a heterodimer of ParB/His-ParB. As controls, ParB and His-ParB were individually denatured and renatured, and both proteins were able to bind parS (Fig. 6B, lanes 3 and 4).

To further demonstrate that the intermediate complex I +

![Figure 5](http://www.jbc.org/content/269/15/8216/F5.large.jpg)

**Fig. 5.** Competition analysis of ParB binding to parS. Gel mobility shift assays were performed with parS-252 (lanes 1–3) or parS-132 (lanes 4–6) DNA fragments (restriction digests as in Figs. 2 and 3) as the 32P-labeled substrates. The reaction mixtures contained 16 fmol of 32P-labeled DNA fragments. When present (+), IHF and ParB were at 400 and 250 nM, respectively. In lanes 7–9, increasing amounts of unlabeled parS-252 (indicated above the lanes in fmol) were added to the reaction mixtures prior to the addition of ParB.

![Figure 6](http://www.jbc.org/content/269/15/8216/F6.large.jpg)

**Fig. 6.** Mobility of ParB and His-ParB homodimers and heterodimers bound to parS DNA. A, DNA binding by native ParB and His-ParB. ParB or His-ParB were incubated with parS-132 DNA fragments (as an XhoI restriction digest of pBEF166) in the presence of IHF (400 nM), and the reaction mixtures were analyzed by electrophoresis in a 5% polyacrylamide gel. The concentrations of ParB and His-ParB (in nM) are indicated above each lane. B, DNA binding by denatured/re-natured ParB, His-ParB, and ParB/His-ParB heterodimers. To form heterodimers, 25 µg each of ParB and His-ParB were mixed, diluted in 150 µl of buffer A (6 M guanidine HCl, 100 mM NaH2PO4, 10 mM Tris, pH 8.0), and then dialyzed against 300 ml of buffer A for 4 h to denature both proteins. They were renatured by successive dialysis steps against decreasing concentrations of guanidine HCl (3, 1, and 0.5 M) followed by a final dialysis against 50 mM sodium phosphate (pH 8.0), 300 mM NaCl, 10% glycerol, 7 mM β-mercaptoethanol. Dialysis was performed at 4 °C. The resulting mixture of homo- and heterodimers (ParB/His-ParB mixture, lanes 5 and 9) was used in DNA binding assays. As controls, 25 µg each of ParB and of His-ParB were independently denatured and renatured (in separate dialysis bags) as above and used in DNA binding assays (ParB, lanes 3 and 7; His-ParB, lanes 4 and 8). To isolate “His-dimers” (dimers in which at least one monomer contains a polyhistidine tag), 15 µg of the denatured/re-natured mixture of ParB and His-ParB were purified over a 20-µl nickel-agarose affinity chromatography column as described in Ref. 23. Bound protein was eluted with 500 mM imidazole, and used in DNA binding assays (His dimers, lanes 6 and 10). ParB protein concentrations (in nM dimers) are indicated above each lane.
B1/hB1 (Fig. 6B, lane 5) was due to heterodimers, the ParB/His-ParB denatured/renatured mixture was repurified by nickel affinity chromatography. ParB protein that bound to the nickel resin, which must contain at least one polyhistidine tag per dimer (His6 dimer), was used in a DNA binding assay (Fig. 6B, lane 6). This protein mix produced primarily the intermediate I + B1/hB1 and upper I + hB1 complexes. Since the latter are identical to those produced by His-ParB homodimers, we conclude that the intermediate complex contains His-ParB/ParB heterodimers. Therefore, the I + B1 complex contains one ParB dimer, and the I + B2 complex contains two ParB dimers.

Chemical Footprint Analysis of the Two ParB Complexes—The parS site contains several DNA binding boxes recognized by ParB (Refs. 14 and 15; see Fig. 1). To examine the location of ParB on the DNA in the I + B1 and I + B2 complexes, we used OP-Cu to footprint complexes directly in the electrophoresis gel. OP-Cu is a chemical nuclease that interacts with DNA through the minor groove, where it cleaves the DNA backbone (26, 27). Access of OP-Cu to the DNA can be blocked sterically by proteins or by an alteration in the geometry of the DNA that narrows the minor groove.

Protein-DNA complexes were formed and resolved by electrophoresis as above (e.g. Fig. 2) and then treated with OP-Cu directly in the gel (see “Experimental Procedures”). After recovery of the cleaved DNA from the gel, the footprint patterns were resolved in a denaturing polyacrylamide gel (Fig. 7A). The radioactive bands were quantified by PhosphorImager analysis. The extent of protection was subtle but reproducible by this technique, particularly when the scans of each footprint were superimposed (Fig. 7B). Protection by IHF and ParB was more visible on the upper strand (Fig. 7A) but was detectable on both strands by quantification of the footprint patterns (Fig. 7B).

IHF protected sequences between boxes A1 and A2 on both DNA strands. In the I + B1 complex, protection due to ParB binding was observed on the left side of the IHF binding site (boxes A1 and B1) and on the right side (boxes A2, A3, and B2) on both strands. ParB also increased the protection at the IHF binding site. This result may reflect that IHF affinity for parS is increased by ParB (16) and/or may suggest that the geometry of the DNA is slightly altered upon ParB binding (see “Discussion”). The protection of parS by ParB was very similar in complex I + B2 compared with complex I + B1, indicating no major difference in ParB’s binding pattern. There were, however, some changes, primarily that the region protected by ParB extended outwards slightly from parS (marked by dots in Fig. 7B). This extra protection included box A4 but also sequences to the left of box B1 (Fig. 7B). The DNA sequence to the left of box B1 is outside the minimal parS region required for parS activity (14). Therefore, the additional contacts made by ParB in complex I + B2 probably represent both specific and nonspecific interactions.

DISCUSSION

The formation of the P1 partition complex is an essential step in the segregation of the unit-copy number P1 plasmid at
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cell division. We have shown that the initial ParB + IHF complex visualized by gel mobility shift assays (I + B1) contains a single dimer of ParB, while the next complex formed (I + B2) contains two dimers of ParB. At higher concentrations of ParB, complexes with increasingly slower migration are observed (I + B3, I + B4, etc.), which presumably result from the loading of additional ParB dimers onto the nucleoprotein complex.

The stoichiometry determined here has interesting implications for the architecture of this partition complex. In particular, the observation that one ParB dimer is responsible for the I + B1 complex is intriguing given the number of specific sequences in parS that are recognized by ParB. parS contains two copies of the box B repeat and four copies of the box A repeat (Fig. 1). We favor a model in which one dimer of ParB is precisely docked to occupy both box B repeats and the box A2 and A3 repeat. First, since both box A2 and A3 repeats are essential for high affinity ParB binding, both must be filled to form the I + B1 complex. Second, although the OP-Cu footprints indicate an additional protection in the region of box A1 (Fig. 6), previous deletion and mutational analyses and DMS interference experiments have shown that boxes A1 and A4 in parS are not essential for partition or for high affinity binding by ParB (14–16). Previous DNase I footprinting experiments showed that ParB and IHF protected the region that contains box A1 from DNase I cleavage even when box A1 was mutated (changed by a 4-bp substitution mutation (14)). This result implies that ParB is in the same position in the complex with or without box A1. Therefore, we propose that this region (box A1) is protected from both OP-Cu and DNase I cleavage by alterations in the geometry of the DNA, such as a compression of the minor groove caused by tightening of the bend for example. The alternative explanation is that the I + B1 complex is a mixture of orientations of ParB binding across parS, in which case the domain of ParB that is responsible for recognition of box A must be quite flexible with respect to the domain responsible for recognition of box B. Given that box A1 cannot substitute for either A2 or A3 (14, 15), this scenario seems less likely.

Our previous data indicate that the bend produced by IHF binding to parS allows ParB to simultaneously contact its DNA recognition sequences that flank this bend (15). Our present results show that it is one dimer of ParB that interacts across this bend. Dimerization of ParB is mediated through a domain located at the C terminus of the protein (20, 23). This region has also been shown to be involved in box B recognition (28). These observations lead to a model in which the dimerized C termini of one ParB dimer interact with both box B sequences simultaneously, perhaps threading the DNA between the monomers. In other words, the extreme ends of the parS site are brought together near or at the dimerization interface. This model is consistent with the biochemical data that indicate that the DNA is wrapped around a core of protein (13, 15). It has been suggested that a putative helix-turn-helix motif in the center of ParB binds the box A motif (28, 29). In this case, these regions of each monomer would be directed toward box A2 and A3 (which make an inverted repeat sequence) but would be positioned on one side of the IHF bend rather than flanking it.

An important question is whether the I + B1 complex is sufficient for partition in vivo. The affinity of ParB for the I + B2 complex is about 100-fold lower than its affinity for the I + B1 complex. ParB exists at relatively high concentrations in the cell (micromolar amounts) (13, 16), and therefore progressive loading of ParB onto the DNA to form higher complexes probably also occurs in vivo. This is supported by the observation that ParB binding can spread a great distance along the DNA on both sides of parS under conditions where ParB can silence genes that are located close to parS (20, 21). In addition, immunofluorescence analysis shows that ParB forms bright, discrete foci at the intracellular locations occupied by P1 plasmids, which suggests that much of the ParB in the cell converges on P1 at the parS site (30). However, the minimal amount of ParB that is necessary for partition in vivo has not been measured. Larger complexes (I + B2, I + B3, etc.) may be necessary to interact with ParA or with host factors. Alternatively, the I + B1 complex may be sufficient for partition, but not for competition (incompatibility). We have observed that weaker par sites (weakened by mutation of parS, for example (15)) are competent for partition but unable to compete with wild-type sites.

Presumably binding of ParB to complex I + B1 to form complex I + B2 is mediated chiefly by protein-protein (dimer-dimer) interactions, which are weaker than the protein-DNA interactions that mediate complex I + B1. Such dimer-dimer interactions may occur via a self-association domain recently identified near the N terminus of ParB (23). In the OP-Cu footprints (Fig. 7), additional DNA on both edges of parS (including box A4) becomes protected in the I + B2 complex, so it seems likely that both specific and nonspecific DNA contacts also contribute to the formation of complex I + B2.

We reported recently that ParA interacts with ParB on the partition complex (24). The nature of this interaction is dependent on ParB concentration. At high ParB concentration, ParA is recruited to the partition complex, whereas at low ParB concentration, ParA interfered with ParB binding to parS. These interactions required magnesium in the gel and running buffer. Under these conditions, the two discrete complexes, corresponding to I + B1 and I + B2, are not observed, although the ParB complex migrates more slowly with increasing ParB concentrations (24). While the experimental conditions are not identical, it is possible, for example, that ParA is recruited only to a partition complex that contains two ParB dimers (complex I + B2 or higher), perhaps explaining a requirement for high concentrations of ParB.

The architecture of ParB binding to parS is intriguing, given the organization of box A and box B motifs (Fig. 1) and the current result that one dimer interacts with these motifs to form the initial partition complex. Our results define the minimal protein requirements for parS binding. The high affinity core (complex I + B1) then recruits more ParB molecules by both protein-DNA and protein-protein interactions, and it will be important to determine how much ParB must bind the core structure in order for the complex to be competent for partition in vivo.

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REFERENCES
1. Williams, D. R., and Thomas, C. M. (1992) J. Gen. Microbiol. 138, 1–16
2. Hiraga, S. (1992) Annu. Rev. Biochem. 61, 283–306
3. Niki, H., and Hiraga, S. (1997) Cell 90, 851–857
4. Gordon, G. S., Sitnikov, D., Webb, C. D., Teleman, A., Straight, A., Losick, R., Murray, A. W., and Wright, A. (1997) Cell 90, 1113–1121
5. Abeles, A. L., Friedman, S. A., and Austin, S. J. (1985) J. Mol. Biol. 183, 261–272
6. Funnell, B. E. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6657–6661
7. Davis, M. A., and Austin, S. J. (1988) EMBO J. 7, 1818–1888
8. Lynch, A. S., and Wang, J. C. (1994) J. Mol. Biol. 236, 679–684
9. Eick, D. P., and Strings, J. (1995) J. Mol. Biol. 246, 388–400
10. Ireton, K., Gunther, N. W., and Grossman, A. D. (1994) J. Bacterial. 176, 5320–5329
11. Lin, D. C.-H., and Grossman, A. D. (1996) Cell 92, 675–685
12. Jensen, R. B., Larr, R., and Gerdes, K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8550–8555
13. Funnell, B. E. (1991) J. Biol. Chem. 266, 14328–14337
14. Davis, M. A., Martin, K. A., and Austin, S. J. (1990) EMBO J. 9, 991–998
15. Funnell, B. E., and Gagnier, L. (1993) J. Biol. Chem. 268, 3616–3624
16. Funnell, B. E., and Gagnier, L. (1994) Biochimia (Par) 76, 924–932
17. Hayes, P., and Austin, S. J. (1994) J. Mol. Biol. 243, 190–198
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18. Yang, C.-C., and Nash, H. A. (1989) *Cell* **57**, 869–880
19. Rice, P. A., Yang, S. W., Mizuuchi, K., and Nash, H. A. (1996) *Cell* **87**, 1295–1306
20. Lobocka, M., and Yarmolinsky, M. (1996) *J. Mol. Biol.* **259**, 366–382
21. Rodionov, O., Lobocka, M., and Yarmolinsky, M. (1999) *Science* **283**, 546–549
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
23. Surtees, J. A., and Funnell, B. E. (1999) *J. Bacteriol.* **181**, 5898–5908
24. Bouet, J.-Y., and Funnell, B. E. (1999) *EMBO J.* **18**, 1415–1424
25. Demczuk, S., Harbers, M., and Vennstrom, B. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 2574–2578
26. Sigman, S. R., Kuwahara, M. D., Chen, C. H., and Bruice, T. W. (1991) *Methods Enzymol.* **208**, 414–433
27. Spassky, D. S., Rimsky, S., Buc, H., and Busby, S. (1988) *EMBO J.* **7**, 1871–1879
28. Radnedge, L., Davis, M. A., and Austin, S. J. (1996) *EMBO J.* **15**, 1155–1162
29. Dodd, I. B., and Egan, J. B. (1990) *Nucleic Acids Res.* **18**, 5019–5026
30. Erdmann, N., Petroff, T., and Funnell, B. E. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 14905–14910
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