Stable maintenance of P1 plasmids in Escherichia coli is mediated by a high affinity nucleoprotein complex called the partition complex, which consists of ParB and the E. coli integration host factor (IHF) bound specifically to the P1 parS site. IHF strongly stimulates ParB binding to parS, and the minimal partition complex contains a single dimer of ParB. To examine the architecture of the partition complex, we have investigated the DNA binding activity of various ParB fragments. Gel mobility shift and DNase I protection assays showed that the first 141 residues of ParB are dispensable for the formation of the minimal, high affinity partition complex. A fragment missing only the last 16 amino acids of ParB bound specifically to parS, but binding was weak and was no longer stimulated by IHF. The ability of IHF to stimulate ParB binding to parS correlated with the ability of ParB to dimerize via its C terminus. Using full and partial parS sites, we show that two regions of ParB, one in the center and the other near the C terminus of the protein, interact with distinct sequences within parS. Based on these data, we have proposed a model of how the ParB dimer binds parS to form the minimal partition complex.

The P1 prophage exists as a low copy number plasmid in Escherichia coli, and its active partition system ensures that P1 is stably maintained in a bacterial population. The P1 par operon encodes two trans-acting factors, ParA and ParB, and a cis-acting centromere-like site, parS. Analogous systems exist in a number of low copy number plasmids, and the genes for homologues of both ParA and ParB have been identified in the chromosomes of several bacterial species (1–7).

Both ParA and ParB are required for and have multiple functions in partition. ParB binds specifically to the parS site (8, 9), along with E. coli integration host factor (IHF). Together, these two proteins bend and wrap the DNA into a structure called the partition complex (10–13). Bright, localized foci of ParB bound to parS in vivo have been visualized by immunofluorescence (14). ParA is an ATPase and binds the par operator region, repressing parA and parB expression (15).

ParA also interacts directly with ParB bound at parS in an ATP-dependent manner (16). ParA is required for proper localization of the ParB-parS complexes (14), although it is not known how ParA promotes positioning of these complexes.

The plasmid centromere, parS, is a multipartite DNA site. An IHF binding site is flanked by two types of distinct sequences, called box A and box B, which are specifically recognized by ParB (11, 13) (Fig. 1A). Previous mutational and deletion analyses have shown that the presence and relative spacing of boxes A2, A3, B1, and B2 are essential for wild-type parS function in vivo, while boxes A1 and A4 are dispensable (11, 13, 17). ParB and IHF enhance each other’s affinity for parS (12, 17). IHF binding creates a large bend in parS (12), and an inherently bent DNA sequence can partially substitute for the IHF site in parS (18). One dimer of ParB binds across the IHF-directed bend in parS (19), forming a structure in which the DNA is wrapped around the protein core (10, 12, 19). Subsequent dimers load onto the partition complex with increasing ParB concentrations via a series of protein-protein interactions and specific and nonspecific protein-DNA interactions that tether increasing amounts of ParB to the partition complex.

Two independent self-association domains have been identified within ParB (Fig. 2A) (20). One domain is located within the last 59 amino acids of ParB and is probably the domain that mediates dimerization in solution. The second domain is located near the N terminus of the protein. Our observations suggested that this domain is involved in oligomerization (i.e. dimer-dimer interactions) but that some type of conformational change in ParB is required for oligomers to form (20). First, the N terminus of ParB is relatively susceptible to proteolysis, suggesting that it is not very stably folded in solution. Second, self-association of the N terminus, measured by chemical cross-linking experiments in vitro, was observed only when the C-terminal half of ParB was removed. One possibility is that DNA binding elicits a conformational change that removes the inhibitory effect of the C terminus on N-terminal self-association, promoting oligomerization (20). The N-terminal oligomerization domain either overlaps or is adjacent to a region of ParB that is required for an interaction with ParA (Fig. 2A) (20, 21).

Previously we began to define the structural domains of ParB by identifying stable fragments generated by limited exposure to trypsin (20). Tryptic digestion resulted in fragments with increasingly C-terminal start sites (Fig. 2A, arrows) that extended to, or close to, the C terminus of ParB. The two longest lived fragments started at residues 142 and 185, respectively (Fig. 2A, arrows C and D). These results suggested that the C-terminal dimerization domain forms the core of a very stably folded C-terminal half of ParB (20).

Two different regions of ParB appear to have a role in its specific DNA binding activity, one near the C terminus of ParB and the second in the middle of the protein (Fig. 2A). Point
mutations near the C terminus disrupt DNA binding activity (22). Proteins with these mutations also fail to dimerize, and it has been suggested that lack of dimerization prevents DNA binding. These mutations all fall within the last 59 amino acids of ParB, a region that encompasses the C-terminal dimerization domain (20). This region also includes the “discriminator recognition sequence” (DRS), defined by domain-swapping experiments (23). When residues 281–302 of P1 ParB were swapped with the equivalent region of the ParB protein of P7, the resulting hybrid protein recognized P1 parS in an in vivo partition assay (23). The P1 parS site is very similar to P1 parS. The P1 and P7 box A sequences are interchangeable, but the box B sequences are not (24). Thus, the swapping experiment defined the DRS as a region that contacts box B sequences are not (24). Thus, the swapping experiment defined the DRS as a region that contacts box B sequence(s) (22). ParB-box B) and protein-protein (dimerization) interactions results suggest a model of how protein-DNA (ParB-box A and ParB-box B) and protein-protein (dimerization) interactions contribute to the architecture of the partition complex.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Media—E. coli DH5 (F endA1 hsdR17 (rK mK) supE44 thi-1 recA1 gyrA96 relA1) was used for all plasmid constructions. E. coli BL21 (F ompT hsdS (rK mK) dcm gal) (DE3), pLysS-parS, and pLysS-parB expression plasmids were generated by PCR from New England Biolabs or Roche Molecular Biochemicals.

Plasmid Construction—The new constructs were used to generate ParB DNA substrates used for gel mobility shift assays as illustrated in Fig. 1B. The lower strand of each pair was labeled at the 5′-end with [γ-32P]ATP and T4 polynucleotide kinase. The single site oligonucleotide substrates used for gel mobility shift assays are shown in Fig. 1A. The labeled oligomers were purified from agarose gels.

Electrophoretic Mobility Shift Assays—The standard reaction mixture (10 μl) contained 0.1 nM 32P-labeled parS substrate in 50 mM HEPES-KOH (pH 7.5), 100 mM NaCl, 0.1 mM EDTA. The mixtures were incubated at room temperature. To stop the cross-linking and precipitate the proteins, samples were mixed with an equal volume of 30% trichloroacetic acid, incubated on ice for 20 min, and centrifuged at 4 °C. The pellets were washed with acetone and resuspended in 20 μl of 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 0.025% bromphenol blue. The samples were incubated at 90 °C for 3 min and were then analyzed by SDS-polyacrylamide gel electrophoresis.

RESULTS

In this work, we have examined the architecture of the partition complex by determining the DNA binding properties of various regions of ParB. ParB is 333 amino acids in length, and we previously examined its domain structure by partial proteolysis mapping and by the self-association activity of various ParB protein fragments (20). Here we have used several of those fragments to examine their interaction with the P1 parS site. All fragments contained a polyhistidine tag to facilitate purification. In addition, we have constructed six new versions of the DNA substrates (25).

The plasmids pBEF165 and pBEF166 contain the P1 parS sequence in opposite orientations in a modified Bluescript vector (22). For gel mobility shift assays with the intact parS site, pBEF166 was digested with HindIII, yielding a 232-bp parS fragment, or XhoI, yielding a 132-bp parS fragment. The DNA fragments were labeled at their 3′-ends with [γ-32P]ATP and DNA polymerase I large fragment and purified with phenol/chloroform extraction and ethanol precipitation (27). For DNase I footprinting reactions, DNA substrates were generated by digesting either pBEF165 or pBEF166 with XhoI and Smal or BglII and BstEEI, generating a 211- or 240-bp parS fragment, respectively. They were 32P-labeled at the 5′-end of the BamHI site. The fragments labeled on the upper strand (as shown in Fig. 1A) were generated from pBEF165, and fragments labeled on the lower strand were generated from pBEF166.

Competitor fragments for the gel mobility shift assays were 270 bp in length and were generated by PCR from pALA207 (28). The parS-containing fragment was amplified using the primers 5′-CTTTCTGGTAGATCCCTTTTCTC and 5′-CTGTTCCAGCAGGATGATG. The nonspecific fragment was amplified with the primers 5′-GCTTCTTGGGAGGATTGCAGT and 5′-CTGTTCCAGCAGGATGATG. The fragments were then purified from agarose gels.

In this study, we have examined the interaction of ParB with its specific sequences in parS by asking how different regions of the protein contribute to its DNA binding activities. Our results suggest a model of how protein-DNA (ParB-box A and ParB-box B) and protein-protein (dimerization) interactions contribute to the architecture of the partition complex.
of ParB (Fig. 2B). Our previous partial proteolysis experiments identified two tryptic fragments that were relatively resistant to further digestion. His-ParB-(142–333) (constructed here) and His-ParB-(187–333) (constructed previously (20)) correspond to these two tryptic fragments (Fig. 2A; Ref. 20). To examine the function of the extreme C terminus of ParB, we removed 3, 8, and 16 residues from its C terminus, generating His-ParB-(1–330), His-ParB-(1–325), and His-ParB-(1–317), respectively. Finally, we made two internal deletions of ParB that removed the HTH motif. The first removed only this predicted motif (residues 166–184), and the second removed residues 143–184, the region between the start sites of the two stable tryptic fragments mentioned above (Fig. 2B).

We first tested the dimerization activity of the new protein fragments in a cross-linking assay (Fig. 3). His-ParB-(142–333) and both proteins with internal deletions (His-ParB-(1Δ166–184) and His-ParB-(1Δ143–184)) were cross-linked to dimerized smears in the presence of DSP, a cross-linking reagent that reacts primarily with lysines. His-ParB-(1–330) and His-ParB-(1–325) were also able to dimerize, as determined by this assay (Fig. 3). His-ParB-(1–317), however, was not cross-linked, indicating that it is predominantly monomorphic in solution. It behaves as His-ParB-(1–312) (Fig. 3), which we previously reported not to be cross-linked by DSP (20). Therefore, the region between residues 317 and 325 of ParB defines the C-terminal boundary of its dimerization domain. The abilities of previously isolated ParB fragments to dimerize are summarized in Fig. 2B (20).

The Region between Residues 142 and 325 Contains All Information Required for Binding to the Full parS Site—In the presence of IHF, one dimer of ParB binds to parS in a very high affinity protein-DNA complex (12, 19). This interaction requires that ParB contact its specific recognition sequences on either side of the IHF-directed bend simultaneously (13). In the absence of IHF, ParB affinity for parS is much lower, and binding is primarily dependent on the box A and box B sequences on the right side of the IHF site (Fig. 1). Therefore, we can distinguish two types of ParB binding: the latter (low affinity), requiring specific contacts but not IHF, and IHF-stimulated DNA binding (high affinity), requiring both specific contacts and the IHF-directed bend in the DNA. In this work, we address the question of what regions of ParB contribute to the different aspects of ParB's DNA binding activities.

We first examined several C-terminal fragments of ParB (i.e. truncated from the N terminus) for their parS binding activity. Full-length ParB (or His-ParB in our assays), and three fragments, His-ParB-(47–333), His-ParB-(67–333), and His-ParB-(142–333), bound to parS, and this binding was stimulated by IHF (Fig. 4, A–C). The affinity of the different ParB fragments for parS differed, but the relative affinity of each ParB fragment for parS was much higher with IHF than without IHF. Therefore, the first 141 residues of ParB are not required for IHF-stimulated parS binding.

Next, we assessed the importance of the central portion of ParB in DNA binding. His-ParB-(187–333) and His-ParB-(275–333) contain both the C-terminal dimerization domains and the DRS but are missing the putative HTH domain located near the center of ParB (Fig. 2). Neither fragment had any parS binding activity, with or without IHF (Figs. 4D and 8 and data not shown). Therefore, a region N-terminal to residue 187 is required for stable parS binding in this assay. Since the first 141 residues were not required for binding (Fig. 4C), the region between amino acids 142 and 187, which includes the putative HTH motif, contains information required for DNA binding. We next examined the activity of ParB fragments lacking only this region, His-ParB-(1Δ166–184) and His-ParB-(1Δ143–184). Neither protein bound parS with or without IHF (Fig. 4, E and F), although some apparently nonspecific or unstable binding activity was observed at the highest protein concentrations. These results indicated that the central region of ParB is required for parS binding and the C-terminal DRS is not sufficient for stable parS binding.

Finally, we were interested in the role of the C terminus of ParB in DNA binding. We deleted 3 and 8 residues from the C terminus of ParB and tested the resulting fragments of ParB for their ability to bind parS in the gel mobility shift assay. His-ParB-(1–330) and His-ParB-(1–325) both exhibited IHF-stimulated DNA binding (Fig. 4, G and H), and both were able to dimerize as judged by cross-linking assays (Fig. 3). DNase I footprinting experiments showed that His-ParB, His-ParB-(47–333), His-ParB-(67–333), and His-ParB-(142–333) exhibited very similar patterns of protection of the parS sequence (Fig. 5A and data not shown). As predicted from the gel mobility shift experiments, these results indicate that the
first 141 amino acids of ParB are not required for minimal partition complex formation. Neither His-ParB-(187–333) nor His-ParB-(275–333) provided any protection of \( \text{parS} \) from DNase I cleavage, also consistent with the results from the gel mobility shift assays (data not shown). Similarly, His-ParB-(D143–184) and His-ParB-(D166–184) afforded no protection from DNase I cleavage (data not shown).

Footprinting experiments with His-ParB-(1–330) produced a pattern of protection similar to that of His-ParB but with additional enhancements (Fig. 5, B and C). First, on the upper strand of \( \text{parS} \), one band in box B1 was strongly enhanced. On the lower strand, one band near the center and one near the right boundary of the IHF binding site were strongly enhanced. These differences indicate changes in the geometry of the complex that make the minor groove more accessible to DNase I in these regions. These enhancements were not observed in the presence of His-ParB-(1–325), whose protection pattern was very similar to that of His-ParB.

C-terminal Dimerization Is Required for High Affinity \( \text{parS} \) Binding—In contrast to the fragments with small C-terminal deletions, His-ParB-(1–317), His-ParB-(1–312), His-ParB-(1–293), and His-ParB-(1–274) bound \( \text{parS} \), but a high protein concentration was required to observe binding (500–1000 nM), and little or no stimulatory effect by IHF was observed (Fig. 4, I and J, and data not shown). The IHF-insensitive binding is relatively weak, as is full-length ParB binding to \( \text{parS} \) in the absence of IHF (12). Therefore, removal of only 16 amino acids from the C terminus removed the ability of IHF to stimulate ParB’s specific DNA binding activity. The appearance of IHF-insensitive binding correlated with the loss of dimerization.
activity (20) (Figs. 2 and 3). Note that the N-terminal fragments with IHF-insensitive binding activity retain the putative HTH domain. His-ParB-(1–317) and His-ParB-(1–312) also retain the DRS.

ParB fragments that exhibited IHF-insensitive DNA binding activity in a gel mobility shift assay showed no detectable protection patterns in parS in DNase I footprinting experiments (Fig. 5D and data not shown). This was unexpected, because we had expected to observe binding to at least the box A motifs, since these fragments retained the HTH motif. We concluded that the parS binding activity of these IHF-insensitive ParB fragments was too weak to be detectable in a DNase
FIG. 5. DNase I footprinting of His-ParB and ParB fragments at parS. The upper and lower strands correspond to the upper and lower strands shown in Fig. 1. The DNA substrates were 211-bp (A and D) or 240-bp (B and C) DNA fragments that were 32P-labeled at the 3'-end of either the upper or lower strand. Protein-DNA complexes were treated briefly with DNase I (see “Experimental Procedures”) and analyzed on 6% sequencing gels. IHF, when present, was at 400 nM. ParB concentrations are reported for monomers. On each gel, Maxam-Gilbert G & A sequencing reactions were included as markers. The box A, box B and IHF sites are shown beside each gel. A, protection of the upper strand of parS from DNase I digestion by His-ParB-(142–333) or His-ParB. B, protection of the upper strand of parS from DNase I by His-ParB-(1–325), His-ParB-(1–330), or His-ParB. C, protection of the lower strand of parS from DNase I by His-ParB or His-ParB-(1–330). The arrows indicate enhancements created by His-ParB-(1–330). D, protection of the upper strand of parS from DNase I digestion by His-ParB, His-ParB-(1–317), or His-ParB-(1–312). The arrow indicates the enhancement generated by His-ParB-(1–317).

FIG. 6. Competition analysis of His-ParB-(1–317) binding to parS. The 32P-labeled DNA substrate was a BamHI restriction digest of pBEF165 and was present at 10 fmol/lane. The fragment containing parS was 252 bp (arrow). Increasing amounts of unlabeled 270-bp DNA fragments containing parS or non-specific sequence (indicated above the lanes in fmol) were added to the reaction mixtures prior to the addition of His-ParB-(1–317) (at 1 μM monomer). No IHF was added, and the mixtures were analyzed by electrophoresis in a 5% polyacrylamide gel (see “Experimental Procedures”).

| parS (fmol) | non-specific (fmol) |
|------------|---------------------|
| 1-317 ParB | + + + + + + + + + |
| 0          | 5 10 20 50 100 300 500 1000 |
| 1000       | + + + + + + + + + |

DNA Binding Domains of P1 ParB Protein
I footprinting assay. However, His-ParB-(1–317) did show evidence of a specific interaction with \( \text{parS} \). The same enhancements observed in the presence of His-ParB-(1–330) were also observed with His-ParB-(1–317) (Fig. 5D).

We tested whether the IHF-insensitive binding activity was still specific for \( \text{parS} \), using competition assays (Fig. 6). The ability of an N-terminal fragment of ParB to bind to a labeled \( \text{parS} \) substrate was challenged by a 270-bp unlabeled DNA fragment containing either the \( \text{parS} \) sequence or a nonspecific sequence. With both His-ParB-(1–317) and His-ParB-(1–312), the \( \text{parS} \) DNA was a better competitor than the nonspecific DNA, indicating that binding was specific (Fig. 6 and data not shown). All ParB fragments that bound \( \text{parS} \) also contained some nonspecific DNA binding activity, a property of the full-length protein (12).

N-terminal fragments smaller than His-ParB-(1–274) had no DNA binding activity (data not shown). His-ParB-(1–234) is not cross-linked by DSP, and His-ParB-(1–189) has some self-association activity through the N-terminal oligomerization domain (20). Neither fragment interacted with \( \text{parS} \). Therefore, a region C-terminal to residue 234 is required for binding, and N-terminal self-association of the protein is not sufficient to promote DNA binding.

**Binding to Individual Box A or Box B Sequences**—While the different DNA binding activities of the ParB fragments used in this study supported the hypothesis that the putative HTH and the DRS regions interact with box A and box B sequences, respectively, we asked whether we could demonstrate direct interactions to isolated box A and/or box B sequences. We designed synthetic oligomer (25-bp) substrates that contained only the box A inverted repeat (i.e. box A2-box A3), only a single box B sequence, or box A2 box A3 combined with box B1 (“box A/B”). The last substrate essentially represents the minimal \( \text{parS} \) site, from box A2 to B1, that is functional in vivo (17, 18, 29). As controls, we designed two nonspecific oligomer substrates. One contained the same sequence as the box B oligomer, except that the box B sequence itself was scrambled, and the second contained an unrelated 25-bp sequence (Fig. 1B). Binding to these oligomeric substrates by His-ParB or His-ParB fragments was weak and often produced smeary complexes. Nonetheless, we could compare binding of the box A/B substrate to that of the single site substrates, which provided
additional insight into ParB's DNA binding activities.

ParB binds to the minimal parS site weakly but specifically, and this site can promote partition in vivo, although it is less efficient than the full wild-type site in the presence of IHF (10–12). We expected, and found, that His-ParB binding to the box A/B substrate better than to either box A or box B substrate, the DNase I footprinting experiment shows an interaction of His-ParB-(1–317) with box B (the enhancements in Fig. 5A) showed weak but detectable binding to the box B substrate, but not to the box A substrate (Fig. 7C). Notably, binding to the box B substrate was about the same as to the box A/B substrate, implying that only the box B sequence contributes to its specific DNA binding activity.

Both His-ParB-(1–330) and His-ParB-(1–317) bound to the box A/B substrate better than to either box A or box B substrate alone (Fig. 7, D and E). As shown also by gel mobility shift assays and DNase I footprinting assays (Figs. 4G and 5C), the removal of 3 amino acids from the C terminus of ParB did not affect its specific DNA binding activities, and His-ParB-(1–330) must contain both the box A and box B recognition domains of the protein. While His-ParB-(1–317) appeared to bind the box A substrate better than the box B substrate, the DNase I footprinting experiment shows an interaction of His-ParB-(1–317) with box B (the enhancements in Fig. 5D). We suggest that removal of 16 residues from the C terminus does not remove but it does destabilize the domain of ParB that recognizes box B. Without proper box B recognition, ParB binding is specific but weak, and it is insensitive to IHF (Figs. 4F and 6).

The Putative Helix-Turn-Helix Domain Can Function as a Monomer—Our experiments indicate that a dimerized C terminus of ParB is necessary but not sufficient for IHF-stimulated DNA binding activity; ParB must contain both the C terminus (including the DRS) and the ITH for this activity. Since HTH domains often function as dimers (30), we asked whether this region in ParB must be dimerized via the C-terminal domain for ParB to bind parS. We formed heterodimers between native ParB (no histidine tag) and His-ParB-(275–333), at a 10:1 molar ratio, by denaturing and then slowly renaturing the protein mixture. This small C-terminal fragment of ParB is able to dimerize but has no DNA binding activity (Figs. 2B and 8A). To confirm that heterodimers were
formed, a portion of the ParB/His-ParB-(275–333) mixture was purified by nickel affinity chromatography. The majority of the mixture was retained on the column (data not shown). ParB dimers that bound the column must contain at least one histidine tag and therefore be present in the form of heterodimers.

The renatured proteins were used in gel mobility shift assays (Fig. 8). In the presence of IHF (Fig. 8A), the ParB/His-ParB-(275–333) heterodimer mix formed a complex that migrated between the parS + IHF (I) complex and the parS + IHF + ParB (I + B1) complex (Fig. 8A). This intermediate complex (I + (B/275)1), represents heterodimers binding to parS. Therefore, a single putative HTH domain, in the presence of a dimerized C terminus is sufficient to allow IHF-stimulated parS binding. Native ParB dimer complexes were also produced from the mixture, even when the heterodimers had been repurified over a nickel affinity column. This suggested that homodimerization of native ParB occurred over the course of the experiment. Finally, no intermediate complexes were observed in the absence of IHF (Fig. 8B); only complexes of the size expected from full-length ParB were seen.

**DISCUSSION**

Assembly of the P1 partition complex first requires the binding of one dimer of ParB and one α/β heterodimer of IHF to parS, forming a very high affinity protein complex. Previous biochemical experiments have shown that ParB simultaneously contacts its recognition sequences on both sides of the IHF-induced bend in the DNA (13). Here, we have used a series of ParB fragments to determine the role of different domains in parS binding activity and to position the different regions of ParB within the nucleoprotein complex. Our results show that dimerization of ParB is essential for the ability of IHF to stimulate ParB binding to parS. Therefore, the ParB-PAR interaction across the IHF-directed bend is at the dimerization interface. In addition, our data support the prediction that the ParB HTH region recognizes the box A motifs and that the DRS is part of the domain that recognizes the box B motifs. While ParB recognizes two distinct sequences with two distinct regions of the protein, both interactions are important to observe maximal parS binding activity.

Fig. 9 illustrates how we think one dimer of ParB interacts with and might be positioned on parS in the presence of IHF. His-ParB-(142–333) interacts with both the box A and box B sequences, and this interaction is stimulated by IHF. Therefore, His-ParB-(142–333) contains all of the information required for assembly of the minimal partition complex that contains one dimer of ParB. The putative HTH motif of ParB is required for stable binding to the intact parS site (Fig. 4, E and F) and is necessary for an interaction with a box A inverted repeat on an oligonucleotide substrate (Fig. 7C). In our model, the HTH region of each monomer interacts with a box A sequence in the A2-A3 inverted repeat, similar to a typical HTH protein such as the λ repressor. Our results also indicate that the C terminus is directly involved in box B binding and that the bend induced by IHF is unable to stimulate ParB binding to parS in the absence of the C-terminal dimerization domain of ParB (Fig. 4, I and J). These observations support our previous prediction that the dimerized C termini of a ParB dimer interact with both box B1 and B2 simultaneously at or near the dimerization interface (19).

Two observations suggest that dimerization and box B binding are at least partially separable functions. First, His-ParB-(1–317), a protein fragment that did not dimerize, was able to recognize the box B sequence, as evidenced by the enhanced observation in its DNase I footprint (Fig. 5C). Second, His-ParB-(1–317) binding to the oligomer substrates was enhanced by the presence of a box B sequence (Fig. 7E), supporting the idea that dimerization and box B binding are separable functions. However, we cannot exclude the possibility that a small amount of dimerization occurs in the presence of the DNA, particularly if this deletion has only partially destabilized the dimerization domain.

**The Putative Helix-Turn-Helix Domain**—Based on sequence alignments, ParB has been predicted to contain a HTH domain between residues 166 and 187 (25). Secondary structure prediction of ParB by PHD (31, 32), shown schematically in Fig. 9A, indicates a high probability that a helix is formed between residues 169 and 174 and between residues 180 and 188 of ParB. Between these two helices is a gap that includes a glycine residue, a residue that is highly conserved in the turn region of a classical HTH motif (30). While our biochemistry does not provide any structural evidence for such a domain, we
have shown that the region spanning this putative motif is required for native DNA binding activity.

Typically, HTH proteins, such as the λ repressor, bind their recognition sites as dimers (30). The arrangement of a ParB dimer interacting with boxes A2 and A3 resembles that of a typical HTH dimer interacting with an inverted repeat binding site. The formation of an IHF-stimulated complex by ParB/His-ParB-(275–333) heterodimers (Fig. 8) shows that the HTH region need not act as a dimer. However, binding of a typical HTH dimer interacting with an inverted repeat ParB dimer interacting with boxes A2 and A3 resembles that of their recognition sites as dimers (30). The arrangement of a half of ParB, but yeast two-hybrid experiments narrowed this domain to residue 142 of ParB or whether these higher order complexes are stabilized by interactions between the N-terminal self-association domains in ParB as well as by protein-DNA interactions. DSP cross-linking experiments showed that this domain was in the N-terminal half of ParB, but yeast two-hybrid experiments narrowed this to within the N-terminal 61 residues (20). Both His-ParB and His-ParB-(47–333) formed higher order complexes in our experiments, while His-ParB-(67–333) did not, consistent with our model. However, His-ParB-(142–333) also appeared to form higher order complexes. We cannot tell whether there are additional self-association contacts that are more C-terminal to residue 142 of ParB or whether these higher order complexes are promoted only by protein-DNA interactions. Another possibility is that the N-terminal oligomerization domain is mainly involved in dimer-dimer interactions that mediate pairing of partition complexes. The role of this domain awaits further structural analyses of ParB in these higher order complexes.

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