Plasmid Partition Mechanisms

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ABSTRACT The stable maintenance of low-copy-number plasmids in bacteria is actively driven by partition mechanisms that are responsible for the positioning of plasmids inside the cell. Partition systems are ubiquitous in the microbial world and are encoded by many bacterial chromosomes as well as plasmids. These systems, although different in sequence and mechanism, typically consist of two proteins and a DNA partition site, or prokaryotic centromere, on the plasmid or chromosome. One protein binds site-specifically to the centromere to form a partition complex, and the other protein uses the energy of nucleotide binding and hydrolysis to transport the plasmid, via interactions with this partition complex inside the cell. For plasmids, this minimal cassette is sufficient to direct proper segregation in bacterial cells. There has been significant progress in the last several years in our understanding of partition mechanisms. Two general areas that have developed are (i) the structural biology of partition proteins and their interactions with DNA and (ii) the action and dynamics of the partition ATPases that drive the process. In addition, systems that use tubulin-like GTPases to partition plasmids have recently been identified. In this chapter, we concentrate on these recent developments since the publication of the first edition of Plasmid Biology in 2004. We will briefly introduce the biology of plasmid partition systems to date; we refer the reader to the earlier chapter on plasmid partition for a comprehensive review of their biology (1).

Partition is a dynamic process; plasmids are moved and positioned inside the cell so that cell division separates at least one copy into each daughter cell. Although there is significant diversity among the types of plasmid systems, their genetic organization and components are remarkably conserved. Plasmid partition systems typically consist of two proteins and one or more partition, or par, sites, which are the DNA sites that direct the action of the segregation machinery. The par sites are considered prokaryotic centromeres because they are required in cis for plasmid stability and because they are the assembly sites of the segregation machinery. One partition protein is a site-specific DNA binding protein that recognizes the par site(s), and is often referred to as the centromere-binding protein, or CBP. The second protein is an ATPase or GTPase, which uses the energy of nucleotide binding and hydrolysis to move plasmid DNA inside the cell. The genes
are typically arranged in an operon, and the proteins regulate their own expression. The location of the par site varies; it can be directly downstream of the par genes, upstream and close to the promoter for the operon, or in multiple locations on the plasmid.

Par systems can be divided into different classes, based on the properties of the NTPase. Type I systems encode Walker ATPases, and the ATP binding site contains a specific variant of the canonical Walker A motif (2–4). ATPases of type II systems contain actin-like ATP binding sites, and the proteins structurally and enzymatically resemble eukaryotic actin (2, 3). Type III systems encode GTPases that resemble tubulin (6–8). Recent structural information for members from all of these classes has validated the classification based on primary sequence data. Therefore, it is likely that since both the sequence and structure of each class of NTPase are conserved, the properties of the NTPase also reflect similar mechanisms of action.

The CBPs fall into two general classes. The first are helix-turn-helix (HTH) DNA binding proteins, and the second are ribbon-helix-helix dimers (RHH2). In addition to site-specific DNA binding activities, they assemble into higher-order oligomers at and around the par site. The interaction of this partition complex with the NTPase is essential for the dynamics and patterning/positioning activities of the NTPase.

To regulate par gene expression, one Par protein directly represses transcription and the other stimulates the repressor activity of its partner (reviewed in references 1, 2, 4). In most partition systems, the CBP is the direct repressor, which acts on operator sites that resemble or act also as centromere sites in the operon. In some type I systems, however, the ATPase is the repressor. Type I ATPases can be divided into two subgroups, Ia and Ib, based on domain analysis. Both contain the core partition activities, whereas type Ia ParAs contain an additional site-specific DNA binding domain, which is responsible for the transcriptional repressor activity.

Many bacterial chromosomes encode plasmid-like partition systems, which have been shown to contribute to bacterial chromosome segregation (reviewed in references 4, 9, 10). These are, so far, invariably type Ib Walker ATPases with an HTH CBP. Chromosomal systems have also been shown to support plasmid partition when cloned into a low-copy-plasmid vector (11). The chromosomal proteins also interact with host replication and other factors, such as DnaA initiator proteins and SMC (structural maintenance of chromosome) condensins, which add another level of complexity to chromosomal segregation in the bacterial cell cycle (12–14). The plasmid systems can be considered minimalist cassettes, which provide excellent context to study the basic biological mechanism of action of these proteins.

In this review we concentrate on recent developments in our understanding of these two broad steps: partition complex assembly at the par site and the mechanisms of the NTPases that move plasmid cargo inside the cell. There are several plasmid systems that have provided significant structural and mechanistic information in the past several years. The paradigms for type Ia partition are Escherichia coli plasmids P1 and F, for type Ib partition they are Salmonella enterica plasmid TP228 and Streptococcus pyogenes plasmid pSM19035, and for type II partition they are E. coli plasmid R1 and Staphylococcus aureus pSK41. In addition, E. coli plasmid pB171 encodes two partition systems, parI (type II) and par2 (type Ib) (15). Both contribute to plasmid stability, and studies of each have added to our understanding of plasmid partition in bacteria. We concentrate on these systems to discuss CBPs and partition mechanisms, but not exclusively, because results from many systems have contributed to our current understanding (Table 1 summarizes specific nomenclature for the main systems discussed in this chapter). Finally, we describe the new class of partition systems that encode tubulin-like GTPases, primarily from studies of the virulence plasmids pXO1 of Bacillus anthracis and pBtoxis of Bacillus thuringiensis.

**PARTITION COMPLEX RECOGNITION AND ASSEMBLY**

The first step in plasmid partition is site-specific DNA binding of the CBP to the par site. There is considerable divergence in sequence among CBPs and par sites in plasmid partition systems. The sites can vary in sequence, organization, number, and arrangement on plasmid chromosomes. The sequence diversity presumably evolved to avoid competition among different, otherwise compatible, plasmids. The par sites vary in length, and the par site typically overlaps with the promoter of the par operon when the CBP is the repressor, which is the case for most type Ib, type II, and type III systems. In contrast, the par sites of type Ia systems are distinct from the operators. They can be downstream of the par genes, for example, in plasmids P1, P7, and F. The RepABC family of plasmids from Agrobacterium and Rhizobium genera (repA and repB are the partition genes) show both variable position and number of par sites (18). The N15 prophage/plasmid in E. coli has four par sites, and plasmid stability
TABLE 1  Plasmid partition system nomenclature

<table>
<thead>
<tr>
<th>Plasmid(s)</th>
<th>Bacterial host</th>
<th>ATPase/GTpace</th>
<th>CBPa</th>
<th>par site</th>
<th>Referenceb</th>
</tr>
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<tbody>
<tr>
<td><strong>Type I ATPase Systems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1, P7</td>
<td><em>E. coli</em></td>
<td>ParA</td>
<td>ParB</td>
<td>parS</td>
<td>27</td>
</tr>
<tr>
<td>F</td>
<td><em>E. coli</em></td>
<td>SopA</td>
<td>SopB</td>
<td>sopC</td>
<td>26</td>
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<tr>
<td>RK2, RP4</td>
<td><em>E. coli/Pseudomonas aeruginosa</em></td>
<td>IncC</td>
<td>KorB</td>
<td>Oβ</td>
<td>29, 36</td>
</tr>
<tr>
<td>TP228</td>
<td><em>S. enterica</em></td>
<td>ParF</td>
<td>ParG</td>
<td>parH</td>
<td>57</td>
</tr>
<tr>
<td>pSM19035</td>
<td><em>S. pyogenes</em></td>
<td>δ</td>
<td>ω</td>
<td>parS</td>
<td>58</td>
</tr>
<tr>
<td>pB171 (par2)</td>
<td><em>E. coli</em></td>
<td>ParA</td>
<td>ParB</td>
<td>parC</td>
<td>15</td>
</tr>
<tr>
<td><strong>Type II ATPase Systems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td><em>E. coli</em></td>
<td>ParM</td>
<td>ParR</td>
<td>parC</td>
<td>15, 22, 61</td>
</tr>
<tr>
<td>pB171 (par1)</td>
<td><em>E. coli</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>pSK41</td>
<td><em>S. aureus</em></td>
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<tr>
<td>pLS20</td>
<td><em>B. subtilis</em></td>
<td>Alp7A</td>
<td>Alp7R</td>
<td>alp7C</td>
<td>16, 65</td>
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<tr>
<td>pLS32</td>
<td><em>B. subtilis</em></td>
<td>AIFa</td>
<td>AIFB</td>
<td>parN</td>
<td>17, 75</td>
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<tr>
<td><strong>Type III GTPase Systems</strong></td>
<td></td>
<td></td>
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<tr>
<td>pXO1</td>
<td><em>B. anthracis</em></td>
<td>TubZ</td>
<td>TubR</td>
<td>tubC</td>
<td>8, 125</td>
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<tr>
<td>pBtoxis</td>
<td><em>B. cereus</em></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td><em>B. thuringiensis</em></td>
<td></td>
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*CBP, Centromere-binding protein
For simplicity, selected studies that define the nomenclature of the partition loci listed here and that are mentioned in this chapter are cited. References 1, 2, and 4 also provide a more extensive review of the original literature.

does depend on the number of sites (19, 20). It is unclear why some plasmids utilize more than one par site, but the answer presumably reflects the architecture of the partition complex.

Although DNA binding specificity differs among CBPs, there are several common themes in partition complex assembly following sequence recognition. First, binding of one CBP is not sufficient for partition, and all partition complexes contain multiple CBPs bound to their respective par sites. This large structure therefore presents many potential binding sites for the cognate NTPase. The modulation of many protein-protein interactions is likely important for the dynamics of partition, so that plasmids are never completely released from their interactions with the NTPase during movement. Second, the interaction of the CBP/plasmid partition complexes stimulates the NTPase activity of the partner, and this modulation of the ATP binding and hydrolysis cycles is necessary for plasmid dynamics. Third, the CBPs are thought to pair (or group) plasmids together. Pairing has been shown to occur in several systems (21–23), although the role of pairing is not understood.

**HTH CBPs**

CBPs with HTH DNA binding motifs are found in type Ia plasmid partition systems and in all known bacterial systems. Again, the sequence conservation among these CBPs is not high, but there is a conservation of domain organization (24–28). Dimerization is mediated by a C-terminal dimerization domain, adjacent to the central HTH DNA binding domain. The N-terminal region of the protein interacts with the ATPase and mediates oligomerization of the CBP at and around the par site. The HTH CBPs have been observed to spread away from their par sites, but the exact molecular nature of this activity is not clear.

The structures of the dimerization and DNA binding domains have been determined for three plasmid HTH ParBs: P1 ParB, F SopB, and RP4 KorB (Table 2) (29–33). All three DNA binding domain structures were solved in complex with their specific DNA binding sites (Fig. 1A–C). For KorB and SopB, the dimerization and DNA binding domains were solved separately. None of the N-termini of these CBPs were amenable to crystallization, consistent with data indicating that they are flexible and not stably folded in solution (24, 26, 34, 35).

The par sites of HTH CBPs contain inverted repeat recognition elements, as is typical of this type of DNA binding motif. RP4 KorB (and that from the related plasmid RK2) is a global transcriptional regulator as well as a CBP, and it binds to 12 related inverted repeat operator sequences called Oβ in different locations in the plasmid (reviewed in reference 4). One, Oβ3, is thought to act as the par site (36). The F sopC partition site consists of 12 copies of a 43-bp repeat, only one of which is essential for partition. A 16-bp inverted repeat within the 43-bp region is the specific binding site for SopB (33, 37), and the remaining sequence is postulated
to be required for proper spacing of the inverted repeats so that they are arranged on the same face of the DNA helix (37). Plasmids such as P1 and P7 in *E. coli* are members of a group of plasmids with bipartite partition sites (reviewed in reference 1). These ParBs recognize two distinct sequence motifs; one is an inverted repeat (the A-box) and the second is a hexamer sequence (the B-box). These motifs are asymmetrically arranged around a binding site for *E. coli* IHF protein. IHF binding strongly stimulates ParB binding to parS in vitro and ParB activity in partition in vivo.

The HTH structures of KorB, SopB, and ParB are all-helical domains that are highly similar to each other (Fig. 1A–C). As predicted from canonical HTH/DNA interactions, the HTH regions of KorB, SopB, and ParB bind to the inverted repeat sequences in DNA (P1 ParB HTH with box-A) such that the “recognition helix” sits in the major groove. Interestingly, the specificity determinants for DNA binding differ among these three proteins. P1 ParB makes its specific contacts with the inverted repeat via residues in this helix (α3) (31, 32). F SopB makes specific contacts via residues in this helix (α3) as well as with a residue, R219, outside of the HTH (33). Genetic and biochemical evidence confirms that R219 is required for sequence-specific binding by SopB (38). KorB makes only nonspecific DNA contacts via the HTH motif (α3–α4), and specificity is determined by residues in helices α6 (T211) and α8 (R240) (30).

### Table 2: Structures of plasmid partition proteins

<table>
<thead>
<tr>
<th>Type</th>
<th>Plasmid protein</th>
<th>Cofactor(s)</th>
<th>PDB identifier</th>
<th>Reference</th>
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<tr>
<td>ATPase</td>
<td>P1 ParA</td>
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<td>3EZ7</td>
<td>97</td>
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<td></td>
<td>ADP</td>
<td>3EZ2, 3EZ6</td>
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<td></td>
<td>P7 ParA</td>
<td>None</td>
<td>3EZ9, 3EF</td>
<td>97</td>
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<td></td>
<td>ATPyS</td>
<td>2OZE</td>
<td></td>
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<tr>
<td></td>
<td>TP228 ParF</td>
<td>ADP</td>
<td>4D77, 4E03</td>
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<td></td>
<td>AMP-PCP</td>
<td>4E07, 4E09</td>
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<td>CBP</td>
<td>RP4 KorB dimer domain</td>
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<td>29, 30</td>
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<td>RP4 DNA binding domain</td>
<td>17-mer Oq</td>
<td>1R71</td>
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<td></td>
<td>P1 ParB</td>
<td>25-mer parS</td>
<td>1ZX4</td>
<td>31, 32</td>
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<tr>
<td></td>
<td>16-mer parS</td>
<td>2NTZ</td>
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<tr>
<td></td>
<td>F SopB dimer domain</td>
<td>18-mer sopC</td>
<td>3MKW, 3MKY, 3MKZ</td>
<td>52, 54</td>
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<tr>
<td></td>
<td>F SopB DNA binding domain</td>
<td>18-mer PcopS</td>
<td>1RQ</td>
<td></td>
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<tr>
<td></td>
<td>pSM19035 ω</td>
<td>17- and 18-mer PcopS</td>
<td>2BNW, 2BNZ</td>
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<tr>
<td></td>
<td>TP228 ParG</td>
<td>None</td>
<td>1P94</td>
<td>53</td>
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<tr>
<td></td>
<td>pCXC100 ParB</td>
<td>None</td>
<td>3NO7</td>
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<td><strong>Type II ATPase Systems</strong></td>
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<tr>
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<td>ADP</td>
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<td>AMP-PNP</td>
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<td>GDP</td>
<td>2ZGY</td>
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<td></td>
<td>GMP-PNP</td>
<td>2ZZZ</td>
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<td>AMP-PNP, ParR101–117</td>
<td>4A6Z</td>
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<tr>
<td></td>
<td>pSK41 ParM</td>
<td>None</td>
<td>3JS6</td>
<td>77</td>
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<td><strong>Type III GTPase Systems</strong></td>
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<tr>
<td>GTPase</td>
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<td>3M8K</td>
<td>125, 127</td>
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<td>GDP</td>
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<td>GTPyS</td>
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<td>GDP</td>
<td>4E17</td>
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<tr>
<td></td>
<td>GTPyS</td>
<td>4E19</td>
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<tr>
<td><strong>CBP</strong></td>
<td>pBtoxis TubR</td>
<td>None</td>
<td>3MBE, 3M8F, 3M9A</td>
<td>125, 129</td>
</tr>
<tr>
<td></td>
<td>24-mer tubC</td>
<td>4ASS</td>
<td></td>
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<tr>
<td></td>
<td>26-mer tubC</td>
<td>4ASS</td>
<td></td>
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<tr>
<td></td>
<td>pBM400 TubR</td>
<td>None</td>
<td>4ASN</td>
<td>129</td>
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</table>

*Bacterial species for most plasmids are listed in Table 1, with the exception of pCXC100 (from *Leifsonia xyli*) and pBM400 (from *Bacillus megaterium*). Models of ParM filaments based on cryo-EM data (70).
All proteins make several contacts with the phosphate backbones of their respective sites.

The presence of another DNA recognition element in parS, the B-box, adds a unique and extra level of complexity to the P1 ParB family of proteins. The secondary DNA binding domain in ParB is an integral part of the C-terminal dimerization domain and requires dimerization to form (31). The dimer domain is a six-stranded β-sheet coiled coil; two loops between β1 and β2 and between β2 and β3 sit in the major groove and make sequence-specific contacts with the B-box sequence (Fig. 1A) (31, 32). Interestingly, the structure of the F SopB dimer domain is very similar to that of ParB except for these extended loops, which explains the lack of DNA binding by this region of SopB (33). Therefore, while the structure is conserved, the DNA binding function is not. In contrast, the dimer domain of KorB

**FIGURE 1** Structures of DNA binding domains of plasmid CBPs. (A–C) The DNA binding domains of HTH CBPs: (A) P1 ParB\textsuperscript{142–335}, (B) F SopB\textsuperscript{157–271}, and (C) RP4 KorB\textsuperscript{139–152} are shown bound to their respective centromere sequences (gray). The HTH motifs are in yellow. Residues that make base-specific contacts with the centromere DNA are shown as sticks (red) and can be found both within the HTH (ParB and SopB) and in the adjacent four-helix bundle (KorB). The dimerization domain of ParB is its second DNA binding domain (to the B-box DNA motif, green DNA). The ParB structure bridges across four DNA molecules (31). Because the dimer domains are absent in the structures of SopB and KorB, two monomers are presented interacting with the one inverted repeat sequence. Additional monomer–monomer contacts (cyan) in the SopB structure suggest it may also bridge across DNA molecules (not shown) (33). (D–H) The DNA binding regions of RHH\textsubscript{2} CBPs. Type Ib CBPs of (D) TP228 ParG\textsuperscript{1–76}, (E) pCXC100 ParG\textsuperscript{69–128}, and (F) pSM19035 ω\textsuperscript{23–71} and type II ParRs of (G) pB171 ParG\textsuperscript{1–96} and (H) pSK41 ParR\textsuperscript{3–48} illustrate the simple β-strand dimerization and DNA binding [represented as red sticks in (F) and (H)] interface stabilized by α-helical interactions. Type-Ib CBPs contain a flexible N-terminal tail (not shown) that interacts with their cognate ATPase. Type II CBPs mediate their cognate ATPase contacts through the C-termini, present in the pB171 ParR structure (G). The C-terminal region of ParRs also promotes higher order assembly on DNA, yielding a super-helical structure, as illustrated by the crystal packing of the pSK41 ParR structure in (I). The electrostatic representation of the superhelical filament shows the electropositive surface (blue) interacting with the DNA and the electronegative surface (red) on the inside of the helical filament. The latter interface interacts with the predominately electropositive surface of its cognate ATPase ParM and serves as a cap for stabilizing ParM filament in the cell. All structural images were generated using PyMOL v1.6.0.0 software (Schrödinger, LLC 2010). PDB information is listed in Table 2. doi:10.1128/microbiolspec.PLAS-0023-2014.f1
is a five-stranded antiparallel all β-sheet structure that strongly resembles the SH3 (Src-homology 3) fold and not that of the ParB/SopB dimerization domains (29). In this case, function (dimerization) is conserved but structure is not.

Because the P1 ParB crystal structure contains both the dimer domain and the HTH domain, it provides unique information on the overall conformation of the protein. In ParB, a short flexible linker connects the HTH α-helical domain with the β-sheet dimer domain. Even with this flexibility, the HTH domains point away from each other, so it is not possible that they contact the same inverted repeat. Indeed, in the structure, a single dimer of ParB bridges across parS sites such that the HTH of one monomer binds to half of the inverted repeat on one DNA molecule while the HTH of the other monomer binds to the other half of the inverted repeat on a different DNA molecule (Fig. 1A). Interestingly, this cross-site arrangement is supported by the SopB/sopC crystal structure even though it did not contain the SopB dimerization domain (33). There were some dimer contacts within the DNA binding domains (Fig. 1B), so in this arrangement, the corresponding DNA binding domains bridged different DNA molecules.

Similarly, one dimer of P1 ParB is able to bridge across four sites due to the extra DNA binding sites (to box-B motifs) in its dimer domains (32). This arrangement gives ParB remarkable flexibility in binding to its various motifs in parS so that multiple ParB dimers can cooperate to bind to a single parS site or across paired sites (Fig. 1A) (31, 39). It is attractive to propose that this flexibility and the ability to pair across sites are conserved among HTH ParBs. Conformational data for KorB suggests similar flexible or disordered linkers (35). Indeed, this flexibility would explain the inability to obtain crystal information for both the HTH and dimer domains in the same structure except in the case of P1, in which the presence of DNA, and binding to both DNA boxes, would constrain this flexibility.

N-termini of HTH CBPs
In general, the N-termini of HTH CBPs contain the regions required for an interaction with the partition ATPase and for oligomerization with itself (24, 26, 27, 40). Interaction with the ATPase is mediated by residues close to the N-terminus, and where tested, a critical arginine is involved in stimulation of ParA ATPase activity. The latter property is shared with type Ib RHH2 CBPs (see below). There are exceptions; for example, sequences in the center of RK2 KorB have also been implicated in interactions with IncC, its cognate ATPase (Table 1) (28). There are no structures of the N-termini of plasmid HTH CBPs, likely because these regions are flexible and somewhat disordered (24, 26, 35). The crystal structure of the N-terminus of an archaeal ParB, *Thermus thermophilus* SpolJ, is a dimer with an extended dimerization interface (41). The relevance of this structure for plasmid CBPs is unclear because this SpolJ fragment does not dimerize in biochemical assays and lacks the strong dimerization domain at its C-terminus (41). Nevertheless, it is attractive to consider that this structure represents the higher-order oligomerization interaction, which is weak in solution by itself but would normally occur in the context of the full-length protein after binding to its cognate par site (42).

After the HTH CBP recognizes and binds to its par site, more CBP molecules are recruited to form higher-order complexes containing many protein molecules. *In vivo*, these can be seen as bright foci of fluorescently labeled CBP that colocalize with plasmids (43–47). F SopB and P1 ParB have been shown to spread away from their binding sites (48, 49), and this is thought to be a general property of this class of CBPs. This activity likely represents the loading of multiple ParBs onto the plasmid DNA so that a highly concentrated focus of multiple proteins is available for interactions with ParA during the partition reaction. The minimal stoichiometry of ParB to plasmid necessary for partition is unknown, except that extensive spreading (>400 bp away from the site) is not required *in vivo* (50). How CBPs interact with each other and with DNA around the par site is an important and as yet unanswered question in partition complex assembly.

**RHH Dimer CBPs**
The RHH2 structure is common in many CBPs (Fig. 1D–H). This DNA binding motif was originally characterized in the Arc/MetJ family of transcriptional repressors (51). Indeed, these CBPs are, in those systems that have been tested, also transcriptional repressors. As opposed to the α helix contacts with DNA by HTH proteins, RHH2 proteins bind to DNA via their antiparallel β-sheets, which insert into the major groove of and make sequence-specific contacts with the DNA (51). The two α helices of each monomer interact with each other to form a tight dimer. In general, the RHH2 CBPs are small proteins but otherwise diverge significantly in their primary sequence. They are common among type Ib and type II partition systems. The primary difference in domain organization among the CBPs of the latter two
classes is in the position of the region that interacts with the ATPase. In type I CBPs, the RHH2 domain is C-terminal, and these proteins interact with the ATPases via a small and flexible N-terminal region. The type II CBPs interact with DNA via an N-terminal RHH2 domain and with the cognate ATPase via the C-terminus.

**Type I CBPs**

Structures of three RHH2 CBPs from type I partition systems have been determined (Fig. 1D-F). They are TP228 ParG, pSM19035 ω (omega), and pCXCI00 ParB (52–53). The first, TP228 ParG, is a 76-amino acid polypeptide consisting of a C-terminal (residues 33 to 76) DNA binding domain and an N-terminal mobile tail (residues 1 to 32). The structure of the C-terminal domain was determined by nuclear magnetic resonance (53) and contains the minimal DNA binding domain and dimerization determinant. The N-terminal extension of ParG is necessary for interactions with the ParF ATPase (56) and also modulates the binding of the C-terminal RHH2 domain to DNA to improve specificity over nonspecific DNA binding (57).

The RHH2 structure of pSM19035 ω bound to its DNA site was solved by X-ray crystallography, providing a direct picture of the assembly of partition complexes (Fig. 1F) (54). ω is a global regulator of transcription as well as a CBP. It is a 71-amino acid protein that binds to series of 7-bp repeats, and affinity depends on the number and arrangement of these heptads. pSM19035 contains three parS sites, each with multiple heptad repeats. The crystal structure of ω20-71 in complex with two heptad repeats, both in direct and inverted orientations, confirmed base-specific contacts with the β-sheets in the DNA major groove. The crystal structure has led to a model in which ω2 dimers bind to successive repeats by wrapping around the parS DNA as a left-handed helix, without bending or otherwise distorting the DNA (23, 54, 58). The stoichiometry is one ω2 per heptad, and these partition complexes do not spread beyond the par site, in contrast to the HTH class of CBPs. As with ParG, the unstructured N-terminal tail of ω2 is necessary for its interaction with the ATPase δ. Another feature that ω2 shares with other CBPs is the capacity to pair plasmids at parS sites, which requires δ and ATP (23, 58).

The crystal structure of the DNA binding domain of pCXCI00 ParB (residues 69 to 128 of 139 total) shows high structural similarity to those of ParG and ω2 (Fig. 1E; Table 2) (55). Its N-terminus is also likely a flexible tail, as it was highly sensitive to proteolytic digestion.

**Type II CBPs**

Type II CBPs are typically named ParR, because the R1 plasmid ParM/ParR/parC system has been the paradigm for functional studies of this class of partition mechanism (Table 1). ParR binds to the partition site, parC, which in R1 consists of 10 11-bp direct repeats upstream of the par genes and overlaps with the promoter (59). ParR can also pair plasmids at parC sites, and pairing is stimulated by the ParM ATPase (22). Unfortunately, R1 ParR was not amenable to crystallization, but the crystal structures of two other ParRs have been solved: pB171 ParR5–95 by itself, and pSK41 ParR1–53 in complex with DNA (Fig. 1G, H) (60, 61). The pSK41 par site consists of two sets of five 10-bp repeats, and two repeats (20 bp) constitute the minimal DNA binding site for ParR (61). pSK41 ParR crystallized as a dimer of RHH2 dimers bound to this 20-bp sequence. The exciting observation for both ParR structures was their super-structure crystal packing, which provides a detailed picture of the higher-order ParR/parC partition complex and how it may interact with the ParM ATPase (Fig. 1I) (60, 61). The proteins assembled into a superhelical structure containing 12 ParR dimers (or 6 pairs of dimers) per 360° turn. This superstructure is consistent with electron microscopy pictures of R1 ParR bound to parC (60). The DNA wraps on the outside of the helix on a positively charged outer surface of the protein helix. The C-termini of the ParR molecules point inward, toward the center cavity, and are negatively charged. Because ParM interacts with the C-termini of these ParR molecules, the resulting models propose that ParM monomers interact with the concave surface of this helical structure as they insert themselves into the growing actin-like ParM filament during partition.

**PARTITION DYNAMICS PROMOTED BY PARTITION NTPases**

ParM and Actin-Like Proteins

A major step toward our understanding of plasmid partition dynamics was first elucidated in the type II partition systems. Several elegant biochemical, cell biology, and structural studies have established that the ParM class of ATPases (named for the R1 plasmid ParM) work as “cytomotive” filaments that resemble the behavior of eukaryotic cytoskeletal elements (Fig. 2) (reviewed in reference 62).

ParM is structurally and biochemically similar to eukaryotic actin, although the overall sequence similarity is low (63). It shares an ATPase motif originally described...
in a variety of proteins, including actin, Hsp70, and hexokinase (5). This group includes MreB, a bacterial protein required for proper cell shape (64). Both ParM and MreB possess the actin-like ATPase fold and subdomains and formed protofilaments reminiscent of F-actin (the polymerized form), and these observations led to the proposal that they represented prokaryotic cytoskeletal elements. A sequence search by Derman et al. has identified over 40 actin-like families of proteins in prokaryotes (65), which were generically called ALPs (actin-like proteins). Evidence is accumulating that these are related families of filament-forming, or cytoskeletal, proteins. They include bacterial, plasmid, and phage-encoded proteins.

The ALPs from several plasmids (R1 and pB171 in E. coli, pSK41 in S. aureus, and pLS32 and pLS20 in Bacillus subtilis) have been examined in detail in a variety of in vivo and in vitro studies. The results support an insertional polymerization, filament-growth mechanism for plasmid transport inside the cell, although there are several structural and mechanistic differences among the families.

The ParMs from R1 and pSK41 are closely related to each other and are the best understood of the type II partition ATPases (Fig. 3). ParM monomers polymerize in the presence of ATP or GTP to form left-handed two-start helices that are dynamically unstable (63, 66–70). The molecules in the filament hydrolyze ATP to ADP but are stable in the filament form as long as they are protected at the end by a cap of molecules in the ATP-bound conformation (Fig. 2A). Loss of this cap results in rapid depolymerization, or catastrophe. The super-
helical ParR/parC structure (Fig. 1I) is thought to be the cap, such that ParM monomers interact with C-termini of ParR on the concave surface of ParR/parC. ParM polymerizes at this interface, effectively pushing the plasmid. Filaments with one end bound to ParR/parC are proposed to search for a second plasmid in cycles of polymerization and catastrophe (67). When the filament captures a second plasmid, and thus has ParB/parC bound at both ends, plasmid separation and movement occurs as the plasmids are pushed to opposite poles of the cell (Fig. 2A, B).

Both in vivo and in vitro experiments support the patterns and the dynamic behavior of ParM and ALPs. Using the R1 system, simultaneous labeling of plasmids and ParMs in vivo revealed filaments of ParM with plasmids attached at the tips, both by immunofluorescence (71) and in live cells (72). The filaments grew between plasmids and then disassembled, showing that polymerization pushes plasmids apart. ParM mutants unable to hydrolyze ATP formed long, stable filaments. This behavior was recapitulated in vitro by TIRF microscopy, which showed filament growth that was coupled to parC-coated beads (67). The filaments went through cycles of growth and shrinkage that depended on ATP binding and hydrolysis. ParM filaments also assemble as bundles, which are visible in vivo by fluorescent microscopy and cryo-electron microscopy (72, 73).

FIGURE 3 ParM structures. The ParM structures from (A) R1 and (B) pSK41 are shown in the absence of nucleotide, the open state of these proteins. ATP binds in the central cleft, and the loops important for binding ATP are highlighted in green. The rest of the structure is colored according to the structural conservation (blue, most conserved; red, least conserved; gray, little to no identifiable conservation) through alignment with the actin structure (PDB 1yag, not shown). Most of the conservation with actin is in domains IA, IIA, and IIB. Domain IB of R1 ParM closes over the ATP binding pocket when nucleotide is present (direction of motion illustrated in (A)). This cap serves to close the structure, forming the pointed end (domains IB and IIB) of the protein. During filament growth, the pointed end interacts with the barbed end (domains IA and IIA), as shown with the closed forms of ParM in (C). Binding of a ParR101–117 peptide induces a closer interaction between the pointed domains, which is proposed to either stabilize the filament to slow down ATP hydrolysis or prevent filament disassembly even in the presence of ATP hydrolysis. ParR binding is confined to the end of the filament because the interaction between the pointed and barbed interfaces of each monomer occludes the ParR binding site (74). All structural images were generated using PyMOL v1.6.0.0 software (Schrödinger, LLC 2010). PDB information is listed in Table 2. doi:10.1128/microbiolspec.PLAS-0023-2014.f3
A central question is the arrangement of ParM filaments in the cell during partition. The individual filaments are polar; that is, they have a pointed end and a barbed end reflecting head to tail polymer arrangement (Fig. 3C). How can these filaments grow bidirectionally, and how does ParR/parC interact with different ends? A recent study that examined ParM growth with TIRF microscopy as well as a cocystal structure of ParM in complex with a peptide from ParR indicated that ParR/parC interacts with the barbed end, resulting in unidirectional growth as new ParM monomers are added to this end (74). In addition, ParM bundles contain antiparallel sets of ParM double-helical filaments (Fig. 2A, B). In this way, one plasmid can be attached at each end of the bundle, or spindle. This scenario further proposes that the pointed end of ParM filaments is protected from catastrophe by pairing with another ParR/parC bound filament.

Other ALPs
Studies of ParMs/ALPs of several other plasmids support the hypothesis that they promote plasmid movement by filamentation (65, 75–78). Interestingly, however, the details of structure and mechanism differ among these proteins. Based on primary sequence, ParM of pSK41, AlfA of pLS32, and Alp7A of pLS20 fall into different classes of prokaryotic ALPs than that of R1 ParM (and those of each other) (65). The proteins also show different architectures and dynamics of their filaments. For example, although the crystal structure of pSK41 ParM confirms its actin-like domains, the protein assembles into one-strand helices, and filaments are not dynamically unstable (77). AlfA forms filaments in vivo and in vitro, which are double-stranded but with a different architecture than that of R1 ParM and are not dynamically unstable (75, 76, 78). Alp7A forms filaments in vivo that colocalize with plasmids, grow and push plasmids apart, are dynamically unstable, but are also capable of treadmill (65). These and other molecular differences in nucleotide specificity, stability, and the seed size for nucleation, for example, have been noted and indicate that the mechanisms by which plasmids use cytotoxic filaments will also differ.

ParAs
By far the most common type of partition system that has been identified in bacterial plasmids uses Walker ATPases as the positioning protein. For simplicity, we will use the most common nomenclature and call these ATPases ParA, and their cognate CBPs ParB, when discussing general members of this class. ParAs contain a deviant version of the Walker A motif (KGGxxK[T/S]) (79), which is the P loop, part of the ATP binding site that interacts with the phosphates of ATP and ADP. The second lysine in the motif is conserved in all Walker ATPases, whereas the first lysine is often called the signature lysine for the ParA class of ATPases. ParA ATPases are also related in sequence, structure, and activity, to bacterial MinD proteins, which are involved in proper positioning of the cell division septum. ParA and MinD are members of a larger class of related ATPases, which include proteins involved in the positioning of other macromolecular structures in bacteria (reviewed in references 80, 81). Studies of MinD support the idea that the ParA/MinD (or “ParA-like”) family of proteins shares common mechanisms of action.

In vivo, fluorescent ParA fusions display dynamic patterns that coincide with the bacterial nucleoid. They have been reported to form broad helical structures, patches, or foci that oscillate over nucleoids, for example (44, 46, 47, 82–85). The oscillatory behavior is also similar to that of E. coli MinD protein, except that the latter movement is on the membrane (86). Oscillation of ParAs requires the presence of cognate ParBs and par sites.

ParA interactions with ATP, with DNA, and with itself
ATP binding and hydrolysis are involved in all properties and interactions of ParAs. Much of our recent understanding of the biochemistry of plasmid ParAs comes from studies with P1 ParA, F SopA, TP228 ParF, pB171 ParA, and pSM19035 δ protein (23, 26, 40, 56, 58, 87–96). Crystal structures of P1 ParA (97), pSM19035 δ (58), and TP228 ParF (98) (Table 2, Fig. 4) have also contributed insight into the action of this class of ParAs.

ParA ATPase activity is strongly stimulated by the cognate CBP and by DNA (40, 56, 58, 100–102). Often this stimulation is highest when the CBP is bound to the par site (40, 58, 101). An arginine residue close to the N-terminus of the CBP is necessary for ATPase stimulation, which has led to the proposal that an “arginine-finger” interaction is responsible (40, 56). In this model, the arginine is inserted into the ATPase active site of ParA to alter catalysis. However, a recent cocystal structure of MinD with MinE (its stimulatory partner) peptide has challenged this model because the comparable region of MinE sits in a cleft on MinD and not within the ATPase active site (103). Nevertheless, the requirement for a critical arginine in the N-terminus of the CBP is emerging as a common feature among these ParAs.
All ParAs form dimers. For most, dimerization is influenced by adenine nucleotides, although the effects of ATP and ADP differ among different members of Walker partition ATPases. TP228 ParF as well as bacterial Soj and MinD are monomers with ADP and dimers with ATP (98, 104, 105). In contrast, pSM19035 δ, another type Ib ParA, forms dimers with or without ATP or ADP (58). Dimerization of type Ia ParAs is stimulated by both ATP and ADP but can occur at high protein concentration without nucleotide (92, 97, 106). Biochemical experiments with P1 ParA show that it exists in a monomer-dimer equilibrium that is shifted toward dimer in the presence of ATP or ADP (106).

Another important property of ParA ATPases, and one that distinguishes them from ALPs, is an ATP-dependent non-specific DNA (nsDNA) binding activity (58, 87, 92, 93). Again, this behavior is shared with B. subtilis Soj (107). The nsDNA binding activity maps to residues in the C-terminus of the ATPase, mutation of which destroys nsDNA binding in vitro and partition activity in vivo (92, 96, 107). In P1 ParA, however, ATP binding is necessary but not sufficient for the nsDNA binding activity. Following ATP binding, which is fast, ParA-ATP then undergoes a slow conformational change to a form that is able to bind DNA nonspecifically, called ParA-ATP* (87). A requirement for ATP to bind DNA nonspecifically is an unusual property of DNA binding proteins and suggests that the ability to control DNA binding is an essential feature of the partition mechanism. The slow conformational change from ParA-ATP to ParA-ATP* is thought to be a key timing step in the mechanism of ParA, and ParA-like, protein action (see below).

A feature of ParAs that is shared with ALPs is a propensity to polymerize (58, 88, 93–95, 97, 102, 108), but the role of this polymerization is not understood. Mutations in TP228 ParF that eliminate polymerization in vitro are defective for partition in vivo, supporting the idea that the activity is necessary for ParA mechanism (98). In vitro, ParAs polymerize, but the conditions and requirements for polymerization differ, and it has not been possible to generalize polymerization dynamics as it has for ParM (for a comparison, see reference 81).

Does polymerization represent formation of structural filaments or the cooperative association of ParA dimers when ParA binds to nsDNA and/or to ParB-DNA complexes? In vivo no filaments of plasmid ParAs that resemble the dynamic behavior of ALPs have been reported. In vitro at physiological concentrations (≤5 μM), neither direct visualization by TIRF microscopy nor size exclusion chromatography of P1 ParA showed significant polymerization (87, 91). The simplest explanation is that cooperativity of ParA, perhaps as the formation of short polymers, is necessary for higher-order complex formation with ParB and DNA, but the resolution to this debate awaits further understanding of the role of this cooperativity in ParA action.

Higher-order complexes with ParBs
How does ParA interact with ParB on DNA? Biochemical experiments have demonstrated that ParA interacts with ParB bound to DNA, particularly ParB bound to the par site. ParA-ParB-DNA complexes have been detected and examined in electrophoretic mobility shift, light scattering, sucrose-density gradient, and electron microscopy assays (23, 58, 88, 96, 109). Complexes form with ATP and not with ADP. They are larger/more stable with ATPyS, indicating that ATP binding is necessary for assembly and ATP hydrolysis promotes disassembly. This conclusion is further strengthened by the behavior of ParA mutants that can bind but not hydrolyze ATP (89, 96). In addition, while these higher-order complexes form with both specific and nsDNA, they are more stable when DNA containing the par site is present (88, 96). Taken together, these observations suggest that these complexes represent the interaction of plasmids via the ParB/parS partition complexes, with ParA bound to the bacterial nucleoid (88, 96).

Structural biology of ParAs
Crystal structures for four plasmid ParAs have been solved: P1 ParA (apoParA and ParA-ADP), P7 ParA (apoParA), pSM19035 δ (δ-ATPyS), and TP228 ParF (ParF-ADP and ParF-AMPPCP) (Table 2; Fig. 4) (58, 97, 98). In addition, structures of an archaeal ParA, T. thermophilus Soj, and bacterial MinD proteins have been determined (103–105, 110, 111). The results illustrate structural features that are common to the ParA-like class of ATPases.

As predicted from the conservation of the Walker A motifs, the structure of the ATPase core is similar among these proteins (Fig. 4G–I). The second lysine in Walker A (conserved in all ParA ATPases) contacts the phosphates of ADP/ATP, and the conserved aspartate residues in Walker A’ (also called switch 1) and Walker B (switch 2) coordinate the Mg^2+ ions. The nucleotide binding site sits at the dimer interface, and several structures are consistent with a “sandwich dimer” in that the nucleotide is bound by residues from both monomers. The signature lysine of ParA-like ATPases crosses the dimer interface to interact with the γ-phosphate of ATP that is bound by the other monomer for ParF, Soj, and MinD.
In P1 ParA, this lysine is positioned at the dimer interface but is unoccupied because only ADP is bound. The exception is δ-ATPγS; however, the signature lysine is on a flexible loop, and in this structure it is not positioned to interact with ATP (Fig. 4B) (58).

Although no DNA/ParA structures have been solved, the nDNA binding region of ParA is in the C-terminus, based on mutagenesis studies (92, 96, 107). The surface charge distribution of the existing ParA structures is consistent with this prediction because areas of positive charge are localized on the surface of the C-termini (Fig. 4D–F).

For ParF, Soj, and MinD, the ADP forms are monomers, and the ATP forms are dimers (98, 104, 105). P1 and P7 ParAs, both type Ia ATPases, are dimers with and without nucleotides at the concentrations used for crystallography. However, the dimerization interfaces differ in the apo (P1 and P7) and ADP-bound (P1) forms (97). The structures indicate a flexible dimer interface in the apo form, allowing several different conformations of dimers. ADP binding appears to lock ParA into one dimer conformation. In addition, ADP promotes the folding of a winged HTH motif in the N-terminus of the protein that is responsible for site-specific DNA binding to the par operator sequence for the repressor activity of ParA.

The ParF-AMPPCP structure also provides intriguing data concerning the polymerization activity of ParAs (98). Within the crystal, linear ParF polymers resulted from dimer-dimer contacts that were rotated 90° relative to each other, rather than the head-to-tail polymerization seen with ALP/ParM proteins. Mutation of key residues in the dimer-dimer interface of ParF eliminated polymerization of ParF in vitro, measured by light scattering, and partition activity in vivo. It will be very interesting to see how this arrangement of ParF is involved in partition activity and whether it is shared by other ParAs as more crystal structures of these proteins become available.

The identification of additional ParA-like molecules in other uncharacterized plasmid systems has also begun to contribute to structural information. A sequence study of four large (≥40 kilobases) plasmids in cyanobacterium Synechocystis sp. PCC 6803 identified a parA homologue common to each plasmid (112), of which one was subsequently crystallized and deposited to the Protein Data Bank (sll6036, PDB ID 3cwq). This structure and its sequence show high similarity to those of TP228 ParF, suggesting a type Ib partition mechanism.

**Type Ia ParAs and transcriptional regulation**

The repressor activity of type Ia ParAs is due to a site-specific DNA binding domain that recognizes and binds an operator sequence in the promoter region of the par operons (100, 113–116). In vivo, however, ParA repressor activity is weak unless stimulated by its cognate ParB protein. In vitro, site-specific DNA binding requires ATP or ADP, which distinguishes this activity from nDNA binding that strictly requires ATP. In the P1 system, evidence from ParAs with mutations in the ATP binding site indicate that ParA-ATP and ParA-

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**FIGURE 4** Structures of ParAs. (A–C) Structures of the nucleotide-bound forms of (A) P1 ParA (ADP), (B) pSM19035 δ (ATPγS), and (C) TP228 ParF (AMPPCP). Nucleotides are shown in red and magnesium, when present, in green. The structures are presented with their dimerization interfaces perpendicular to the viewing plane. (D–F) Surface charge electrostatics for monomer views of the above proteins are presented as electropositive (blue) or electronegative (red) and were generated with the Adaptive Poisson-Boltzmann Solver software (99). Structures were superimposed with the PyMOL software package, with similar orientations of each monomer [(D) ParA, (E) δ, and (F) ParF] to expose the nucleotide binding pocket and dimerization surface. DNA binding regions are characteristically electropositive, such as the winged HTH in ParA (D). (G–I) Alignments of the nucleotide binding pockets of ParA, δ, and ParF. The structure of the protein backbone is shown, with the side-chains of critical residues represented as sticks. Nucleotides are in red and magnesium ions are in green. The phosphate-binding regions consist of three conserved motifs: (G) Walker A and A′, in purple and Walker B in orange in (H). Also shown in (G) is the signature lysine (blue) of ParA-like Walker A motifs. (I) The region highlighted in tan is structurally conserved among ParA proteins and provides multiple specific contacts with the adenine moiety. (J) The sequences of the nucleotide binding regions above are aligned with each other and with the overall secondary structure for ParA, δ, and ParF (α-helices in red, β-strands in blue). The Walker A, A′, and B motifs are indicated above the sequence alignment. All structural images were generated using PyMOL v1.6.0.0 software (Schrödinger, LLC 2010). PDB information is listed in Table 2. doi:10.1128/microbiolspec.PLAS-0023-2014.f4
ADP, but not ParA-ATP*, are the forms competent for transcriptional repression (87, 89, 114). Mutations that prevent the ParA-ATP* transition act as super-repressors; that is, they repress much more strongly than wild-type ParA represses, and their repression is insensitive to ParB. This observation also suggests an explanation for the corepressor activity of ParB; that is, ParB converts ParA-ATP* to ParA-ADP and/or ParA-ATP. An intriguing question concerning the type Ia class of ParAs is why repressor activity is integrated into the ATP cycle of these proteins.

Models for ParA action

What is the mechanistic force that drives plasmid segregation? Two general models have been proposed, which arise from (i) similarities among the ParA/MinD class of proteins and (ii) polymerization properties of ParA and ParM-like proteins.

The first model, based on reaction-diffusion-type mechanisms and the mechanism of MinD action, is supported by growing biochemical, genetic, and cell biology evidence. Reaction-diffusion mechanisms, originally proposed by Alan Turing (117), depend on the interactions between two components to set up biological patterns. In this model for partition, interactions of ParB with ParA set up dynamic patterns of ParAs on the bacterial nucleoid. In essence, ParB both creates and follows a wave of ParA on the nucleoid. This model, termed diffusion-ratchet, proposes that plasmids, via the ParB/parS complex, ratchet along the surface of the bacterial nucleoid following an oscillating wave of ParA (Fig. 5) (87).

The first critical parameter is the ATP-dependent nsDNA binding activity of ParAs. Because the bacterial nucleoid is the major nsDNA in the cell, it is proposed as the surface along which the plasmids move, in an ATP-dependent fashion. The second key parameter is the slow interconversion of the DNA binding and nonbinding forms of ParA, which essentially creates two pools of ParA in the cell: one that is bound to the nucleoid and one that is free to diffuse in the cytoplasm. Many ParB molecules bind at and around parS on the plasmid, which creates a high local concentration of ParB. ParB on the plasmid binds to ParA on the nucleoid (a positive interaction) and then acts to remove ParA from the nucleoid (a negative interaction) via ATP hydrolysis. Because of the slow timing step, ParA cannot immediately rebind the nucleoid and is free to diffuse throughout the cell, losing its positional memory. The latter interaction creates a void of ParA on the nucleoid, which means that ParB must move toward adjacent ParA on the nucleoid for the next interaction. Repeated cycles of these interactions result in plasmid ratcheting along the surface of the nucleoid. In effect, ParB is following the gradient of ParA that it creates. When two plasmid/ParA complexes are present, they will move away from each other toward the nearest high concentration of ParA because the ParA between them has been depleted.

As in the above situation, a slow timing step is crucial for this type of patterning mechanism to yield two populations of ParA, bound and freely diffusing. This step has been identified biochemically for P1 ParA (the slow ParA-ATP to ParA-ATP* conformational transition), but not yet for other ParAs. In principle, it could be any step in the ATP cycle of the protein, so this property may vary among different ParAs. Indeed, this timing step is a critical feature for the dynamic mechanism of MinD movement and has been proposed to be at the step of nucleotide exchange in this system (118). Another possibility may be a slow dimerization step for those ParAs that dimerize only with ATP.

The oscillatory patterns of ParAs over the bacterial nucleoid seen in vivo are consistent with this model (46, 47, 85). The dynamic interactions of ParB/plasmid complexes with ParA-coated DNA (as a mimic of the nucleoid) have also recently been recapitulated in vitro using TIRF microscopy (90, 91) and support the ATP-dependent steps proposed for this model of ParA-mediated plasmid partition.

The alternative model for ParA action proposes that filaments of ParA either push or pull plasmids and was suggested based on the polymerization properties of ParAs (85, 95). The energetics of movement would be provided by insertion or removal of ParA molecules at the end of a filament to which the ParB/plasmid complexes are attached, conceptually similar to ParM action. The model was subsequently modified to suggest that ParA filaments polymerized on DNA, to explain the involvement of nsDNA in ParA action, and that filament disassembly (retraction) pulled plasmids inside the cell (85). However, the in vitro and in vivo behavior of fluorescent plasmid ParA molecules does not detect discrete filaments (46, 47, 85, 87, 90, 91, 102). The debate about these two models continues (for example, see references 119, 120), and its resolution will likely depend on further in vitro reconstruction of the partition reaction as well as a better understanding of the role of polymerization in ParA action.

ParA-like ATPases in other processes

ParA-like ATPases participate in the transport of a variety of subcellular structures and organelles, as well
as DNA, in bacteria (reviewed in references 80, 81, 119). These ParA-like proteins, including MinD, use ATP-dependent patterning on the surface of either the nucleoid (ParA, gray ovals) and one not, which is diffusible in the cytoplasm (ParA, white squares). The conversion between these two forms is slow and depends on the ATP binding cycle of ParA. In P1 ParA, the slow step is a specific conformational change after it binds ATP (ParA-ATP to ParA-ATP* [87], not shown). (B) ParB/plasmid complexes interact with ParA on the nucleoid, and this interaction stimulates the conversion of active ParA back to the diffusible form. Because the conversion to the active form is slow, the inactive form diffuses away from its original location, leaving a void of ParA on the nucleoid. (C–D) This movement continues as ParA rebinds ATP and is then converted back to the DNA binding active form. (E–F) When two plasmid complexes are present, they move toward the nearest, high concentration of ParA on the nucleoid, away from each other. doi:10.1128/microbiolspec.PLAS-0023-2014.f5

FIGURE 5 Diffusion-ratchet mechanism of plasmid partition. (A) ParA exists in two forms, one active to bind the nucleoid (ParA*, gray ovals) and one not, which is diffusible in the cytoplasm (ParA, white squares). The conversion between these two forms is slow and depends on the ATP binding cycle of ParA. In P1 ParA, the slow step is a specific conformational change after it binds ATP (ParA-ATP to ParA-ATP* [87], not shown). (B) ParB/plasmid complexes interact with ParA on the nucleoid, and this interaction stimulates the conversion of active ParA back to the diffusible form. Because the conversion to the active form is slow, the inactive form diffuses away from its original location, leaving a void of ParA on the nucleoid. (C–D) This movement continues as ParA rebinds ATP and is then converted back to the DNA binding active form. (E–F) When two plasmid complexes are present, they move toward the nearest, high concentration of ParA on the nucleoid, away from each other. doi:10.1128/microbiolspec.PLAS-0023-2014.f5

TYPE III (TUBULIN-LIKE) PARTITION SYSTEMS

TubZ

Relatively new to the realm of plasmid partition systems are members of the tubulin-like superfamily of bacterial proteins. The first of these proteins identified was RepX, a protein necessary for replication and maintenance of the virulence plasmid pXO1 of B. anthracis (6). RepX
bore sequence homology to FtsZ, a GTPase and tubulin-like protein necessary for cell division in bacteria. A similar gene and function was characterized from the endotoxin-encoding plasmid pBtoxis of *B. thuringiensis* subsp. *israelensis* (2). The protein, renamed TubZ (for tubulin), from both *B. anthracis* (Ba-TubZ) and *B. thuringiensis* (Br-TubZ), was shown to form filaments in *vivo* and to be necessary for plasmid maintenance (8, 123). In pBtoxis, the genetic arrangement of tubZ also supported that it belonged to a partition system. An additional open reading frame, now called tubR, and a set of repeat sequences as a putative partition site, tubC, were upstream of tubZ. TubR is a small HTH protein that binds to tubC and interacts with TubZ (124, 125). The wrinkle in this system is its apparent requirement during plasmid DNA replication as well as segregation, which has yet to be explained. Nevertheless, the properties of TubRZ/tubC strongly suggest that it is a segregation system, and its coordination with replication of these plasmids is an intriguing unanswered question.

The biochemistry, cell biology, and structural biology of TubZ and TubR support a filamentation mechanism for partition of these plasmids (Fig. 2C). TubZ is a GTPase that polymerizes into filaments in the presence of GTP (8, 123, 124, 126). A critical concentration of TubZ is necessary to initiate filamentation, and mutations that prevent GTP hydrolysis lead to longer filaments and plasmid instability in *vivo*. Several crystal structures of TubZ (Table 2) have been reported and illustrate that these proteins do share the FtsZ and tubulin folds predicted from their sequences (125, 127, 128). The initial Br-TubZ structure confirmed the expected nucleotide-binding Rossman fold seen in both FtsZ and tubulin structures (127). Electron microscopy of TubZ filaments along with the crystal structures of TubZ bound to GDP and GTPyS indicate that TubZ forms a parallel, double helical filament (126–128). TubZ in the filaments is almost all in the GDP form, suggesting that a GTP cap must be present to prevent depolymerization (126). This pattern is reminiscent of ParM filaments. However, there is one important distinction about filamentation dynamics. In *vivo*, Br TubZ-GFP forms filaments that grow from one (the plus) end and disassemble from the other (minus) end, a behavior known as treadmilling (8) (Fig. 2C). The latter property has led to a cable-car model in which plasmids, via TubR, move along the growing TubZ filament (125) (Fig. 2C). Interestingly, in *vivo* when TubZ filament growth reaches a cell pole, it curves and continues along the inside of the cell. One proposal is that this bending stress acts as a switch for the plasmid to “hop off” the cable (125).

**TubR/tubC**

The tubC partition site in pBtoxis contains four imperfect 12-bp repeats, to which TubR binds (124, 125). A closer inspection based on TubR binding further refined the region to seven repeats in two sets, to which TubR binds cooperatively (129). This arrangement, two sets of direct repeats, is also reminiscent of the type II par site, R1 parC.

CBPs in type III partition systems take structural features from both type I and type II systems. Crystallization of TubR from both *Bacillus megaterium* pBM400 and *B. thuringiensis* pBtoxis revealed a compact, dimerized CBP that oligomerizes on centromeric DNA similarly to ParR of the type II partition systems (125, 129). Unlike the compact type Ib and type II CBPs, however, TubR possesses a winged-HTH structure instead of the RHH$_2$ motif. Interestingly, DNA recognition is different than that by canonical HTH proteins. The recognition helix of the HTH makes up a majority of the dimerization interface, and the N-termini of these paired helices project into a single major groove of tubC. A basic patch of residues on the wing and short helix of the HTH (Arg$^{74}$, Lys$^{43}$, and Lys$^{79}$ in Br-TubR) further contribute to DNA binding by interacting with the phosphate backbone; mutation of these residues abrogates DNA binding activity (125, 129). The positioning of dimers on DNA is such that the wings of one dimer are inserted into successive minor grooves, but the wings of adjacent dimers are tightly paired in the same minor groove (129). In this way, TubR forms an oligomerized helical filament with the DNA bound around the surface of the protein in a fashion also reminiscent of type II ParR (Fig. 2C).

**WHAT NEXT?**

Our understanding of plasmid partition has advanced considerably, yet many important questions still remain. At the forefront of these is the mechanism of type I ParAs. What is the role of ParA polymerization, for example? How does the ATP binding and hydrolysis cycle of these proteins dictate the steps in the patterning reactions? What is the role of ATP hydrolysis by ParA, and ParB stimulation thereof, in the partition mechanism? A recent study of the F Sop system concluded that SopB stimulation of SopA ATPase activity modulated, but was not required for, the oscillatory patterning of SopA in the cell (130). In addition, evidence suggested that this stimulation was involved in separation of plasmid pairs or complexes during the cell cycle. Indeed, how plasmids count to make sure the required number...
of plasmids are partitioned is unknown. How do ParA-ParB and ParB-DNA interactions mediate pairing and unpairing? What is the biochemical basis for longitudinal and bidirectional movement? Another important question concerns the role of the nucleoid and its shape on mechanism as well as on the cell biology patterns in vivo.

There are variations within any one class of partition system that we have discussed here, for example, the different architectures and dynamics of plasmid ALPs. Therefore, although general models have been developed, we anticipate there will also be significant variation in the molecular interactions involved. This variation is an exciting aspect of plasmid partition research. We expect that as more protein (and protein-DNA) structures are determined, as in vitro reconstitution of partition dynamics is achieved, and as additional genetic analyses identify novel phenotypes, our understanding of plasmid partition will progress rapidly.

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REFERENCES


KorB-binding site O(B)3 but other KorB-binding sites form destabilizing activity of the RK2 central control region requires only


Plasmid Partition Mechanisms


