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Plasmid Partition System of the P1*par* Family from the pWR100 Virulence Plasmid of *Shigella flexneri*

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P1*par* family members promote the active segregation of a variety of plasmids and plasmid prophages in gram-negative bacteria. Each has genes for ParA and ParB proteins, followed by a *parS* partition site. The large virulence plasmid pWR100 of *Shigella flexneri* contains a new P1*par* family member: pWR100*par*. Although typical *parA* and *parB* genes are present, the putative pWR100*parS* site is atypical in sequence and organization. However, pWR100*parS* promoted accurate plasmid partition in *Escherichia coli* when the pWR100 Par proteins were supplied. Unique BoxB hexamer motifs within *parS* define species specificities among previously described family members. Although substantially different from P1*parS* from the P1 plasmid prophage of *E. coli*, pWR100*parS* has the same BoxB sequence. As predicted, the species specificity of the two types proved identical. They also shared partition-mediated incompatibility, consistent with the proposed mechanistic link between incompatibility and species specificity. Among several informative sequence differences between pWR100*parS* and P1*parS* is the presence of a 21-bp insert at the center of the pWR100*parS* site. Deletion of this insert left much of the *parS* activity intact. Tolerance of central inserts with integral numbers of helical DNA turns reflects the critical topology of these sites, which are bent by binding the host IHF protein.

The enteric bacterium *Shigella flexneri* is the causative agent of shigellosis, a severe human enteric disease. It is one of the leading causes of death in Third World countries. Pathogenic strains of *S. flexneri* contain a large (200-kb) plasmid, pWR100, which encodes many of the factors essential for virulence (17). The sequence of this plasmid has been determined (3) (accession number AL391753). It revealed a homolog of the partition region of the plasmid prophage of bacteriophage P1.

The P1*par* (partition) region consists of an operon containing *parA* and *parB* genes and a centromere analog site, *parS*, that lies downstream (1). Like similar systems found in other low-copy-number plasmids, it assures the faithful distribution of the plasmids between dividing cells by an active process akin to mitosis (10, 19). The *parA* gene encodes a Walker-type ATPase essential for plasmid movement during partition (6, 15). The *parB* gene encodes a protein that can bind tightly to the partition site, *parS* (5, 8). It is required for capture of the plasmid at the cell center prior to partition (15).

There are now six identified members of the P1*par* family. New loci have been identified in the large virulence plasmids of *Salmonella* and *Shigella* species and the Rts1 plasmid of *Proteus vulgaris* (A. Dabrazhynetskaya, K. Sergueev, and S. Austin, unpublished data). In addition to P1*par*, two others have been previously studied: those found in bacteriophage P7 from *Escherichia coli*, and the virulence plasmid pMT1 from *Yersinia pestis* (25; Dabrazhynetskaya et al., unpublished). These two have a similar structure and organization to P1*par*. Each has a unique species specificity. The Par protein of each species works only with the cognate *parS* site from the same species (12, 25; Dabrazhynetskaya et al., unpublished). The three previously studied species also have unique specificities for partition-mediated incompatibility. Two plasmids with identical *par* systems are incompatible because they displace each other from the cell. This is due to competition for selection of the plasmid as a substrate for partition (2). In the cases studied so far, incompatibility and species specificity appear to correlate: each family member exerts incompatibility against plasmids with its own type of *par* element but does not interfere with the activity of the other *par* family members (12, 25; Dabrazhynetskaya et al., unpublished). The P1*parS* site consists of a central integration host factor (IHF) binding region flanked by two arms that bind ParB. Within the arms lie a specifically arranged set of motifs, the BoxA and BoxB sequences (Fig. 1B). BoxA sequences are the principle binding sites for ParB (5, 9). The BoxB sequences also contact ParB. They are essential for partition and control the species specificity by allowing P1 ParB to bind and by excluding the binding of P7 ParB (20, 23). P7*parS* is organized similarly to P1*parS* but has a different BoxB motif. Substitution of the P7 BoxB sequences into an otherwise-intact P1 *parS* site switches the specificity, so that the site then works exclusively with the P7 Par proteins (12, 13). Similarly, the unique BoxB sequences of the pMT1*parS* site also determine species specificity (Dabrazhynetskaya et al., unpublished). There are two hexamer BoxB sequences in each *parS* site. They generally differ from those of another species by only one or two bases. The species-specific information in the cognate ParB protein also appears to be limited to a small region: a run of less than 11 amino acids near the carboxy terminus in the case of P7 ParB (20). Changes in a small amount of information in BoxB and its contact point on the ParB protein appear to be capable of defining a number of species specificities for the system.

Here, we characterize the pWR100*par* element from *S. flex-*

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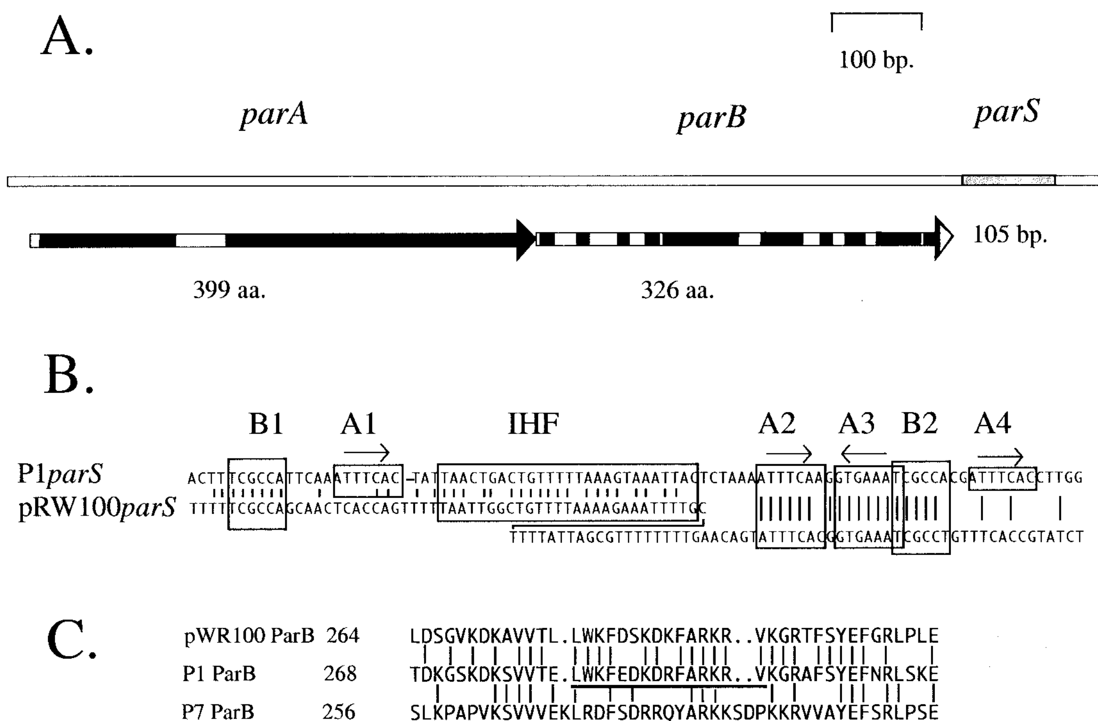

FIG. 1. The pWR100 *par* region. A. Map of the pWR100*par* region showing the *parA* and *parB* open reading frames and the *parS* site. Black boxes on the open reading frame arrows show regions of very high similarity to the amino acid sequences of the equivalent P1 Par proteins. Blocks of >10 amino acids with >90% similarity to the P1 proteins are included. B. Alignment of the *parS* sequences of pWR100 and P1. The pWR100*parS* site is displayed in a staggered configuration in order to accommodate an additional 21-bp sequence that is absent from P1*parS*. Vertical lines join identities. The boxed sequences B1 and B2 are the discriminator hexamers whose variants determine the species specificity differences for *parS* between various P1*par* family members. The boxes A1 through A4 are repeats of the heptamer BoxA sequence with the consensus ATTTCAC. They are the principle binding motifs for the ParB protein. Boxes A1 and A4 are conserved in previously characterized P1*par* family members but appear to be absent in pWR100*parS*. C. Alignment of amino acid sequences from near the C termini of the ParB proteins of pWR100, P1, and P7. Vertical lines join identities. The underlined P1 and P7 sequences are the regions within which resides the information thought to be involved in the specific recognition of the respective P1 and P7*parS* BoxB motifs (20).

neri and show that its sequence and specificity confirm the importance of the BoxB-ParB contact in species specificity. The results provide further support for the hypothesis that species specificity governs partition-mediated incompatibility and constitutes a special mechanism for plasmid speciation and evolution. The results also confirm that two prominent sequence features that are conserved in the previously characterized family members are either unnecessary or redundant.

MATERIALS AND METHODS

General methods and materials. General methods and materials were as previously described (20).

Bacterial strains. Strain DH5α was used for general DNA manipulations and for tests of operon regulation in β-galactosidase assays (22). Strain CC2056 *recA56* trp_{am} *thi* $lacZ_{am}$ λW82 (24) was used for the colony color assay and for incompatibility tests.

Plasmids. Plasmids pALA1513 and pALA1514, which carry the P1 and P7 *par* operons, respectively, were produced by taking the purified EcoRI-XbaI fragment of pALA1413 or pALA1414 (20) and inserting it between the EcoRI and BamHI sites of the pGB2 vector (4). Ligation was carried out in the presence of a BamHI-XbaI linker formed by annealing the oligonucleotides 5′-GATCCGA ACCCCTTCG and 5′-CTAGCGAAGGGGTTCG. Plasmid pALA2601, which contains the pWR100 *parA-parB* operon, consists of the pWR100 sequence bp 28818 to 31240 (accession number AL391753). The *par* operon was obtained as a PCR product with BamHI and SalI extensions at the left and right ends, cut with SalI and BamHI, and inserted between the homologous sites in the vector pBR322. The PCR primers used were 5′-CCTGTGGATCCTCTACGCTGAA CTGGATTATTCGGGGGAA and 5′-CATCGGTCGACGCTCTCCAATTAA AAACTGGTGAGTTGC; the underlined sequences are complementary to pWR100.

Plasmid pALA2602 contains a 255-bp pWR100 fragment (bp 31200 to 31454) that includes the *parS* site. The *parS* site was obtained as a PCR product with BamHI extensions on both ends. The PCR used the primers 5′-TGTCCGGAT CCAAATTCAAGTTTTTCGCCAGC and 5′-CGAGGATCCGGTATCCCGC TTACTTTTGTTG, where the underlined sequences are complementary to pWR100. The PCR product was then cut with BamHI and inserted in the homologous sites of pALA1991 (25). Plasmid pALA2602 was recombined with λ-P1:5RΔ1005 to give λ-P1:5RΔ1005::pALA2602 as previously described (24).

Plasmid pALA2609 was constructed by excising the BamHI fragment of pALA2602 and inserting it into the BclI site of pACYC184. The smaller PvuII fragment of plasmid pALA2609 was removed, and the remainder was religated to produce pALA2611. This deleted the C-terminal portion of the chloramphenicol resistance cassette.

A PCR fragment containing the promoter of the pWR100 *par* operon was inserted as an EcoRI-BamHI fragment between the equivalent sites of the *lacZ* reporter plasmid pALA1426 (14) to give the plasmid pALA2615. The promoter region was obtained as a PCR product using the primers 5′-GATCGGAATTC CCGGGGTGAACTGGATTATTCGGGGG and 5′-TGTCCGGATCCCCGG GTAGGGCGAGCAACATTTTAT, where the underlined sequences are complementary to the pWR100 template.

Plasmid pALA2620 is the result of cloning of the BamHI-SalI fragment from pALA2601 into the same sites in the pGB2 vector (4). A 21-bp deletion within the *parS* site of plasmid pALA2602 was made to give pALA2621. This was generated using the GeneTailor site-directed mutagenesis system (Invitrogen) using 5′-TAAAAGAAATTTTGCTTTTAATTTCACGGTGAAAT and 5′-TA AAGCAAAATTTCTTTTAAAACAGCCAATTA as mutagenic primers. The

deletion starts at position 31268 and extends to position 31288 of the pWR100 sequence.

Colony color partition assay. The colony color partition assays were performed in strain CC2056 (20) using high-titer lysates as described elsewhere (24). The appropriate mini-P1 plasmid containing the respective *parS* site was recombined into a λ-P1:5RΔ*par* phage vector and introduced by infection into cells supplying the P1, P7, or pWR100 Par proteins. The recombined elements replicate as low-copy-number plasmids driven by the P1 replicon. Their maintenance stability was measured after 25 generations of unselected growth, scoring for the ability of the *supF* marker that they carry to suppress the *lacZ* amber mutation in the strain and hence give a red colony on lactose MacConkey indicator plates (20).

Incompatibility tests. The ability of supernumerary *parS* sites to exert incompatibility against plasmids maintained by a *par* system was carried out as follows. Colony color partition tests were carried out as described above, except that each strain carried an additional plasmid derived from the vector pACYC184 that carried the *parS* site from P1 (pALA1849), P7 (pALA1850), or pWR100 (pALA2611). Tetracycline (5 μg/ml) was added to the medium to ensure retention of pACYC184 derivatives.

Assays of β-galactosidase activity. Assays of β-galactosidase activity were performed on cells permeabilized with sodium dodecyl sulfate (18). The DH5α cells contained the plasmid pALA2615, which has the *lacZ* gene under control of the pWR100*par* promoter-operator region, and plasmid pBR322 or its derivative pALA2601, which produced the pWR100 Par proteins. The β-galactosidase values presented are the averages from assays done in triplicate.

RESULTS

pWR100 *par* region. The putative ParA and ParB proteins of *S. flexneri* pWR100 are similar in size and sequence to their bacteriophage P1 counterparts (3). ParA has 399 residues and shows 86% similarity to P1 ParA. ParB has 326 residues and is 76% similar to P1 ParB (Fig. 1A). The stop codon of the *parA* open reading frame overlaps the start codon of *parB* by 1 nucleotide, a feature seen in P7 and pMT1 *par* operons, but not in P1*par* (16, 25).

The organization of the putative pWR100*parS* sequence differs considerably from those of the previously characterized family members (Dabrazhynetskaya et al., unpublished) (Fig. 1B). It begins 13 bp downstream of the *parB* open reading frame. The right arm of the site is P1 like, with boxes A2, A3, and B2 clearly present (3; Dabrazhynetskaya et al., unpublished). However, there is no recognizable BoxA4 sequence, and the central region contains a 21-bp sequence, not present in P1*parS*, that acts as a spacer between the right-arm sequences and a sequence clearly homologous to the P1 IHF binding site. The left arm has no recognizable BoxA1 sequence but has a BoxB1 that is a perfect repeat of BoxB2. The distance from the BoxB1 sequence to the start of the IHF site is similar to those of other family members (Fig. 1B).

The pWR100*par* system is functional in *E. coli*. We used the colony color partition test to study the activity of the pWR100*par* region in *E. coli*. This assay measures the stability of the maintenance of a λ-mini-P1 plasmid carrying the *parS* partition site when Par proteins are supplied in *trans.* The proteins were produced from a pGB2 plasmid vector carrying the *par* genes under control of their endogenous promoter. The putative pWR100*par* operon and *parS* partition site were separately amplified by PCR, using primers that introduced new restriction sites at the ends of the pWR100 sequences. The *parS* site and the *par* operon were then cloned into λ-mini-P1 and pGB2 plasmid vectors, respectively, as described in Materials and Methods. Table 1 shows that the λ-mini-P1pWR100*parS* test plasmid was faithfully maintained, but

TABLE 1. Colony color partition assays[a]

Plasmid	*par* operon carried on plasmid	Test plasmid[b] *parS* site	% Retention (25 generations)
pGB2	None	pWR100*parS*	<2
pALA2620	pWR100 *parA-parB*	None	<2
pALA2620	pWR100 *parA-parB*	pWR100*parS*	70
pALA2620	pWR100 *parA-parB*	P1*parS*	30
pALA2620	pWR100 *parA-parB*	P7*parS*	<2
pALA1513	P1*parA-parB*	P1*parS*	97
pALA1513	P1*parA-parB*	pWR100*parS*	92
pALA1514	P7*parA-parB*	P7*parS*	93
pALA1514	P7*parA-parB*	pWR100*parS*	<2

[a] Retention of a test plasmid carrying the appropriate *parS* site was measured when a second, pGB2-based plasmid supplying the Par proteins from the appropriate *par* operon was present.

[b] The test plasmids were λ-P1:5RΔ1005::pALA1952 (P1*parS*), λ-P1:5R Δ1005::pALA1991 (no *parS* site), λ-P1:5RΔ1005::pALA1993 (P7*parS*), or λ-P15R:5RΔ1005::pALA2602 (pWR100 *parS*).

only when the pWR100 Par proteins were supplied. The presence of the pWR100*parS* site on the target plasmid was required for this effect. We conclude that the pWR100*par* system is functional in *E. coli*. Its efficiency in this assay was slightly less than those of the P1 and P7*par* systems assayed under similar conditions (Table 1).

The pWR100*par* genes are autoregulated at the level of transcription. We constructed a transcriptional fusion consisting of 264 bp of the pWR100*par* operon linked to the *lacZ* gene of the vector pRS415 (pALA2615). The sequence begins 201 bp upstream from the start of the *parA* open reading frame. This pWR100*parA-lacZ* fusion expressed high levels of β-galactosidase activity in the absence of Par proteins but was repressed eightfold when both pWR100 ParA and ParB proteins were supplied in *trans* from a compatible plasmid (Table 2). Thus, expression of the pWR100*par* operon is autoregulated at the level of transcription by interaction of the Par proteins with control sequences located near the start of the ParA open reading frame, as is the case with other members of the P1*par* family (7).

The pWR100 and P1 *par* systems have the same species specificity for the action of the Par proteins at *parS*. The partition assay used here has the *parS* site and the *par* operon on two different plasmids. On substituting one or the other plasmid with its equivalent from different plasmid species, the species specificities of the site and proteins can be assessed. Table 1 shows that the P1 Par proteins were able to function efficiently with the pWR100*parS* site. Also, the test plasmid carrying the P1*parS* site was partitioned when the pWR100 Par proteins were supplied, although the efficiency was significantly less than when the native P1 proteins were used. There was no apparent interaction between pWR100 Par components and

TABLE 2. Autoregulation of the pWR100*par* operon[a]

Supplying plasmid	Par proteins supplied	β-Galactosidase activity (Miller units)
pBR322	None	12,074 +/− 407
pALA2601	pWR100 ParA, ParB	1,575 +/− 305

[a] The DH5α cells contained the plasmid pALA2615 that has the *lacZ* gene under control of the pWR100*par* promoter-operator region.

TABLE 3. Incompatibility assays

parS site on resident target plasmid[a]	Par protein(s) supplied[b]	Supernumerary *parS* sites carried by incoming pACYC184 derivative[c]	% Retention of target plasmid (25 generations)
P7	P7	P7	3
P1	P1	P1	3
pWR100	pWR100	pWR100	5
P1	P1	pWR100	10
P7	P7	pWR100	95
pWR100	pWR100	P1	5
pWR100	pWR100	P7	60
P1	P1	P7	95
P7	P7	P1	95

[a] The target plasmids were λ-P1::5RΔ1005::pALA1993 (P7*parS*), λ-P1::5RΔ1005::pALA1952 (P1*parS*), or λ-P1::5RΔ1005::pALA2602 (pWR100*parS*).
[b] The Par proteins were supplied from pALA1414 (P7 ParA and ParB), pALA1413 (P1 ParA and ParB), or pALA2620 (pMT1 ParA and ParB).
[c] Supernumerary *parS* sites were provided by the presence of the following pACYC184 derivatives: pALA1850 (P7*parS*), pALA1849 (P1*parS*), or pALA2609 (pWR100*parS*).

TABLE 4. Effect of deleting the noncanonical 21-bp sequence from the pWR100*parS* site

Plasmid	*par* operon carried	Test plasmid *parS*[a] site	% Retention[b] (25 generations)
pGB2	None	pWR100*parS*Δ21	<2
pALA2620	pWR100*parA-parB*	pWR100*parS*	72
pALA1413	P1*parA-parB*	pWR100*parS*	96
pGB2	None	pWR100*parS*Δ21	<2
pALA2620	pWR100*parA-parB*	pWR100*parS*Δ21	28
pALA1413	P1*parA-parB*	pWR100*parS*Δ21	78

[a] The test plasmids were λ-P1::5RΔ1005::pALA2602 (pWR100*parS*) or λ-P1::5RΔ1005::pALA2621 (pWR100*parS*Δ21).
[b] All values were obtained from a single experiment. Duplicate experiments gave similar results. The controls using the wild-type pWR100*parS* are duplications of tests reported in Table 1, where similar but not identical values were obtained.

those of the P7 plasmid (Table 1). We conclude that the Par proteins and *parS* sites of pWR100 and P1 are substantially interchangeable. They share the same species specificity for this interaction, despite considerable sequence differences.

The pWR100 and P1*par* systems are mutually incompatible. When two identical *par* regions are present on two different plasmids in the same cell, the plasmids become incompatible with each other. This partition-mediated incompatibility is caused by like *parS* sites competing with each other during partition. It is a major factor in determining whether two plasmids can coexist in the same cell (2). A convenient assay for this effect consists of the introduction of supernumerary *parS* sites on an otherwise-compatible cloning vector into cells containing the relevant partitioning plasmid. Partition-mediated incompatibility is seen when the resident plasmid is displaced from the cell. Using this assay, we have previously shown that the P1, P7, and pMT1*par* systems each show unique incompatibility properties: their *parS* sites exerted incompatibility against *par* plasmids of their own species, but not against those of two other species (2, 12, 25).

The pWR100*parS* site was inserted into a pACYC184 vector (see Materials and Methods), and the resulting plasmid was transformed into the partition assay strain containing a test plasmid maintained by the pWR100*par* system. On selecting only for the incoming plasmid, the test plasmid was rapidly lost from the cells (Table 3). Thus, pWR100*parS* exerts an incompatibility effect against its own *par* system similar to that exerted by P1, P7, or pMT1 *parS* sites against their respective *par* systems. We then tested the ability of other *parS* species to exert incompatibility against the pWR100*par* system. The P1*parS* site was able to exert incompatibility against pWR100*par*. Likewise, the pWR100*parS* site exerted incompatibility against the P1*par* system (Table 3). There was no comparable effect exerted between P7 and pWR100 *par* components, although the P7*parS* site may have a small destabilizing effect on the pWR100*par* system (a reduction from 70% to 60% retention) (Tables 1 and 4). Thus, the pWR100 and P1 systems exhibit the same incompatibility specificity as well as sharing species specificity for *parS* recognition by the Par proteins. This further supports the theory that species specificity and incompatibility have a common mechanistic basis in members of the P1*par* family of partition elements (Dabrazhynetskaya et al., unpublished).

Deletion of the noncanonical 21-bp sequence in the pWR100*parS* site. The most prominent difference between pWR100 and P1 *parS* sites is the presence in pWR100*parS* of a 21-bp extension between the IHF binding site and BoxA2 (Fig. 1). In order to investigate the significance of this sequence, the 21 nucleotides were deleted. The mutated *parS* site was inserted in the λ mini-P1 test plasmid, and its stability was checked when pWR100 or P1 ParA and ParB proteins were supplied in *trans*. As shown in Table 4, the deletion retained significant function, although the maintenance efficiency of the plasmid was reduced almost threefold. The deleted pWR100*parS* site also functioned when the P1 ParA and ParB proteins were supplied. Notably, the degree of the stabilization by the P1 proteins remained high when the deletion was introduced. The mutant site worked considerably better with the P1 proteins than with its native pWR100 proteins (Table 4). Thus, although the specificities of the pWR100 and P1 sites substantially overlap, the pWR100 proteins appear to have adapted to function with the longer *parS* site so that they work less well without this extra sequence element.

DISCUSSION

The P1*par* family of partition loci is a group of versatile elements that are employed in a wide variety of plasmid types. They have presumably been disseminated to these plasmids by lateral exchange. Lateral exchanges of DNA elements (cassettes) containing various plasmid functions are common (19). The currently described P1*par* family members are from plasmid prophages (P1 and P7), large virulence plasmids (pMT1, pSLT, and pWR100), and antibiotic resistance plasmids (Rts1). These general plasmid types have little else in common. They are found in five different bacterial genera: *Escherichia*, *Salmonella*, *Yersinia*, *Shigella*, and *Proteus* (Dabrazhynetskaya et al., unpublished). Thus, by associating with different cassettes containing a variety of different replication systems, virulence factors, phage genes, etc., the P1*par* family members have achieved a broad distribution in nature as elements in diverse plasmids and hosts.

The pWR100 ParA and ParB proteins are typical of the P1*par* family, with the highest degree of similarity to the ParA and ParB proteins of P1*par* and pSLT*par* (Dabrazhynetskaya

et al., unpublished). However, the pWR100*parS* site is atypical, with a 21-bp inclusion between the IHF and A2 boxes and the absence of any obvious A1 or A4 box (Fig. 1). The inclusion is not essential for function, as it can be deleted. The P1*parS* site tolerates inserts with integral numbers of helical turns in a similar position (11). Shorter or longer inserts are not tolerated. This was interpreted as a requirement for the two arms of the site to have specific faces of the helix aligned with each other when brought together by the severe bending imposed by IHF binding (11). The 21-bp inclusion in the pWR100*parS* site constitutes two turns of the helix. By analogy with the P1 case, its existence and dispensability can therefore be understood.

The P1 Par proteins work efficiently with the pWR100*parS* site that lacks the A1 and A4 boxes, even though their cognate P1*parS* site has them. Also, the pWR100 Par proteins work with the P1*parS* site that has these features. This is consistent with the A1 and A4 boxes being redundant or nonfunctional in P1 and also, perhaps, in the other P1*par* family members that have them. The A4 boxes of P1and P7*parS* can be deleted without affecting partition (11, 12). Multiple mutations in BoxA1 reduced, but did not eliminate, the partition activity of P7*parS* (13).

The P1 species specificity difference from P7 is determined by the interaction of a short tract of amino acids in ParB with a unique BoxB motif (21). The species specificity of pMT1 also proved to reside in a unique BoxB motif. Changing just a single base in one of the pMT1*parS* BoxB repeats was sufficient to change the species specificity of the pMT1 site to that of P1 (Dabrazhynetskaya et al., unpublished). Based on these findings, it appears that the BoxB-ParB contact is a special mechanism for defining several unique specificities. Minor variations in the BoxB motif, in conjunction with minor differences in a small region of the cognate ParB protein, can generate several different exclusive specificities for ParB action at *parS*. This appears to lend the system considerable flexibility for rapid speciation during plasmid evolution.

The properties of pWR100*par* extend support for BoxB as a unique determinant of species specificity. The pWR100*par* system showed the same species specificity as P1*par.* Although pWR100*parS* is an atypical *parS*, with considerable divergence from the form and sequence of other family members, it has recognizable BoxB sequences that are identical to those of P1. The ParB regions thought to contain the contact with BoxB are also very similar in the two species (Fig. 1C). In contrast, the equivalent P7 ParB, which fails to function with pWR100 or P1 *parS*, shows little similarity (Fig. 1C).

For the P1, P7, and pMT1 systems, it has been shown that species specificity correlates with partition-mediated incompatibility. Also, BoxB alterations that change species specificity can change the incompatibility specificity correspondingly (Dabrazhynetskaya et al., unpublished). This correlation is also extended here: pWR100*parS* and P1*parS* have identical BoxB sequences and the same species specificity and show the same incompatibility type, despite considerable differences in sequence and organization of other features. Thus, species specificity for Par protein action at *parS* and partition-mediated incompatibility appear to have a common mechanistic basis. Two plasmids with like ParB-*parS* complexes compete with each other during partition, leading to incompatibility. Unlike ones fail to compete and thus are compatible. The basis of this competition might be the ability to form mixed plasmid pairs via the bound ParB proteins prior to segregation (2).

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