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DOMAIN 7 GENETICS AND GENETIC TOOLS

Plasmid Localization and Partition in *Enterobacteriaceae*

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ABSTRACT Plasmids are ubiquitous in the microbial world and have been identified in almost all species of bacteria that have been examined. Their localization inside the bacterial cell has been examined for about two decades; typically, they are not randomly distributed, and their positioning depends on copy number and their mode of segregation. Low-copy-number plasmids promote their own stable inheritance in their bacterial hosts by encoding active partition systems, which ensure that copies are positioned in both halves of a dividing cell. High-copy plasmids rely on passive diffusion of some copies, but many remain clustered together in the nucleoid-free regions of the cell. Here we review plasmid localization and partition (Par) systems, with particular emphasis on plasmids from *Enterobacteriaceae* and on recent results describing the *in vivo* localization properties and molecular mechanisms of each system. Partition systems also cause plasmid incompatibility such that distinct plasmids (with different replicons) with the same Par system cannot be stably maintained in the same cells. We discuss how partition-mediated incompatibility is a consequence of the partition mechanism.

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INTRODUCTION

Naturally occurring plasmids usually exist in a symbiotic relationship with their host bacterial cell; the host provides energy and machinery for DNA replication, and plasmids often provide genes that confer an advantage to host survival in the competitive environments in which they live. Of particular interest to human health are plasmid genes that confer resistance to antibiotics or pathogenesis determinants. Plasmids have evolved several strategies to promote their stable maintenance in bacterial populations, including replication control that maintains their copy number, site-specific recombination that reduces multimerization, toxin-antitoxin systems that counterselect plasmid-free cells, and partition that acts to faithfully segregate plasmids to daughter cells at cell division. The intracellular location of plasmids is restricted by partition systems, when present, and by the presence of the bacterial nucleoid, which takes up much of the volume of the cell. Here we focus on plasmid partition and its influence on the localization of plasmids in the cell.

Many natural (nonengineered) bacterial plasmids are low copy. The low copy number necessitates active partition to avoid loss from random segregation. Segregation follows DNA replication (1) and involves the separation and transportation of the copies in opposite directions along the longitudinal cell axis, which ensures that every daughter cell receives at least one copy of the plasmid. Almost all partition systems identified to date consist of three components, one or more copies of a partition site (the "centromere"), a centromere-binding protein (CBP), and an NTPase (ATPase or GTPase). The genes encoding the NTPase and the CBP are arranged together in an operon (Fig. 1), and both protein products contribute to the regulation of their own gene expression. The centromere is often either just downstream or upstream of the genes and can be present in more than one copy in some plasmids (Fig. 1). Plasmid partition systems exert incompatibility; that is, different plasmids with compatible replicons are unstable if they contain the same partition system or some of its components. Here we review the partition process, incompatibility, and gene expression properties of plasmid partition systems, with a primary but not exclusive focus on plasmids from *Enterobacteriaceae*. We note that most bacteria, although interestingly neither *Escherichia coli* nor *Salmonella enterica* serovar Typhimurium, encode partition proteins that are homologous to those from some plasmids and contribute to segregation of the bacterial chromosome. Although chromosomally encoded systems

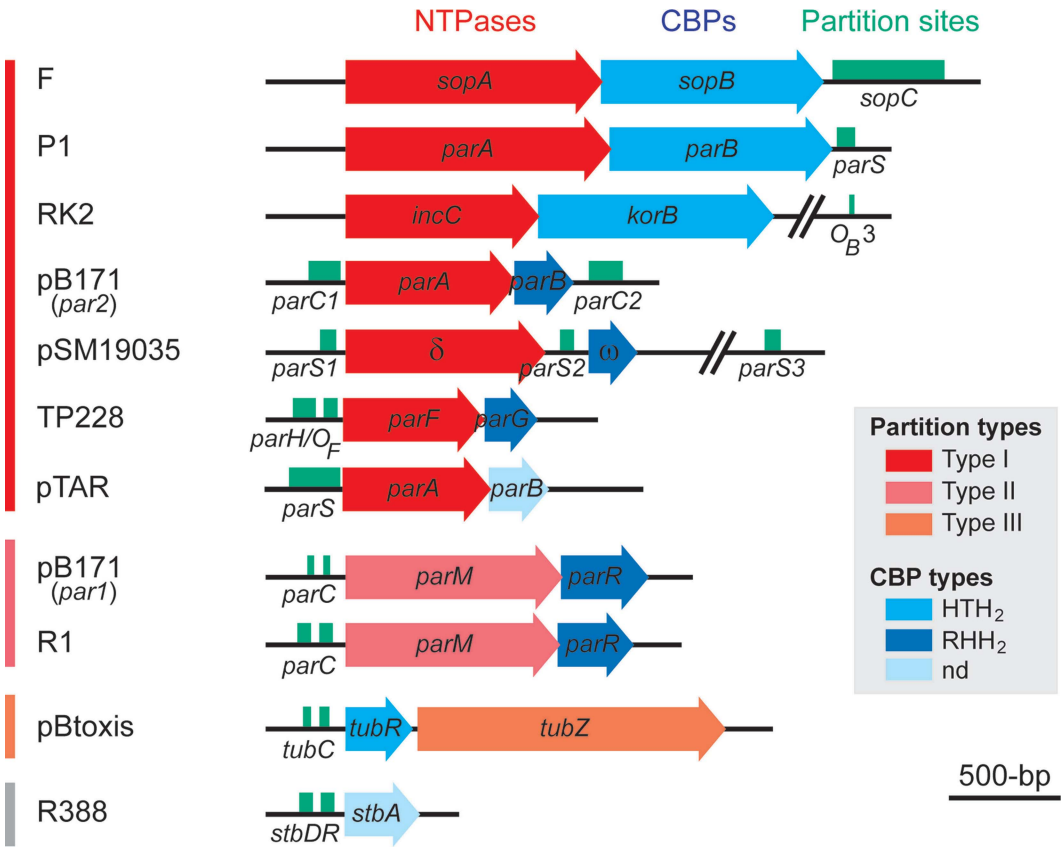

Figure 1 Genetic organization of plasmid partition loci. Genes encoding the NTPases and the CBPs are depicted by red and blue arrows, respectively, and the centromere sites are displayed by green boxes. The type I (*parABS*), type II (*parMRC*), and type III (*tubRZC*) partition loci are distinguished by their NTPase signatures, Walker-A (light red), actin-like (dark red), and tubulin-like (orange red), respectively. The CBPs harbor either an HTH_2 (light blue) or an RHH_2 (dark blue) DNA binding motif. The partition system of R388 of yet-undetermined partition type (gray) encodes only a CBP (purple arrow) with an undetermined (nd) DNA binding motif. For type III, note that the order of the NTPase and CBP is inverse from that of types I and II. All plasmids diagrammed are found in *Enterobacteriaceae* except pTAR, pSM19035, and pBtoxis, which are included since their properties are discussed in this review. The historical names of genes and centromeres are indicated within the arrows and below the boxes, respectively (reviewed in reference 14). Note that in the body of the review we have simplified this nomenclature for type I systems and use ParA, ParB, and *parS* (with subscripts indicating the plasmid) for the ATPase, CBP, and centromere, respectively. The schematic representation is drawn at the indicated scale.

are reviewed elsewhere (2, 3), we mention results from studies that have informed our understanding of the action of plasmid partition components.

EARLY HISTORY

The replicon model, proposed by Jacob et al. over 50 years ago, provided a conceptual framework for our understanding of and our approaches to studying both plasmid replication and partition (4). From studies of the replication and segregation of the F plasmid in *E. coli*, they proposed that replication was promoted by an initiator (protein) acting on a replicator (origin DNA). They further suggested that the plasmid replicon would be attached to the bacterial membrane and that new membrane growth between replicated copies would separate them into daughter cells. The replicon model essentially was also the first partition model and suggested that partition was a problem of plasmid positioning during the bacterial cell cycle.

Subsequent studies established that membrane material did not grow by selective insertion at the cell center but instead was distributed throughout the cell surface (5), ruling out the membrane growth explanation for plasmid partition. The idea that plasmids would be tethered to some sites on or at the inner membrane remained attractive, in part because it provided a simple explanation for incompatibility (competition for attachment sites). However, recent advances in cell biology, genetics, biochemistry, and structural biology, which we discuss below, have indicated that plasmid partition systems can segregate plasmids without tethering to any specifically localized host proteins.

Early studies of plasmid biology recognized that plasmid stability would be intimately dependent on plasmid copy number, which is controlled by the replication system. For random distribution, plasmid loss is directly proportional to the copy number at cell division and can be estimated as follows: $P_{loss} = 2^{(1-n)}$, where n is the copy number at cell division. High-copy-number plasmids can rely on passive diffusion for segregation; the frequency of loss of ColE1 plasmid (~20 copies per chromosome) was estimated to be $<5 \times 10^{-6}$ (6), in agreement with this formula. Therefore, it was proposed that random diffusion would explain the extreme stability of plasmids with copy numbers over about 10 per host chromosome (6, 7). Several recent studies have revisited and reaffirmed the idea of random distribution and segregation of plasmids, using high-resolution microscopy to visualize them and modeling studies to explain them (reviewed in reference 8), although there are unexpected and interesting observations that plasmids are not dispersed randomly throughout the cell (see below). For low-copy-number plasmids, however, an active partition process is critical to prevent plasmid loss at cell division.

PLASMID PARTITION SYSTEMS AND PARADIGMS

Partition systems are essentially transport and positioning systems with plasmid DNA as the cargo. The partition complex, formed by binding of the CBP to the plasmid centromere(s), interacts with the system's NTPase, whose action is responsible for both plasmid transport and positioning. Three general types of partition systems have been identified, defined by the type of partition NTPase that promotes plasmid localization (9, 10). Type I systems employ a Walker-type ATPase, which promotes segregation by forming dynamic patterns on the bacterial nucleoid. Type I systems are the most prevalent in sequenced plasmid genomes, and those from plasmids F, P1, RK2, and pB171 in *E. coli* and TP228 from *Salmonella enterica* serovar Newport have been the most extensively studied for *Enterobacteriaceae*. Chromosomally encoded Par systems are also exclusively type I. Early classification of type I partition systems created subtypes based on the type of CBP, the position of the centromere(s) relative to the *par* operon, and the size of the ATPase (9). However, these distinctions are no longer completely consistent so we have avoided them; instead, we describe the differing properties of their components where relevant.

Type II partition systems use an actin-like ATPase, which polymerizes into dynamic filaments that effectively push plasmids apart. The paradigm for this system is the R1 plasmid of *Enterobacteriaceae*. Type III systems encode a tubulin-like GTPase, which also works via a dynamic polymerization mechanism. So far, type III systems have been found in plasmids of certain *Bacillus* and *Clostridium* species and in some bacteriophages, but not in *Enterobacteriaceae*. Finally, a new kind of plasmid partition system involving only one protein and a centromere site has recently been described for the broad-host-range plasmid R388 from the IncW plasmid family (11). In contrast to the NTPases, the CBPs of type I, II, and III systems generally do not show significant sequence similarity even among members of the same type. They do, however, share structural similarities (see below). They

are dimers of either helix-turn-helix (HTH_2) or ribbon-helix-helix (RHH_2) DNA binding proteins.

Partition systems are also not mutually exclusive. A number of plasmids found in nature contain two different partition systems, but generally one of each type, and in some cases, both have been shown to contribute to plasmid stability. For example, pB171 and R27 from *E. coli* each contain one type I and one type II system, and their roles in plasmid stability have been examined independently and in combination (12, 13). For both pB171 and R27, the type I system makes the larger contribution to plasmid stabilization.

We have simplified the nomenclature of partition components in this review and use the most common or typical names from paradigm systems (and with subscripts indicating the plasmid, where relevant). For a more extensive reference to specific nomenclature, see reference 14.

VISUALIZATION OF PLASMID DYNAMICS IN CELLS

Cell biology has proven an exceptionally powerful approach to examine plasmid segregation, from early days with simple fluorescent DNA stains and immunofluorescence to rapidly emerging and sophisticated subcellular epifluorescence, superresolution, and three-dimensional (3D) microscopy. The field recognized early that direct visualization of plasmids inside cells would be necessary to bridge the gap between genetic and biochemical dissection of partition components and their mechanism *in vivo*. A breakthrough discovery arose from two studies reporting the intracellular localization of specific DNA loci in bacterial cells (15, 16). They revealed that F and P1 plasmids are preferentially found around mid- or quarter-cell positions and confirmed that the partition process is a positioning process within the cell. Further technological improvements in live-cell imaging, high-resolution and superresolution fluorescence microcopy, and cryo-electron microscopy (cryo-EM), have led to analyses of spatial localization of proteins and DNA in their native environment with nanometer precision (Fig. 2). They have also allowed visualization of plasmid and protein dynamics with high temporal resolution (for review on fluorescence imaging techniques, see reference 17). In addition, evidence indicates that the bacterial chromosome compacted in the nucleoid contributes both passively (as the major structure occupying space inside a bacterial cell) and actively (as a support and/or matrix for plasmid movement) to plasmid partition mechanisms (18–23; also see below).

Imaging Plasmids with Type I Partition Systems

As mentioned above, studies using F and P1 plasmids showed preferential mid-cell and quarter-cell positioning in small and larger cells, respectively (15, 16). Plasmids colocalized with the bacterial nucleoid or nucleoids. When more than two plasmid foci were present, they were positioned approximately equidistantly from each other along the cell length (24). This localization pattern was dependent on the partition system because in its absence, plasmids were typically found in the nucleoid-free areas of the cell (18, 19). Subsequent time-lapse fluorescence microscopy studies showed that type I Par^+ plasmids moved around but that their dynamics were restrained around the mid-and quarter-cell positions (25, 26).

The positions and patterns of partition proteins and their complexes have informed as well as supported the mechanisms derived from genetics and biochemistry. HTH_2 CBPs of type I partition systems, typically called ParB, are present in large amounts in cells (27, 28) and are observed as bright foci that depend on the partition sites and correspond to the plasmid positions (Fig. 2A) (18, 24, 29–32). Recent advances in high-resolution microscopy have further resolved the composition of these complexes. Single-molecule photo-activated localization microscopy (PALM) has shown that over 90% of individual $ParB_F$ (ParB from the F plasmid) proteins are present in clusters composed of several hundred ParB molecules nucleated at the F partition site, $parS_F$ (33). 3D fluorescence microscopy, namely, structured illumination microscopy (3D-SIM) with resolution to ~80 nm, revealed that F plasmid partition complexes are intimately associated with the bacterial nucleoid and localized within its mass (22) rather than simply on its surface. Recently, the RHH_2 CBP of the type I system of plasmid TP228 has also been imaged, and it forms large foci with plasmid DNA, which dynamically associate with the cognate ParA in live *E. coli* cells (23).

Epifluorescence microscopy of type I ATPases (typically called ParA) has revealed interesting and sometimes conflicting dynamic patterns. Some, such as $ParA_{pB171}$, $ParA_F$, and $ParA_{TP228}$, displayed an oscillatory behavior (Fig. 2B and C) (12, 23, 34–37). Others, such as $ParA_{P1}$, did not oscillate but did form dynamic gradients (38). In all cases, the dynamic patterns coincided with the bacterial nucleoid. Early reports of helical and filamentous structures

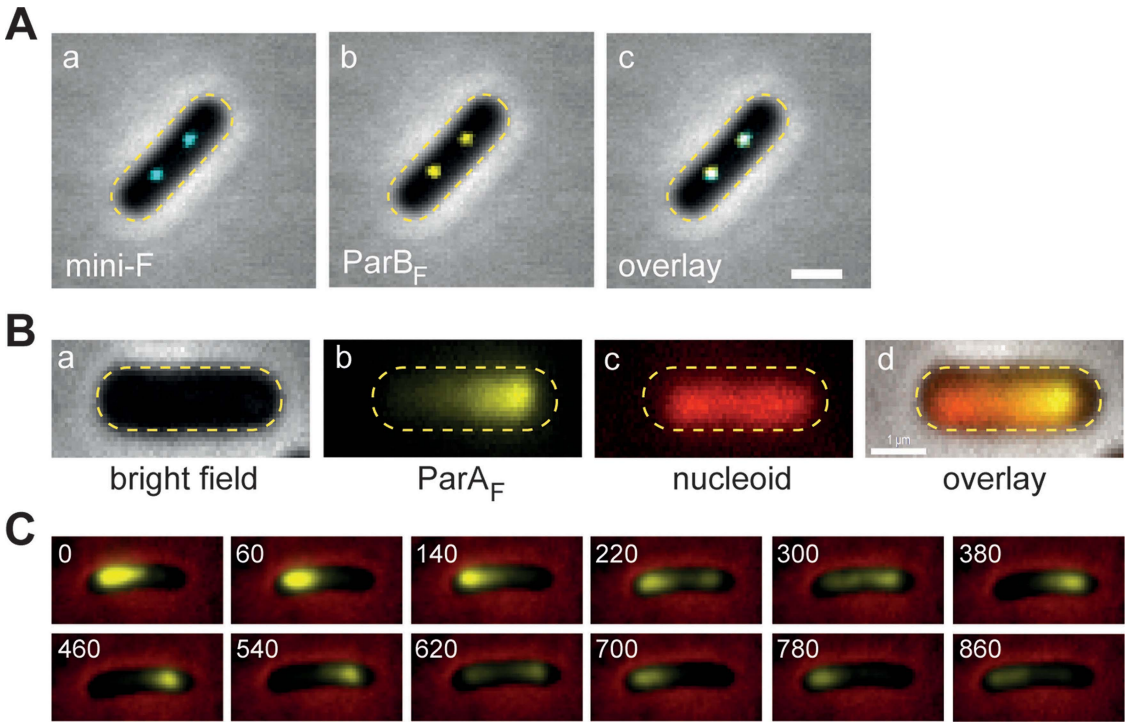

Figure 2 Mini-F plasmids, partition proteins, and nucleoid localization. **(A)** Colocalization of mini-F plasmids and $ParB_F$ clusters in a growing *E. coli* cell. **(a)** The mini-F plasmid pJYB273 is visualized with the $ParB_{pMT1}$-mTurquoise2/$parS_{pMT1}$ labeling system. **(b)** $ParB_F$-mVenus fusion protein is expressed from its endogenous locus on pJYB273. **(c)** The overlay displays the phase-contrast along with the two fluorescent channels. The dashed yellow lines represent the contour length of the cell. **(B)** Dual localization of $ParA_F$ and the nucleoid. The *E. coli* strain DLT3057 expresses the HU-mCherry fusion that labels the nucleoid and carries a mini-F with the *parA*$_F$-*mVenus* allele (pJYB243). The growing cell was observed in phase contrast **(a)** and in the yellow **(b)** and red **(c)** channels to image $ParA_F$ and the nucleoid, respectively. The overlay **(d)** displays the combination of all three channels, showing that $ParA_F$ localized over the nucleoid. **C** $ParA_F$ oscillates from pole to pole. An *E. coli* cell carrying the mini-F *parA*$_F$-*mVenus* (pJYB243) was imaged every 20 s for 15 min. A selection of images (times in seconds) were displayed showing that $ParA_F$-mVenus proteins oscillate in a collective and coordinated fashion. Scale bars: 1 µm.

(19, 34, 36) have not been reproduced using high-resolution microscopy, and this may be due to problems with protein overexpression, fusion behavior, or the limited resolution of conventional light microscopy, for example. Recent 3D-SIM and single-particle tracking PALM imaging indicated that $ParA_F$ does not form filaments *in vivo* but follows two distinct behaviors: it appears as a few concentrated patches bound to some dense regions of the nucleoid, and it oscillates within the nucleoid (22). Current subcellular imaging supports biochemical data that Brownian ratchet mechanisms (described below) explain dynamic ParA localization and activity in partition (20, 22, 23, 39–43).

Imaging Plasmids with Type II and Type III Partition Systems

Imaging of the R1 plasmid CBP, $ParR_{R1}$, did not detect visible or localized complexes (44), presumably because they were too small to be detected by conventional microscopy. In contrast, imaging of the ATPase, $ParM_{R1}$, was instrumental in the molecular understanding of type II plasmid partition. Epifluorescence microscopy illustrated that the actin-like $ParM_{R1}$ protein forms long filaments *in vivo* (45). ParM filaments were also detected by cryo-EM of *E. coli* cells (46). A combination of phase-contrast and immunofluorescence microscopy was used to show that plasmids are present on both tips of the ParM polymers (47). Along with biochemical and genetic data (see below), these observations supported the idea that movement of plasmid DNA to opposite cell poles is driven by ParM polymerization between sister ParR-*parC* (the R1 centromere) complexes.

Similarly, imaging of type III tubulin-like GTPases from plasmids of Gram-positive bacteria shows that they form dynamic and cytoskeletal-like filaments, which supports the conclusions that type III systems also work by filamentation mechanisms to distribute plasmids inside cells (reviewed in reference 48). The TubZ GTPase of pBtoxis

from *Bacillus thuringiensis* was shown *in vivo* by epifluorescence microscopy to assemble into linear and dynamic polymers that exhibit directional polymerization with plus and minus ends, resulting in a treadmilling displacement (10).

Imaging the Bacterial Nucleoid for Its Role in Plasmid Partition

The nucleoid is the largest structure in a bacterial cell, containing a highly compacted and topologically constrained but organized bacterial chromosome (reviewed in references 3 and 49). Although a comprehensive description of chromosome architecture is beyond the scope of this review, its properties must be considered for the development of plasmid partition models. Plasmids without Par systems are excluded from the nucleoid, but Par$^+$ plasmids are not (18, 19, 21, 22). Why plasmids are excluded from the nucleoid is not understood. Nucleoid structure is especially relevant for type I partition, which requires ATP-dependent nonspecific DNA binding of the partition ATPase to nucleoid DNA to drive plasmid localization. Recent improvements of fluorescence microscopy reagents and techniques have allowed preliminary visualization of the nucleoid at high resolution in time and space in living *E. coli* cells. The nucleoid appears as a dynamic helical ellipsoid with overall low DNA density (50, 51). Some areas of the nucleoid display locally higher-density regions (HDRs) within in its central mass, and these HDRs have been proposed to play a role in type I plasmid partition (22, 52). It is, however, not known how HDRs are formed or how they contribute to chromosome biology. Nevertheless, recent evidence shows that at least some plasmids move within the nucleoid structure (22, 23). Detailed high-resolution images of the dynamics of nucleoid architecture will be necessary to fully understand how plasmids interact with and respond to the nucleoid during the cell cycle.

Imaging of High-Copy-Number Plasmid Distribution and Segregation

An interesting paradox emerged from the initial visualization of high-copy-number plasmids in living cells, which indicated that they appeared to cluster in groups rather than exist as many independently localized units (53, 54). Clustering would reduce the number of segregating units, which should decrease plasmid stability as calculated solely by random distribution formulae that had been consistent with measured loss rates (55, 56). Recently, quantitative fluorescence microscopy analyses in live cells (21) and in fixed cells by single-molecule fluorescence *in situ* hybridization in superresolution (57) have revisited and resolved this apparent paradox. In these studies, plasmids were mainly found at the cell poles because they are excluded from mid-cell by the nucleoid. Importantly, however, some individuals do occasionally move around (21). Due to the low resolution of conventional microscopy (~300 nm), plasmids appear misleadingly as a few clusters localized in the nucleoid free space. Single-molecule microscopy along with the use of monomeric variants of fluorescent proteins revealed that a significant number of plasmids were not present in the polar compartments but constituted the diffuse fluorescent background observed by conventional microscopy (57). Analyses of the radial distribution of the fluorescent signal indicated that the plasmid distribution follows a donut shape, suggesting that the freely diffusing plasmids are mainly located outside the nucleoid at the inner periphery of the bacteria. Taken together, these data indicate that high-copy-number plasmids exist both in multiple clusters at the cell poles and randomly distributed throughout the cell volume with a low frequency inside the nucleoid. They support a mixed distribution model which involves both plasmids clustering and random diffusion of individual high-copy-number plasmids (8). The nature of the clustering remains to be uncovered.

GENETIC AND MOLECULAR DISSECTION OF PLASMID PARTITION COMPONENTS AND MECHANISMS

Plasmid Centromeres

Plasmid centromeres vary considerably in sequence, complexity, number, and position (Fig. 1 and 3). The variation reflects the selective advantage for plasmids with different Par systems to be compatible with each other, and only small differences in DNA sequence are sufficient to ensure that related plasmids do not compete with each other. Centromeres of type I and II systems can be grouped into those recognized by HTH_2 CBPs or by RHH_2 CBPs. In the former group, centromeres, often called *parS*, typically contain one or more 13- to 16-bp inverted repeat sequences (Fig. 3). The RK2 *parS* (O_B3) consists of one inverted repeat (58). The F plasmid centromere, $parS_F$, is composed of 12 direct repeats of 43 bp (59), 10 of which contain the 16-bp inverted repeat to which its CBP ($ParB_F$) binds (60). Only one, however, is absolutely essential for centromere activity (61). Close homologs of $parS_F$, harboring the same ParB binding sequence, are widely found on *Enterobacteriaceae* plasmids, with a var-

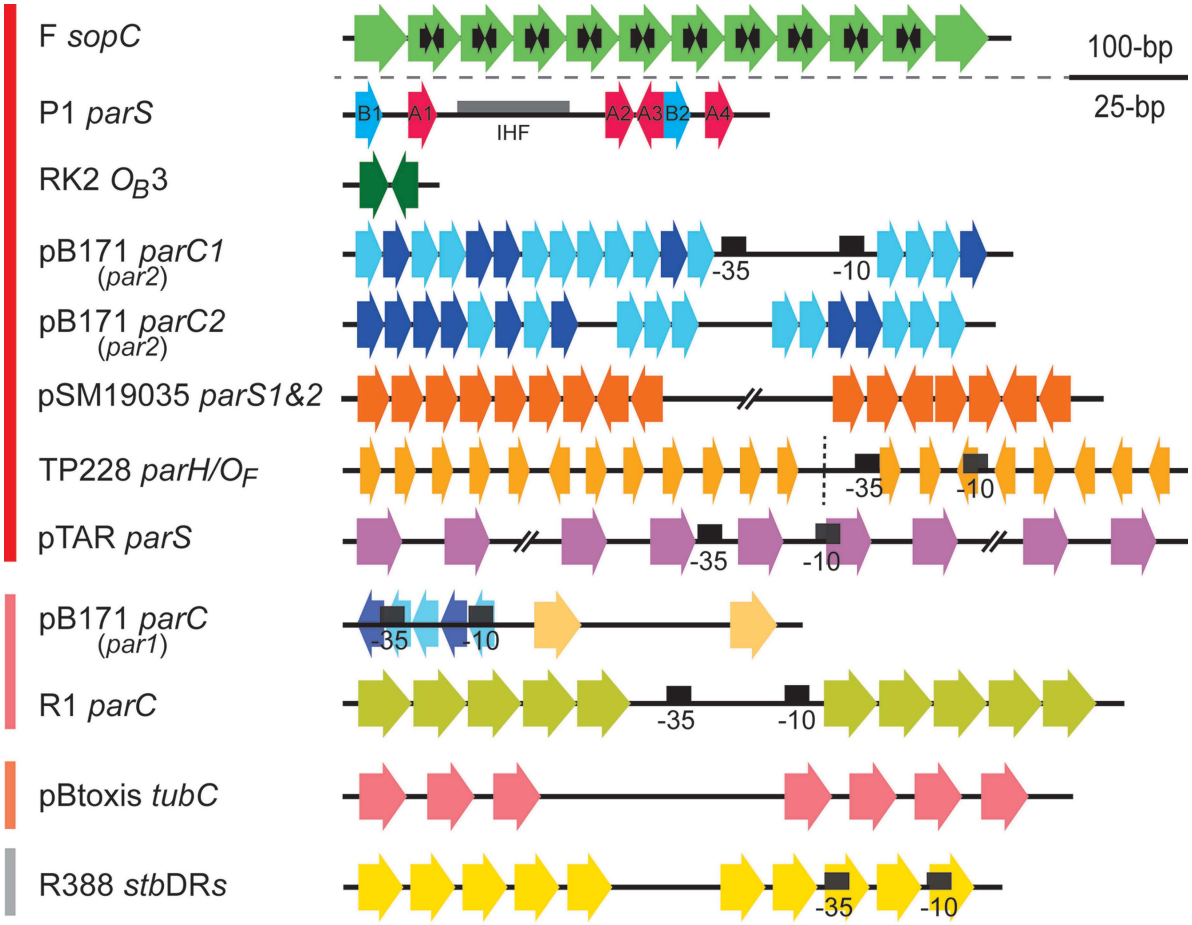

Figure 3 The organization and composition of centromeres are highly diverse. Direct and inverted repeats are depicted by oriented arrows with the same color, indicating conserved motifs. For $parS_F$, the black inverted arrows represent the 16-bp inverted repeat ParB binding sites within the 43-bp direct repeats (green arrows). The centromere of P1 is composed of two 6-bp box B (blue) and four 7-bp box A (red) motifs present on both sides of an integration host factor (IHF) binding site (gray rectangle). The RK2 centromere (O_B3) consists of one inverted repeat of 13 bp. For pB171, the *parC1* and *parC2* regions of *par2* are composed of 17 (2 clusters) and 18 (3 clusters) repeats, respectively, of a 6-bp motif. The *parS* site of *par1* comprises only two identical 10-bp motifs (orange arrows) in direct orientation separated by a 31-bp direct repeat; the blue arrows overlapping the −35 and −10 promoter sequences correspond to the beginning of *parC1* from the *par2* locus involved in the cross-regulation between the two Par systems of pB171. pSM19035 carries three *parS* loci composed of contiguous repeats of 7 bp in direct or inverse orientations (only *parS1* and *parS2* are depicted). For $parS_{TP228}$, the two centromere regions, *parH* (left) and O_F (right), delineated by a vertical dashed line, are composed of 12 and 8 degenerated repeats (4 bp) separated by AT-rich spacers (4 bp), respectively. The pTAR centromere contains 13 repeats of 9 bp, each separated by 8 bp, encompassing the −35 and −10 promoter boxes. The centromere of plasmid R1 comprises two arrays, spaced by 39 bp, composed of five direct repeats of 11 bp. The pBtoxis centromere, *tubC*, comprises two arrays of three and four 12-bp motifs, separated by 54 bp. For $parS_{R388}$, the two arrays spaced by 43 bp are each composed of five direct repeats of 9 bp separated by 2 bp; the putative −35 and −10 promoter sequences are deduced from the sequence. Note that (i) the scale for the large *sopC* centromeres is different from all others (separated by the horizontal gray dashed line), and (ii) only 9 out of 13 repeats of the pTAR *parS* are drawn. The *parS* centromeres are depicted in the same order with the same color code (colored vertical lines on the left) as the partition loci to which they belong (Fig. 1).

iable number of direct repeats ranging from 4 (pETEC-74) to 16 (p1658) (60). A subgroup of type I plasmids, which includes P1 and P7 from *E. coli* and pMT1 from *Yersinia pestis*, contain *parS* sites with multiple DNA sequence motifs (62–65). These *parS* sites are composed of two arms with nonsymmetrical hexamer (box B) and inverted-repeat heptamer (box A) DNA motifs separated by a central binding site for the host-encoded integration host factor (IHF) (Fig. 3). Their CBPs ($ParB_{P1/P7/pMT1}$) contain two independent DNA binding domains, which recognize the A and B boxes (see below).

Centromeres that are recognized by RHH_2 CBPs generally contain several direct repeats that vary in length from 4 to 11 bp, arranged in one or several arrays (Fig. 3). Their arrangements in different systems vary consider-

ably, and arrays can be spaced far apart (12, 66, 67). Minimal centromere activity can be elastic, such that different geometries of subsites may be sufficient. For example, the centromere of TP228 is composed of the *parH* and O_F loci, each containing multiple repeats of a degenerate 5′-ACTC sequence separated by AT-rich spacers (67). Although *parH* is sufficient for full centromere activity, its repeats can be reoriented or they can be replaced by those of the O_F locus at lower efficiency. One possible distinction between centromeres that are recognized by type I versus type II RHH_2 CBPs is intrinsic curvature of the DNA site, which may reflect the final architecture of the partition complex that is formed. The R1 plasmid *parC* site (type II) is curved (68), whereas TP228 *parH* is not (67).

Centromeres of type III partition systems, called *tubC*, are also upstream of the *tubRZ* genes (Fig. 1). *tubC* has been most extensively examined for pBtoxis from *B. thuringiensis* and is composed of seven 12-bp direct repeats, arranged in two clusters (69) (Fig. 3). Centromere binding analysis of TubR from pBtoxis has revealed a superstructure similar to that for type II and suggests a convergent evolution for type II and III centromeres (69).

CBPs and Partition Complex Assembly

The CBPs are responsible for recognition of the partition site and bind in multiple copies to form higher-order partition complexes, although the architecture or "superstructure" of these complexes varies and depends on the type of CBP (Table 1). The CBPs also play a direct role in the partition reaction by recruiting as well as influencing the activity of their cognate NTPase.

Type I HTH_2 CBPs and dynamic assembly of higher-order partition complexes

HTH_2 CBPs do not share extensive sequence similarity, but the domain organization is generally conserved, with some interesting variations in function and specificities. The protein is often called ParB, which we use here for simplicity. ParBs contain a C-terminal dimerization domain, a central HTH domain that binds to inverted repeats in DNA, and a flexible N-terminal domain responsible for protein oligomerization and for interactions with its cognate ATPase (70–73). The structures of the HTH domains of $ParB_{P1}$, $ParB_F$, and $ParB_{RK2}$ (KorB) bound to their specific sites are highly similar to each other and show that the recognition helix sits in the major groove of the DNA (74–76). DNA specificity is determined slightly differently, however, depending on the ParB: by contacts both with the recognition helix and with residues outside of the HTH motif. For example, both $ParB_F$ and $ParB_{RK2}$ also make specific base contacts with residues outside of the HTH (75–77). Another variation is that P1 ParB and its relatives contain a second specific DNA binding region within the C-terminal domain that requires dimerization to form and is responsible for binding to a second DNA sequence motif (called box B [Fig. 3]) within the P1 *parS* site (63, 71, 74). Interestingly, recent evidence suggests that a chromosomally encoded ParB (*Bacillus subtilis* Spo0J) binds DNA nonspecifically via its C-terminal dimerization domain and that this interaction is necessary for higher-order Spo0J partition complex assembly called "spreading" (78) (discussed below). It is not known whether nonspecific DNA binding by the C-terminal dimerization domain is a common feature of HTH_2 ParBs.

The N termini of ParBs are generally flexible and have been difficult to analyze structurally, although two structures of these regions of chromosomal ParBs have been determined (79, 80). Higher-order complex assembly requires the N-terminal regions of ParBs. This region contains a conserved motif called box II (81), which is implicated in ParB dimer-dimer interactions that are essential for the assembly of higher-order dynamic partition complexes (33, 80, 82, 83).

Table 1 CPBs and their partition complexes

Par system[a] (of cognate NTPase)	CBP	Representative member(s)[a]	Partition complex superstructure
Type I	HTH_2	$ParB_{P1}$, $ParB_F$, $ParB_{RK2}$	Nucleation at *parS* and dynamic spreading/bridging/caging that builds large complexes with many ParB molecules
	RHH_2	$ParG_{TP228}$, $\omega_{pSM19035}$	Unknown; multiple direct repeats in *parH*/*parS*, predicted to direct CBP binding on straight DNA
Type II	RHH_2	$ParR_{R1}$, $ParR_{pSK41}$	Curved superhelical filament and ring structure at *parC*
Type III	HTH_2	$TubR_{pBtoxis}$	Curved superhelical filament and ring structure at *parC*

[a]Representative members of each type of plasmid partition system are represented by their CBPs; the plasmids are indicated by the subscript following the name of the CBP.

Early evidence that HTH_2 ParBs form large, oligomeric assemblies came from observations that these ParBs "spread" on the DNA away from their specific binding sites, that complex formation altered the topology of the DNA to which ParBs were bound, and that large fluorescent ParB foci formed at *parS* sites inside cells. Spreading was inferred from chromatin immunoprecipitation (ChIP) experiments that demonstrated $ParB_{P1}$ binding several kilobases away from *parS* (84). Subsequent ChIP or ChIP sequencing (ChIP-seq) analyses of $ParB_F$ and several chromosomal ParBs have confirmed that spreading is a general property of HTH_2 ParBs (33, 85–88). Further, $ParB_F$ binding to one or multiple copies of its *parS* site induced a strong deficit in plasmid supercoiling (61, 89, 90). The results showed that higher-order nucleoprotein complexes form on *parS* sites and extend on adjacent DNA, and led to the initial proposal that spreading occurred laterally and unidimensionally (1D) along DNA extending away from *parS* (61, 84).

This simple model was subsequently challenged with biochemical and modeling studies (reviewed in reference 91). The large size of the spreading zone was not compatible with the number of ParB molecules present inside the cell, and direct observations of ParB binding to tethered DNA molecules by total internal reflection fluorescence (TIRF) microscopy showed that ParB (from both chromosomes and plasmids) could bridge DNA segments when it formed higher-order complexes (82). These results, in conjunction with physical modeling studies, led to a model in which patches of ParB spreading (1D) coupled to their bridging (3D) explain spreading and ParB complex assembly (92). However, the DNA binding activity observed in TIRF was not *parS* specific, and the "spreading and bridging" model did not fully solve the question of how small ParB patches on nonspecific DNA are stable enough to support bridging with other ParB patches on *parS* or non-*parS* sites. A subsequent model termed nucleation and caging proposed that the strong specific binding of a few ParB dimers to *parS* sites would nucleate the attraction of many other ParBs through a combination of weak but synergistic interactions between ParB molecules and between ParB and nonspecific DNA. The latter model is supported by *in vivo* single-molecule PALM and assays of fluorescence recovery after photobleaching in live cells and by physical modeling of the ChIP-seq patterns of $ParB_F$ binding to DNA (33, 83). These studies demonstrated, for example, that over 90% of ParB dimers exist within highly confined clusters at the centromere sites and that ParB dimers can rapidly exchange between clusters. Modeling data support stochastic binding of the "caged" ParB over nonspecific DNA. Further analyses of the molecular nature of these ParB-ParB and ParB-DNA interactions should help to distinguish and refine these models. In all cases, the HTH_2-ParB partition complexes form large, highly dynamic structures that would provide numerous interactions with their cognate ParAs.

Type I and II RHH_2 CBPs and assembly of partition complexes

In general, RHH_2 CBPs are small proteins that share little similarity in primary sequence but share the common fold and DNA binding properties of the Arc/MetJ family of transcriptional repressors. In early studies, their genes were often overlooked because the open reading frames were smaller than the computational cutoffs for protein-encoding genes. Where studied, the RHH_2 CBPs are also transcriptional repressors (of the *par* operons; see below). All partition RHH_2 CBPs also contain flexible tails that contain the residues that interact with their cognate partition NTPases. A major distinction between the CBPs of the type I and type II plasmid partition systems is the relative orientation of the DNA binding domain and the region that interacts with the ATPase. In type I CBPs, the RHH_2 domain is C terminal and the ATPase interaction is N terminal. In type II CBPs, this orientation is switched.

Type I and II RHH_2 CBPs have similar DNA binding domains (93–97); however, the assembly into higher-order structures is known only for type II CBPs, which are typically called ParR. Crystal structures of $ParR_{pB171}$ and of $ParR_{pSK41}$ bound to its centromere site (pSK41 from *Staphylococcus aureus*) have been used to build models of the higher-order structure of the partition complex (96, 97). $ParR_{pB171}$ assembles through dimer-dimer interactions to form a helical structure with the basic patches of the RHH_2 domains regularly spaced on the helix exterior and with the negatively charged C-terminal tails pointing inward toward a center cavity (97). Twelve ParR dimers, stabilized by cooperative interdimer contacts, are present per full 360° turn to form a helical DNA-binding scaffold in which the centromere DNA region wraps on the positively charged outside surface of the ParR helix. This model is supported by EM visualization of $ParR_{R1}$, which shows rings of ParR bound to DNA (96), and by the intrinsic curvature of the R1 partition site (68). The internal cavity of this superstructure is proposed

to be the insertion site of the ParM ATPase filament via its interactions with the ParR C-terminal domain (see below).

Type I RHH_2 CBP structures have been solved for TP228, pCXC100 (*Leifsonia xyli*), and pSM19035 (*Streptococcus pyogenes*), but only the latter in complex with its centromere site (93–95, 98). The pSM19035 CBP binds to 10 7-bp repeats in *parS* without inducing bends (Fig. 3) (99). The absence of intrinsic curvature in the $parS_{TP228}$ region fits with this model (67). It is still unknown whether all type I RHH_2 partition complexes assemble in a similar straight structure, but these observations imply that type I superstructures are very different from the ring-like architecture of those from type II systems. This distinction may reflect differences in the mechanisms of their cognate ATPases (see below).

The CBPs of type III partition systems are called TubR, and current evidence suggests that they share characteristics with both HTH_2 and RHH_2 CBPs. TubR from *B. thuringiensis* plasmid pBtoxis is an HTH_2 DNA binding protein but binds DNA in a noncanonical fashion. Its recognition helix is part of the dimerization interface, and its binding site (*tubC*) contains direct repeats (Fig. 3) (69, 100). The paired recognition helices interact in one major groove of DNA. The superstructure of TubR-*tubC* complexes is reminiscent of the ParR-*parC* ones in that TubR forms a ring-like structural filament around a superhelical DNA core (69, 101). This TubR-*tubC* complex stimulates the polymerization of TubZ *in vitro* (69, 102).

PARTITION NTPases AND MECHANISMS OF PARTITION

The motors for plasmid segregation are the ATPases or GTPases encoded by the partition systems. They do the work; that is, they provide the energy for plasmid movement. As introduced above, current evidence indicates that they fall into two general classes of mechanism (Fig. 4) and can be distinguished by the sequence conservation of their ATP binding sites.

Filamentation-Driven Mechanisms

The first type of plasmid partition mechanism that was uncovered was that of the type II system of plasmid R1 and uses "cytomotive" filaments to distribute plasmids dynamically in the cell (Fig. 4A). Type II partition ATPases fall into a general class of "actin-like proteins" (ALPs) designated by sequence and/or structural similarities to eukaryotic actin (103–105). Many cell biology and biochemical observations have established that they act by ATP-dependent insertional polymerization, effectively pushing plasmids apart along the longitudinal axis of a rod-shaped cell (reviewed in reference 48). *In vivo*, fluorescently labeled $ParM_{R1}$ filaments are observed with plasmids attached at opposite ends (47, 106). The filaments grow between the plasmids and then disassemble, so that prior to cell division, plasmids are positioned on opposite ends of the cell.

The biochemical properties and structural biology of $ParM_{R1}$ and $ParM_{pB171}$ established these dynamics of filament growth and polarity (45, 103, 107–110). *In vitro* in the presence of ATP (or GTP), monomers of ParM polymerize in a head-to-tail arrangement, forming left-handed double helices that are polar because asymmetry in each monomer creates a pointed and a barbed end on each filament (Fig. 4A). The plasmid partition complex composed of ParR bound to *parC* binds to the barbed end, and "caps" the filament. This cap prevents filament disassembly even though the individual monomers in the filament may hydrolyze ATP. New ATP-bound monomers of ParM are inserted at the ParR/*parC* concave interface, and as the filament grows, the plasmid moves in the cell. TIRF microscopy of fluorescently labeled proteins has also directly visualized these dynamics *in vitro*, using *parC*-coated beads (108). Filaments bound to these beads undergo cycles of growth and shrinkage that depend on ATP binding and hydrolysis. Studies using transmission and cryo-EM have visualized ParM filaments both *in vitro* and *in vivo* (107, 109, 111). High-resolution reconstructions from cryo-EM analyses support a model in which polar ParM filaments associate longitudinally as antiparallel bundles (109), so that plasmid-mediated filament growth occurs bidirectionally, at both ends of the bundle, effectively separating the plasmids.

Genes for a large number of ALPs have been identified in bacterial and plasmid genomes, which have been proposed to play roles in spatial arrangement of the bacterial cell as well as in plasmid distribution (105, 112). A variety of studies support the notion that these ALPs also work by insertional polymerization, although there are differences in the structural and mechanistic details among them (104, 113–118). The architecture of the filaments also varies among members of the ALPs. For example,

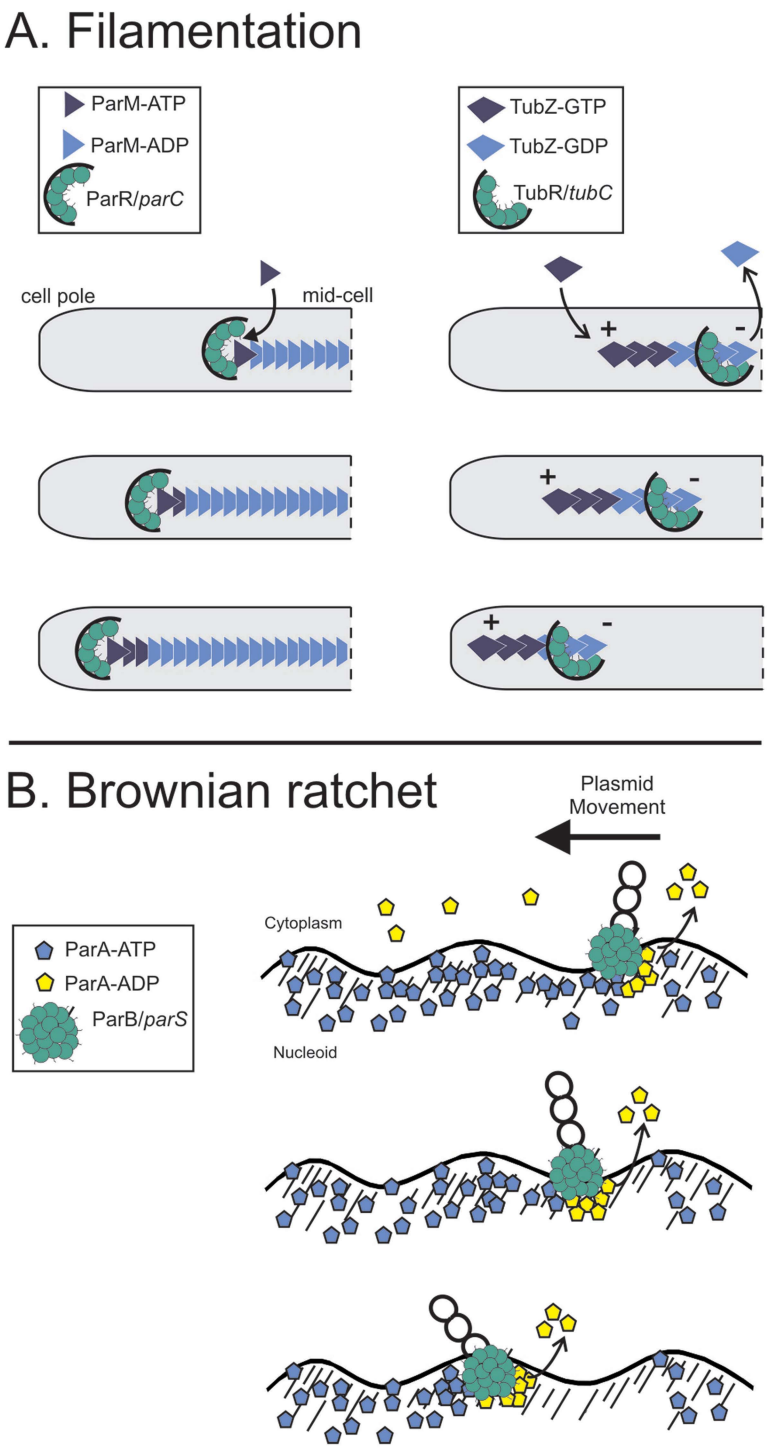

Figure 4 Mechanisms of partition NTPase-driven plasmid movement. (A) Filamentation. (Left) Filament growth and catastrophe. Plasmid R1, the paradigm for type II plasmid partition, uses ATP-dependent polymerization of the actin-like ParM ATPase to push plasmids towards the poles. Plasmids (via their ParR/*parC* partition complexes) are inserted at the growing end of the filaments, which are polar, by associating with the barbed end of a ParM molecule. Filaments are capped by partition complexes and ATP subunits, while individual monomers within the filaments hydrolyze ATP to ADP. The other "pointed" end of the filament is proposed to be capped by association with an antiparallel ParM filament (not shown), which itself associates with another plasmid via ParR/*parC* complexes for bidirectional plasmid movement (111). Loss of the cap results in "catastrophe," or rapid filament disassembly (not shown). (Right) Treadmilling. In type III partition systems such as that of pBtoxis, the tubulin-like TubZ GTPase polymerizes by addition of TubZ-GTP to the plus end and depolymerizes by loss of TubZ-GDP from the minus end, a behavior known as treadmilling. The plasmid (via its TubR/*tubC* partition complex) tracks with the minus end, so it is pulled from midcell to the cell pole. **(B)** Brownian ratchet partition systems rely on the ATP-dependent nonspecific DNA binding activity of the partition ATPase (ParA), which binds to the bacterial nucleoid. The plasmid (via the ParB/*parS* partition complex) attaches to ParA on the nucleoid and then stimulates ParA release from DNA by ATP hydrolysis or conformational change. Because ParA rebinding to the nucleoid is slow (40), a void of ParA is created on the bacterial chromosome, which serves as a barrier to motion so that the ParB/*parS*/plasmid complex moves towards the remaining ParA on the nucleoid. Further details and variations of this mechanism are described in the main text.

$ParM_{pSK41}$ forms a single rather than double helical filament (104).

Type III partition systems also work by polymerization but use tubulin-like GTPases to form the cytomotive filaments (reviewed in references 14 and 119). This family of partition proteins has been identified in plasmids from *Bacillus* and *Clostridium* species; however, other tubulin-like proteins do participate as cytoskeletal elements in *Enterobacterioceae*, such as the FtsZ cell division protein. During type III partition, TubZ polymerizes in the presence of GTP to form helical 2-stranded and 4-stranded

filaments, which are stabilized by a GTP cap (100, 120–123). During partition, TubR assembles at *tubC* and interacts with a long and critical C-terminal tail of TubZ. This C-terminal tail is also involved in interactions with TubZ in the filament lattice, which are thought to contribute to the dynamics of filament assembly and disassembly (123). TubZ polymers are polar; they grow at the plus end and disassemble at the minus end, a behavior called treadmilling (Fig. 4A) (10, 124). The TubRC complex tracks with the minus end of the TubZ filament, which, in essence, pulls the plasmids (rather than pushes during insertional polymerization as with ParM). However, how pulling results in plasmid separation is still not clear since TubZ filaments are not bipolar.

Patterning/Brownian Ratchet Mechanisms

Type I partition systems are the most common type of plasmid partition system. Although nomenclature varies among the members, for simplicity we call this type of partition ATPase ParA. ParAs carry a specific version of the Walker A box (part of the P loop) with the sequence KGGxxK(T/S) (125), and they show high structural similarities among their ATP binding domains (126–130). The ParA-like class of ATPases includes other dynamic proteins involved in positioning events in bacteria, which likely share common mechanisms of action (14, 41, 131). Examples for which dynamic behavior has been observed include *E. coli* MinD, involved in placement of the cell division septum (132), and *Synechococcus elongatus* McdA, necessary for localization of carboxysomes (133). Because of the properties of type II partition systems, early models proposed that ParAs would also work via a cytomotive filamentation mechanism (24, 134, 135). However, more recent data have led to models in which ParA proteins form dynamic gradients in the cell due to interactions with the bacterial nucleoid as well as with their cognate ParB partners, which determine the asymmetry of the ParA-nucleoid interaction (20, 22, 40, 43, 136–138). The ParB partner, with its plasmid cargo in tow, follows the gradient of ParA that it promotes (Fig. 4B). Several versions of this model have been suggested, but they share the basic principle of a Brownian ratchet (compared in reference 139). Very simplistically, directionality of diffusive movement is imposed by barriers in one dimension so that the cargo moves away from the barrier. The act of movement itself creates the barrier behind it, and the cargo ratchets "forward." In the case of plasmid partition, the barrier is a low concentration of ParA behind the ParB/plasmid complex compared to a high concentration in front.

Key molecular properties include the ATP-dependent nonspecific DNA binding activity of ParA, the stimulation of ParA ATPase activity by ParB and DNA, and the high concentration of ParB molecules bound at and in the vicinity of *parS*. All of these parameters have been established for several type I ParAs (reviewed in reference 14). In the presence of ATP, ParA binds to the DNA of the nucleoid (Fig. 4B). ParB (bound to *parS*) associates with ParA on the nucleoid, which then ejects ParA from the nucleoid. This occurs by two pathways, one dependent on and one independent of ParB stimulation of ParA ATPase activity (37, 40). The displaced ParA diffuses in the cytoplasm until it is able to rebind ATP and regain its DNA-binding activity (a slow step [40]), which leaves a void of ParA on the nucleoid. For a single plasmid, directionality is imposed when ParB associates with adjacent ParA on the nucleoid and, in turn, removes that ParA from DNA (Fig. 4B). Movement is thus biased away from the lower concentration (or void) of ParA. Bidirectional motion would occur when two plasmids separate after replication; they would move apart because the concentration of ParA would be lowest between them.

Biochemical, structural, and cell biology data support this general model and argue against a filamentation mechanism such as those of ParM or TubZ (20, 22, 40, 42, 43, 130). Notably, the basic mechanism has been partially recapitulated *in vitro* and observed by TIRF microscopy using slides coated with nonspecific DNA as a mimetic for the bacterial chromosome, as well as fluorescent components of the partition systems from P1 and F (20, 42, 43). In these experiments, ParA-ATP bound to the DNA carpet and ParB (in complex with *parS* plasmids) associated with this ParA. ParB subsequently promoted dissociation of ParA from the DNA, leaving a void of ParA due to a low refilling rate. Magnetic beads coated with $parS_F$ sites (the beads were prevented from dissociating vertically off the slide because of a low magnetic field) were observed to move over the DNA carpet, leaving a transient void of ParA in their wake (43). Filaments of ParA were not observed in these experiments (43).

How ParB (when bound to *parS*) attaches to and detaches from the ParA carpet is also an important component of the model. For the HTH_2 ParBs, many molecules of ParB associate with the plasmid, so that many ParB-ParA bonds, although weak, can work in concert. In principle, as some ParBs dissociate following ejection of ParA, some are still attached and others are newly forming (Fig. 4B).

In this way, the cargo is dynamically tethered to the carpet (thus limiting passive diffusion) and moves by (simplistically) rolling along the surface.

Mutants of $ParB_F$ that can no longer stimulate $ParA_F$ ATPase are still able to promote the dynamic patterns of ParA movement on the nucleoid, but with altered (increased) oscillation properties (37). These mutants, however, promote fewer clusters of F plasmids *in vivo*, leading to the conclusion that ParB stimulation of ParA ATPase is necessary to separate pairs of plasmids before they move apart. Further, the results suggest that ParB can promote ParA dissociation from DNA independently of its ATPase activity, that is, by altering ParA's DNA binding conformation directly.

Several versions of the Brownian ratchet, or "burnt-bridge" concept, have been proposed, based on cell biology, biochemical, and modeling studies (reviewed in reference 139). These include "diffusion-ratchet" (as originally proposed [40]), "DNA-relay," "hitchhiking," and "Venus flytrap" models (22, 23, 137, 140–142). They all rely on asymmetry of ParA distribution that is generated by interactions with ParB bound to *parS*; that is, when ParB and its plasmid cargo move, they burn the ParA bridge behind them. The models differ in the details of how this asymmetry generates the energy necessary to drive movement, the role of the bacterial chromosome, the role of ParA oligomerization, and the effects of ParA-ParB bonds on cargo mobility.

An important player is the bacterial nucleoid, as its shape and organization within a bacterial cell are relevant to understanding plasmid localization. The simplest versions of this model envision the nucleoid as a surface or DNA carpet, over which the plasmids were transported (20, 43). Recent superresolution microscopy has revealed that plasmids, partition complexes, and components can be found within the matrix of the nucleoid (22, 23). The hitchhiking and Venus flytrap models both propose a 3D ParA distribution within the nucleoid volume through oligomerization or limited polymerization of ParA, forming concentrated patches or a meshwork of ParA that serve as a sink of ParA to capture plasmids and ParB complexes. The hitchhiking idea posits that these patches form in HDRs of the nucleoid and that ParB (with its plasmid cargo) depletes ParA from one HDR and then diffuses to the next HDR (22). The Venus flytrap model envisions a dynamic meshwork of branched ParA polymers that grows to sample the nucleoid and shrinks with the passage of ParB/plasmid complexes (23). Finally, the DNA relay model proposes that the intrinsic torsional strain of the supercoiled bacterial chromosome provides energy to propel the cargo along the nucleoid (137). The differences between the mechanisms proposed may also reflect differences between the partition systems for which they were developed. Although it is highly likely that all type I partition ATPases use a Brownian ratchet patterning mechanism, the details and thus relevant model may depend on different biochemical properties and levels of expression of the ParAs and ParBs.

Other Partition Systems

Recently, a noncanonical partition system has been uncovered on the broad-host-range plasmid R388 (Fig. 1 and 3). One single DNA binding protein, StbA, was shown to be sufficient for R388 stability (11). StbA binds to a *cis*-acting region, the *stbDR*s, composed of two arrays of five 9-bp direct repeats spaced by 2-bp present upstream of *stbA* (143). The *stbA* gene is in an operon with *stbB* and *stbC*, which are not necessary for partition. This genetic organization is conserved on plasmids of different incompatibility groups such as pSa, R46, RP4, and R721, indicating that this noncanonical partition system is widespread (11). Sequence analyses are unable to predict whether StbA is an HTH_2, RHH_2, or other type of CBP. How StbA promotes plasmid stability without a cognate NTPase is unknown.

Host Factors

The replicon model described by Jacob et al. (4) proposed the existence of tethers that anchored or restricted the location of plasmids in the cell. Despite the efforts of many laboratories over several decades, no such protein tethers have been identified for plasmid partition systems. The only host protein identified has been for P1 and P1-like partition, which uses *E. coli* IHF as an accessory factor to promote high-affinity ParB binding to *parS* (144). In type I partition, the host chromosome is essentially a dynamic host factor, providing the matrix on which ParA patterning occurs. The recent finding that dense regions of the nucleoid, termed HDRs (see above and reference 52), colocalize with both ParB clusters and ParA patches suggests that they may serve as transient (but non-sequence-specific) anchors, thus reducing the mobility of the partition complexes (22). Permanent separation would, in principle, be achieved when the bacterial chromosome itself segregates into two nucleoids prior to cell division. Interestingly, chromosomally encoded ParAB partition systems do interact with other bacterial

proteins, and tethers have been identified that anchor the chromosomal *parS* sites to cell poles, such as the PopZ protein, which interacts with ParB in *Caulobacter crescentus* (2, 3).

PARTITION-MEDIATED INCOMPATIBILITY AND MECHANISMS

Two distinct plasmids unable to coexist stably in a growing bacterial population are termed incompatible (Fig. 5A). Competition arises from the presence of shared determinants between the two plasmids. These determinants, either identical or closely related phylogenetically, belong either to the replication or to the partition processes (145). Partition-mediated incompatibility has been observed for both type I and type II partition (146–149), although it has been more extensively examined in the former. Interestingly, the presence of two distinct partition systems on the same plasmid, either of the same or of different types, does not result in incompatibility (12, 150). Importantly, the mechanisms and strength by which plasmids exert incompatibility are different depending on (i) the Par element involved and (ii) the nature of the competing plasmids (151) (Fig. 5B).

ParA-Mediated Incompatibility

The expression of a *parA* gene on a high-copy-number plasmid provokes the destabilization of a coresident plasmid that employs a Par system with the same *parA*. This was first observed by the rapid loss of a mini-P1 plasmid after the introduction of a high-copy-number vector carrying the P1 *parA* gene (152). A similar phenomenon, termed IncI, was reported with the destabilization of a mini-F by $ParA_F$ (153). Quantitative measurement of the mini-F loss rate indicated that overexpression of $ParA_F$ provokes a dose-dependent increase of incompatibility, up to a maximum loss rate corresponding to random inheritance as for a mini-F without its *par* locus (154). The excess $ParA_F$ was shown to interfere specifically with the formation and/or the stability of the partition complex. Likewise, an excess of $ParA_{P1}$ was able to disrupt the P1 plasmid partition complex *in vitro* (155), highlighting the need to maintain the relative amounts of ParA and ParB within fairly strict limits for faithful partition. In addition, the Brownian ratchet mechanism itself could be perturbed by too much ParA (or ParB), which would manifest as incompatibility. Modeling studies suggest that excess ParA or ParB would disturb the dynamics and change behavior or interfere with movement (141). For example, high ParA could increase the "refilling rate" of the nucleoid, which would reduce the barrier created by ParA voids, and plasmid movement would not occur.

ParB-Mediated Incompatibility

Incompatibility mediated by ParB was first shown for $parB_F$; a high-copy pBR322 plasmid expressing $parB_F$ destabilized a low-copy mini-F (par^+) plasmid (156). This incompatibility, termed IncG in the F system (146), was also reported for $parB_{P1}$ and shown to be strictly dependent on the presence of *parS* (157). Depending on the ParB overexpression level, the strength of incompatibility is variable, from mild to severe, and several mechanisms, which may occur simultaneously, have been proposed.

When ParB expression levels are very high, the destabilization of a *parS*-carrying plasmid is more severe than expected for random inheritance of the same plasmid without its active Par system (157). This observation has suggested that the *parS* plasmids are sequestered together, which lowers the number of free plasmid units able to segregate by random diffusion within the cytoplasm. This sequestration has led to the hypothesis that in the regular partition process, one of the roles of ParB was to pair plasmids via their *parS* sites after plasmid replication, to provide the substrate for a ParA-mediated separation step. This idea was consistent with the explanation for ParA-mediated incompatibility. In other words, if ParB levels are too high, ParA cannot separate partition complexes and release plasmids, and if ParA levels are too high, ParB complexes are disrupted inappropriately.

Another mechanism for the incompatibility has been proposed to explain the IncG phenotype that occurs with a physiological level of $ParB_F$ (158, 159). An intrinsically stable medium-copy plasmid carrying $parS_F$ was destabilized by moderate (5-fold) overproduction of $ParB_F$ (under these conditions, the ratio of $ParB_F$ to $parS_F$ sites was 0.7 relative to that in low-copy par^+ F plasmids). However, similar levels of $ParB_F$ resulted in only a small destabilization of low-copy mini-F. This inconsistency was resolved by the finding that $ParB_F$ induces RecFOR-dependent recombination events between sibling plasmids, probably at the onset of replication, resulting in a high rate of multimerization (159). Therefore, the reduction of the number of segregating units (but not copy number) led to missegregation events that increased proportionally to the level of multimerization. The results

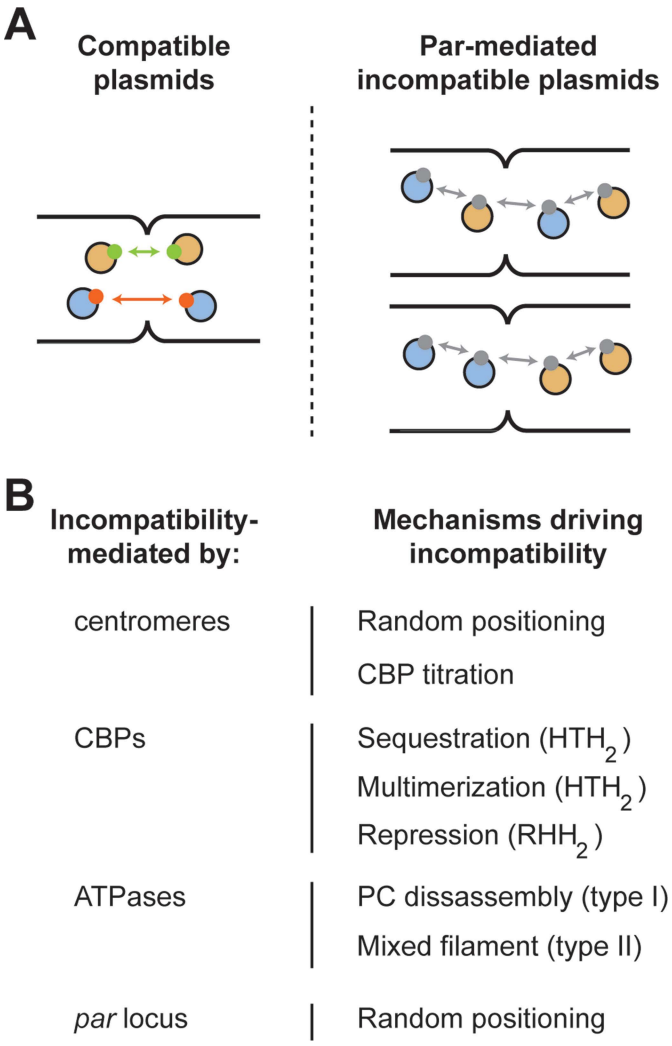

Figure 5 Partition-mediated incompatibility phenotypes. **(A)** Schematic representation of the incompatibility at the onset of cell division. Two different replicons, represented with orange and blue colors, are fully compatible if their partition systems (red and green circles) are distinct (left) or are incompatible if their partition systems cross-react (gray circles) (right). In the latter case, sibling plasmids would frequently be inherited in the same daughter cell, leading to mutual exclusion. **(B)** Mechanisms driving incompatibility phenotypes. Each partition component leads to mutual exclusion of plasmids sharing parts of the partition locus (see main text for details).

highlight the requirement for dimer resolution in plasmid stability, such as the ResD/*rfsF* system on the F plasmid.

Centromere-Mediated Incompatibility

Two distinct plasmids (different replicons) that contain the same partition site are generally incompatible (146, 156, 160, 161). The replicon model (4) envisioned that they would compete for a limited number of partition-specific host/cellular sites. However, the lack of specific host-specific factors involved in plasmid partition has led to other proposals, such as mixed pairing, random positioning, and ParB titration (151).

Titration of ParB has been shown to occur when extra *parS* sites are present on a high-copy-number plasmid (28). Indeed, increasing the intracellular amount of ParB reduces the severity of *parS*-mediated incompatibility. However, high ParB levels can never fully restore a compatible state, which suggests that other mechanisms are at play. It was proposed that the formation of mixed pairs, i.e., pairing between the two different plasmids via the partition complexes assembled on *parS*, leads to random segregation by the partition apparatus (reviewed in references 151 and 162). However, visualization of plasmid position in cells by fluorescence microscopy revealed that mixed pairing did not occur (163). Rather, all competing *parS*-carrying plasmids were separated and spaced relatively evenly along the cell length but randomly distributed with respect to each other. In other words, the partition system properly distributed plasmids along the cell length but did not discriminate different plasmids because they all contained the same *parS*. This randomization of plasmid positioning relative to each other explained the extent of *parS*-mediated incompatibility that is observed with medium-copy-number plasmids.

When a competing *parS* site is present on a low-copy-number plasmid, incompatibility is very strong and leads to a loss rate that is higher than random (28). As described above, mixed pairing was excluded because fluorescence imaging showed plasmids spaced equally but randomly along the cell length (32). An alternative mechanism based on the timing of replication has been proposed to explain the strong incompatibility. Plasmid replication occurs anytime during the cell cycle (164), so when one plasmid replicates late (close to cell division), its two copies would not redistribute with the other *parS*-containing plasmids, leading to their inheritance in the same daughter cell and thus to strong mutual exclusion (32).

Type II partition systems also induce incompatibility. Extra copies of a truncated R1 centromere *parC* on high- or medium- but not low-copy-number plasmids would destabilize R1 (148). Titration of ParR by extra *parC* sites may explain these observations. A strong incompatibility was observed between two plasmids, pB171 and pCP301, whose Par operons showed substantial sequence diver-

gence (149). However, $ParR_{pB171}$ was shown to bind to the *parC* sequence from both plasmids, and it was proposed that ParM filaments could form with two different plasmids at each end, leading to missegregation.

REGULATION OF *par* GENE EXPRESSION

All the *par* operons are autoregulated, and their misregulation interferes with plasmid stability. One protein is a transcriptional repressor, and often the other acts as a corepressor. However, the identity of the repressor varies: in some type I systems, it is the ParA ATPase, and in all others, it is the CBP. In some systems, the repressor also regulates other genes on the plasmid in addition to the *par* genes.

ParAs that function as repressors (sometimes called type Ia) possess an extra N-terminal HTH site-specific DNA binding domain, which recognizes operator sequences in the promoter region of the *par* operon (89, 128, 165–170). The best-studied examples are ParAs from P1, P7, and F plasmids. The repression is stimulated by the cognate ParB (89, 165) and is further enhanced by the centromere site (171, 172). The exact sequences necessary for operator function have not been fully defined. The promoter regions of the P1 and P7 *par* operons contain large inverted repeats that are proposed to be the recognition elements, and mutations in these repeats diminish ParA repressor activity (166, 168). *In vitro*, P1 ParA binds to a large region of DNA that centers over these repeats in DNase I footprinting experiments (166, 167). For plasmid F, a region overlapping the promoter that contains four direct 6-bp repeats is protected from DNase I *in vitro* (169). They are involved in $ParA_F$ binding, since mutating all four motifs prevents this interaction (173), but the exact recognition elements are yet to be identified.

Another interesting feature of the ParA repressors is that their activity is dependent on or influenced by ADP and ATP. $ParA_{P1}$ requires ADP or ATP to bind to the *par* operator with high affinity, and ADP is a better cofactor than ATP (155, 167). $ParA_F$ in its apo or ADP-bound form, but not the ATP-bound form, will bind to the F *par* operator region (39). Both observations are consistent with the idea that the ATP-dependent nonspecific DNA binding activity of ParA that is necessary for partition and resides in the C-terminal region of ParA (174) outcompetes the N-terminal site-specific DNA binding activity when ATP is present. They suggest that the repressor and partition forms of ParA are different, and the difference is regulated by ATP and ADP (155, 175). How ParB acts as a corepressor and stimulates ParA's repressor activity is not known, but an attractive model is that ParB decreases the ATP-bound conformation of ParA that is necessary for ParA's nonspecific DNA binding activity. Indeed, certain $ParA_{P1}$ variants that can bind but not hydrolyze ATP are locked in the repressor form because they have lost the nonspecific DNA binding activity of ParA (40, 175). They behave as "superrepressors" that no longer require the stimulation by ParB.

The broad-host-range plasmid RK2 has a different regulatory arrangement in that $ParB_{RK2}$ (KorB) regulates many genes on the plasmid, including the partition genes *incC* ($parA_{RK2}$) and *korB* (58, 176). The partition site, O_B3, 1 of 12 O_B sites to which KorB binds, is not involved in the regulation of the expression of the partition genes. The repression is achieved through KorB binding to O_B1, located immediately upstream of the −35 sequence. IncC stimulates the repressor activity of KorB but, interestingly, requires a longer version of IncC (called IncC1) than is necessary for IncC/ParA partition activity (177). Another example of partition proteins that control the regulation of other genes is in the RepABC family of plasmids in *Rhizobiaceae* and other α-proteobacteria (178, 179). RepA and RepB correspond to ParA and ParB, respectively, and regulate the expression of the third gene in their operon, *repC*, which encodes the replication initiator. The misregulation of replication genes is also a potential cause of incompatibility when the Par proteins are overexpressed. Where tested, RHH_2 CBPs act as the repressors of *par* genes by binding to centromere repeat sequences that are present in promoter regions (Fig. 3). For example, in R1 *par*, two sets of five direct repeats in *parC* flank the −10 and −35 core promoter sequences (180). This promoter was shown to be negatively regulated by $ParR_{R1}$; however, $ParM_{R1}$ plays no role in this regulation (180). The type II Par system (Par1) of pB171 is also negatively regulated by the binding of $ParR_{pB171}$ to the two 10-bp motifs present between the promoter and the first codon of the *parM* gene (66). The type I partition system of pB171 (Par2) is negatively regulated by $ParB_{pB171}$ binding to the *parC1* region containing the two arrays of direct repeats that flank the −10 and −35 promoter sequences of *parAB* (66). Plasmids TP228 and pTAR (from *Agrobacterium tumefaciens*) are both type I partition systems with RHH_2 CBPs that repress their cognate *par* genes (181, 182). In the case of pTAR, the cognate ParA does stimulate repression by the CBP (181).

In plasmid R388, StbA may control the expression of the *stbABC* operon, to which it belongs. Although autoregulation remains to be demonstrated, the *StbDR*s motifs that constitute the centromere overlap the putative −10 and −35 core promoter sequences (Fig. 3).

CONCLUDING REMARKS

Partition systems have developed strategies to build a complex and highly controlled system using only two proteins and a few short DNA motifs. They share several principles to promote efficient DNA partitioning, including (i) the self-organization of large structures for both partition complexes and NTPase assemblies, each using a single protein with oligomerization or dynamic and concerted interactions; (ii) the modulation of protein activities, which are finely regulated through interaction with the cognate partition proteins, nucleotide or DNA; and (iii) the use of a cyclical rather than linear pattern of behavior, which is achieved by binding, hydrolyzing, and releasing nucleotides.

Our knowledge of the mechanisms of plasmid partition has improved dramatically since the original model from Jacob et al. (4), yet many interesting questions remain. For example, plasmids that encode only one partition protein, such as R388, may conform to some or all of the above-mentioned principles, perhaps with the participation of unidentified partners, or may act in novel ways. Why some plasmids have acquired and utilize more than one partition system, and why they do not interfere with each other, are not known. In this review we have focused on the role of partition in plasmid localization, but what other processes contribute to localization? Are there host factors involved at other steps or as accessory factors? The bacterial nucleoid serves both as a matrix for type I partition and as a passive barrier to all plasmids via nucleoid exclusion. What are the important features of nucleoid and chromosome architecture that dictate each of these activities? Researchers continue to develop creative ways to image the steps in plasmid partition, as well as to advance our understanding of the structural biology and enzymology of its components and complexes. Plasmid partition is a fascinating problem of genome dynamics, which must be integrated with their general lifestyle as well as those of their bacterial hosts.

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