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Mechanisms of plasmid segregation: have multicopy plasmids been overlooked?

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Abstract

Plasmids are self-replicating pieces of DNA that can help dissemination of non-essential genes. Given that plasmids represent a metabolic burden to the host, mechanisms ensuring plasmid transmission to daughter cells are critical for their stable maintenance in the population. Here we review these mechanisms, focusing on two active partition strategies common to low-copy plasmids: *par* systems type I and II. Both involve three components: an adaptor protein, a motor protein, and a centromere, which is a sequence area in the plasmid that is recognized by the adaptor protein. The centromere-bound adaptor nucleates polymerization of the motor, leading to filament formation, which can pull plasmids apart (*par I*) or push them towards opposite poles of the cell (*par II*). No such active partition mechanisms are known to occur in high copy number plasmids. In this case, vertical transmission is generally considered stochastic, due to the random distribution of plasmids in the cytoplasm. We discuss conceptual and experimental lines of evidence questioning the random distribution model and posit the existence of a mechanism for segregation in high copy number plasmids that moves plasmids to cell poles to facilitate transmission to daughter cells. This mechanism would involve chromosomally-encoded proteins and the plasmid origin of replication. Modulation of this proposed mechanism of segregation could provide new ways to enhance plasmid stability in the context of recombinant gene expression, which is limiting for large-scale protein production and for bioremediation.

Keywords

partition; segregation; cytoskeleton; motors; DNA-binding; replication

Plasmids are self-replicating, extra-chromosomal pieces of DNA that help their hosts face environmental challenges or adapt to specific niches through the expression of selected sets of genes (1-3). Plasmid-borne genes are typically dispensable, although there is a class of large plasmids known as “chromids” that frequently encode essential genes for core physiology (4). Plasmids also contribute to evolution by facilitating horizontal dissemination of the genes they bear, frequently across species (5) .

Plasmids are typically present in multiple copies, and the copy number is relatively stable. Plasmid copy number varies widely depending on the plasmid, ranging from as few as 1-2

copies per cell for F or R1 plasmids (6) to up to 200 copies per cell for ColE1 plasmid derivatives used for recombinant gene expression (7).

Plasmids represent a significant metabolic burden for their hosts (8). The metabolic burden associated with a particular plasmid is determined both by expression of plasmid-borne products and the number of copies of plasmids within a cell (8-11). High-copy number (hcn) plasmids such as cloning vectors are typically lost from populations at a high rate in the absence of selection, likely because these constructs both exert a large metabolic burden and lack plasmid maintenance factors found on natural plasmids. Plasmid loss has been identified as a key factor limiting yield of large-scale recombinant gene expression (12), leading to substantial efforts directed at understanding the mechanisms involved in regulation of plasmid copy number and plasmid loss (13-15).

Given that expression of plasmid-borne genes only confers a selective advantage in specific environments, in order to prevent loss when conditions change, plasmids need mechanisms to ensure their transmission to daughter cells. Mechanisms for vertical plasmid propagation among bacterial hosts fall into the following three categories:

1. Partitioning systems: Low copy number plasmids often harbor genes whose function is to physically distribute plasmids so that each daughter cell receives at least one plasmid upon cell division (16, 17). Examples include the *parRMC* system of R-plasmids found within gammaproteobacteria (18, 19), the *sopABC* system of F-plasmids (20) and the *Rep/mob* systems found in Gram-positive bacteria (21). The first part of the present review describes two partitioning systems that use cytoskeletal-like structures to direct plasmid distribution.
2. Random segregation: For plasmids that are kept at high copy number (>15 copies/cell), the theoretical probability of plasmid-less segregation is vanishingly small (22, 23), so it has been proposed that these plasmids would not require active segregation; instead, each daughter cell receives at least one plasmid as a result of random diffusion (23). Random plasmid distribution is facilitated by multimer resolution systems; these systems use site-specific DNA recombination to resolve plasmid multimers (which reduce the effective copy number) into monomers (24, 25). The second part of the present review discusses emerging evidence questioning this random distribution model and proposes that hcn plasmid transmission does involve segregation, but that in this case partition is dependent on chromosomal, rather than plasmid-intrinsic, factors.
3. Post-segregational killing: Some plasmids ensure their maintenance in the population through mechanisms that selectively kill plasmid-free daughter cells (26, 27). Examples include the CcdA/B system found on F plasmids (28) and the colicin system of ColE1 plasmids (27). The CcdA/B system depends on a highly-stable cytoplasmic toxin (CcdB, a topoisomerase inhibitor) and a short-lived cognate immunity protein (CcdA) (29, 30). Plasmid-free daughter cells arising upon cell division are killed by CcdB once the immunity protein CcdA degrades (28). Thus the CcdA/B system ensures vertical transmission of plasmids to daughter cells. Similarly, colicins are plasmid-borne cytotoxic peptides and

immunity proteins produced by certain strains of *E. coli*. Colicins kill cells lacking immunity proteins by a variety of mechanisms including nuclease activity, pore-formation in the outer membrane, and inhibition of cell wall metabolism (31). Unlike CcdB, however, colicins are secreted, which means they eliminate all plasmid-free cells in a population; not only plasmid-free progeny.

In addition to mechanisms dedicated to ensuring vertical transmission, conjugative transfer can also be considered a mechanism promoting plasmid stability, as it allows some plasmids to reestablish themselves in plasmid-less cells (32).

Active plasmid partitioning

Numerous plasmids of *E. coli* harbor active partitioning mechanisms, aimed at ensuring proper segregation between daughter cells. Of note, plasmid partitioning systems also serve as compatibility determinants for plasmids, excluding foreign plasmids by ensuring that only plasmids arising from pre-existing copies are faithfully segregated into daughter cells (33).

To illustrate mechanisms of active plasmid segregation, we will focus on *par* systems as general paradigms for plasmid partitioning. *Par* systems, such as the Rep/*mob* system found in plasmids of gram-positive bacteria, the RepABC system of alpha-proteobacteria (21, 34), or the *sopABC* locus of the conjugative plasmid F, are well characterized and share some commonalities with other partitioning mechanisms (16, 17).

Par systems are made up of three components: (1) a *cis*-acting centromeric sequence element, (2) a motor protein that derives energy from hydrolysis of nucleotide triphosphates, and (3) a DNA-binding protein that serves as an adaptor between the motor and the centromere. Adaptor binding of the centromere nucleates polymerization of the motor; polymerization and filament formation are essential for segregation.

Par systems are auto-regulated by their own DNA-binding protein (35), which may also play a role in regulation of plasmid-encoded genes by binding and silencing the promoter regions of centromere-proximal genes (36). Disruption of the regulation of these systems leads to segregation defects, and over-expression of any individual component causes plasmids to be lost at a high rate (35, 37). *Par*-mediated plasmid localization and segregation is independent of the cell cycle or chromosomal replication, as demonstrated in numerous studies using cephalosporin treatment or cells blocked for chromosomal replication (38, 39).

The centromeric sequence varies between different *par* systems and is composed of tandem arrays of either direct or inverted repeats (40-42) that establish DNA curvature (43). This curvature is likely critical for its function, as inversion of the repeated sequences (presumably altering the local topology) leads to reduced binding affinity for the adaptor protein (43). The *CEN* sequences in budding yeast also display curvature (44), suggesting that this property may be universal to centromeric DNA.

Par systems are classified based upon the motor proteins they encode. Type I *par* systems feature Walker-A P-Loop ATPases (43), whereas type II *par* systems are driven by actin-

like ATPases (45). For both systems, polymerization is energetically favorable when monomers of the motor protein are in the ATP-bound state (46). Filaments rapidly disassemble upon ATP hydrolysis. Type I *par* systems use the filament disassembly to pull plasmids to the quarter-cell position prior to cell division. The *sopABC* locus found on the conjugative plasmid F represents a typical type I *par* system. Type II *par* systems, by contrast, bind and then separate plasmids to the cell poles by a pushing mechanism, in a process analogous to actin filament dynamics (19). The model for type II *par* systems is the *parRMC* locus found on the R1 multidrug resistance plasmid of *E. coli* (47).

Type I *par* systems appear to have been co-opted for chromosomal segregation by numerous bacterial species, as orthologous genes to *sopABC* are found in *Bacillus subtilis*, and *Caulobacter crescentus*, where they are critical for proper chromosomal segregation (48, 49). Type I-like centromeric sequences have been detected in prokaryotic genomes across many phyla: indeed, up to 70% of bacterial genomes may contain these sequence elements (50). Type II *par* systems appear to be more restricted to plasmids. However, the ParR protein is analogous to MreB and the ParR protein shares homology with FtsA, two important proteins for chromosomal segregation indicating that these systems may be evolutionarily related (51).

Type I *par* systems: The F-plasmid *sopABC* locus

Type I *par* systems have three elements: two protein-coding genes (*sopA* and *sopB* for the system encoded by the F-plasmid) and the centromeric sequence (*sopC* for the F-plasmid) (Fig. 1a). Consistent with *par* systems' regulatory independence from chromosomal replication, plasmids harboring *sopABC* genes are faithfully segregated and stably maintained in *E. coli* mutant backgrounds deficient for chromosome partitioning (52).

The motor protein, SopA, is a Walker-A type P-loop ATPase with a non-specific DNA-binding domain (20). In the absence of DNA or SopB, the kinetics of ATP hydrolysis by SopA are extremely slow (20). SopA forms filamentous structures *in vitro* and *in vivo* only in the presence of ATP (53). Both filament assembly and disassembly are required for proper segregation by type I *par* systems, as plasmids encoding mutant alleles of SopA that are deficient in ATP-binding (54) and also mutants that cannot hydrolyze ATP (54) are lost at a high rate.

The interaction between SopA and SopB is necessary for proper segregation (55). The ATPase activity of SopA is cooperative and stimulated both by naked DNA and by interaction with SopB in complex with *sopC* DNA (56). Paradoxically, even though SopB-*sopC* complexes stimulate SopA ATPase activity, SopA filament formation in the presence of DNA requires SopB both *in vitro* and in living cells (53). This may be because SopB shields naked DNA, preventing it from activating the ATPase of SopA (57). Thus, SopB serves both to promote and to destabilize filament formation by SopA and this dynamic process drives plasmid partition.

Type I *par* systems segregate plasmids by a pulling mechanism, using de-polymerization of the motor protein filament to separate plasmids between daughter cells (19). This process of segregation is reminiscent of kinetochore motion during eukaryotic mitosis. Initially, SopB

pairs two plasmids together at mid-cell by binding *sopC* as a dimer (Fig 1a, panel ii). SopB multimerizes, coating naked plasmid DNA surrounding *sopC* in a process referred to as “spreading” (58, 59) (Fig 1a, panel iii). SopB in complex with non-centromeric DNA promotes polymerization of SopA (57). SopA filaments extend radially outward from the plasmids in both directions (Fig 1a, panel iv), until they encounter SopB monomers in complex with *sopC* (56), which stimulates the ATPase of SopA, leading to a wave of ATP hydrolysis that destabilizes the filaments (57) (Fig 1a, panel v). Disassembling SopA filaments exert tension on SopB, pulling the plasmids apart to the quarter-cell positions (53) (Fig 1a, panel vi). The opposing forces exerted by individual radial filaments may serve to center the plasmids with respect to each other and the short axis of the cell, akin to the localization the mitotic spindle due to the force exerted by microtubule de-polymerization in higher eukaryotes (60).

The dynamics of partitioning by *sopABC* have been observed *in vivo* using microscopy. Plasmids harboring a *sopABC* cassette initially localize at mid-cell. Upon replication, the resulting plasmids migrate symmetrically to the quarter-cell positions (61). In the absence of SopB, SopA filaments oscillate between cell poles *in vivo*, displaying dynamics analogous to the localization of the septation-determinant MinD (62); it is thought that this oscillation contributes to the pulling force that drives plasmid motion (63).

SopA filaments may be tethered at one end to some yet uncharacterized cellular structure. Initially it was thought that the nucleoid could serve as an anchor to ensure segregation by *sopABC*, however faithful segregation of F plasmids into anucleate cells argues against this possibility (52). It is possible that the cell membrane or a cytoskeletal protein is responsible for this tethering (64). Other studies have proposed that the mechanism of segregation by SopA does not depend on filament formation or polymerization, but rather on a gradient of SopA-ATP monomers distributed throughout the cytosol (65). In this model, SopA monomers close to the nucleoid are in the ADP-bound state, and SopA monomers distal from the nucleoid are ATP-bound leading to a gradient. SopB has a much higher affinity for the ATP- than the ADP-bound state of SopA, and SopB stimulates the ATPase activity of SopA (57). SopB may sequentially bind SopA monomers, dissociate upon ATP hydrolysis, and subsequently bind the next monomer, pulling plasmids apart by a diffusion-ratchet rather than by a depolymerizing filament (65). *In vitro* studies have demonstrated SopA-mediated pulling of SopB on a DNA via this diffusion-ratchet, indicating that filaments and a tether may not be required for pulling cargo (66). However, it is unclear whether these conditions are applicable to cellular physiology. Additionally, microscopic studies indicate that SopA does indeed form filamentous structures in living cells (62, 63). The mechanics of segregation, including whether a host-cell derived anchor is required, the regulation of the organization of SopA filaments, and how SopB stimulates the ATPase activity of SopA in type I *par* systems remain ongoing topics of study.

Type II *par* systems: The R1-plasmid *parRMC* locus

The type II *par* system encoded by the *E. coli* multi-drug antibiotic resistance plasmid R1 was discovered in 1986 (47). This represents the archetypal member of this family of partitioning systems.

The type II *par* system of R1 possesses two protein-coding genes: *parR*, which encodes the adaptor protein (67), and *parM*, which encodes the actin-like ATPase (68) (Fig. 1b). The centromeric element, *parC*, is a 160 base pair sequence found upstream of the open reading frames for ParRM. This region consists of two tandem arrays of five direct-repeats, flanking the promoter for the *parRM* genes (41). ParR preferentially binds *parC* sequences in supercoiled DNA, and its binding induces significant bending of the DNA (69). All three components are necessary for symmetrical distribution of plasmids between daughter cells (38)

The actin-like motor protein, ParM, forms polar, left-handed double-stranded filaments *in vivo* (70) and *in vitro* (71). ParM cooperatively forms filaments in the ATP-bound state. Oligomerization promotes the ATPase activity of ParM. ParM monomers are added to the ends of filaments in the ATP-bound state, and lost from the ends upon hydrolysis. Although the organization of ParM monomers within a filament is unidirectional, assembly and disassembly occurs at equal rates from either end (72). In the absence of ParR, ParM forms transient, short filaments (73). ParM filaments are stabilized by the presence of an ATP-bound ParM monomer at either end, however the rapid kinetics of the ATPase activity leads to frequent catastrophic disassembly (74). Association with ParR in complex with *parC* stabilizes the ends of ParM filaments (72). ParM monomers are added to the ends of ParR-bound filaments, leading to bi-directional elongation (72). The bi-directional elongation of ParM filaments drives plasmids to opposite poles of the cell during segregation. Thus, unlike type I *par* systems, type II *par* systems use polymerization of the motor protein to push plasmids apart.

The dynamics of type II *par* segregation has been observed *in vivo* using R1 plasmids carrying tandem *lac* operator arrays in combination with fluorescent fusions to the LacI repressor protein (73). Single R1 plasmids initially diffuse freely within accessible areas of the cytoplasm (Fig. 1b panel i). When two plasmids encounter each other in the cell, they become tethered and move as a single unit (Fig. 1b panel ii). Filament formation by ParM leads to rapid separation along the long axis of the cell over the course of 10-30 seconds (Fig. 1b panels iii, iv, v). ATP hydrolysis triggers ParM filament disassembly (Fig. 1b panel vi). After separation, plasmids again diffuse freely until they encounter each other and pair again (Fig. 1b panels vii, i, and ii). This pairing and separation occurs repeatedly, both pre- and post- cell division. The temporal dynamics of repeated pairing and separation of plasmids is recapitulated *in vitro* (75). Because the time scale of plasmid separation by ParM filaments is much shorter than that of cell division, repeated pushing of plasmids to opposite cell poles maximizes the likelihood that each daughter cell will inherit a copy of the plasmid.

The random distribution hypothesis of plasmid segregation for high-copy number plasmids

Hcn plasmids typically lack genes encoding canonical active partition systems. These plasmids are associated with an elevated metabolic burden both because of elevated gene expression and because of the cost associated with replicating to a high copy number. Therefore (in the absence of positive selection for plasmid-borne genes, or of negative

selection against plasmid loss) plasmid-free daughters that stochastically arise following cell division would be expected to have a significant fitness advantage, threatening plasmid maintenance in the population (8).

Hcn plasmids, however, show considerable stability in the absence of positive selection (8), which has been attributed to infrequent generation of plasmid-free cells. This interpretation, known as the random distribution model, assumes free diffusion of plasmids throughout the cytoplasm before cell division and random segregation during cell division.

If plasmids segregate randomly between cells, the probability of a plasmid-free daughter cell arising (ρ_0) upon each round of cell division is given by $\rho_0=2^{(1-n)}$, where n =plasmid copy number (25). Based on this equation, the theoretical frequency loss of a plasmid is given by $L_{th}=(1/2)^n$ (22). Because hcn plasmids have a large n , the estimated ρ_0 for these plasmids is low. For example, if a hypothetical plasmid is carried at greater than 15 copies per cell, the predicted loss frequency per round of cell division is less than 0.003%. Thus, in the absence of selection, fewer than 0.3% of cells would be predicted to have lost this plasmid after 100 generations in culture, suggesting that hcn plasmids can be retained long-term absent selection or active partitioning.

Evidence against random distribution model

The calculations above assume a normal distribution of plasmid copy number in the population. For a given average copy number the estimated fraction of plasmid-free cells per generation varies substantially depending on the standard deviation. This explains the paradoxical finding that mutations increasing plasmid copy number through deregulation of control of plasmid copy number often lead to increased plasmid instability (76). A second assumption is absence of negative selection against plasmid-carrying cells. As explained above, this is an unrealistic scenario given the metabolic burden represented by gene expression and plasmid replication, and the result is that rare, plasmid-less cells are expected to have a substantial fitness advantage that would eventually lead to plasmid loss in the population. In addition to these two conceptual considerations, more recently two experimental lines of evidence further question the random distribution model:

1. Non-random distribution of plasmids within the cell: One key assumption behind the random distribution model for hcn plasmid segregation is that plasmid molecules diffuse freely throughout the cell. However, microscopic studies using fluorescently labeled plasmids show that ColE1 plasmids are excluded from the nucleoid region (77-79), localizing preferentially at cell poles (79). *In vivo* microscopy studies showed that during replication, fluorescent foci corresponding to the studied plasmid (a pUC19-derivative) appeared to split and subsequently localize to the quarter-cell positions prior to septation and division (78). This was confirmed in a second study, which showed that a ColE1 plasmid localized both at the pole and mid-cell, with the mid-cell position of the mother cell becoming the cell pole in one of the daughters (80). Overall, these observations evidence that plasmid diffusion within living cells is strongly constrained.

2. Effective reduction in plasmid copy number through clustering: in addition to limiting the cellular volume available for diffusion, the specific subcellular localization of plasmids also reduces the effective number of units available for segregation (81). Indeed, the studies mentioned above found hcn plasmids co-localizing as clusters (78, 80). According to the prediction of the random distribution model, where the probability of plasmid-free daughters arising is $\rho_0 = 2^{(1/n)}$, clustering and multimerization has a strong effect on ρ_0 for high-copy plasmids. For ColE1, which is maintained at roughly 15 copies per cell, dimerization increases the probability of plasmid loss each division by 256 fold. Plasmid clustering within cells was recognized earlier as a challenge to the random distribution model, and an attempt was made to reconcile the two (81). Deletion of site-specific recombination systems that resolve hcn plasmid multimers and catenanes (17) lead to decreased stability (82), demonstrating the impact on plasmid stability of decreasing the number of plasmid units available for segregation at cell division.

Alternative hypothesis

The repeated observation that hcn plasmids localize to the cell poles (78, 80) and that they accumulate and replicate at cell poles or the mid-cell position (78-80) is consistent with a process of regulated distribution aimed at ensuring vertical transmission of these plasmids during cell division.

The preferential localization to cell poles has alternatively been attributed to displacement of the plasmid by the nucleoid (79), which also appears to be the default pathway for plasmids with active partition systems (83-86). Indeed, the rearrangements in subcellular localization for hcn plasmids described above parallel the observed reorganization of the nucleoid prior to cell division and septation (79).

Despite the growing evidence pointing to a role of nucleoid exclusion in hcn plasmid segregation, the observation of discrete, multifocal clusters (78, 80) is more consistent with an actively regulated process. Here we posit the existence of a segregation mechanism for hcn plasmids, although we can only speculate on the nature of this mechanism. This mechanism would depend on proteins encoded in the chromosome. Two key differences with the nucleoid exclusion model are that hcn plasmid segregation could be subject to regulation and also that it would involve recognition of plasmid sequence by the segregation machinery. Modifications in plasmid *ori* sequence introduced in recombinant gene expression vectors or alterations in the host cell induced by recombinant gene expression could contribute to plasmid instability by directly interfering with segregation, generating more plasmid-free cells per generation than expected based on average plasmid copy number.

Following replication, the chromosomal origin of replication migrates bi-directionally towards either pole (87, 88) and then migrates to the replication factory at mid-cell (89). These chromosomal origin of replication migrations are reminiscent of relocations associated with plasmid segregation and could represent direct or indirect physical interactions between chromosomal and plasmid replication. However, it is hard to envision

how the two processes would be coordinated in time, as the kinetics of the two processes are very different, with plasmid replication being much faster (10-20 s compared to 30-40 min for chromosomal replication). The observation that plasmid replication initiation occurs randomly *in vivo* (90, 91) and the absence of co-localization between chromosomal and plasmid replication in time-lapse microscopy experiments (79) further argue against a link between chromosomal replication and plasmid partition.

Known plasmid-partitioning systems rely on a sequence motif to anchor the motor protein responsible for partition to the plasmid. Such a motif may also be present, in cryptic form, in hcn plasmids. Given that most recombinant expression vectors contain only the minimal sequence for plasmid replication initiation, yet still show evidence for segregation (notably clustering) this motif would likely be found within this minimal sequence. Additional signals for partitioning existing in the native plasmids may have been deleted in vectors designed for recombinant gene expression, much like the loss of *cer*, which affects the ability of these plasmids to resolve concatemers (82).

In sum, although it is formally possible that segregation of high-copy plasmids is entirely driven by a combination of random diffusion and nucleoid exclusion, clustering and dynamics of plasmid loss associated with recombinant gene expression point to the existence of a regulated process of segregation for hcn plasmids. While these plasmids do not bear canonical active partitioning systems, segregation could depend on chromosomally-encoded proteins. Unlike the type-I and type-II partitioning systems described above, segregation in hcn plasmids could be tied to some other process associated with cell division. Alternatively, hcn plasmid could have coopted chromosomal partition mechanisms to ensure proper spatial regulation and segregation, just as DNA-containing organelles have (51), although no recognizable centromeric motifs have been reported in hcn plasmid origins of replication .

The possibility that hcn plasmid segregation is a regulated process should therefore be investigated further. Specifically, looking for cellular factors that physically interact with hcn plasmids could offer clues to the mechanisms contributing to hcn plasmid stability. Also, the plasmid origin of replication should be revisited not only as the site orchestrating initiation of plasmid replication but also as a possible site for recruitment of factors involved in controlling plasmid distribution in the cell. However, the multifunctional nature of the plasmid origin of replication and the role of secondary structures (which include three stem loops mediating stable complex formation between antisense and primer RNA, three stems mediating action at a distance of the antisense RNA, and two hairpins toward the 3' end of *ori* sequence associated with R-loop formation and recruitment of RNaseH, (reviewed in (92)) would complicate these studies .

Understanding plasmid segregation in hcn plasmids as a regulated process could open the door to new approaches for enhancing the stability of vectors used for recombinant gene expression. Plasmid loss has been recognized as a limiting factor for large-scale recombinant gene expression and for bioremediation because the use of antibiotics to ensure plasmid retention is not practical in these settings (13, 14). Available approaches for increasing plasmid stability depend on a particular strain or culture condition (plasmid addition

systems) (93, 94), or on inducing growth arrest through massive metabolic burden (95, 96), or through induction of a quiescent state (97). Approaches aimed at increasing plasmid stability based on modifying the plasmid origin of replication are particularly appealing because they would be largely autonomous, *i.e.* would be largely independent on a particular strains or culture conditions.

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Fig. 1. *Par*-mediated plasmid segregation

Type-I (a) or type-II (b) *par* mechanisms of segregation. *Par* systems have three functional elements: (1) a *cis*-acting centromeric sequence element, (2) an ATPase motor protein, and (3) a DNA-binding protein. The DNA-binding protein serves as an adaptor between the motor and the centromere; this protein nucleates polymerization of the motor.

Polymerization is energetically favorable when monomers of the motor protein are in the ATP-bound state. Filaments rapidly disassemble upon ATP hydrolysis. (a) **Type I *par* systems.** Centromere: *sopC* (gray rectangle); adaptor: SopB (black circle); motor protein:

SopA (ATP-bound, rectangle; ADP-bound, pentagon). (i) Single F plasmids initially diffuse freely within accessible areas of the cytoplasm. (ii) SopB binds *sopC* from two F plasmids, bringing them together. (iii) SopB extends outward onto naked plasmid DNA. (iv) SopA nucleates onto SopB in areas where SopB is not complexed with centromeric DNA. (v) SopA polymerizes and filaments extend outward in both directions until they encounter SopB monomers in complex with *sopC*, (56). (vi) SopB monomers bound to *sopC* stimulate the ATPase of SopA, leading to a wave of ATP hydrolysis that destabilizes the filaments (57). Disassembling SopA filaments exert tension on SopB, pulling the plasmids apart in opposite directions (53). (vii) Filament disassembly drives plasmids to opposite poles of the cell. (b) **Type II *par* systems.** Centromere: *parC* (gray rectangle); adapter: ParR (black circle); motor protein: ParM (ATP-bound, rectangle; ADP-bound, pentagon). (i) Single R1 plasmids initially diffuse freely within a confined area. (ii) When two plasmids encounter each other in the cell, they become tethered and move as a single unit, through binding of ParR to *parC*. (iii) This association between ParR and *parC* stabilizes the ends of ParM filaments. (iv) ParM monomers are added to the ends of ParR-bound filaments. (v) The bi-directional elongation of ParM filaments drives plasmids to opposite poles of the cell during segregation. (vi) ATP hydrolysis triggers ParM filament disassembly. (vii) Pairs of plasmids end up in opposite ends of the cell. Thus, whereas in type I systems, the plasmids are “pulled” by filament disassembly (process akin to kinetochore motion during eukaryotic mitosis), in type II *par* systems, plasmid pairs are “pushed” by filament polymerization in a process akin to actin filament dynamics.