Plasmid Segregation Mechanisms

Gitte Ebersbach and Kenn Gerdes

Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK-5230 Odense M, Denmark; email: kgerdes@bmb.sdu.dk

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Annu. Rev. Genet.
2005. 39:453–79

The *Annual Review of Genetics* is online at http://genet.annualreviews.org

doi: 10.1146/annurev.genet.38.072902.091252

Copyright © 2005 by Annual Reviews. All rights reserved

0066-4197/05/1215-0453$20.00

Key Words

actin, oscillation, ParM, ParA, ParB

Abstract

Bacterial plasmids encode partitioning (*par*) loci that ensure ordered plasmid segregation prior to cell division. *par* loci come in two types: those that encode actin-like ATPases and those that encode deviant Walker-type ATPases. ParM, the actin-like ATPase of plasmid R1, forms dynamic filaments that segregate plasmids paired at mid-cell to daughter cells. Like microtubules, ParM filaments exhibit dynamic instability (i.e., catastrophic decay) whose regulation is an important component of the DNA segregation process. The Walker box ParA ATPases are related to MinD and form highly dynamic, oscillating filaments that are required for the subcellular movement and positioning of plasmids. The role of the observed ATPase oscillation is not yet understood. However, we propose a simple model that couples plasmid segregation to ParA oscillation. The model is consistent with the observed movement and localization patterns of plasmid foci and does not require the involvement of plasmid-specific host-encoded factors.

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Contents

INTRODUCTION 454
FACTORS AND GENES INFLUENCING PLASMID MAINTENANCE 455
PLASMID PARTITIONING LOCI . 455
TYPE II PARTITIONING LOCI ... 456
ParR-MEDIATED PLASMID PAIRING 456
THE ATPase ACTIVITY OF ParM . 456
ParM FORMS DYNAMIC FILAMENTS 456
ParM SHARES THE ACTIN FOLD 458
ParM EXHIBITS DYNAMIC INSTABILITY 458
MODEL FOR *parMRC*-MEDIATED PLASMID SEGREGATION 458
MreB, ANOTHER BACTERIAL ACTIN HOMOLOG, IS REQUIRED FOR CHROMOSOME SEGREGATION 459
TYPE I PARTITIONING LOCI 459
THE PARTITIONING COMPLEX 459
LONG-DISTANCE GENE SILENCING BY ParB/*parS* OF P1 461
PLASMID POSITIONING BY TYPE I *par* LOCI 462
ROLES OF DEVIANT WALKER-TYPE ATPases IN PLASMID PARTITIONING 462
Promoter Regulation 462
Separation of Plasmids Paired Via Their Partitioning Complexes . 463
Segregation of Plasmids into Daughter Cells 464
HOST FACTORS AND PLASMID PARTITIONING 464
A SIMPLE MODEL THAT COUPLES PLASMID SEGREGATION TO ParA OSCILLATION 465
A NEW EXPLANATION FOR PARTITION-ASSOCIATED PLASMID INCOMPATIBILITY 466
PLASMID SEGREGATION AND THE PARADOX OF PLASMID CLUSTERING 467
CHROMOSOME-ENCODED TYPE I *par* LOCI 467
COMPARISON OF THE OSCILLATING PROTEINS MinD, ParA, AND Soj 469
CONCLUDING REMARKS 470

INTRODUCTION

In prokaryotes, active segregation of low-copy-number plasmids into daughter cells relies on the function of partitioning (*par*) loci encoded by the plasmids themselves. The early discovery that *par* loci encode *trans*-acting proteins that act on *cis*-acting regions on the plasmids prompted investigators to designate these regions as centromere-like sites, by analogy to the centromeres of eukaryotic chromosomes. Recent discoveries that *par* loci encode cytoskeletal spindle-like structures that separate and distribute plasmids to daughter cells have extended the parallels between eukaryotic chromosome segregation and prokaryotic plasmid partitioning, and the proposed functional analogy between these processes has turned out to be even more justified than initially anticipated. In particular, the discovery that the Type II partitioning ATPase, ParM, forms actin-like filaments that segregate plasmids has greatly changed the view of the plasmid partitioning process. The unrelated and more common Type I *par* loci also encode filament-forming ATPases that play a central role in plasmid

segregation. These filaments oscillate over the nucleoid. Here we review recent advances in the mechanistic understanding of how plasmid-encoded partitioning loci secure ordered segregation of their replicons and compare them to chromosome-encoded partitioning loci. We also present a working model that explains how oscillating, filament-forming proteins might mediate plasmid segregation.

FACTORS AND GENES INFLUENCING PLASMID MAINTENANCE

Several factors influence the genetic stability of prokaryotic plasmids. Obviously, one important factor is copy-number control. The copy-number-control systems in natural plasmids ensure that each plasmid, on average, replicates once per cell cycle. In general, high-copy-number plasmids are relatively small and their genetic stability is thought to rely on random (i.e., binomial) segregation of the individual plasmid molecules at cell division. However, not all high-copy-number plasmids are as stable as would be expected from random assortment. This is usually attributed to factors that reduce the number of segregating units, such as fluctuations in plasmid copy-number in individual cells, plasmid clustering (discussed below), or plasmid multimerization arising from homologous recombination (101a, 119, 135). Accordingly, most natural plasmids encode site-specific resolution systems that resolve plasmid multimers into monomers (5, 19, 87, 136).

In general, low-copy-number plasmids are larger than high-copy-number plasmids, a relationship that may help to minimize the metabolic burden imposed by the plasmids on their host cells. Obviously, a low-copy-number raises another problem: low-copy-number plasmids cannot rely on random segregation to ensure stable inheritance. Accordingly, plasmids have evolved true partitioning (*par*) loci that actively segregate plasmid copies to daughter cells before cell division. In addition, many low-copy-number plasmids encode so-called post-segregational killing (PSK) systems that increase plasmid maintenance by killing plasmid-free cells. These systems may be viewed as providing a backup stabilization mechanism of last resort that is executed when a true *par* locus fails to function properly (44, 71)].

PSK: post-segregational killing

PLASMID PARTITIONING LOCI

All known plasmid-encoded partitioning loci encode two *trans*-acting proteins expressed from an operon and one or more *cis*-acting centromere-like sites, at which the proteins act (36, 45, 58, 116). All three components of a *par* locus, i.e., two proteins and a centromere-like *cis*-acting site, are essential (1, 20, 36, 57, 116). Furthermore, the amounts of the partitioning proteins need to be carefully regulated as excess, or shortage, of either partitioning protein is detrimental to *par* function (20, 36, 38, 85, 91). Accordingly, transcription of *par* operons is tightly autoregulated by the *par* proteins themselves (36, 58, 60, 70). Generally, *par* loci function as cassettes independently of the replicon on which they reside (2, 28, 45, 116).

The first gene of a *par* operon encodes an ATPase that belongs to one of two different superfamilies of proteins. This property was used to divide *par* loci into two types (46). Thus, Type I loci encode Walker box ATPases, and Type II loci encode actin-like ATPases (14, 81). Members of each of the two ATPase families form filamentous structures central to plasmid partitioning (8, 27, 108).

The second gene of a *par* operon encodes a DNA-binding protein that recognizes varying numbers of direct or inverted repeats within a cognate centromere-like site (23, 74, 107, 110). Binding of the protein to these sites results in the formation of a nucleoprotein complex, also known as the partitioning complex. The partitioning complex is the substrate for plasmid segregation, in which replicated plasmid molecules, often located at the mid-cell position, are moved in

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

opposite directions. There is evidence that the partitioning complex pairs plasmid molecules in a process analogous to pairing of eukaryotic chromosomes before segregation occurs (29, 74).

Generally, plasmids harbor only one *par* locus, and Type I loci are by far the most common. However, plasmids carrying two *par* loci are known: *Escherichia coli* plasmid pB171 and *Salmonella enterica* plasmid R27 both carry two functional *par* loci, one of each type (26, 88).

TYPE II PARTITIONING LOCI

The best investigated Type II *par* locus is encoded by plasmid R1 (see **Figure 1**). Here, the ATPase was designated ParM (motor), whereas the DNA-binding protein was called ParR (repressor) (20). The *cis*-acting site, *parC* (centromere), consists of ten 11-bp direct repeats located in two clusters of five repeats on either side of the −10 and −35 sequences of the *par* promoter (20). All ten direct repeats were required to obtain maximal *parC*-mediated plasmid stabilization (18). Gel-shift and footprinting analyses showed that ParR binds cooperatively to the ten direct repeats within *parC*, forming a large nucleoprotein-complex, which also includes the promoter sequences located between the two sets of repeats (107). Consistent with these findings, ParR is the main regulator of *par* promoter activity (70). ParM is not involved in regulating *par* operon transcription (70).

ParR-MEDIATED PLASMID PAIRING

ParR binds to *parC* in vivo and in vitro (20, 70, 107). The specific interaction of ParR with *parC* was investigated by electron microscopy (74). When supercoiled *parC*-containing DNA was mixed with purified ParR, binding of ParR resulted in a shortening of the DNA fragment, indicating that the *parC* DNA was wrapped around a core of ParR. Most importantly, ParR mediated *parC*-dependent pairing of plasmid molecules in vitro. The frequency of pairing was highest with supercoiled plasmid DNA, and the presence of ParM and ATP increased the pairing frequency. A ligation kinetics assay showed that ParR-dependent pairing of DNA molecules at *parC* was nonrandom with respect to orientation. Hence, the DNA fragments appeared to be ligated in a head-to-head fashion, suggesting that oriented pairing might yield the directionality required for ordered plasmid segregation (74).

THE ATPase ACTIVITY OF ParM

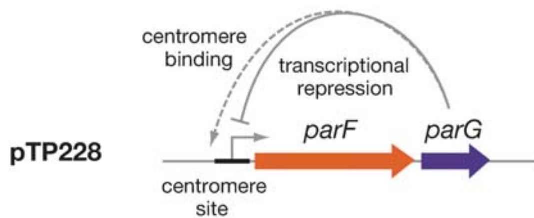
ParM has moderate, cooperative ATPase activity (that is, its specific activity increases with increasing concentrations of ParM). In addition, the ATPase activity is stimulated by ParR bound to *parC* DNA (72, 108). Mutations that changed the conserved Asp170 in the ATPase site of ParM (14) simultaneously reduced the ParM ATPase activity and the ability of ParM to support plasmid partitioning (72).

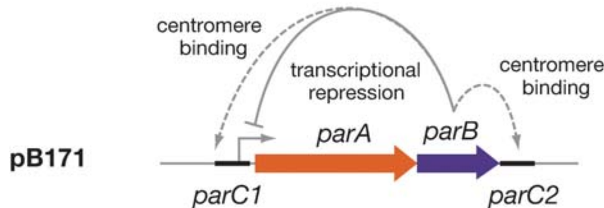
ParM FORMS DYNAMIC FILAMENTS

Cytological studies employing immunofluorescence microscopy (IFM) of ParM revealed a striking pattern: In some cells, ParM formed long, curved filaments extending from one pole to the other, or two filaments extending from the middle of the cell to opposite poles. In other cells, ParM appeared as foci, and in yet another subpopulation of cells, the ParM signal was diffusively located throughout the cell. These results show that ParM forms dynamic polymers that alternate between phases of polymerization and depolymerization. Estimation of the number of ParM molecules per cell (15,000–18,000) furthermore suggested that each filament observed by IFM most likely consisted of several parallel protofilaments (108). The formation of dynamic ParM filaments depended on the presence of both ParR and *parC*. Thus, even massive overproduction of

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Type I *par* loci





Type II *par* loci

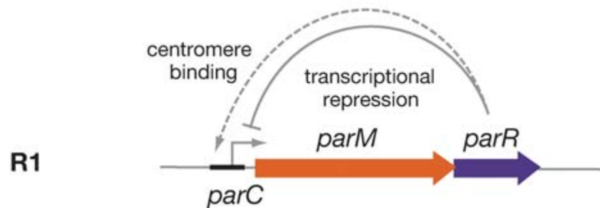


Figure 1

Genetic organization of representative Type I (plasmids P1, F, pTP228 and pB171) and Type II (plasmid R1) *par* loci, respectively. Solid arrows represent genes that encode ATPases (*red*) and centromere-binding proteins (*blue*), respectively. Centromere-like sites are shown as black bars. Arcs indicate DNA-binding properties of *par* gene products: solid arcs, regulation of promoter activity; dashed arcs, formation of partitioning complex.

ParM alone did not yield detectable formation of filaments (108). In contrast, the ATPase and partition-deficient ParM mutant proteins described above formed straight, rod-like filaments along the long axis of almost all cells. This "hyper-filamentation" phenotype exhibited by the ParM mutant proteins did not depend on ParR and *parC* and showed that the ATPase activity of ParM is not required for filament formation

per se; however, it is important for ParM dynamics. The detrimental ParM ATPase mutants were *trans*-dominant, indicating that the ParM mutant proteins interfered with proper function (i.e., filamentation) of wild-type ParM, most likely by protein-protein contact.

ParM SHARES THE ACTIN FOLD

ParM belongs to a superfamily of ATPases that, besides eukaryotic actins, comprises a number of diverse proteins such as heat shock proteins (Hsc70), sugar kinases, and prokaryotic cell cycle proteins (FtsA and MreB). Despite the relatively low overall sequence identity among these proteins, they all exhibit similarity in five separate, colinear sequence motifs. In the folded proteins, these motifs together form the characteristic three-dimensional structure of actin that binds and hydrolyzes ATP (14). The crystal structure of ParM revealed a three-dimensional architecture very similar to that of actin (140). Moreover, like actin, ParM forms double helical protofilaments that twist gently around each other (140).

ParM EXHIBITS DYNAMIC INSTABILITY

When eukaryotic cells enter mitosis, microtubules are reorganized into the mitotic spindle and attach to the kinetochores of paired sister chromatids, thereby promoting their alignment and subsequent partition into daughter cells. Before the spindle apparatus is assembled, tubulin polymers switch between phases of elongation and rapid shortening, a property known as dynamic instability (104). Dynamic instability plays an important role in the search and capture of chromosomes for correct bipolar alignment (80, 105). Recently, in vitro studies of ParM polymerization showed that ParM exhibits a very similar dynamic instability (43). However, unlike tubulin and actin filaments, ParM filaments appeared to grow symmetrically with equal rates of assembly at the two filament ends. After some time, filament growth switched to rapid unidirectional decay. The switch from growing to shortening appeared to be stochastic, and once disassembly of the filaments had begun, the disintegration process proceeded to completion. The dynamic instability of ParM filaments was linked to the ability of ParM to hydrolyze ATP as ParM proteins carrying mutations in their ATP-binding site formed stable filaments. Moreover, ADP-ParM filaments were extremely unstable, whereas ATP-γ-S-ParM filaments were highly stable (43). These findings are consistent with the previous immunolocalization experiments in which ParM ATPase mutants exhibited a hyperfilamentation phenotype (108). In mixing experiments, mutant ATP-bound ParM proteins stabilized wild-type ParM filaments, indicating that the filaments are stable as long as an ATP-bound cap of ParM is present at both filament ends (43).

MODEL FOR *parMRC*-MEDIATED PLASMID SEGREGATION

In actin filaments, nucleation as well as depolymerization and nucleotide exchange is regulated by a number of exogenous factors (131). For ParM, very fast rates of nucleation, filament disintegration, and dissociation of ADP from ParM monomers suggest that such regulatory factors might not be needed (43). Furthermore, the fact that the steady-state critical concentration (2.3 μM) needed for ATP-ParM polymerization appears to be far below the intracellular concentration (12 to 14 μM) of ParM suggests that polymerization does not require a nucleation factor (43). Based on these and previous findings, Dyche Mullins and co-workers proposed a model for R1 partitioning, according to which ParM filaments form spontaneously within the cell. ATP hydrolysis inside the ParM polymers leads to destabilization of the filaments that eventually disintegrate.

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

However, when a growing filament encounters a set of paired plasmids, the ParR-*parC* complex, present on each plasmid molecule, acts to stabilize both ends of the ParM filament. In this way, the dynamic instability enables ParM to find and capture plasmids paired by ParR, analogous to the way in which eukaryotic tubulin probes through the cell to locate and bind kinetochores of unattached chromosomes. Once plasmids have been captured, insertional polymerization of ATP-bound ParM monomers at the ParM-ParR interface causes the ParM filaments to grow bidirectionally, thereby pushing the two plasmids apart toward opposite ends of the cell. This model is consistent with cytological observations showing that ParM filaments always have ParB/*parC* carrying plasmids at their ends (107).

MreB, ANOTHER BACTERIAL ACTIN HOMOLOG, IS REQUIRED FOR CHROMOSOME SEGREGATION

Almost all rod-shaped bacteria encode one or more actin-like homologs that are required to maintain cell shape (76). Thus, in the three model organisms *E. coli*, *Bacillus subtilis* and *Caulobacter crescentus*, MreB is essential in that its depletion leads to the formation of spherical cells and eventually cell lysis (33, 76, 83). MreB assembles into filaments that are located beneath the inner cell membrane (76, 84). The first indication that MreB might be involved in chromosome segregation came from the observation that in *E. coli*, overproduction of ATPase-defective MreB mutant proteins prevented nucleoid separation and severely perturbed localization of the origin and terminus regions of the chromosome (84). More recently, MreB of *C. crescentus* was found to interact, directly or indirectly, with a region close to the origin of replication (47). Inactivation of MreB prevented normal segregation of this region of the chromosome. Whether origin movement is driven by dynamics within the polymerized MreB spirals, reminiscent of the proposed mechanism of ParM-mediated plasmid segregation, or whether motor proteins use MreB cables as a track for pulling the newly replicated origin regions still needs to be elucidated.

IHF: integration host factor

TYPE I PARTITIONING LOCI

Type I partitioning ATPases belong to a family of ATPases that have a so-called deviant Walker A box (also called P-loop) motif (81). This family of Walker box proteins also includes the MinD cell division proteins. Phylogenetic investigations of the deviant Walker-type ATPases involved in plasmid partitioning resulted in a sub-division of the proteins into Type Ia and Ib (46). The Type Ia subgroup is represented by two of the best-studied partitioning ATPases, namely ParA from prophage P1 and SopA from plasmid F. The Type Ib subgroup includes ParF from *Salmonella newport* plasmid pTP228, ParA from *Agrobacterium tumefaciens* plasmid pTAR, and ParA from *E. coli* plasmid pB171 (46, 57). Comparison of protein sequences and genetic organization of *par* loci encoding Type Ia and Type Ib ATPases, respectively, revealed several differences, which are summarized in **Table 1**. The organization of Type Ib loci appears to be more reminiscent of Type II loci than of Type Ia loci, an observation that may reflect convergent evolution at the level of gene organization (see **Figure 1**) (46).

THE PARTITIONING COMPLEX

The *par* locus of P1 encodes ParA and ParB and the *cis*-acting site *parS* (see **Figure 1**) (3). *parS* contains four heptameric (box A) and two hexameric (box B) sequences to which ParB binds (41). The ParB-binding sites are arranged asymmetrically on either side of a 29-bp DNA sequence recognized by the small heat-stable protein integration host factor (IHF) (40, 41). A minimal *parS* site capable of stabilizing a P1 plasmid in the presence of ParA and ParB consists of no more

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Table 1 Comparison between Type Ia and Type Ib *par* loci

	Type Ia	Type Ib
A proteins	Size: 321–420 aa Contain DNA-binding HTH-motif in the N terminus Main transcriptional regulator of the *par* operon	Size: 192–308 aa Do not contain HTH-motif
B proteins	Size: 312–342 aa Show mutual homology	Size: 46–131 aa Diverse group of proteins Few homologues Main transcriptional regulator of the *par* operon
Cis-acting[a] site	Located downstream of the *par* operon	Located upstream of the *par* operon
Model systems	*parABS* of P1 *sopABC* of F	*par2* of pB171 *parFG* of pTP228 *parAB* of pTAR

[a]Some *par* loci contain more than one *cis*-acting region, e.g., *par2* of pB171 has two *cis*-acting sites (see **Figure 1**) (26, 46). Furthermore, the *E. coli* linear plasmid N15 encodes a Type Ia *par* locus very similar to the *sop* locus of F but lacks the *cis*-acting site located downstream of the *sopAB* operon in F. Instead, N15 contains 4 *sopC*-like inverted repeats, scattered on 13 kb of the N15 sequence (54, 127). Plasmid RK2 is another example of a plasmid that might encode a Type Ia *par* system with multiple *cis*-acting sites as KorB (the ParB homologue) recognizes a sequence repeated 12 times in the RK2 genome. However, most of the 12 sites might not function primarily as partitioning sites in that KorB also acts as a global transcriptional repressor (145).

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

HTH: helix-turn-helix

than 34 bp, containing only the ParB-binding sites to the right of the IHF site (103). Thus, IHF is not essential for *par* function, but binding of IHF to *parS* greatly increases the affinity of ParB for *parS* (39, 40). ParB interacts with *parS* in the form of a dimer. Dimerization is mediated by a domain located in the C terminus of the protein (99, 137). Binding to DNA is most likely mediated by a helix-turn-helix (HTH) motif situated in the central part of the linear sequence of ParB. The ParB HTH-motif is believed to bind to the box A sequences of *parS* (99, 137). ParB has a second DNA-binding domain that overlaps with the dimerization domain located in the C terminus. The ParB-ParB dimerization interface recognizes the two box B sequences in *parS* located on both sites of the IHF-binding site (99, 123, 138). Thus, formation of the ParB-*parS* partitioning complex probably initiates with one dimer of ParB interacting simultaneously with its recognition sequences on both sites of an IHF-induced bend in the DNA (17, 138). Subsequently, more ParB dimers are loaded onto the complex. These additional dimers are thought to bind primarily by protein-protein interactions, probably via an additional self-interaction domain located in the N-terminal part of ParB, and by nonspecific DNA-protein interactions. The end result is a higher-order structure in which *parS* is wrapped around a core of IHF and ParB (17, 137, 138).

The *sop* (stability of plasmid) locus of F encodes SopA and SopB and the *cis*-acting site, *sopC* (see **Figure 1**) (116). *sopC* consists of twelve 43-bp direct repeats, each containing a pair of 7-bp inverted repeats that are recognized by SopB (109, 110). A single 43-bp repeat is sufficient to allow stable maintenance of a mini-F plasmid (12). Like ParB, SopB binds to DNA via its HTH-motif (55, 110, 128). Alignment of the amino acid sequences of ParB/SopB homologous proteins from a number of Type Ia *par* loci including ParB of prophage P7, KorB of

the broad host range plasmid RK2, and ParB of *Salmonella typhimurium* plasmid pSLT has shown that despite an overall low sequence similarity, these proteins all contain a putative HTH-domain, indicating that these proteins might bind their *cis*-acting sites in a way analogous to ParB and SopB (55, 99).

In F, plasmid supercoiling is affected by the partitioning complex (12, 91). Thus, a single *sopC* repeat significantly increases the linking number of a mini-F plasmid in the presence of high concentrations of SopB in vivo (100). As the change in linking number is too large to originate solely from structural changes within the *sopC* repeat, it was suggested that binding of SopB to *sopC* nucleates the formation of a higher-order wrapped nucleoprotein complex that involves the DNA adjacent to *sopC* and protein-protein interactions between SopB proteins (12, 100). Using fluorescence microscopy, the subcellular localization of SopB was examined. One study used antibodies against SopB (60), while another used a SopB-GFP fusion protein (78). Both studies reported the presence of SopB foci; however, in the former study, formation and localization of SopB foci required SopA and *sopC*, whereas in the latter study, SopB localization seemed to be independent of the presence of these components.

In the case of the Type Ib *par* loci of pB171, pTP228, and pTAR, the B proteins bind to *cis*-acting regions located upstream of their cognate *par* operons. Like ParB and SopB, the B proteins of the Type Ib loci form dimers in solution. However, they do not contain HTH-motifs (7, 35). The structure of the ParG dimer from plasmid pTP228 was solved (50). The ParG dimer consists of a folded domain made up of the intertwined C-terminal regions of each ParG subunit and a flexible domain consisting of the unstructured N-terminal regions. The folded C-terminal part of the dimer, which forms the putative DNA-binding domain of ParG, has a ribbon-helix-helix (RHH) architecture similar to the well-described transcriptional repressors of the Arc/MetJ/CopG superfamily (51, 126).

GFP: green fluorescent protein

RHH: ribbon-helix-helix

LONG-DISTANCE GENE SILENCING BY ParB/*parS* OF P1

Specific binding of P1 ParB to *parS* can function as a nucleation site for unspecific binding of ParB to several kilobases of DNA on both sides of *parS*, a phenomenon called spreading (129). A similar behavior has been reported for the chromosome-encoded ParB homologue of *Streptomyces coelicolor*, suggesting that spreading might be a characteristic of many of these DNA-binding proteins (69). The role of ParB spreading in partitioning is not clear. Initially, analysis of selected spreading-defective P1 ParB mutants revealed that these mutants were also partition defective, and thus led to the suggestion that spreading might play a role in plasmid segregation (129). Nevertheless, a more recent study, in which plasmid stability was assayed after ParB spreading had been restricted by the introduction of roadblocks on both sites of *parS* (RepA or GAL4 bound to their cognate recognition sites), indicated that even though limited spreading might assist the partition process, perhaps during plasmid pairing, extensive ParB spreading is probably not essential (130).

Spreading of ParB results in silencing of genes several or even many kilobases away from *parS*, presumably because the formation of a ParB-DNA nucleoprotein filament impedes binding of RNA polymerase to the region covered by the nucleofilaments (129). Similarly, SopB of F can silence genes located far away from *sopC*. Simultaneously, the DNA becomes inaccessible to cellular proteins such as DNA gyrase and DNA adenosine methylase (101). However, in the case of F, it is not known if SopB forms a nucleoprotein filament and SopB does not polymerize on DNA outside *sopC* in vitro (55). In the F case, gene silencing was explained by SopB-mediated sequestration of the *sopC* region to specific

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

subcellular positions near the cell poles (79, 101). This proposal is consistent with the finding that the N-terminal region of SopB, which apparently is involved in specific subcellular localization of the protein, is essential for SopB-mediated gene silencing (55, 78, 79).

aa: amino acids

PLASMID POSITIONING BY TYPE I *par* LOCI

Plasmids without a *par* locus are more or less randomly localized within the bacterial cell, preferably at positions not occupied by the nucleoid (27, 96, 111). In contrast, the presence of a Type I *par* locus leads to specific subcellular localization of plasmids to mid-cell and quarter-cell positions (13, 27, 52, 59, 61, 88, 95, 96, 111). The plasmid localization pattern is consistent with a model in which plasmids are replicated at mid-cell, separated and actively moved to quarter-cell positions. However, there is some controversy regarding the timing of the plasmid separation process. In one study, synchronized replication of a mini-F plasmid showed that sister plasmid copies could be separated and moved to quarter-cell positions within 5 min after replication had completed, independently of cell division (117). In two other studies in which time-lapse microscopy was used to show the dynamic movements of P1 plasmids and plasmids carrying *par2* of pB171, respectively, plasmid foci positioned at mid-cell usually did not segregate until very late in the cell cycle (27, 95). The apparent temporal coupling of plasmid segregation and cell division observed in the latter studies raises the possibility that plasmid partitioning and cell division might be co-ordinated. In the P1 study, it was suggested that plasmids might be capable of delaying cell division until segregation is complete (95). However, several studies have shown that plasmids carrying Type I *par* loci are regularly distributed along the long axis of filamentous cells in which cell division has been inhibited by treatment with cephalexin (27, 30, 52). The latter observations indicate that plasmid segregation does not depend on completion of cell division.

ROLES OF DEVIANT WALKER-TYPE ATPases IN PLASMID PARTITIONING

Promoter Regulation

Type Ia ATPases function both in plasmid partitioning and in repression of *par* operon transcription (**Figure 1**). ParA of P1 and SopA of F bind to operator sites in their cognate promoter regions via HTH-motifs in their N termini (24, 58, 110, 128). In the case of P1 ParA, ATP and ADP both promote ParA dimerization and it is the dimer that binds operator DNA (21). However, ADP and nonhydrolyzable ATP analogs stimulated operator binding much more than ATP (15). ParA proteins with aa changes in the Walker box motifs that reduced ATPase activity behaved as superrepressors of transcription (37). These results support the notion that the catalytic event itself triggers a conformational change in ParA that is deleterious to DNA binding.

In vivo, ParB and SopB greatly enhance transcriptional repression by ParA and SopA (36, 60), respectively, in agreement with in vitro data showing that ParB/SopB stimulates ParA/SopA operator DNA binding (22, 110). In vivo, *parS* and *sopC* act as ParB/SopB dependent corepressors of their cognate *par* promoters, and deletion of *parS*/*sopC* in both cases leads to significant increases in *par* operon transcription. *parS*/*sopC* also act as co-repressors in *trans*, implying that transcriptional repression is not due to spreading of ParB/SopB from the centromere site (56, 148). In support of this notion, Hao & Yarmolinsky found that silencing of the *par* operon by spreading of ParB from *parS* in its natural position downstream of ParB probably does not play any major role in autoregulation of the *par* promoter (56). The fact that a single *parS* site in the chromosome was able to repress the *par* promoter carried by a high-copy-number reporter plasmid led to

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

the suggestion that *parS* might act catalytically by stimulating ATP hydrolysis and thereby induce a conformational change in free ParA that would make it a better repressor (56).

For F, it was suggested that the *sopC*-mediated enhancement of SopB co-repressor activity could be the result of a direct contact between the SopB-*sopC* partitioning complex and the SopA protein bound at the *par* promoter (148). Such an interaction might involve intramolecular looping of plasmid DNA or alternatively, the SopB-*sopC* complex on one plasmid might interact with ParA bound on another plasmid in an intermolecular looping reaction. This would agree with the proposal that pairing of plasmids is an important step in the partitioning process.

Separation of Plasmids Paired Via Their Partitioning Complexes

Austin & Abeles were the first to suggest a model for P1 partitioning involving pairing of plasmids at *parS* (3). Excess ParB protein destabilizes a P1 plasmid carrying either the entire *par* locus or the *parS* site (38). Plasmid destabilization was more severe than would be expected from random distribution of individual plasmids at cell division, consistent with the proposal that the plasmids segregated in pairs or even in clusters. Recently, the question of plasmid pairing was addressed by in vivo experiments in which the topoisomer distribution of *parS*-containing plasmids was analyzed in the presence and absence of ParB. The results were consistent with ParB-mediated plasmid pairing at *parS*; however, they did not prove that pairing actually occurs (29).

A series of experiments, performed with different *par* loci, raised the possibility that the Walker Box ATPases might play a role in the separation of plasmid molecules paired at their centromere sites. In vitro experiments performed with the components of the P1 *par* locus have shown that at high ParA/ParB ratios, ParA destabilizes the partitioning complex formed at *parS* (15). A similar observation was made in the case of the Type Ib *par* locus of pTP228 (7). Moreover, ectopic overexpression of ParA has been shown to destabilize a P1 plasmid (1). The same was true for plasmid F, in which case an excess of SopA interfered with plasmid partitioning. Investigation of this phenomenon revealed that excess SopA is capable of counteracting the increase in plasmid linking-number normally induced by binding of SopB to *sopC*, suggesting that SopA disrupts the partitioning complex (91). The SopA effect required ATP-binding and/or hydrolysis and is probably mediated by direct interaction of SopA with SopB. In another study, overproduction of a mutant SopA protein, carrying an aa substitution of a conserved lysine in the ATP-binding site, apparently had no effect on the linking number of a mini-F plasmid (97). Moreover, in mini-F plasmid stability tests, expression of the mutant SopA protein instead of wild-type SopA resulted in a hyper-instability phenotype. One possible interpretation of these data is that the mutant SopA protein fails to separate F plasmids paired via their SopB/*sopC* complex. Consequently, the plasmids cannot segregate as independent units and severe plasmid instability follows (97). Certain aa substitutions in the Walker A box of ParA of P1 also conferred a hyper-instability phenotype (37).

Another clue that the Type I ATPases might function in separation of plasmids during partitioning came from cytological studies. In the case of the P1 plasmid, absence of ParA resulted in a decreased number of cells with a plasmid focus at mid-cell, and foci localized to the cell center failed to divide before cell division (96). Further support for this contention came from a study of aa changes in the Walker A box of ParA of pB171 that rendered the protein defective in plasmid stabilization. The mutant protein still mediated mid-cell localization of plasmid foci in a significant number of cells but the foci often failed to divide, thus explaining why plasmids carrying this mutation were unstable (27). Finally, inactivation of the IncC ATPase of plasmid RK2 resulted in a decrease in the number of

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

plasmid foci that could be observed by IFM with KorB-specific antibodies, consistent with impaired plasmid separation (13).

Segregation of Plasmids into Daughter Cells

The partitioning ATPases studied so far all have a weak intrinsic Mg^{2+}-dependent ATPase activity that is stimulated by DNA and even more so by centromere DNA bound with the cognate B protein (8, 24, 141). Mutations in the ATPase domains of the ParAs of P1 and pB171 and ParF of pTP228 revealed a correlation between the ability of these proteins to hydrolyze ATP and to mediate plasmid stability, implying that the ATPase activity plays a role in the DNA segregation process (8, 26, 37).

Recent cytological observations showed that the Walker-type ATPases are required for specific positioning of plasmids at subcellular locations (27, 30, 96). Moreover, experiments with ParA of pB171 revealed that a ParA-GFP fusion protein oscillated in spiral-shaped structures over the nucleoid region (26, 27). Oscillation, but not spiral formation, required ParB and *parC*, showing that the partitioning complex regulates the dynamic properties of ParA. Change of a conserved amino acid residue in the Walker A box motif revealed a correlation between ParA oscillation, subcellular plasmid positioning, and plasmid stability, suggesting a role for ParA oscillation in plasmid segregation (26, 27). The dynamic movement of ParA ATPases in filamentous structures led to the proposal that the ATPases play a direct role in plasmid segregation, perhaps by providing the motive force required for active plasmid segregation.

In vitro, ParF of pTP228 forms extensive filaments very similar to those formed by MinD of *E. coli*. This is important because MinD of *E. coli* also oscillates in filamentous structures in vivo (132). Both ParF and MinD form bundles of parallel protofilaments in which one end is compact and the other end has a frayed appearance consistent with filament polarity (8, 134). ParF polymerization was stimulated by ATP but suppressed by ADP. Non-hydrolyzable ATP analogues had a stimulating effect similar to ATP, indicating that ATP hydrolysis is not required for polymerization. Moreover, ParG, which stimulates the ATPase activity of ParF, was shown to associate with ParF filaments and regulate polymerization: At low ParG/ParF ratios, ParG appeared to enhance polymerization and filament bundling, whereas high ParG concentrations resulted in decreased ParF polymerization. These observations suggest that ParG mediates its effect on ParF polymerization through stimulation of the ATPase activity of ParF. Analysis of partition-deficient ParF Walker A box mutants showed that these proteins had an altered filamentation pattern (8). Based on the observations described above, it is tempting to speculate that dynamic oscillation may be a general property of Walker box partitioning proteins.

HOST FACTORS AND PLASMID PARTITIONING

Several elegant genetic screens have been devised to search for host cell-encoded factors involved in plasmid partitioning (11, 66, 112, 133). So far, such screens have led to the identification of factors involved in recombination and plasmid replication, but not in plasmid segregation. IHF is the only host factor known to play a direct role in plasmid partitioning (39, 40). Initially, it was suggested that the process of plasmid segregation itself might be carried out by binding of plasmids to the growing plasma membrane or to the bacterial chromosome (34, 68). However, the rapid subcellular movement of plasmids (27, 95, 117) and the fact that both F and P1 plasmids segregate into anucleate cells of *mukB* mutant cells argue against these ideas (32, 42). The alternative suggestion that plasmids might segregate by direct attachment to a cytoskeletal machinery encoded by the host also seems unlikely, especially in light of more recent findings that partitioning ATPases of both types have the

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

ability to form dynamic, filamentous structures (27, 107, 140).

Host factors have also been proposed to participate in maintaining or tethering of plasmids at distinct cellular locations. The similar localization patterns of plasmids carrying Type I *par* loci seem to support this idea. However, in cytological studies in which F plasmids were detected simultaneously with P1 plasmids or RK2 plasmids (61) or with an R1 plasmid stabilized by *par2* (28), the different plasmid foci occupied similar but clearly distinct positions. For these observations to agree with the receptor hypothesis requires that each *par* locus should have its own receptor. Furthermore, *par2* distributes plasmid foci along the long axis of the cell irrespective of the focus number (28). Thus, as many as 9 plasmid foci were more or less regularly distributed along the long axis of the cell, an observation that is most readily reconciled with a receptor-independent plasmid localization mechanism.

A SIMPLE MODEL THAT COUPLES PLASMID SEGREGATION TO ParA OSCILLATION

It is not yet known how the oscillating, filament-forming Walker box ATPases mediate plasmid segregation. However, in analogy to the Type II partitioning loci, it is tempting to suggest that polymerization of ParA acts to physically push or pull plasmid molecules apart. In the "jumping ParM model" shown in **Figure 2*a***, we envisage that ParA polymerizes between two adjacent plasmid molecules (or clusters of molecules) and pushes them apart in a mechanism similar to that of ParM of plasmid R1. At a particular moment, ParA concentration will be highest between two foci, say F1 and F2. These two foci will be pushed apart due to polymerization of ParA that interacts with the ParB/*parC* complexes of F1 and F2. Due to oscillation of ParA, ParA concentration at a later moment is highest between F2 and F3 that now will be pushed apart. Thus, the oscillation of ParA functions to average, over the cell cycle, the force exerted by ParA filaments between any two given juxtaposed plasmid foci such that, over a time period of a full oscillation cycle, all plasmid foci will experience a force that distributes them along the length of the cell. The model assumes that ParA oscillates over the nucleoid and that the polar borders of the nucleoid function as toeholds for ParA. The model predicts that, in cells with one plasmid focus, the focus will end up at mid-cell and in cells with two foci, the foci will end up approximately at quarter-cell positions (**Figure 2*b***).

All cytological observations obtained with *par2* of pB171 are consistent with the model. ParA of pB171 oscillates over the nucleoid in filamentous structures (27). Oscillation depends on ParB bound to *parC*, meaning that ParA "senses" the presence of plasmid substrates for the segregation process (26). Most important, in cells with many plasmid foci, the foci are evenly distributed along the length of the cells (28), as predicted by the model. The model explains in a simple way how ParA oscillation might lead to plasmid positioning. Moreover, the model predicts that even though *par*-carrying plasmids are found at mid-cell or quarter-cell positions there is no requirement for host cell receptors to tether the plasmids at those positions: they simply end up there due to oscillation of ParA. The lack of specific host receptors at those positions is also consistent with the observation that two compatible plasmids carrying different Type I *par* loci localize at similar subcellular positions but do not exhibit colocalization (that is, the plasmids are detected as distinct nonoverlapping foci at those positions) (28, 120). Moreover, the jumping ParM model is consistent with the puzzling observation that chromosome-encoded Type I *par* loci from distantly related organisms (i.e., *B. subtilis*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*) can stabilize plasmids replicating in *E. coli* (9, 49, 147). The observation that the Type I *par* locus from *B. subtilis*, which encodes the

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

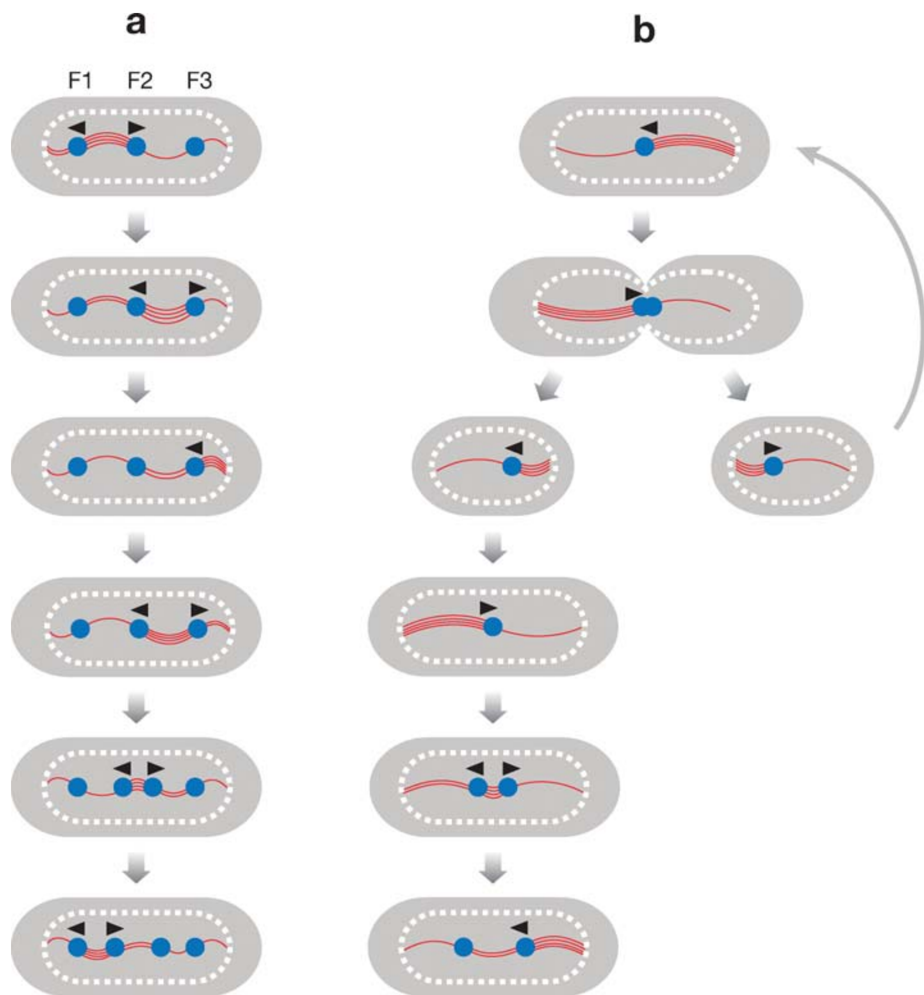


Figure 2
Model that explains the coupling of plasmid segregation to ParA oscillation. (*a*) Schematic showing the "jumping ParM model" that explains how oscillating ParA filaments might confer a regular plasmid focus pattern as observed (28). See the text for details. (*b*) Schematic showing how ParA oscillation might lead to focus positioning at mid-cell in cells with one focus and movement of newly divided foci to the approximate quarter-cell positions.

oscillating Soj protein, positioned an *E. coli* plasmid at mid-cell and quarter-cell positions is also consistent with the model (147).

A NEW EXPLANATION FOR PARTITION-ASSOCIATED PLASMID INCOMPATIBILITY

The centromere-like sites of Type I and II *par* loci exert partition-associated plasmid incompatibility (4, 16, 26, 116). Thus, when present on an otherwise compatible plasmid, *parC* of R1 destabilizes a mini-R1 stabilized by *parMRC*. Several models have been proposed to explain the phenomenon (4, 113). One of the most popular models states that *par*-specific plasmid pairing of heterologous replicons would readily explain partition-associated incompatibility (74, 113). As described above, plasmid pairing by the *parMRC*-encoded components occurs in vitro. However, in vivo, *parC*-mediated incompatibility was weak, even when *parC* was present on a high-copy-number plasmid (20). Furthermore, titration of ParR by *parC* was also modest (70). One explanation for these observations is that ParR is preferentially *cis*-acting and that plasmid pairing is coupled to replication such that sister plasmid molecules have a greater chance to pair than non-sisters,

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

that is, pairing occurs between two newly replicated plasmid molecules. If this is the case, then the plasmids that pair would not be randomly selected from a pool and the incompatibility phenotype therefore would be weaker than expected from random selection and pairing.

To investigate the plasmid incompatibility phenotype associated with Type I *par* loci in vivo, we used a GFP-based dual labeling technique to simultaneously visualize two different plasmids. As expected, the presence of an F plasmid carrying *parC1*, *parC2*, or *par2* of pB171 interfered with proper partition of an R1 plasmid carrying *par2*. Remarkably, however, fluorescence microscopy of the incompatible plasmids did not show any indication of heterologous plasmid pairing. Instead, pure clusters of R1 and F plasmids appeared to be distributed in a random order along the long axis of the cell (28). This striking observation raises the possibility that incompatibility in Type I partition loci can be explained by random assortment of pure plasmid clusters. This mechanism does not involve pairing of heterologous plasmids carrying the same *par* locus. On the other hand, cytological data show clearly that homologous plasmid molecules do pair or cluster (see below). These results are most readily explained by the assumption that plasmid pairing occurs preferentially between newly replicated plasmid molecules.

PLASMID SEGREGATION AND THE PARADOX OF PLASMID CLUSTERING

Accumulating evidence indicates that low-copy-number plasmids are non-covalently clustered within bacterial cells. The average copy number of plasmid R1 is 4-5 per cell (115) but the average number of plasmid foci detected by FISH or GFP-tagging was considerably lower (73, 144). Convincing evidence of the clustering phenomenon came from the observation that an R1 plasmid copy-number mutant (4 times the wild-type copy number) had an average focus number similar to the wild-type control plasmid (144). Plasmid P1 is also located in clusters (53) and even high-copy-number plasmids can be found in clusters (75, 119). Plasmid clustering, although not understood, is not a result of plasmid multimer formation and does not depend on the presence of a *par* locus (27, 144). Inhibition of DNA replication or DNA gyrase alters plasmid focus localization but does not prevent plasmid clustering, suggesting that normal superhelicity and DNA replication are not required for clustering (75). Double labeling experiments showed that clustering is replicon specific, that is, clusters normally consist of one type of replicon only (28, 61). This observation raises the possibility that plasmid clustering depends on sequence homology between the origins of replication, a hypothesis that should now be tested.

The clustering phenomenon raises a paradox: Normally, low-copy-number plasmids devoid of their *par* loci are characterized by a loss frequency compatible with each plasmid molecule behaving like one segregating unit. Thus, if the plasmid cluster behaves as the unit of segregation a much larger loss frequency would be expected (114). One possible solution to this paradox is that the replication machinery located at mid-cell (31, 82, 90) recruits plasmids one at a time from the plasmid clusters for replication. If, after replication, the two new plasmids distribute themselves randomly between the cell halves, then the clustering phenomenon would not be inconsistent with a binomial distribution of the plasmid molecules between daughter cells (114). However, the clustering phenomenon raises a number of questions; among others is how a partitioning machinery copes with assemblies of more than two plasmid copies.

CHROMOSOME-ENCODED TYPE I *par* LOCI

Most bacterial chromosomes encode Type I *par* loci. Type II loci have not yet been found on bacterial chromosomes (46, 57). Some of

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

the better studied examples of chromosomal Type I loci are from *Bacillus subtilis*, *C. crescentus*, *P. putida* and *P. aeruginosa* (9, 49, 98, 106). The large linear chromosome of *Streptomyces coelicolor* A3 also encodes a Type I *par* locus (77). However, *E. coli* and some of its close relatives such as *Haemophilus influenzae* do not contain Type I loci (46).

Like plasmid-encoded *par* loci, chromosomal *par* loci encode two *trans*-acting proteins that act on a number of *cis*-acting sites scattered on the chromosome. The number and distribution of the *cis*-acting sites vary among species, and they need not all be closely linked to the *par* operon. In general, the *cis*-acting sites are clustered around the origin-proximal region of the chromosome (9, 49, 69, 98). Consistent with the notion that the chromosomal *par* loci, like their plasmid-encoded counterparts, might be involved in DNA segregation, mutations in or deletion of chromosomal *par* loci result in chromosome segregation defects. However, these effects often are modest or even difficult to detect as they might appear only during specific growth conditions or during certain time points in the developmental life-cycle of a cell (49, 67, 77, 94). Moreover, a number of indirect observations indicate that Type I loci are not the main players in bacterial chromosome segregation. For example, movement of the origin region of *B. subtilis* does not depend on *spo0J* (142, 143). However, as described below, chromosome-encoded *par* loci may play central roles in chromosome segregation during specialized conditions.

The chromosomal *par* locus *soj-spo0J* of *B. subtilis* has been studied in detail. Like its plasmid-encoded counterparts, the *B. subtilis* ParB analogue, Spo0J, contains a HTH-motif (93) that mediates specific binding of Spo0J to 8 *cis*-acting *parS* sites in the origin-proximal 20% of the chromosome (98). Fluorescence microscopy experiments have shown that binding of Spo0J to *parS* creates small nucleoprotein complexes that, in the presence of Soj, condense into one discrete focus at each origin region (48, 102). During vegetative growth, Spo0J foci follow the replicated origin regions as they move apart and segregate to positions near the borders of the nucleoid located at the quarter-cell positions (48, 89, 102). This finding, combined with the observation that deletion of Spo0J results in elongated cells with abnormal nucleoid morphology in addition to an increased percentage of anucleate cells, led to the suggestion that Spo0J-Soj might be involved in origin organization and/or segregation (6, 67). Consistently, comparison of origin positions revealed that origin regions were located closer together in *spo0J* than in wild-type cells (89). This suggested that Spo0J plays a role in origin positioning. However, introduction of *parS* sites into different chromosomal locations did not allow Spo0J to bring these sites to the quarter-cell positions. Alternatively, it was proposed that Spo0J might play a role in separation of sister origin regions or in maintenance of these regions at specific cellular positions (89). Curiously, deletion of Soj does not seem to affect cell length or nucleoid appearance (67), indicating that unlike the plasmid-encoded ParA homologues, Soj does not appear to be essential for chromosome partitioning during vegetative growth. Nevertheless, as Spo0J and Soj are both required for the stabilization of a *parS*-carrying plasmid in *B. subtilis*, Soj may play an as yet unknown role in chromosome segregation (98).

Recent evidence points to a more direct role for Soj-Spo0J in chromosome segregation during sporulation. At the onset of sporulation, before formation of the asymmetric septum, the chromosomal DNA forms an elongated structure called the axial filament, in which the origin proximal region of the chromosome moves to the cell pole where it forms a condensed structure (120). Investigations of this process have shown that Spo0J and Soj might play a role in the correct positioning of the origin region at the cell pole (10, 146). However, the experiments also show that Spo0J-Soj probably do not act alone but are complemented by an independent origin segregation mechanism that involves the

RacA protein expressed early in sporulation (10, 146). Apparently, RacA binds to a region to the left of the origin of the chromosome and recruits it to the pole (10). Both Soj-Spo0J and RacA require the polarly sequestered DivIVA protein as an anchor for the origin DNA. Consistent with the proposal that RacA and Soj-Spo0J play partially redundant roles in origin segregation during sporulation, deletion of both systems at the same time results in strains in which most cells fail to trap the origin region in the prespore (10, 146).

Deletions in the *parAB* locus of *S. coelicolor* also result in chromosome segregation defects during formation of spore chains. In agreement with a role of ParAB of *S. coelicolor* in chromosome segregation during sporulation, transcription of *parAB* from one of two operon promoters is greatly stimulated at the time of sporulation (77). The free-living rod-shaped bacterium *P. putida* does not pass through developmental phases. However, in this organism the effect of ParAB on chromosome segregation depends on the culture medium and growth phase, as mutations in the *parAB* locus result in chromosome loss only during the transition from exponential growth to stationary phase of cells grown in minimal medium (49, 94).

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

COMPARISON OF THE OSCILLATING PROTEINS MinD, ParA, AND Soj

Like ParA of pB171, the deviant Walker-type ATPases Soj of *B. subtilis* and MinD of *E. coli* exhibit dynamic movements. Apparently, Soj forms polarly located patches that oscillate or jump from nucleoid to nucleoid within living cells of *B. subtilis* (102, 122). Soj jumping requires the presence of Spo0J and probably *parS*. Hence, when Spo0J is absent, Soj relocates to the chromosome, where it binds and represses sporulation-specific promoters (102, 121, 122), which explains the sporulation-defective phenotype of *spo0J* cells. Examination of the ability of Soj ATPase mutants to localize to the pole and bind to the nucleoid led to the proposal that the ATP-bound form of Soj interacts with Spo0J and the ADP-bound form binds DNA and regulates promoter activity (122). More recently, in vitro studies of Soj from *Thermus thermophilus* have shown that Soj forms ATP-dependent dimers in solution. Dimerization of Soj facilitates cooperative and non-specific binding of the protein to DNA, thereby mediating the formation of a nucleoprotein filament (92). Spo0J activates the ATPase activity of Soj, which is thought to result in dissociation of Soj dimers followed by release of Soj from the DNA. The hypothesis that Spo0J regulates Soj oscillation is consistent with the observation that a mutation in Spo0J resulted in an increased Soj oscillation frequency (6).

Whereas ParA and Soj oscillate over the nucleoid, *E. coli* MinD is located at the inner surface of the cell membrane where it undergoes cooperative polymerization (86, 139). Like Soj, MinD undergoes ATP-dependent dimerization. The MinD dimer forms a complex with MinC and thereby recruits it to the membrane where MinC acts to prevent Z-ring formation (62, 64, 65, 118). MinCD-mediated inhibition of cell division is controlled by MinE, a topological specificity factor that constrains the MinCD inhibitory action to the cell poles (25, 149). Hence, in the presence of MinE, the MinCD complex oscillates in a timescale of seconds from cell pole to cell pole within extended coiled structures that wind around the cell cylinder (64, 124, 125, 132). Analogous to Spo0J-mediated regulation of Soj jumping, MinE is thought to regulate MinD oscillation by stimulating the ATPase activity of membrane-bound MinD, thereby releasing MinD into the cytoplasm (62, 63, 86, 134).

The properties of MinD, Soj, ParF, and pB171 ParA are in all cases modulated by ATP and ADP. In the case of MinD and Soj, nucleotide-dependent dimerization appears to be required for DNA/membrane-binding (65, 92), whereas Spo0J/MinE-mediated stimulation of the ATPase activity regulates dynamic movement of the proteins

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

(86, 92). As oscillation of ParA of pB171 apparently requires ATP-binding or hydrolysis in addition to ParB and *parC*, a similar regulation might control the oscillation of this protein (26, 27). Also, assembly and disassembly of the ParF filaments is modulated by the nucleotide state of the protein, which again seems to be regulated by ParG (8). Together, these observations are consistent with the proposal that nucleotide binding and hydrolysis act as a molecular switch that controls the properties of all these proteins in vitro as well as their function in vivo. In the case of the P1 *par* system, ADP stimulates the promoter regulatory activity of ParA, as mentioned above. Recognition of the partitioning complex by ParA, on the other hand, requires ATP but is not dependent on hydrolysis (15). Thus, also in this case, ADP and ATP might function as a molecular switch that controls the dual functions of ParA in autoregulation and partition.

CONCLUDING REMARKS

The DNA segregation mechanisms specified by plasmid partitioning loci have been difficult to uncover. However, recent cytological observations have opened the door to the problem and coherent models are emerging. Especially in the case of the R1 Type II *par* locus, an understanding of the molecular mechanisms underlying plasmid segregation appears within reach. Until recently, the phenomenon of dynamic instability was associated solely with eukaryotic microtubules, where the repeated cycles of growth and shrinkage of the tubulin polymers are thought to play an essential role, particularly in DNA segregation. However, the finding that the actin-like partitioning ATPase, ParM, exhibits a similar behavior indicates that eukaryotic and prokaryotic cells have evolved functionally very similar ways of dealing with an essential process in the cellular life cycle, namely DNA segregation. For Type I *par* loci, thorough examinations of the P1 and F loci in particular have contributed extensive information on transcriptional regulation of the *par* operons, formation of partitioning complexes, in addition to biochemical properties of the individual *par* proteins. Of the questions still remaining the most important seems to be the role of the ATPases in plasmid distribution. A clue to an understanding of this central question came from the discovery that two Type Ib partitioning ATPases form filamentous structures that play a vital role in plasmid segregation. It is now within experimental reach to elucidate this type of DNA segregation mechanism as well.

SUMMARY POINTS

1. The *parMRC* locus segregates plasmids by a mitotic-like mechanism.
2. ParM forms actin-like filaments reminiscent of the eukaryotic spindle.
3. ParM filaments exhibit dynamic instability, a property hitherto only seen with eukaryotic tubulin.
4. ParA ATPase of pB171 forms spiral-shaped structures that oscillate on the nucleoid.
5. ParF of pTP228, a ParA homolog, forms MinD-like filaments in vitro.
6. *par2* of pB171 distributes plasmids along the long axis of the cell.
7. A model that explains the apparent coupling between ParA filament oscillation and ordered plasmid segregation is presented here.
8. Cytological observations allow us to propose an unexpected explanation of partition locus associated plasmid incompatibility.

UNRESOLVED ISSUES

1. Does the ParR/*parC* complex regulate dynamic instability of ParM filaments?
2. Can the coupling between plasmid segregation and ParA filament oscillation be elucidated, including a challenge of the jumping ParM model?
3. Is plasmid pairing an essential intermediate in the plasmid segregation process mediated by Type I partitioning loci?
4. What are the roles of ParA ATPases in plasmid pairing and separation?
5. What is the molecular basis of plasmid clustering?
6. What is the molecular basis of ParA/Soj oscillation?

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

ACKNOWLEDGMENTS

This work was supported by The Center for Bacterial Genetics under The Danish Natural Science Research Council and The Carlsberg Foundation.

LITERATURE CITED

1. Abeles AL, Friedman SA, Austin SJ. 1985. Partition of unit-copy miniplasmids to daughter cells. III. The DNA sequence and functional organization of the P1 partition region. *J. Mol. Biol.* 185:261–72
2. Austin S, Abeles A. 1983. Partition of unit-copy miniplasmids to daughter cells. I. P1 and F miniplasmids contain discrete, interchangeable sequences sufficient to promote equipartition. *J. Mol. Biol.* 169:353–72
3. Austin S, Abeles A. 1983. Partition of unit-copy miniplasmids to daughter cells. II. The partition region of miniplasmid P1 encodes an essential protein and a centromere-like site at which it acts. *J. Mol. Biol.* 169:373–87
4. Austin S, Nordström K. 1990. Partition-mediated incompatibility of bacterial plasmids. *Cell* 60:351–54
5. Austin S, Ziese M, Sternberg N. 1981. A novel role for site-specific recombination in maintenance of bacterial replicons. *Cell* 25:729–36
6. Autret S, Nair R, Errington J. 2001. Genetic analysis of the chromosome segregation protein Spo0J of *Bacillus subtilis*: evidence for separate domains involved in DNA binding and interactions with Soj protein. *Mol. Microbiol.* 41:743–55
7. Barillà D, Hayes F. 2003. Architecture of the ParF-ParG protein complex involved in prokaryotic DNA segregation. *Mol. Microbiol.* 49:487–99
8. Barillà D, Rosenberg MF, Nobbmann U, Hayes F. 2005. Bacterial DNA segregation dynamics mediated by the polymerizing protein ParF. *EMBO J.* 24:1453–64
9. Bartosik AA, Lasocki K, Mierzejewska J, Thomas CM, Jagura-Burdzy G. 2004. ParB of *Pseudomonas aeruginosa*: interactions with its partner ParA and its target parS and specific effects on bacterial growth. *J. Bacteriol.* 186:6983–98
10. Ben Yehuda S, Rudner DZ, Losick R. 2003. RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science* 299:532–36

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

11. Biek DP, Cohen SN. 1986. Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in *Escherichia coli*. *J. Bacteriol.* 167:594–603
12. Biek DP, Shi J. 1994. A single 43-bp sopC repeat of plasmid mini-F is sufficient to allow assembly of a functional nucleoprotein partition complex. *Proc. Natl. Acad. Sci. USA* 91:8027–31
13. Bignell CR, Haines AS, Khare D, Thomas CM. 1999. Effect of growth rate and incC mutation on symmetric plasmid distribution by the incP-1 partitioning apparatus. *Mol. Microbiol.* 34:205–16
14. Bork P, Sander C, Valencia A. 1992. An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. *Proc. Natl. Acad. Sci. USA* 89:7290–94
15. Bouet JY, Funnell BE. 1999. P1 ParA interacts with the P1 partition complex at parS and an ATP-ADP switch controls ParA activities. *EMBO J.* 18:1415–24
16. Bouet JY, Rech J, Egloff S, Biek DP, Lane D. 2005. Probing plasmid partition with centromere-based incompatibility. *Mol. Microbiol.* 55:511–25
17. Bouet JY, Surtees JA, Funnell BE. 2000. Stoichiometry of P1 plasmid partition complexes. *J. Biol. Chem.* 275:8213–19
18. Breuner A, Jensen RB, Dam M, Pedersen S, Gerdes K. 1996. The centromere-like parC locus of plasmid R1. *Mol. Microbiol.* 20:581–92
19. Colloms SD, Bath J, Sherratt DJ. 1997. Topological selectivity in Xer site-specific recombination. *Cell* 88:855–64
20. Dam M, Gerdes K. 1994. Partitioning of plasmid R1. Ten direct repeats flanking the parA promoter constitute a centromere-like partition site parC, that expresses incompatibility. *J. Mol. Biol.* 236:1289–98
21. Davey MJ, Funnell BE. 1994. The P1 plasmid partition protein ParA. A role for ATP in site-specific DNA binding. *J. Biol. Chem.* 269:29908–13
22. Davey MJ, Funnell BE. 1997. Modulation of the P1 plasmid partition protein ParA by ATP, ADP, and P1 ParB. *J. Biol. Chem.* 272:15286–92
23. Davis MA, Austin SJ. 1988. Recognition of the P1 plasmid centromere analog involves binding of the ParB protein and is modified by a specific host factor. *EMBO J.* 7:1881–88
24. Davis MA, Martin KA, Austin SJ. 1992. Biochemical activities of the parA partition protein of the P1 plasmid. *Mol. Microbiol.* 6:1141–47
25. de Boer PAJ, Crossley RE, Rothfield LI. 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*. *Cell* 56:641–49
26. Ebersbach G, Gerdes K. 2001. The double par locus of virulence factor pB171: DNA segregation is correlated with oscillation of ParA. *Proc. Natl. Acad. Sci. USA* 98:15078–83
27. Ebersbach G, Gerdes K. 2004. Bacterial mitosis: Partitioning protein ParA oscillates in spiral-shaped structures and positions plasmids at mid-cell. *Mol. Microbiol.* 52:385–98
28. Ebersbach G, Sherratt D, Gerdes K. 2005. Partition-associated incompatibility caused by random assortment of pure plasmid clusters. *Mol. Microbiol*. 56:1430–40
29. Edgar R, Chattoraj DK, Yarmolinsky M. 2001. Pairing of P1 plasmid partition sites by ParB. *Mol. Microbiol.* 42:1363–70
30. Erdmann N, Petroff T, Funnell BE. 1999. Intracellular localization of P1 ParB protein depends on ParA and parS. *Proc. Natl. Acad. Sci. USA* 96:14905–10

31. Espeli O, Levine C, Hassing H, Marians KJ. 2003. Temporal regulation of topoisomerase IV activity in *E. coli*. *Mol. Cell* 11:189–201
32. Ezaki B, Ogura T, Niki H, Hiraga S. 1991. Partitioning of a mini-F plasmid into anucleate cells of the mukB null mutant. *J. Bacteriol.* 173:6643–46
33. Figge RM, Divakaruni AV, Gober JW. 2004. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. *Mol. Microbiol.* 51:1321–32
34. Firshein W, Kim P. 1997. Plasmid replication and partition in *Escherichia coli* : Is the cell membrane the key? *Mol. Microbiol.* 23:1–10
35. Fothergill TJG, Barillà D, Hayes F. 2005. Protein diversity confers specificity in plasmid segregation. *J. Bacteriol.* 187:2651–61
36. Friedman SA, Austin SJ. 1988. The P1 plasmid-partition system synthesizes two essential proteins from an autoregulated operon. *Plasmid* 19:103–12
37. Fung E, Bouet JY, Funnell BE. 2001. Probing the ATP-binding site of P1 ParA: Partition and repression have different requirements for ATP binding and hydrolysis. *EMBO J.* 20:4901–11
38. Funnell BE. 1988. Mini-P1 plasmid partitioning: Excess ParB protein destabilizes plasmids containing the centromere parS. *J. Bacteriol.* 170:954–60
39. Funnell BE. 1988. Participation of *Escherichia coli* integration host factor in the P1 plasmid partition system. *Proc. Natl. Acad. Sci. USA* 85:6657–61
40. Funnell BE. 1991. The P1 plasmid partition complex at parS. The influence of *Escherichia coli* integration host factor and of substrate topology. *J. Biol. Chem.* 266:14328–37
41. Funnell BE, Gagnier L. 1993. The P1 plasmid partition complex at parS. II. Analysis of ParB protein binding activity and specificity. *J. Biol. Chem.* 268:3616–24
42. Funnell BE, Gagnier L. 1995. Partition of P1 plasmids in *Escherichia coli* mukB chromosomal partition mutants. *J. Bacteriol.* 177:2381–86
43. Garner EC, Campbell CS, Mullins RD. 2004. Dynamic instability in a DNA-segregating prokaryotic actin homolog. *Science* 306:1021–25
44. Gerdes K, Gultyaev AP, Franch T, Pedersen K, Mikkelsen ND. 1997. Antisense RNA-regulated programmed cell death. *Annu. Rev. Genet.* 31:1–31
45. Gerdes K, Molin S. 1986. Partitioning of plasmid R1. Structural and functional analysis of the parA locus. *J. Mol. Biol.* 190:269–79
46. Gerdes K, Møller-Jensen J, Jensen RB. 2000. Plasmid and chromosome partitioning: surprises from phylogeny. *Mol. Microbiol.* 37:455–66
47. Gitai Z, Dye NA, Reisenauer A, Wachi M, Shapiro L. 2005. MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell* 120:329–41
48. Glaser P, Sharpe ME, Raether B, Perego M, Ohlsen K, Errington J. 1997. Dynamic, mitotic-like behavior of a bacterial protein required for accurate chromosome partitioning. *Genes Dev.* 11:1160–68
49. Godfrin-Estevenon AM, Pasta F, Lane D. 2002. The parAB gene products of *Pseudomonas putida* exhibit partition activity in both *P. putida* and *Escherichia coli*. *Mol. Microbiol*. 43:39–49
50. Golovanov AP, Barillà D, Golovanova M, Hayes F, Lian L-Y. 2005. ParG, a protein required for active partition of bacterial plasmids, has a dimeric ribbon-helix-helix structure. *Mol. Microbiol.* 50:1141–53
51. Gomis-Ruth FX, Sola M, Acebo P, Parraga A, Guasch A, et al. 1998. The structure of plasmid-encoded transcriptional repressor CopG unliganded and bound to its operator. *EMBO J.* 17:7404–15

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

52. Gordon GS, Sitnikov D, Webb CD, Teleman A, Straight A, et al. 1997. Chromosome and low copy plasmid segregation in *E. coli*: visual evidence for distinct mechanisms. *Cell* 90:1113–21
53. Gordon S, Rech J, Lane D, Wright A. 2004. Kinetics of plasmid segregation in *Escherichia coli*. *Mol. Microbiol.* 51:461–69
54. Grigoriev PS, Lobocka MB. 2001. Determinants of segregational stability of the linear plasmid-prophage N15 of *Escherichia coli*. *Mol. Microbiol.* 42:355–68
55. Hanai R, Liu R, Benedetti P, Caron PR, Lynch AS, Wang JC. 1996. Molecular dissection of a protein SopB essential for *Escherichia coli* F plasmid partition. *J. Biol. Chem.* 271:17469–75
56. Hao JJ, Yarmolinsky M. 2002. Effects of the P1 plasmid centromere on expression of P1 partition genes. *J. Bacteriol.* 184:4857–67
57. Hayes F. 2000. The partition system of multidrug resistance plasmid TP228 includes a novel protein that epitomizes an evolutionarily distinct subgroup of the ParA superfamily. *Mol. Microbiol.* 37:528–41
58. Hayes F, Radnedge L, Davis MA, Austin SJ. 1994. The homologous operons for P1 and P7 plasmid partition are autoregulated from dissimilar operator sites. *Mol. Microbiol.* 11:249–60
59. Hiraga S. 1992. Chromosome and plasmid partition in *Escherichia coli*. *Annu. Rev. Biochem.* 61:283–306
60. Hirano M, Mori H, Onogi T, Yamazoe M, Niki H, et al. 1998. Autoregulation of the partition genes of the mini-F plasmid and the intracellular localization of their products in *Escherichia coli*. *Mol. Gen. Genet.* 257:392–403
61. Ho TQ, Zhong Z, Aung S, Pogliano J. 2002. Compatible bacterial plasmids are targeted to independent cellular locations in *Escherichia coli*. *EMBO J.* 21:1864–72
62. Hu Z, Gogol EP, Lutkenhaus J. 2002. Dynamic assembly of MinD on phospholipid vesicles regulated by ATP and MinE. *Proc. Natl. Acad. Sci. USA* 99:6761–66
63. Hu Z, Lutkenhaus J. 2001. Topological regulation of cell division in *E. coli*: Spatiotemporal oscillation of MinD requires stimulation of its ATPase by MinE and phospholipid. *Mol. Cell* 7:1337–43
64. Hu Z, Lutkenhaus J. 1999. Topological regulation of cell division in *Escherichia coli* involves rapid pole to pole oscillation of the division inhibitor MinC under the control of MinD and MinE. *Mol. Microbiol.* 34:82–90
65. Hu Z, Saez C, Lutkenhaus J. 2003. Recruitment of MinC, an inhibitor of Z-ring formation, to the membrane in *Escherichia coli*: role of MinD and MinE. *J. Bacteriol.* 185:196–203
66. Ingmer H, Miller CA, Cohen SN. 1998. Destabilized inheritance of pSC101 and other *Escherichia coli* plasmids by DpiA, a novel two-component system regulator. *Mol. Microbiol.* 29:49–59
67. Ireton K, Gunther NW, Grossman AD. 1994. spo0J is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J. Bacteriol.* 176:5320–29
68. Jacob F, Brenner S, Cuzin F. 1963. On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symp. Quant. Biol.* 23:329–48
69. Jakimowicz D, Chater K, Zakrzewska-Czerwinska J. 2002. The ParB protein of *Streptomyces coelicolor* A3(2) recognizes a cluster of parS sequences within the origin-proximal region of the linear chromosome. *Mol. Microbiol.* 45:1365–77

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

70. Jensen RB, Dam M, Gerdes K. 1994. Partitioning of plasmid R1. The parA operon is autoregulated by ParR and its transcription is highly stimulated by a downstream activating element. *J. Mol. Biol.* 236:1299–309
71. Jensen RB, Gerdes K. 1995. Programmed cell death in bacteria: proteic plasmid stabilization systems. *Mol. Microbiol.* 17:205–10
72. Jensen RB, Gerdes K. 1997. Partitioning of plasmid R1. The ParM protein exhibits ATPase activity and interacts with the centromere-like ParR-parC complex. *J. Mol. Biol.* 269:505–13
73. Jensen RB, Gerdes K. 1999. Mechanism of DNA segregation in prokaryotes: ParM partitioning protein of plasmid R1 co-localizes with its replicon during the cell cycle. *EMBO J.* 18:4076–84
74. Jensen RB, Lurz R, Gerdes K. 1998. Mechanism of DNA segregation in prokaryotes: replicon pairing by parC of plasmid R1. *Proc. Natl. Acad. Sci. USA* 95:8550–55
75. Johnson EP, Yao S, Helinski DR. 2005. Gyrase inhibitors and thymine starvation disrupt the normal pattern of plasmid RK2 localization in *Escherichia coli*. *J. Bacteriol.* 187:3538–47
76. Jones LJ, Carballido-Lopez R, Errington J. 2001. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* 104:913–22
77. Kim HJ, Calcutt MJ, Schmidt FJ, Chater KF. 2000. Partitioning of the linear chromosome during sporulation of *Streptomyces coelicolor* A3(2) involves an oriC-linked parAB locus. *J. Bacteriol.* 182:1313–20
78. Kim SK, Wang JC. 1998. Localization of F plasmid SopB protein to positions near the poles of *Escherichia coli* cells. *Proc. Natl. Acad. Sci. USA* 95:1523–27
79. Kim SK, Wang JC. 1999. Gene silencing via protein-mediated subcellular localization of DNA. *Proc. Natl. Acad. Sci. USA* 96:8557–61
80. Kline-Smith SL, Walczak C. 2004. Mitotic spindle assembly and chromosome segregation: refocusing on microtubule dynamics. *Mol. Cell* 15:317–27
81. Koonin EV. 1993. A superfamily of ATPases with diverse functions containing either classical or deviant ATP-binding motif. *J. Mol. Biol.* 229:1165–74
82. Koppes LJ, Woldringh CL, Nanninga N. 1999. *Escherichia coli* contains a DNA replication compartment in the cell center. *Biochimie* 81:803–10
83. Kruse T, Bork-Jensen J, Gerdes K. 2005. The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. *Mol. Microbiol.* 55:78–89
84. Kruse T, Møller-Jensen J, Løbner-Olesen A, Gerdes K. 2003. Dysfunctional MreB inhibits chromosome segregation in *Escherichia coli*. *EMBO J.* 22:5283–92
85. Kusukawa N, Mori H, Kondo A, Hiraga S. 1987. Partitioning of the F plasmid: Overproduction of an essential protein for partition inhibits plasmid maintenance. *Mol. Gen. Genet.* 208:365–72
86. Lackner LL, Raskin DM, de Boer PA. 2003. ATP-dependent interactions between *Escherichia coli* Min proteins and the phospholipid membrane in vitro. *J. Bacteriol.* 185:735–49
87. Lane D, de Feyter R, Kennedy M, Phua SH, Semon D. 1986. D protein of mini-F plasmid acts as a repressor of transcription and as a site-specific resolvase. *Nucleic Acids Res.* 14:9713–28
88. Lawley TD, Taylor DE. 2003. Characterization of the double-partitioning modules of R27: correlating plasmid stability with plasmid localization. *J. Bacteriol.* 185:3060–67

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org by HARVARD UNIVERSITY on 03/30/10. For personal use only.

89. Lee PS, Lin DC, Moriya S, Grossman AD. 2003. Effects of the chromosome partitioning protein Spo0J (ParB) on oriC positioning and replication initiation in *Bacillus subtilis*. *J. Bacteriol.* 185:1326–37
90. Lemon KP, Grossman AD. 1998. Localization of bacterial DNA polymerase: evidence for a factory model of replication. *Science* 282:1516–19
91. Lemonnier M, Bouet JY, Libante V, Lane D. 2000. Disruption of the F plasmid partition complex in vivo by partition protein SopA. *Mol. Microbiol.* 38:493–505
92. Leonard TA, Butler PJ, Lowe J. 2005. Bacterial chromosome segregation: structure and DNA binding of the Soj dimer—a conserved biological switch. *EMBO J.* 24:270–82
93. Leonard TA, Butler PJ, Lowe J. 2004. Structural analysis of the chromosome segregation protein Spo0J from *Thermus thermophilus*. *Mol. Microbiol.* 53:419–32
94. Lewis RA, Bignell CR, Zeng W, Jones AC, Thomas CM. 2002. Chromosome loss from par mutants of *Pseudomonas putida* depends on growth medium and phase of growth. *Microbiology* 148:537–48
95. Li Y, Austin S. 2002. The P1 plasmid is segregated to daughter cells by a 'capture and ejection' mechanism coordinated with *Escherichia coli* cell division. *Mol. Microbiol.* 46:63–74
96. Li Y, Dabrazhynetskaya A, Youngren B, Austin S. 2004. The role of Par proteins in the active segregation of the P1 plasmid. *Mol. Microbiol.* 53:93–102
97. Libante V, Thion L, Lane D. 2001. Role of the ATP-binding site of SopA protein in partition of the F plasmid. *J. Mol. Biol.* 314:387–99
98. Lin DC, Grossman AD. 1998. Identification and characterization of a bacterial chromosome partitioning site. *Cell* 92:675–85
99. Lobocka M, Yarmolinsky M. 1996. P1 plasmid partition: a mutational analysis of ParB. *J. Mol. Biol.* 259:366–82
100. Lynch AS, Wang JC. 1994. Use of an inducible site-specific recombinase to probe the structure of protein-DNA complexes involved in F plasmid partition in *Escherichia coli*. *J. Mol. Biol.* 236:679–84
101. Lynch AS, Wang JC. 1995. SopB protein-mediated silencing of genes linked to the sopC locus of *Escherichia coli* F plasmid. *Proc. Natl. Acad. Sci. USA* 92:1896–900
101a. Løbner-Olesen A. 1999. Distribution of minichromosomes in individual *Escherichia coli* cells: implications for replication control. *EMBO J.* 18:1712–21
102. Marston AL, Errington J. 1999. Dynamic movement of the ParA-like Soj protein of *B. subtilis* and its dual role in nucleoid organization and developmental regulation. *Mol. Cell* 4:673–82
103. Martin KA, Friedman SA, Austin SJ. 1987. Partition site of the P1 plasmid. *Proc. Natl. Acad. Sci. USA* 84:8544–47
104. Mitchison T, Kirschner M. 1984. Dynamic instability of microtubule growth. *Nature* 312:237–42
105. Mitchison TJ, Salmon ED. 2001. Mitosis: a history of division. *Nat. Cell Biol.* 3:E17–21
106. Mohl DA, Gober JW. 1997. Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* 88:675–84
107. Møller-Jensen J, Borch J, Dam M, Jensen RB, Roepstorff P, Gerdes K. 2003. Bacterial Mitosis: ParM of plasmid R1 moves DNA by an actin-like insertional polymerization mechanism. *Mol. Cell* 12:1477–87
108. Møller-Jensen J, Jensen RB, Lowe J, Gerdes K. 2002. Prokaryotic DNA segregation by an actin-like filament. *EMBO J.* 21:3119–27

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

109. Mori H, Kondo A, Ohshima A, Ogura T, Hiraga S. 1986. Structure and function of the F plasmid genes essential for partitioning. *J. Mol. Biol.* 192:1–15
110. Mori H, Mori Y, Ichinose C, Niki H, Ogura T, et al. 1989. Purification and characterization of SopA and SopB proteins essential for F plasmid partitioning. *J. Biol. Chem.* 264:15535–41
111. Niki H, Hiraga S. 1997. Subcellular distribution of actively partitioning F plasmid during the cell division cycle in *E. coli*. *Cell* 90:951–57
112. Niki H, Ichinose C, Ogura T, Mori H, Morita M, et al. 1988. Chromosomal genes essential for stable maintenance of the mini-F plasmid in *Escherichia coli*. *J. Bacteriol.* 170:5272–78
113. Nordström K, Austin SJ. 1989. Mechanisms that contribute to the stable segregation of plasmids. *Annu. Rev. Genet.* 23:37–69
114. Nordström K, Gerdes K. 2003. Clustering versus random segregation of plasmids lacking a partitioning function: a plasmid paradox? *Plasmid* 50:95–101
115. Nordström K, Molin S, agaard-Hansen H. 1980. Partitioning of plasmid R1 in *Escherichia coli*. I. Kinetics of loss of plasmid derivatives deleted of the par region. *Plasmid* 4:215–27
116. Ogura T, Hiraga S. 1983. Partition mechanism of F plasmid: two plasmid gene-encoded products and a *cis*-acting region are involved in partition. *Cell* 32:351–60
117. Onogi T, Miki T, Hiraga S. 2002. Behavior of sister copies of mini-F plasmid after synchronized plasmid replication in *Escherichia coli* cells. *J. Bacteriol.* 184:3142–45
118. Pichoff S, Lutkenhaus J. 2001. *Escherichia coli* division inhibitor MinCD blocks septation by preventing Z-ring formation. *J. Bacteriol.* 183:6630–35
119. Pogliano J, Ho TQ, Zhong Z, Helinski DR. 2001. Multicopy plasmids are clustered and localized in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 98:4486–91
120. Pogliano J, Sharp MD, Pogliano K. 2002. Partitioning of chromosomal DNA during establishment of cellular asymmetry in *Bacillus subtilis*. *J. Bacteriol.* 184:1743–49
121. Quisel JD, Grossman AD. 2000. Control of sporulation gene expression in *Bacillus subtilis* by the chromosome partitioning proteins Soj (ParA) and Spo0J (ParB). *J. Bacteriol.* 182:3446–51
122. Quisel JD, Lin DC, Grossman AD. 1999. Control of development by altered localization of a transcription factor in *B. subtilis*. *Mol. Cell* 4:665–72
123. Radnedge L, Davis MA, Austin SJ. 1996. P1 and P7 plasmid partition: ParB protein bound to its partition site makes a separate discriminator contact with the DNA that determines species specificity. *EMBO J.* 15:1155–62
124. Raskin DM, de Boer PA. 1999. MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in *Escherichia coli*. *J. Bacteriol.* 181:6419–24
125. Raskin DM, de Boer PA. 1999. Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 96:4971–76
126. Raumann BE, Rould MA, Pabo CO, Sauer RT. 1994. DNA recognition by beta-sheets in the Arc repressor-operator crystal structure. *Nature* 367:754–57
127. Ravin N, Lane D. 1999. Partition of the linear plasmid N15: Interactions of N15 partition functions with the sop locus of the F plasmid. *J. Bacteriol.* 181:6898–906
128. Ravin NV, Rech J, Lane D. 2003. Mapping of functional domains in F plasmid partition proteins reveals a bipartite SopB-recognition domain in SopA. *J. Mol. Biol.* 329:875–89

129. Rodionov O, Lobocka M, Yarmolinsky M. 1999. Silencing of genes flanking the P1 plasmid centromere. *Science* 283:546–49
130. Rodionov O, Yarmolinsky M. 2004. Plasmid partitioning and the spreading of P1 partition protein ParB. *Mol. Microbiol.* 52:1215–23
131. Schmidt A, Hall MN. 1998. Signalling to the actin cytoskeleton. *Annu. Rev. Cell. Dev. Biol.* 14:305–38
132. Shih YL, Le T, Rothfield L. 2003. Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. *Proc. Natl. Acad. Sci. USA* 100:7865–70
133. Slavcev RA, Funnell B. 2005. Identification and characterization of a novel allele of *Escherichia coli* dnaB helicase that compromises the stability of plasmid P1. *J. Bacteriol.* 187:1227–37
134. Suefuji K, Valluzzi R, RayChaudhuri D. 2002. Dynamic assembly of MinD into filament bundles modulated by ATP, phospholipids, and MinE. *Proc. Natl. Acad. Sci. USA* 99:16776–81
135. Summers D. 1998. Timing, self-control and a sense of direction are the secrets of multicopy plasmid stability. *Mol. Microbiol.* 29:1137–45
136. Summers DK, Sherratt DJ. 1984. Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell* 36:1097–103
137. Surtees JA, Funnell BE. 1999. P1 ParB domain structure includes two independent multimerization domains. *J. Bacteriol.* 181:5898–908
138. Surtees JA, Funnell BE. 2001. The DNA binding domains of P1 ParB and the architecture of the P1 plasmid partition complex. *J. Biol. Chem.* 276:12385–94
139. Szeto TH, Rowland SL, Rothfield LI, King GF. 2002. Membrane localization of MinD is mediated by a C-terminal motif that is conserved across eubacteria, archaea, and chloroplasts. *Proc. Natl. Acad. Sci. USA* 99:15693–98
140. van den Ent F, Møller-Jensen J, Amos LA, Gerdes K, Löwe J. 2002. F-actin-like filaments formed by plasmid segregation protein ParM. *EMBO J.* 21:6935–43
141. Watanabe E, Wachi M, Yamasaki M, Nagai K. 1992. ATPase activity of SopA, a protein essential for active partitioning of F plasmid. *Mol. Gen. Genet.* 234:346–52
142. Webb CD, Graumann PL, Kahana JA, Teleman AA, Silver PA, Losick R. 1998. Use of time-lapse microscopy to visualize rapid movement of the replication origin region of the chromosome during the cell cycle in *Bacillus subtilis*. *Mol. Microbiol.* 28:883–92
143. Webb CD, Teleman A, Gordon S, Straight A, Belmont A, et al. 1997. Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. *Cell* 88:667–74
144. Weitao T, Dasgupta S, Nordstrom K. 2000. Plasmid R1 is present as clusters in the cells of *Escherichia coli*. *Plasmid* 43:200–4
145. Williams DR, Macartney DP, Thomas CM. 1998. The partitioning activity of the RK2 central control region requires only incC, korB and KorB-binding site OB3 but other KorB-binding sites form destabilizing complexes in the absence of OB3. *Microbiology* 144:3369–78
146. Wu LJ, Errington J. 2003. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating *Bacillus subtilis*. *Mol. Microbiol.* 49:1463–75
147. Yamaichi Y, Niki H. 2000. Active segregation by the *Bacillus subtilis* partitioning system in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 97:14656–61

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.

148. Yates P, Lane D, Biek DP. 1999. The F plasmid centromere, sopC, is required for full repression of the sopAB operon. *J. Mol. Biol.* 290:627–38
149. Zhao C-R, de Boer PAJ, Rothfield LI. 1995. Proper placement of the *E. coli* division site requires two functions that are associated with different domains of the MinE protein. *Proc. Natl. Acad. Sci. USA* 92:4313–17

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Annual Review of Genetics

Volume 39, 2005

Contents

John Maynard Smith
Richard E. Michod 1

The Genetics of Hearing and Balance in Zebrafish
Teresa Nicolson 9

Immunoglobulin Gene Diversification
Nancy Maizels 23

Complexity in Regulation of Tryptophan Biosynthesis in *Bacillus subtilis*
Paul Gollnick, Paul Babitzke, Alfred Antson, and Charles Yanofsky 47

Cell-Cycle Control of Gene Expression in Budding and Fission Yeast
Jürg Bähler 69

Comparative Developmental Genetics and the Evolution of Arthropod Body Plans
David R. Angelini and Thomas C. Kaufman 95

Concerted and Birth-and-Death Evolution of Multigene Families
Masatoshi Nei and Alejandro P. Rooney 121

Drosophila as a Model for Human Neurodegenerative Disease
Julide Bilen and Nancy M. Bonini 153

Molecular Mechanisms of Germline Stem Cell Regulation
Marco D. Wong, Zhigang Jin, and Ting Xie 173

Molecular Signatures of Natural Selection
Rasmus Nielsen 197

T-Box Genes in Vertebrate Development
L.A. Naiche, Zachary Harrelson, Robert G. Kelly, and Virginia E. Papaioannou 219

Connecting Mammalian Genome with Phenome by ENU Mouse Mutagenesis: Gene Combinations Specifying the Immune System
Peter Papathanasiou and Christopher C. Goodnow 241

Evolutionary Genetics of Reproductive Behavior in *Drosophila*: Connecting the Dots
Patrick M. O'Grady and Therese Anne Markow 263

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org by HARVARD UNIVERSITY on 03/30/10. For personal use only.

Sex Determination in the Teleost Medaka, *Oryzias latipes*
Masura Matsuda 293

Orthologs, Paralogs, and Evolutionary Genomics
Eugene V. Koonin 309

The Moss *Physcomitrella patens*
David Cove 339

A Mitochondrial Paradigm of Metabolic and Degenerative Diseases, Aging, and Cancer: A Dawn for Evolutionary Medicine
Douglas C. Wallace 359

Switches in Bacteriophage Lambda Development
Amos B. Oppenheim, Oren Kobiler, Joel Stavans, Donald L. Court, and Sankar Adhya 409

Nonhomologous End Joining in Yeast
James M. Daley, Phillip L. Palmbos, Dongliang Wu, and Thomas E. Wilson 431

Plasmid Segregation Mechanisms
Gitte Ebersbach and Kenn Gerdes 453

Use of the Zebrafish System to Study Primitive and Definitive Hematopoiesis
Jill L.O. de Jong and Leonard I. Zon 481

Mitochondrial Morphology and Dynamics in Yeast and Multicellular Eukaryotes
Koji Okamoto and Janet M. Shaw 503

RNA-Guided DNA Deletion in Tetrahymena: An RNAi-Based Mechanism for Programmed Genome Rearrangements
Meng-Chao Yao and Ju-Lan Chao 537

Molecular Genetics of Axis Formation in Zebrafish
Alexander F. Schier and William S. Talbot 561

Chromatin Remodeling in Dosage Compensation
John C. Lucchesi, William G. Kelly, and Barbara Panning 615

INDEXES

Subject Index 653

ERRATA

An online log of corrections to *Annual Review of Genetics* chapters may be found at http://genet.annualreviews.org/errata.shtml

Annu. Rev. Genet. 2005.39:453-479. Downloaded from arjournals.annualreviews.org
by HARVARD UNIVERSITY on 03/30/10. For personal use only.