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BACTERIAL CHROMOSOME SEGREGATION

Geoffrey C. Draper and James W. Gober
Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California 90095-1569; e-mail: gober@chem.ucla.edu

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■ **Abstract** Recent studies have made great strides toward our understanding of the mechanisms of microbial chromosome segregation and partitioning. This review first describes the mechanisms that function to segregate newly replicated chromosomes, generating daughter molecules that are viable substrates for partitioning. Then experiments that address the mechanisms of bulk chromosome movement are summarized. Recent evidence indicates that a stationary DNA replication factory may be responsible for supplying the force necessary to move newly duplicated DNA toward the cell poles. Some factors contributing to the directionality of chromosome movement probably include centromere-like-binding proteins, DNA condensation proteins, and DNA translocation proteins.

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INTRODUCTION

Bacterial cells possess a host of functions that replicate, maintain, and segregate their chromosomal DNA. Although the underlying mechanisms of bacterial DNA replication and repair are well known, our understanding of the mechanisms of chromosomal DNA partitioning is largely incomplete. While the relatively small size of the bacterial cell has hampered efforts to visualize specific structures within the cell that might constitute a mitotic apparatus, the advent of powerful immunocytological and genetic techniques has allowed the visualization of the subcellular localization of a variety of other structures within the bacterial cell. In particular, it is now known that the bacterial nucleoid has a specific orientation within the cell, with origin of replication (*oriC*) regions being located toward the cell poles for much of the cell cycle. The foundations of the bacterial DNA partitioning field has its roots in genetic screens that identified mutants defective in chromosome partitioning. Most of these mutants were later found to possess mutations in genes encoding DNA gyrase and topoisomerases, which do not function to move replicated chromosomes per se but facilitate the production of substrates that are viable for segregation.

For many years it was accepted that newly replicated chromosomes were segregated into their daughter cell compartments by a passive, membrane-bound process. This "replicon model" of chromosome segregation, which was originally applied to explain the fidelity of F-factor partitioning in *Escherichia coli*, suggested that the origin regions of the daughter nucleoids were anchored to the cell membrane at a central position of the cell. DNA replication would result in daughter chromosomes also positioned at the midcell. Cell elongation and addition of new cell membrane specifically between the attachment sites of the two chromosomes to the membrane were proposed to provide the motive force to segregate the nucleoids into the daughter cell compartments (63). It would later be shown that the F and P1 plasmids encode specific determinants, named *par* (or *sop*) genes, that facilitate their efficient partitioning [reviewed in (41)]. Chromosomally encoded homologs of the *par* system have since been discovered in a wide range of bacteria including *Bacillus subtilis* (Soj and Spo0J) and *Caulobacter crescentus* (ParA and ParB) and may influence the inheritance of newly replicated chromosomes (see section below). ParB and Spo0J bind specific regions of DNA located close to the

origin of replication. Thus, this system appears to be functionally related to the eukaryotic kinetochore and centromere. ParB (Spo0J) has a dynamic localization pattern in vivo. Early in the cell cycle, ParB is localized to the poles of the cell. During chromosome replication another region of ParB localization can be seen (presumably from ParB binding to the duplicated origin region), which then migrates to the opposite pole of the cell. Upon completion of chromosome replication and just prior to cell division, ParB is localized to both poles of the cell. Division results in daughter cells with ParB localized to one of the cell poles.

Other factors that influence the partitioning of bacterial chromosomes include topoisomerases and recombinases that separate physically linked and dimerized chromosomes. Proteins homologous to the eukaryotic SMC (structural maintenance of chromosomes) proteins act to condense the nucleoid and show a dynamic pattern of localization through the cell cycle (see section below).

In this review we summarize recent findings that may lead to an understanding of bacterial chromosome segregation (i.e., the physical separation of newly replicated chromosomes). We then detail efforts to illuminate the spatial organization of the nucleoid within the bacterial cell, particularly recent exciting experiments in which the dynamic localization of proteins thought to contribute to partitioning are assayed in living cells. These same groundbreaking techniques have also been used to visualize the subcellular location of specific regions of the genome. We discuss factors likely to contribute to chromosome movement, including the DNA-condensing proteins, MukB and SMC, the process of DNA replication itself, and DNA translocation by cell division–related proteins. Last we report on the role of the cellular homologs of plasmid-partitioning proteins in coupling chromosome dynamics to developmentally or cell cycle–regulated pathways.

GENERATION OF MONOMERIC CHROMOSOME SUBSTRATES FOR PARTITIONING

The partitioning of newly replicated chromosomes can only take place after DNA segregation, which includes (*a*) resolution of dimeric chromosomes formed by odd numbers of recombination events and (*b*) successful decatenation of the two covalently closed circular DNA molecules. In *E. coli*, resolution of chromosome dimers is accomplished by the *dif*/XerCD system. The unlinking of the catenated chromosomes formed upon completion of DNA replication requires the concerted action of the DNA gyrase and topoisomerase IV.

dif and XerCD: Resolution of Chromosome Dimers Formed by Sister Chromosome Exchange

Daughter chromosomes are prone to recombination that produces a circular dimer (76). Such dimers must be resolved into monomeric chromosomes prior to segregation. In *E. coli*, resolution occurs at a specific locus, known as *dif* (deletion induced filamentation) (16, 76). *dif* is a 28-bp sequence located in the central region

of the replication terminus (10, 76). Strains carrying a *dif* deletion fail to resolve chromosome dimers and elicit a characteristic Dif phenotype, typified by an induction of the SOS response, aberrant nucleoid morphology, filamentation, and reduced viability and growth rate (19, 76). Although the SOS response is induced, filamentation observed in *dif* mutants is independent of the SOS-induced division inhibitor, SulA, suggesting that it is the lack of resolution of chromosome dimers itself that blocks cell division, possibly by nucleoid occlusion of the division site (46, 76, 157). The core *dif* resolvase site functions in the absence of any accessory sequences (141). A 173-kb deletion of the terminus region can be replaced with a 33-bp *dif* sequence that is competent for resolution. However, the position of the *dif* site is important; the Dif phenotype is not suppressed when the only copy of *dif* is moved 30 kb from the normal *dif* locus (19, 75, 141).

Recombination at *dif* is independent of RecA and requires the XerC and XerD proteins (10, 11). The XerC and XerD resolvases belong to the lambda integrase family (37% identity) (10, 11) and bind to 11-bp sequences within the *dif* site opposite a 6- to 8-bp central region (130). Strains carrying mutations in one or both *xer* genes result in the Dif phenotype (10, 76, 141). Individually, the Xer proteins act as type I topoisomerases and relax supercoils by nicking one strand of the *dif* site. The rate of nicking is, however, low and is not thought to be of any biological importance (18). Homologs of XerC and XerD have been described from a number of organisms, including *Pseudomonas aeruginosa* (55), *Salmonella typhimurium* (44), *B. subtilis* (125, 132), and several species of *Enterobacteriaceae* (131), which suggests that this mechanism of dimer resolution is highly conserved.

Topoisomerases: Unlinking the Circles

Early investigations in *E. coli* gave rise to a number of mutants defective in chromosome partitioning (52, 54, 70, 71, 89, 124, 133). These *par* mutant strains exhibited a temperature-sensitive, filamentous cell morphology, which contains nonsegregating nucleoids at the nonpermissive temperature. These strains were later shown to carry mutations in genes encoding either the subunits of DNA gyrase or topoisomerase IV. The *parA* allele mapped to the *gyrB* gene, which encodes the B subunit of DNA gyrase (70). *parD* was originally thought to be a novel gene (60) but this was later shown to be unlikely, as the strain also carried an amber mutation in the gene that encodes the gyrase A subunit, *gyrA* (61). The *parC* allele mapped to a gene encoding a homolog of the A subunit of DNA gyrase (69). The *parE* gene was mapped and cloned, and the deduced protein was shown to have a significant degree of similarity to the B subunit of DNA gyrase (133). Crude cell extracts from strains overexpressing *parC* and *parE* were shown to relax supercoiled DNA. Increasing the copy number of both the *parC* and *parE* genes suppressed a mutation in *topA*, the gene-encoding topoisomerase I. Together, these experiments showed the presence of a second type II topoisomerase system in *E. coli*, encoded by *parC* and *parE*, which was named topoisomerase IV (69). Purified topoisomerase

IV was also demonstrated to be capable of catalyzing the efficient decatenation of circular DNA molecules in vitro (2, 72, 114, 115). Pulse-labeling experiments showed that nearly all newly replicated plasmid DNA is catenated under conditions in which topo IV is not active (168). These experiments also revealed that DNA gyrase activity could slowly process catenanes formed as a consequence of DNA replication in the absence of topo IV activity. Therefore, the combined activities of topo IV and gyrase are responsible for the decatenation of chromosomal DNA formed during DNA replication, although the rate of decatenation by gyrase alone is 100-fold less than in the presence of both proteins (168, 169). A possible function of DNA gyrase in the unlinking of chromosomes is to introduce negative supercoils into the DNA, creating a better substrate for decatenation by topo IV than for relaxed DNA (169).

In contrast, in *Caulobacter crescentus*, cells lacking topo IV (*parE*) did not form long filaments, but rather possessed multiple sites of pinching along the length of the cell, indicating a defect in a later stage of cell division (150). Furthermore, unlike enteric bacteria, *C. crescentus* topo IV mutants exhibited no defect in chromosome segregation. However, DNA segregation defects could be readily demonstrated if the *parE* mutation was combined with a conditional mutation in the early cell division gene, *ftsA*, thus providing a genetic link between the chromosome segregation and cell division (150). This difference in phenotypes between *C. crescentus* and enteric bacteria probably reflects the tight coupling of cell cycle–regulated events in *Caulobacter*.

Interestingly, ParC of *B. subtilis* has been localized to the cell poles (58). In the absence of ParE, ParC is localized to the nucleoid instead of the poles. ParE had no specific localization pattern and could be detected throughout the cytoplasm (58). The same authors also provided evidence that both the DNA gyrase A and B subunits are generally associated with the nucleoid, consistent with the established idea that DNA gyrase is required for higher-order DNA structure. The results of these localization experiments are consistent with the findings that topo IV functions to decatenate linked chromosomes prior to partitioning and, in agreement with Zechiedrich et al. (169), that DNA gyrase creates a DNA topology favorable for topo IV function. A previous study used immunogold-labeled antibodies to show that *E. coli* GyrA and GyrB were randomly localized in the cytoplasm (143). The difference in localization patterns may indicate that *B. subtilis* and *E. coli* use different mechanisms to segregate their nucleoids.

Other Mutations Affecting Chromosome Segregation in *Escherichia coli*

Many other mutants of *E. coli* that possess observed chromosomal DNA segregation or partitioning defects have been identified. Mutations in these genes result in either recombination defects or global cell cycle defects, which in turn generate a DNA segregation defect. None of these is likely to be directly involved with segregation.

1. One of the *parB* mutants described in the preceding section was found to be an allele of the gene-encoding DNA primase, *dnaG* (53, 111). Later experiments showed that the *parB* allele of *dnaG* retained the ability to synthesize functional RNA primers for DNA replication (147). There is a distinct possibility that the *par* phenotype observed in strains carrying the *parB* allele of *dnaG* could be due to a loss of interaction of DnaG with a putative mitotic apparatus (147).
2. *E. coli* strains bearing mutations in the *ruvC*, *recBC*, and *sbcCD* genes also form filamentous cells with chromosome segregation defects (167). The phenotype is due to incomplete recombination events initiated by RecA that cannot progress into mature recombinants owing to the loss of *ruvC* function. Because functional RuvABC is required for resolution of Holliday intermediates formed by RecA-mediated strand exchange, it follows that in these mutants the chromosomes are physically linked at multiple sites via Holliday intermediates (167).
3. *E. coli* mutants lacking the histone-like proteins H-NS and HU produce anucleate cells possibly because of the loss of higher-order structure of the nucleoid (31, 59, 64, 68, 148). These proteins bind and bend DNA and have been implicated in DNA replication, transposition, and DNA and chromosome segregation [reviewed in (32)]. An insertionally inactivated *hupA* gene, which encodes HU2 (one of the subunits of the heterodimeric HU protein), could not be combined with a null-allele of *mukB*, indicating that a global loss of chromosome organization and condensation is lethal (64).
4. In a genetic screen designed to detect anucleate cell production, a number of mutations were generated in the *min* locus (see section below) (3, 49, 22). The *min* locus consists of three genes, *minC*, *minD*, and *minE*, whose products exert control over cell division site selection in *E. coli* (25–27). Mutations in the *min* locus result in the loss of division site specificity and divisions can take place at both the central (normal) division site and at the cell poles, which are remnants of previous cell divisions. Polar divisions can lead to the production of anucleate minicells, and thus it is not unexpected that mutations in the *min* genes could be generated in the screen designed to isolate partitioning mutants (see *mukB* section below) (49). Interestingly, Mulder et al. (101) found that plasmids segregated into minicells show reduced levels of supercoiling, a phenomenon that could reflect decreased topoisomerase activity in minicells, exacerbating the partitioning defect.

SPATIAL ORGANIZATION OF THE BACTERIAL CHROMOSOME

In order for partitioning to operate with efficiency, there must exist mechanisms that function to orient each daughter chromosome to opposite poles of the cell. The circular chromosome of bacteria, although compacted by supercoiling and the

binding of histone-like proteins, still occupies much of the internal volume of the cell. Surprisingly, however, a significant fraction of the bulk chromosomal DNA resides at specific locations within the cell. Below, we summarize experiments that first described the spatial organization of the bacterial chromosome.

Sporulating Cells Have a Stringent Requirement for Efficient Chromosome Partitioning

Initial indications that the bacterial chromosome might have a distinct orientation within the cell came from studies on sporulation in *Bacillus subtilis*. In response to starvation, *B. subtilis* undergoes a program of development that results in the formation of a dormant spore [reviewed in (35, 81)]. The initial morphogenetic event in this developmental pathway is the formation of an asymmetric septum near the pole of the cell. The smaller compartment thus formed, the prespore, eventually becomes the spore. Both the prespore compartment and the larger adjacent mother cell compartment each contain a complete chromosome, and differential programs of gene expression within each compartment directs the successful development of the spore. The SpoIIIE protein was shown to be required for complete segregation of chromosomal DNA into the prespore (159). During the asymmetrical cell division, the closure of the septum results in the bisection of the newly replicated chromosomes. At this point approximately 30% of the chromosome is located in the prespore with the remainder in the mother cell compartment. Mutations in *spoIIIE* prevent the completion of DNA segregation into the prespore (159). SpoIIIE is localized to the prespore septum where it is proposed to move the remaining 70% of the chromosome from the mother cell compartment into the prespore via a conjugation-like mechanism (160). Using elegant genetic and physical methods, it was determined that the DNA located in the prespore in a *spoIIIE* mutant always consisted of the same region of chromosome (159, 161). This region corresponded to a 500-kb region of DNA surrounding the origin of replication, *oriC* (127, 159, 160). The observation that a specific chromosomal region arrives first in the prespore compartment pointed toward the presence of a mitotic-like apparatus responsible for the orientation of the origin to the cell pole during asymmetric cell division.

The gene encoding SpoIIIE is expressed constitutively and has the effect of enhancing the fidelity of vegetative chromosome segregation in *B. subtilis* by translocating trapped DNA from the closing septum during cell division (126). Indeed, biochemical experiments have demonstrated that the conserved carboxyl-terminal domain of SpoIIIE possesses an ATPase activity that can be stimulated 10-fold by the presence of double-stranded DNA (7). Furthermore, by probing the topology of the DNA substrate following ATP hydrolysis, these experiments show that DNA tracks along SpoIIIE or vice versa (7). Taken together, these results indicate that SpoIIIE functions as a DNA pump that translocates unpartitioned chromosomal DNA across the prespore division septum. The homologs of SpoIIIE, such as FtsK, found in nonspore-forming bacterium may have a similar role in chromosome movement.

Localization of the Origin and Terminus Regions of the Bacterial Nucleoid

Specific orientation of the chromosome was subsequently discovered in *E. coli* and in vegetative cells of *B. subtilis* (40, 105, 128, 153, 154). An ingenious reporter system, originally used to visualize chromosomal regions in *Saccharomyces cerevisiae* (137), showed that the origin regions of both the *B. subtilis* (137) and *E. coli* (154) chromosomes are dynamically positioned. The technique relies on a tandem repeat of 256 *E. coli lac* operator (*lacO*) sites that are inserted into the chromosome at specific loci, in these cases near either the origin or terminus. A *lac* repressor-green fluorescent protein chimera (LacI-Gfp) expressed in these cells bound the operator sites, and the localization of the complex was detected by fluorescence microscopy (40, 153, 154). Fluorescent in situ hybridization (FISH) has also been employed in *E. coli* (105) and *C. crescentus* (65) to assay location of *oriC* in the cell. The reports had similar findings; in newborn *E. coli* cells with ongoing DNA replication (i.e., fast-growing cells), the two copies of the origin are located at opposite cell poles (Figure 1). Before DNA replication is reinitiated, the origin regions move from the cell pole toward the midcell (the presumptive division site). After replication, one of the origins from each pair of chromosomes moves toward the cell pole while the other remains localized toward the cell center. Formation of a septum and subsequent cell division results in a daughter cell with a chromosome origin at each pole. *B. subtilis* origin placement is similar to that observed in *E. coli* (153, 128). Origin regions of the *E. coli* chromosome detected by FISH are localized to the pole proximal border of the nucleoid (110, 128). The bulk-segregated nucleoids in the postseptational but predivisional cell occupy the quarter positions of the cell (29, 50). In *B. subtilis*, nucleoids are separated by a fixed distance that is not dependent on the cell division machinery (128). The presence of a hypothetical chromosomal "ruler" that dictates the positioning of segregated nucleoids is an attractive theory that could explain the regularity observed in nucleoid spacing (129).

The location of the terminus regions of *B. subtilis* and *E. coli* were also visualized by *lacO*/LacI-GFP (40, 154) or FISH in *E. coli* (105) and *C. crescentus* (65). The terminus initially appears at the center of the cell, and completion of DNA replication results in the formation of two termini that separate. The termini move toward the middle of the two nascent daughter cells as the second copy of origin migrates to the potential division site. Based on these observations, Niki & Hiraga (105) proposed the existence of an active mechanism that caused the movement of the terminus to the midcell. However, the replisome model of chromosome replication and dynamics could suggest a more passive mechanism of terminus mobility. The dynamics of the origin and terminus regions in slow-growing *E. coli* is somewhat different from that observed in fast-growing cells. Mononuclear newborn cells have their single origin and terminus regions at opposite poles of the cell (Figure 2). Later in the cell cycle the origin and terminus move to the center of the cell and DNA replication ensues. The daughter origin regions migrate to the cell poles and the terminus regions remain in the center of the cell. Cell division results in daughter cells with polar origin and terminus placement.

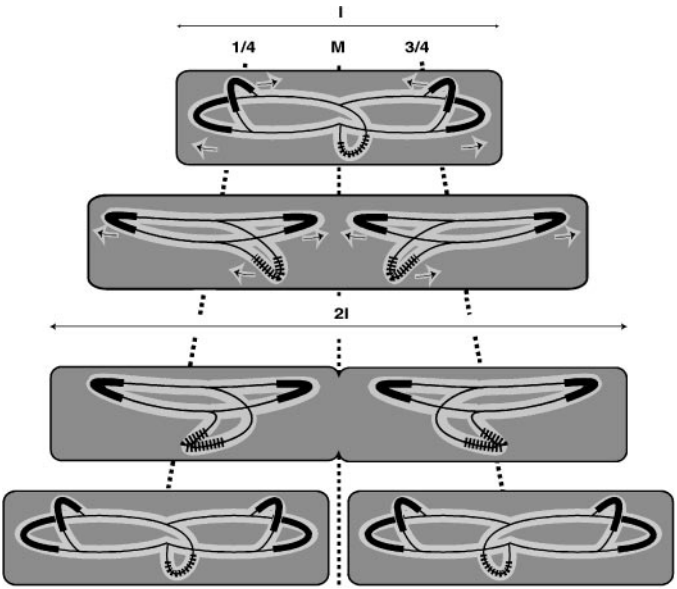

Figure 1 Origin of replication (*oriC*) and terminus localization in fast-growing *E. coli* cells. Depicted is a schematic showing a newly born *E. coli* cell in rich medium. The *bar* above the cell indicates the cell length (l) immediately following division. The midcell (M) and one-quarter and three-quarter positions are indicated, as are the *oriC* regions (highlighted as *dark*) and the terminus (*hatched line*). Immediately following division in fast-growing cells there are four *oriC* regions. Two are located closer to the cell poles than the other two, which reside near the one-quarter and three-quarter positions. At this stage, the terminus region is located at the midcell. As the cell grows and eventually reaches a length that is twice that of the newly born cell (2l), the *oriC* regions that occupied both the one-quarter and three-quarter positions, migrate to the midcell. The termini migrate to both quarter positions at this stage. Subsequent cell division results in the formation of daughter cells each containing *oriC* regions at their poles and a terminus at the midcell position.

CHROMOSOME MOBILITY AND PLACEMENT

There are two proposed opposing views on the mechanism and rate of bulk chromosome movement. One body of work reported that nucleoids partition slowly as the cell elongates (145, 158). This is thought to result from the coupled transcription/translation of membrane proteins. In growing cells these processes are proposed to link active regions of the chromosome to the cell membrane, which in turn slowly drag the chromosome apart as the cell elongates [reviewed in (156)]. This model of nucleoid partitioning implicitly requires a zonal, outward expansion of the cell membrane during cell elongation. In *B. subtilis*, there is evidence that DNA is associated with the peptidoglycan layer, and outward growth of the cell

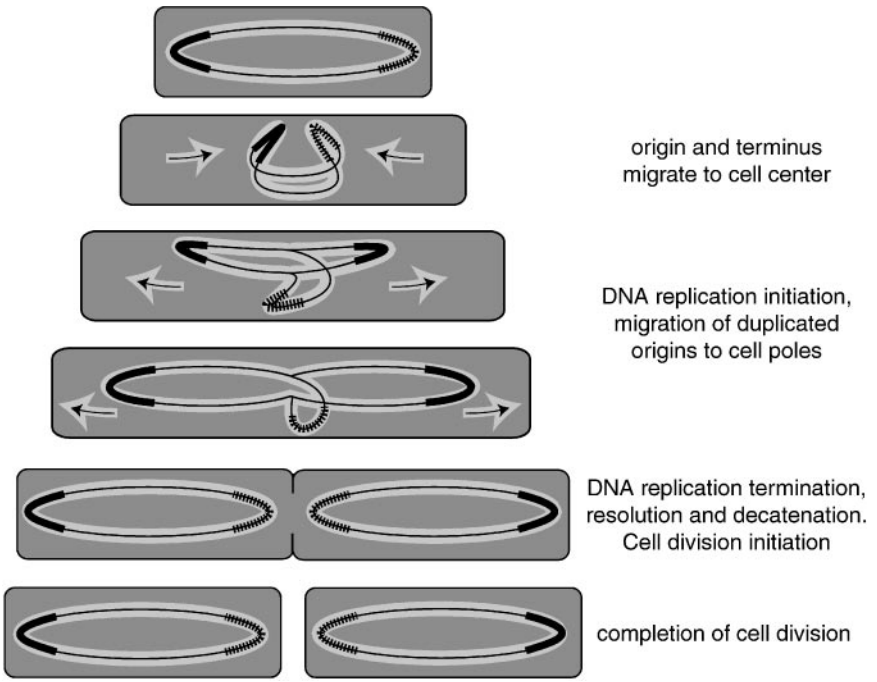

Figure 2 Origin of replication (*oriC*) and terminus localization in slow-growing *E. coli* cells. Depicted is a schematic view showing a newly born *E. coli* cell. The *oriC* regions (highlighted as *dark*) and the terminus (*hatched line*) are indicated. Immediately following division in slow-growing cells there is one *oriC* region and a single terminus. These migrate to the center of the cell, where replication initiates and duplicates the *oriC* region. The duplicated *oriC* regions rapidly migrate toward the pole of the cell. Replication then terminates, with subsequent resolution of chromosome dimers and the removal of catenanes. This is followed by the completion of cell division. Note that in at least one organism (*C. crescentus*) the localization of the origin region and ParB is required for the initiation of cell division (see text for details).

could lead to partitioning of the nucleoid via such attachments (122, 123). This, however, is not the case in *E. coli*, where extension of the cell occurs in a nonzonal manner (103, 128).

In contrast other experimental results suggest that chromosomes partition to predetermined sites in the daughter cell compartments quite rapidly, mediated by an active mechanism (9, 29, 50). Indeed, upon resumption of protein synthesis after a period of inhibition, fused chromosomes reoccupy DNA-free regions of filaments without any appreciable increase in cell length, supporting the idea of a mitotic apparatus that functions to actively segregate chromosomal DNA (145). Webb et al. (153) estimated the velocity of segregating origin regions of

B. subtilis to be 0.17 mm min^{-1}, whereas the rate of cell elongation was only 0.011–0.025 mm min^{-1}. As the rate of origin movement exceeds that of cell elongation, this finding is in conflict with those who propose that chromosome partitioning is mediated by nucleoid association with the cell membrane. Van Helvoort et al. (144) have attempted to reconcile their proposal that DNA partitioning is mediated by interactions of the transcription/translation machinery with the cell membrane and as such is a gradual process with the discovery of active DNA-partitioning proteins and centromere-like regions of DNA in *B. subtilis* (154) and *C. crescentus* (95). The authors suggest that the function of the Soj/Spo0J (*B. subtilis*) and ParA/ParB (*C. crescentus*) systems is to orient the newly replicated origin regions to different ends of the cell, after which the transcription-mediated partitioning system actually moves the bulk of the nucleoid. This is an insightful interpretation of their data, which relies on a gradual movement of nucleoids, but is not in harmony with the data of other groups that demonstrate active and rapid chromosome movements during DNA partitioning.

DNA Replication: Giving Chromosomes a Helpful Push

Replication of the circular bacterial chromosome initiates at the origin of replication and proceeds in a bidirectional manner, until halting in a region opposite the origin called the terminus. The loading of DnaA onto the origin results in localized unwinding, a process continued by DNA helicase. The unwinding of the origin permits formation of the DNA replication complex, or replisome. It was generally accepted that the replisome was mobile and replicated chromosomal DNA by moving along the template DNA in a processive manner. It has recently been shown, however, that the replisome is a structure that has a limited amount of mobility, as it is located toward the cell center (77). To demonstrate the localization of the replisome, the δ', τ, and PolC components of the DNA polymerase holoenzyme from *B. subtilis* were independently fused to GFP. The resultant fusion proteins were functional. Fluorescence microscopy revealed that the replisome is localized to the midcell for much of the cell cycle (77). Fast-growing cells with multiple chromosomes had additional foci, located at the cell one-quarter and three-quarter positions. Under conditions where DNA replication was blocked there were no observable replisome foci, supporting the proposal that the foci demarcated the position of the replisome and not just aggregations of excess replisome components. Furthermore, it has been demonstrated that the localized DNA polymerase is associated with replication forks and that replication effectively extrudes DNA following synthesis (78).

The authors propose that the spooling effect of the nascent daughter chromosomes from the replisome could impart a motive force on the DNA to aid segregation (77–79). This pushing action could be complemented by the pulling force of DNA condensation activity of the SMC proteins on the newly replicated regions of the chromosome (Figure 3). Other factors, perhaps the Par system, could impart the directionality of chromosome movement (i.e., each newly replicated origin toward opposite cell poles). Terminus mobility could also be directly

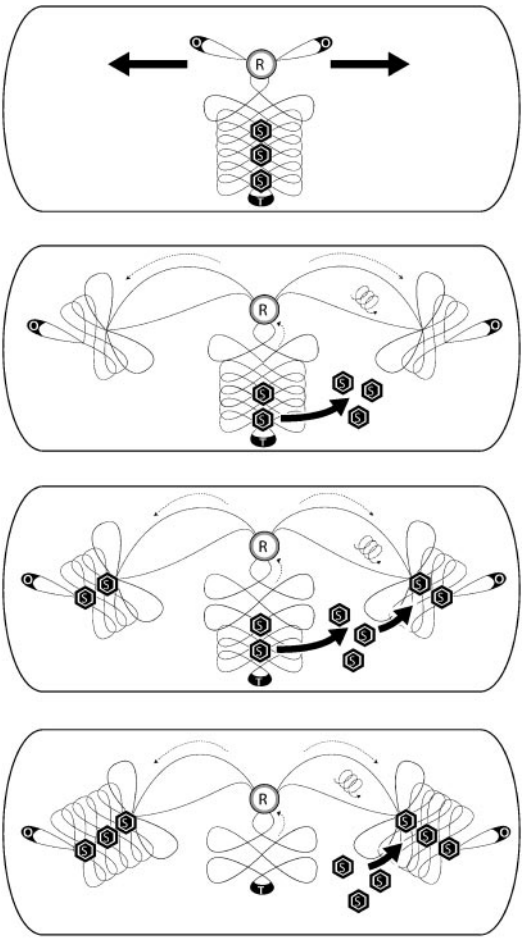

Figure 3 Model summarizing the factors contributing to bacterial chromosome partitioning. At the top of the diagram is a schematic view of a cell shortly following the initiation of DNA replication. A stationary DNA replication factory (replisome) located at the midcell is indicated (R). The newly duplicated *oriC* regions, indicated as (O), then migrate relatively rapidly toward the opposite poles of the cell. Data indicate that the driving force for movement at this stage may be the polymerization of DNA by the stationary replisome. Also shown is the dynamic role of DNA condensation proteins (S), such as MukB or SMC, in chromosome partitioning. These proteins probably disassemble from the DNA to permit the passage of replication forks and then possibly reassemble on the newly duplicated regions of the chromosome. This would lead to condensation and perhaps, in conjunction with a tethered *oriC*, result in a bulk movement of the newly replicated DNA toward opposite cell poles. The completion of DNA replication results in the localization of the terminus region at the midcell. At this location, chromosome dimers are resolved by XerCD homologs via a FtsK-stimulated recombination reaction (not shown) (see text for details), thus uniting cell division and chromosome segregation.

influenced by the replisome. As chromosomal DNA is spooled through the replication complex, the terminus would inevitably be dragged toward the midcell as the amount of initial template DNA decreases.

Experiments with *E. coli* indirectly indicate that the replisome may also be localized at the midcell. The localization of the SeqA protein was followed in synchronized populations of cells (48). SeqA is responsible for sequestering newly replicated hemimethylated DNA in *E. coli* (87). These experiments have demonstrated that foci of SeqA exhibit a bidirectional migration from the midcell distinct from the observed migration of the duplicated *oriC* regions (47, 48, 113). Because SeqA preferentially binds to newly replicated, hemimethylated DNA, this result suggests that newly replicated regions of the *E. coli* chromosome migrate from the midcell in a concerted fashion and suggests the possibility of a midcell localized replisome in *E. coli*. In contrast, in *C. crescentus*, the replisome migrates from the pole of the cell to the midcell shortly after the initiation of DNA replication (66). This variation could be a consequence of the fact that the *C. crescentus oriC* (*Cori*) remains localized at the cell pole following partitioning (65). The authors provide evidence that the replisome movement requires DNA replication, suggesting that migration to the midcell is passively mediated through displacement by newly replicated DNA.

ROLE OF CHROMOSOME CONDENSATION IN PARTITIONING

SMC and MukB are DNA Condensation Proteins

The SMC proteins of *B. subtilis* and *C. crescentus* and the MukB protein of *E. coli* are functionally and structurally similar proteins that participate in chromosome partitioning (14, 65, 94, 99, 109, 107). The two proteins belong to the larger SMC family of proteins whose functions include sister chromatid cohesion, DNA repair, and chromosome condensation and segregation in eukaryotes [reviewed in (138)]. Eukaryotic SMC proteins function as heterodimers, whereas their eubacterial counterparts are homodimers, as there is only one *smc* (*mukB*) gene per genome. Due to the relatively large size of the *B. subtilis* SMC (BsSMC) and *E. coli* MukB proteins (135 kD and 176 kD), it has been possible to view their structure using the electron microscope (94, 107). The dimeric complexes consist of two globular domains separated by an atypical antiparallel coiled-coil domain (94, 107). In the central portion of the coiled-coil domain a flexible hinge region allows the complex to adopt many conformations ranging from a straight, rod-like form to a V-like structure to a point where the two arms are completely closed (94, 107). Purified MukB binds DNA, and its amino-terminal region contains a nucleotide-binding domain that binds both ATP and GTP (94). The binding and hydrolysis of ATP is not required for MukB to bind DNA, but incubation of MukB with single-stranded DNA results in an increase in ATPase activity (107). The dynamic structure of MukB, coupled with its DNA and nucleotide-binding

properties, has led to the suggestion that this protein acts as a motor that, in part, drives chromosome segregation (107, 109).

MukB, SMC, and Chromosome Segregation

The *mukB* gene was originally isolated in a genetic screen to detect mutants defective in chromosome segregation (49, 109). This screen employed a plasmid-borne copy of the *lacZ* gene, the expression of which was repressed by a chromosomally expressed *lac* repressor. Mutated cells were plated onto indicator plates containing X-Gal. Those mutants that elicited chromosome partitioning defects (i.e., anucleate cell production) produced a blue pigment due to the β-galactosidase expression from the randomly segregated *lacZ*-containing plasmid into the anucleate cells in which no *lac* repressor was being expressed. Another mutant generated in the same screen, *mukA*, was mapped to the *tolC* gene, mutations in which are pleiotropic and exhibit reduced resistance to colicins and antibiotics, abnormal OmpF and OmpC expression, and increased sensitivity to SDS (49, 108, 155). At present, the involvement of TolC in chromosome segregation remains unclear. The two genes immediately upstream of the *mukB* in *E. coli* are also involved in chromosome partitioning (163). These genes, *mukE* and *mukF*, are essential for accurate chromosome segregation, and MukB, MukE, and MukF interact, possibly via the carboxyl terminus of MukB (163, 164).

Mutations in *mukB* or *B. subtilis smc* result in a chromosome partitioning defect. MukB point mutations result in slow growth, 5% anucleate cell production at 22°C and highly restrict growth at 42°C in rich media. Both *mukB* and *smc* null-mutants do not form colonies in rich media at elevated temperatures (14, 99, 109). At 22°C the *mukB* null-mutant produces a mixture of anucleate cells, cells with bisected nucleoids and septated pairs of cells, one of which is anucleate (109). Incubation of this *mukB* mutant at 42°C causes the cells to elongate and chromosome positioning becomes erratic; extended incubation at 42°C results in cell division, with anucleate cells comprising 40%–60% of the population (109). Similarly, null mutations in both the *B. subtilis* (14, 99) and *C. crescentus* (66) *smc* genes result in the inability to form colonies in rich media at elevated temperatures. A significant fraction (10%–15%) of the cells in *B. subtilis* strains bearing *smc* null-alleles was anucleate (14, 99). Moriya et al. (99) described how a strain with the *smc* gene under control of the IPTG inducible *spac* promoter showed an increase in anucleate cell production that correlated with decreasing concentrations of inducer. A *C. crescentus* strain in which the *smc* gene was insertionally inactivated did not produce a significant amount of anucleate cells at either the permissive or the elevated temperatures (65). Instead, a majority of the cells were unable to divide, arresting at a predivisional stage, and did not elongate. Examination of stained chromosomes in these "stalled" cells showed that the distribution of the nucleoids was impaired.

In *B. subtilis*, SMC is localized to the nucleoid (14, 42). Britton et al. (14) also described 1 to 2 foci of BsSMC at polar, although chromosome associated, sites and suggested that these sites might represent regions where nucleoid condensation

was active. In contrast, Graumann et al. (42) described these polar foci as being separate from the nucleoid. The same group also indicated that the polar foci were present in only newborn cells. In older cells with segregated chromosomes there were no detectable polar BsSMC foci, suggesting that BsSMC moves from the polar sites to the chromosome prior to or during nucleoid partitioning. The methods used to generate the above data could explain why similar experiments yielded (albeit slightly) different results. Britton et al. (14) used a BsSMC-GFP chimera to detect BsSMC localization, whereas Graumann et al. (42) employed immunofluorescence microscopy with BsSMC-specific antibodies. Either the BsSMC-GFP fusion protein has different localization properties than the wild-type protein, or the fixation protocol required for IFM resulted in a dissociation of the polar foci from the chromosome borders. The carboxyl-terminal region of BsSMC appears to be required for nucleoid association but not formation of polar foci (42). Both the polar and nucleoid-associated forms of BsSMC foci were visible in aseptate filaments generated by the depletion of the essential cell division protein FtsZ from the cell. BsSMC, therefore, does not require the cell division apparatus to dictate its pattern of localization.

Another phenotype associated with a disruption of MukB/BsSMC function is abberrant chromosome condensation [(42, 56, 99); reviewed in (138)]. *smc* knockouts in *B. subtilis* and *C. crescentus* result in a decondensation or relaxation of the nucleoid (14, 65, 99). Analysis of the localization of the *oriC*-associated Spo0J protein in *smc* mutants of *B. subtilis* revealed that the origin region was mislocalized in the absence of BsSMC (14, 99). It is possible that bacterial MukB/SMC proteins condense the nucleoid during or prior to segregation to provide tension that allows the chromosomes to partition into daughter cell compartments. The presence of a mitotic apparatus linking the origin region of the chromosome to a polar structure could act as an anchor to maintain the tension imparted by chromosome condensation, thus ensuring the direction the chromosomes will recoil upon decatenation and resolution. Alternatively, nascent replicated chromosomes emerging from the centrally positioned replisome (77) could be directed toward the cell poles by the origin region localization systems (the *par* system) and continuously condensed at the polar regions (see Figure 3). As discussed above, a *mukB* null-allele cannot be combined with an inactivated *hupA* gene, which encodes the HU2 subunit of the *E. coli* DNA-binding protein HU, again stressing the importance of nucleoid structure and condensation in chromosome segregation (64).

A *mukB* null mutation also cannot be combined with an *ftsK* allele that expresses only the amino terminus (cell division domain) of the protein in *E. coli* (165, 166). In *B. subtilis*, an *smc spoIIIE* double-deletion mutant grows poorly and accumulates suppressor mutations (13). In a *B. subtilis* strain with an inducible copy of *smc* and either a *spoIIIE* null-allele or the *spoIIIE36* allele, which harbors three missense mutations, growth ceased after several generations in media without inducer. BsSMC depletion also caused the appearance of cells with nucleoids bisected by invaginating septa (13). The trapping of nucleoids at the closing septum is associated with a loss of function of SMC and SpoIIIE. Britton & Grossman

(13) show that when the *spoIIIE* and *smc* mutations are combined, the proportion of cells with the bisected nucleoid phenotype is 38% compared to ~10% of cells with the *smc* knockout alone. The earlier work of Sharpe & Errington (126) also showed that in a *spoIIIE* mutant, chromosomes could be trapped by septa. Thus, in situations where the nucleoid is decondensed (by virtue of a mutation in *smc* or *mukB*) and cannot move straggling regions of the chromosome (because of the lack of functional SpoIIIE or the SpoIIIE-like domain of *E. coli* FtsK), the trapped nucleoid phenomenon is exacerbated, strengthening the case for a role for each protein in chromosome segregation.

Interestingly, a spontaneous mutation that suppressed the growth defect phenotype of *E. coli* cells bearing a *mukB* deletion was shown to map to the *topA* gene (encoding topoisomerase I) (121). This and other mutations in *topA* were found to be general suppressors in a variety of *mukB*, *mukE*, and *mukF* mutants. The authors propose a mechanism by which the increased negative supercoiling imparted on the chromosomes by the unrestricted activity of DNA gyrase in the *topA*/*muk* mutant strains is responsible for increasing the compactness of the nucleoid and thus aiding segregation into daughter cells. Inhibition of DNA gyrase activity by coumermycin reverses this suppression and a typical *mukB*$^-$ phenotype was observed. Some gyrase mutants are defective in segregating their chromosomal DNA (134). Possibly both MukBEF and negative supercoiling act in concert to promote a compact nucleoid structure (121) that favors efficient partitioning.

INTEGRATION OF CHROMOSOME PARTITIONING WITH THE CELL CYCLE

A Link Between Cell Division and Replication Termination Events

It has been demonstrated that resolution of chromosome dimers via the *dif*-XerCD system requires cell division (136). It was subsequently discovered that it was not the lack of cell division that resulted in the loss of dimer resolution but that the carboxyl terminus of the cell division protein FtsK was required for resolution of dimers (120, 135). FtsK is a member of the SpoIIIE family of DNA translocases that localize to the septum during cell division (8, 149, 159, 161, 166). The amino-terminal domain of FtsK contains a membrane-spanning region that is essential for cell division (8, 30, 149, 166), and the carboxyl terminus contains an ATP-binding site and is required for efficient chromosome partitioning (8, 86, 165). Strains carrying deletions in the carboxyl-terminal region of FtsK do not resolve chromosomal or plasmid-borne *dif* sites, but there is no effect on the resolution of the plasmid recombination sites *cer* and *psi*, which also require XerCD for resolution (19, 120, 135, 139). It has been demonstrated that FtsK functions to activate resolution of chromosome dimers via XerCD late in the cell cycle (120). Dimer resolution via XerCD is a sequential process where a Holliday junction intermediate between *dif* sites is formed by the XerC-mediated exchange of a first

pair of DNA strands. The carboxyl-terminal domain of FtsK functions to activate the XerD-catalyzed exchange of the second pair of strands (6). The localization of FtsK to the midcell, through association with other cell division proteins, would effectively deliver it to dimer chromosomes that have been trapped or guillotined by the closing septum. This would insure that resolution takes place immediately prior to completion of cell division and explain the requirement for cell division for *dif*-mediated resolution of dimeric chromosomes (120, 135). Another perhaps complementary role for FtsK in nucleoid partitioning is in DNA translocation. The similarity of the carboxyl terminus of FtsK with SpoIIIE suggests that FtsK could remove trapped chromosomes into the daughter cell compartments at the last stages of septal closure (86, 120, 135).

A similar relationship between replication termination events and partitioning has been discovered in *B. subtilis* (80). As in *E. coli*, chromosome dimers are resolved by a XerD homolog, RipX. Mutations in the gene encoding RipX result in the generation of a significant fraction of anucleate cells. These authors discovered a synthetic effect when a *ripX* mutation was combined with a null mutation in *rtp*, a gene encoding a replication termination DNA-binding protein that blocks replication forks in the terminus region. A *ripX rtp* double mutant generated twice as many anucleate cells as the strain bearing the single *ripX* mutation. Notably a strain containing only a *rtp* null mutation did not exhibit a partitioning defect. Interestingly, the *rtp* null mutation, when combined with a *spoIIIE* mutation, also exhibited a synthetic partitioning defect (80). The authors suggest that these effects result from overreplication of the terminus region in the absence of *rtp*, which would generate a double-stranded end that is highly recombinogenic. In the *rtp spoIIIE* double mutant, an increase in anucleate cells is observed because SpoIIIE, in a role analogous to FtsK in *E. coli*, is probably required to enhance or facilitate the dimer resolution catalyzed by RipX.

ParA AND ParB: CELL CYCLE REGULATORS OR COMPONENTS OF A BACTERIAL MITOTIC APPARATUS?

The positioning of the origin regions and the regular mobility of newly replicated origins suggested that an active mechanism is responsible for the movement and positioning of the bacterial nucleoid. However, no structure similar to the mitotic spindle has been observed or detected in the eubacteria. The origin of replication is the first region of chromosomal DNA to be replicated (by definition) and it displays a dramatic dynamic localization pattern (see section above). Therefore, it is possible that the determinant for chromosomal DNA polarity is the origin. However, experiments have shown that *oriC* is not the actual determinant of bulk chromosome positioning. For example, in *E. coli oriC*-containing plasmids are not stably inherited (106, 112). Introduction of the partitioning functions of F-factor into such a plasmid, however, can confer stable inheritance (106). One fifth of the chromosome, centered around the origin region, colocalizes with the origin. Likewise one fifth of the chromosome, which contains the terminus, appears to

take the same position as the terminus itself (110). Thus these extended regions of chromosomal origin and terminus DNA contain the regions partly responsible for chromosome placement. Given these findings, it is apparent that there must exist a mechanism that recognizes and partitions copies of the origin to the poles of the predivisional cell. The discovery that many species of bacterial cells contain homologs of the well-characterized partitioning proteins of low-copy-number plasmids (F-factor) and bacteriophage (P1) suggests the existence of a similar, simple mitotic apparatus. Below, we briefly review the role of these proteins in the partitioning of episomes. [For a more detailed review of partitioning mechanisms in these and other unit-copy plasmids, the reader is referred to (41)]. We then summarize the experimental evidence for the role of the cellular homologs of plasmid partitioning genes in chromosome partitioning.

Plasmid and Bacteriophage DNA Segregation

Low-copy-number plasmids, like chromosomes, must possess mechanisms to ensure that efficient replicon partitioning occurs following DNA replication. In particular, experiments with bacteriophage P1, which can exist as a single-copy episome when acting as a lysogen, and F-factor have provided insights into the function of the widely occurring partitioning proteins ParA and ParB. The plasmid-encoded *parA* (*sopA*) and *parB* (*sopB*) gene products from bacteriophage P1 and P7 (1, 4, 5, 88) or F-factor (97, 98, 112) have been demonstrated to be essential for efficient partitioning. Conserved homologs of *parA* have been sequenced from at least 10 different plasmids or prophages. The deduced amino acid sequence of *parA* shows that it is homologous to a large distinct family of ATPases (74, 100), including the *minD* gene product of *E. coli* and *B. subtilis*. MinD, in concert with other *min* gene products such as MinC and MinE (in *E. coli*)/DivIVA (in *B. subtilis*), specifies the topological position of the cell division septum (27, 82, 91, 146). The plasmid *parB/sopB* gene encodes a sequence-specific DNA-binding protein (23, 24, 98, 151). For example, the *parB* gene product from the prophage form of *E. coli* bacteriophage P1 binds to repeated sequences of dyad symmetry that lie immediately following the end of the *parB* coding region (23, 38, 93). This *cis*-acting sequence, called *parS* (sopC in F-factor), is required for efficient plasmid partitioning and is regarded to function as the plasmid equivalent of a centromere. Sequences of this type have been identified adjacent to the *parAB* operons of several different plasmids, and mutant plasmids with deletions in this *cis*-acting region cannot be stably maintained. In addition, P1 plasmids containing only the *parS* sequence do not partition efficiently unless the *parA* and *parB* gene products are supplied in *trans* (93). Therefore, these experiments demonstrate that the *parS* sequence functions as the plasmid equivalent of a centromere during the partitioning process; the binding of ParB to the *parS* centromere sequence is hypothesized to function in daughter replicon pairing as a prelude to partitioning (33, 92).

Experiments with purified ParA and SopA have demonstrated that they possess a weak ATPase activity in vitro (20, 24, 152). ATPase activity can be stimulated in the presence of purified *parB* gene product and *parS* DNA, indicating that these

two proteins interact with each other in vivo (21, 24). Experiments with P1 and P7 proteins have identified the sequences in ParA and ParB that are essential for this interaction (118). The *parA* gene products of plasmid and phage bind to DNA sequences within their own promoter and function to regulate expression of the *parAB* operon (36, 37, 45, 51). This autoregulation is critical for proper partitioning, as overexpression of *parA* or *parB* leads to partitioning defects (1, 38). The precise biochemical function fulfilled by ParA ATPase activity is not clear. It has been proposed that the ATP hydrolysis functions to break apart paired sister replicons as a necessary prelude for partitioning. Alternatively, it has been suggested that the hydrolysis of ATP is required for movement of the sister plasmids toward the cell poles. Experiments have shown that both ATP and ADP can be bound to ParA (21). Biochemical experiments have demonstrated that the double-stranded DNA-binding activity is influenced by the nucleotide-bound state of ParA (12). When bound to ADP ParA represses transcription by binding to the *parAB* operon promoter region, and when bound to ATP it interacts with the ParB-*parS* complex (12). ParB presumably regulates this transition between these two activities by stimulating ATPase activity.

Subcellular localization experiments have demonstrated that both P1 (40) and F-factor (40, 104) are located at the midcell of newly formed cells. When they are duplicated in growing cells, each daughter episome migrates to the one-quarter and three-quarter positions of the predivisional cell. Plasmids without the partitioning proteins exhibited a somewhat random localization, occupying regions of the cell that lacked nucleoid material (104). Taken together these results show that ParA (SopA) and ParB (SopB) are responsible for directing the orientation and partitioning of bacteriophage P1 and F-factor. An important question is whether these proteins, in particular the centromere-binding protein ParB/SopB, bind to the *parS*/*sopC* sequence and travel with the segregating plasmid, or rather exist in preformed localized foci that function as receptors for the migrating daughter plasmids. In the case of P1, experiments have demonstrated that the formation of localized foci depends on the presence of *parS* sequences (34) thus supporting the idea that ParB migrates with the plasmid DNA during partitioning. The data are not as clear for F-factor. Experiments using immunofluorescence microscopy have shown that the formation of SopB foci depends on the plasmid centromere sequence (*sopC*) (51). However, using a SopB-Gfp fusion, Kim & Wang (73) have shown that foci of this fusion protein form near the poles of the cell even in the absence of *sopC* sequences. These authors even demonstrated that a fusion lacking the SopB DNA-binding sequences exhibited a similar pattern of localization (73), indicating the existence of a cellular receptor for SopB.

Role of the Cellular Homologs of ParA and ParB in the Partitioning of Chromosomal DNA

Chromosomally encoded homologs of the plasmid partitioning genes *parA* and *parB* have been identified in the genome sequences of at least 29 different eubacterial species (162). Most of our knowledge regarding the role of these proteins

in partitioning is mainly derived from experiments with *B. subtilis* and *C. crescentus*. These proteins, Soj/Spo0J in *B. subtilis* and ParA/ParB in *C. crescentus*, possess biochemical properties that are similar to those described for the plasmid homologs.

Genetic experiments in *B. subtilis* were the first to implicate the chromosomally encoded *par* homologs in the partitioning of chromosomal DNA. The gene encoding the ParB homolog, *spo0J*, was originally identified as being required for an early stage of the sporulation pathway (102). Specifically, *spo0J* is required for the expression of relatively early-acting sporulation genes, which require the Spo0A transcription factor for expression. The gene encoding the ParA homolog, *soj*, can suppress the sporulation defect in *spo0J* mutant strains by restoring Spo0A-activated transcription (62). Mutations in *spo0J* result in a 100-fold increase in the generation of anucleate cells, suggesting an active role in chromosome partitioning (62). Like its plasmid homolog, Spo0J possesses double-stranded DNA-binding activity. Spo0J binds to relatively conserved 16-bp *parS* sequences, clustered around the *soj spo0J* operon (84). These sequences occur approximately ten times within the origin-proximal 20% of the *B. subtilis* genome (84).

Foci of Spo0J colocalize with the origin of replication region (39, 83, 85). Furthermore, experiments that assayed prespore-compartment-specific gene expression indicated that *spo0J* mutants had defects in the orientation of the prespore chromosome (127). One important experiment revealed that an unstable plasmid can be stabilized by the presence of a Spo0J-binding site. This stabilization was also dependent on the presence of Spo0J and Soj (84). These findings prompted the hypothesis that Spo0J was involved in spatial organization of the origin proximal regions of the chromosome and thus is one component of the *B. subtilis* mitotic apparatus. In support of this idea, Soj and Spo0J can function to partition a plasmid bearing *parS* sequences in *E. coli* (162). In contrast to these findings, which indicate a role for Spo0J in partitioning, it appears that Spo0J is not required for proper positioning or movement of the chromosomal origin of replication in *B. subtilis* (153). It is possible that this result indicates that *B. subtilis* may have an additional system for directing the *oriC* to the cell poles. Indeed, recent evidence has shown that the division site-selection protein (91) DivIVA has an additional role in orienting the *oriC* region of the *B. subtilis* chromosome (142). These authors found that *divIVA* mutants displayed a defect in both the expression of early sporulation genes and in DNA partitioning during sporulation. An alteration in prespore-specific transcription indicated that the *divIVA* mutant strain had a defect in the positioning of *oriC* at the prespore pole (142). The authors speculate that the widespread distribution of *divIVA* homologs in other gram-positive organisms, particularly those lacking a *min* system, possibly indicates a primary role for this protein in chromosome partitioning (142).

Deletion of *soj* (*parA* homolog) does not result in a DNA segregation defect, but it is required for the stability of *parS*-containing plasmids (162). Biochemical experiments have shown that Soj inhibits Spo0A-activated transcription by binding to single-stranded DNA in the transcription open complex (15). This unusual mechanism of repression is most evident in the absence of Spo0J; using in vivo

crosslinking followed by immunoprecipitation, Soj preferentially associated with early (*spoII* class) sporulation promoters in a *spo0J* mutant (117). Soj also reinforces this negative regulation of early sporulation promoters by repressing the expression of *spo0A*, the gene encoding their transcriptional activator (116).

Thus the Soj/Spo0J system of *B. subtilis* operates a checkpoint that couples chromosome partitioning to developmental gene expression. When partitioning is incomplete, it is likely that Soj represses the expression of Spo0A-dependent promoters. The completion of partitioning results in Spo0J inactivating the repressive activities of Soj. Although the details are not yet known, this regulation involves the bipolar localization of Spo0J. In other words, partitioning is probably sensed by this signal transduction system when the chromosomal origin of replication and thus Spo0J arrives at the poles of the cell.

How does the spatial location of Spo0J regulate Soj activity? Soj displays a remarkable pattern of subcellular localization. Soj oscillates from one cell pole to the other within a period of approximately 20 sec. Such oscillation is dependent on Spo0J, and in its absence Soj localizes to the nucleoid (90, 117). It can be inferred from these observations that the oscillating form of Soj does not repress early-sporulation promoters and that the nonoscillating, nucleoid-associated form does direct repression. The conversion between these two forms may be linked to Soj ATPase activity. In the related ATPase, MinD, which regulates division site selection in *E. coli*, polar oscillation is regulated by the MinE protein (43, 57, 119), which stimulates MinD ATPase activity (57). In support of this idea, a Soj mutant predicted to be defective in ATPase activity failed to exhibit bipolar oscillation (117).

The ParA/ParB system has also been studied in some detail in *C. crescentus*. Like *B. subtilis*, the operon containing *parA* and *parB* is near the origin of replication region (*Cori*) (95). ParB binds to several *parS* sequences adjacent to the *par* operon and the *Cori* [(95); J. Easter, R.M. Figge & J.W. Gober, unpublished data). Cell cycle subcellular localization experiments have shown that a single focus of ParB is found at the poles of cells containing a single chromosome (95, 96). Initiation of DNA replication results in the relatively rapid formation of an additional ParB focus at the opposite pole of the cell. This dynamic pattern of localization reflects the movement of the newly duplicated *Cori* region during the cell cycle (65). ParA localizes at the cell poles; however, it has not been assayed for polar oscillation in living cells (95). Overexpression of either ParA or ParB causes cell filamentation and a marked chromosome partitioning defect. Simultaneous overproduction of both proteins has little effect on cell division but still results in a relatively severe partitioning defect (95). These results suggest that a proper balance of ParA and ParB concentration in the cell is required for proper progression of the cell cycle. In contrast to *B. subtilis*, inactivation of either *parA* or *parB* in *C. crescentus* is lethal to the cell. In ParB depletion experiments, loss of the protein leads to cell filamentation and eventual death (96). Under these conditions, filamentation is the result of the inability to form FtsZ rings, the earliest cytological event in bacterial cell division. An identical phenotype with regard to FtsZ ring formation is observed when ParA is overexpressed. Thus increasing the ratio of ParA to ParB inhibits cell division (96). Based on these results it has been hypothesized

that ParA and ParB in *C. crescentus* operate a cell cycle checkpoint that couples partitioning to cytokinesis (96). With analogy to *B. subtilis*, it is proposed that a ParA cell division block is probably relieved upon the arrival of ParB to opposite poles of the cell. Indeed in this regard the formation of bipolar ParB foci precedes the formation of an FtsZ ring by 20 min (96). The role of ParA and ParB in directing chromosome partitioning still remains unclear.

PERSPECTIVES

The question of how the bacteria segregate their chromosomes with such a high degree of fidelity is now being addressed from a number of approaches. Origin regions are segregated and positioned by an active mechanism. The ParA/ParB system or an analogous but elusive centromere-binding protein may be partly responsible for the localization of the origin to the cell pole. Bulk chromosome polarity could be imparted simply by the order in which the chromosomal DNA is replicated; with the first regions to be duplicated, the origins, being pushed and pulled to the cell poles by the action of the replisome and SMC proteins (Figure 3). Postreplication, poleward mobility of the origin, has been described in many species and can be explained by the above mechanisms, but what of the movement of the origin to the center of the cell prior to replication in slow-growing cells? Does SMC play a role in relocalizing chromosomal DNA prior to DNA replication? Is there a scaffold protein present in the cell in which the origin, and perhaps bulk chromosomal DNA, can track along? What are the cues that trigger SMC to associate with the nucleoid, condense the nucleoid, and presumably permit decondensation of the nucleoid prior to DNA replication?

One additional persistent question is: What determines the location of the cell poles and the midcell? Insights from cell division studies in *E. coli* have implicated the *min* system in determining that the central potential division site should be used for division (rod-shaped organisms can be thought of as having three potential division sites, one at the cell center and one at either pole, which are relics of previous division events). This question remains: What is the descriptor that the cell employs to indicate a pole? The periseptal annulus, a ring-like structure positioned on either side of the midcell potential division site that, upon cell division, results in a single annulus at the cell pole, is a candidate for a pole "tagging" structure (17). Other landmarks such as polar peptidoglycan structure(s) or membrane domains could also help the cell determine its poles.

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