Chromosome segregation in Eubacteria
Kit Pogliano*, Joe Pogliano and Eric Becker

It is now clear that bacterial chromosomes rapidly separate in a manner independent of cell elongation, suggesting the existence of a mitotic apparatus in bacteria. Recent studies of bacterial cells reveal filamentous structures similar to the eukaryotic cytoskeleton, proteins that mediate polar chromosome anchoring during Bacillus subtilis sporulation, and SMC interacting proteins that are involved in chromosome condensation. A picture is thereby developing of how bacterial chromosomes are organized within the cell, how they are separated following duplication, and how these processes are coordinated with the cell cycle.

Introduction
Forty years ago, Jacob, Brenner and Cuzin presented a characteristically elegant model for bacterial chromosome segregation, in which the chromosomes, attached to the cell envelope near mid-cell, were separated by localized envelope growth between them [1]. Although this compelling model still appears in textbooks, recent cell biological experiments have revealed that bacterial chromosomes rapidly separate in a manner independent of cell elongation, rendering it unlikely that the model of Jacob et al. [2–6] is entirely correct. Central to the renewed debate has been the ability to precisely determine the subcellular distribution of proteins and DNA molecules within bacterial cells. In slowly growing Escherichia coli [7], Bacillus subtilis [8,9] and Caulobacter crescentus [10], origins and termini localize towards opposite poles of newborn cells (Figure 1). In B. subtilis [11,12] and E. coli [13,14], both regions move toward the cell center, where a stationary DNA replication factory catalyzes both leading and lagging strand synthesis [11]. Following origin duplication, and likely at a specific time in the cell cycle, the origins migrate towards opposite cell poles, while the termini separate close to or shortly after cell division, following chromosome decatenation [15**]. There are interesting variations on this emerging picture. For example, in C. crescentus, the DNA replication factory slowly marches from the cell pole to the mid-cell [16], while one replicated origin stays at the pole and the other migrates to the opposite pole [10]. When multiple chromosomes and plasmids co-exist in the same cell, similar replicons typically target to distinct sites within the same general region of the cell. For example, in Agrobacterium tumefaciens, which has one circular and one linear chromosome and two plasmids, each chromosomal origin and plasmid occupies distinct sites near the cell pole [17*].

For simplicity here, we will consider bacterial chromosome segregation to consist of four steps, (i) the movement of newly replicated origins from mid-cell towards the cell poles, (ii) the anchoring of the origin to specific locations within the cell, (iii) the separate condensation of newly replicated chromosomes, and (iv) the resolution of dimeric and catenated chromosomes, although we recognize that these steps might not be completely distinct. These events are almost certainly coordinated with specific steps of cell division, and we will end this review by touching on this nearly uncharted territory. In this review, we discuss recent experiments that have shed light on each of these topics.

Origin movement
The rapid separation of origins through the viscous cytoplasm is likely to require a force-generating mechanism. Although the recently discovered cytoskeleton-like structures within bacterial cells (some of which are involved in plasmid segregation) raise the possibility that similar structures might be involved in chromosome segregation [18**–20**], the observation that DNA polymerase is a motor protein which resides in a stationary replisome, has led to the ‘extrusion-capture’ model for chromosome segregation [4*,11,21]. This model proposes that DNA replication itself pushes newly replicated DNA towards opposite sides of the cell, where the origins are captured by an as yet unidentified origin-binding protein. Vectoral origin movement has been proposed to be maintained by coupled transcription, translation and protein export, as well as factors involved in chromosome compaction, such as SMC (structural maintenance of chromosomes) [22,23]. Although the extrusion-capture model is appealing, certain results contradict with its most simple version. For example, in E. coli chromosomes engineered to initiate replication from integrated plasmid origins instead of the chromosomal origin, the chromosomal origin still localizes to the cell poles before origin-distal regions, emphasizing...
the importance of capturing chromosomal regions at specific locations within the cell [24].

**Chromosome condensation**

In growing bacteria, the replicating chromosome assumes a bi-lobed structure that occupies a small fraction of its volume in solution. This compaction crucially depends on negative superhelicity, which is maintained by topoisomerases, and also by the bacterial orthologs of the SMC proteins [25]. SMC proteins have a centrally located coiled-coil domain flanked by large globular domains that interact upon dimerization to form an ATPase. A flexible hinge region within the coiled-coil domain allows the protein to bend into V-shape molecules, perhaps regulating SMC dimerization and DNA binding activity [26,27,28**,29]. Eukaryotes typically have multiple forms
of SMC that participate in many different aspects of chromosome dynamics, including sister chromosome cohesion, DNA condensation and DNA repair (reviewed in [30,31]), whereas bacteria usually have either a single SMC, or a functionally equivalent distant relative, MukB (as in the case of E. coli), that appear to act primarily in DNA condensation [32]. In B. subtilis, C. crescentus and E. coli, smc or mukB mutants have decondensed chromosomes and chromosome segregation defects [10,33–35], but in B. subtilis, the newly replicated origins still move rapidly towards the cell poles [36]. Importantly, these defects can be suppressed by increasing the negative superhelicity of the chromosome, emphasizing the importance of supercoiling in chromosome compaction [21,25,37].

In many bacteria, as in eukaryotes, SMC interacts with accessory proteins such as E. coli MukE and MukF [38] and the recently identified and highly conserved B. subtilis ScpA and ScpB [39**,40**]. Mutants lacking either ScpA or ScpB have phenotypes identical to the smc mutant, and ScpA, ScpB, and SMC form a complex frequently located near mid-cell or quarter-cell positions [39**,41,42**]. The similarity in the position of this complex with the location of the DNA replication machinery suggested that SMC complexes might be closely associated and co-localized with the replisome, which may facilitate their ability to organize and condense newly replicated chromosomes. However, the picture that has emerged from localization studies is more complex. Although SMC proteins do form foci near replisomes in some cells, they are frequently located away from the replisome [38,42**,43–45]. Furthermore, chromatin immunoprecipitation experiments demonstrate that SMC binds many chromosomal regions, including sequences that have not yet been replicated in synchronized cell cultures [42**]. Thus, it is likely that SMC complexes both refold the chromosome after replication and maintain global chromosome organization.

Resolution of catenated chromosomes: the peculiarities of circularity

The final stages of chromosome segregation in bacteria with circular chromosomes (the majority of bacteria) include the resolution of chromosome dimers, which form when circular daughter chromosomes undergo an odd number of homologous recombination events [46], and the decatenation of topologically linked circular DNA molecules. Remarkably, in E. coli, both events are spatially and temporally regulated, occurring specifically at mid-cell during the final stages of cell division. This regulation is conferred by FtsK, an essential and conserved cell division protein consisting of two domains, an amino-terminal membrane domain involved in cell division, and a carboxy-terminal ATPase domain capable of moving along DNA in an ATP-dependent manner. This DNA tracking domain localizes the enzyme responsible for chromosome decatenation (TopoIV) to the division site, and also acts on XerC and XerD [47,48], the site-specific recombinase that mediates chromosome dimer resolution. It appears that FtsK aligns the dif sites at which recombination occurs [49], modifies the XerC/ XerD/dif site interaction to promote XerC-mediated resolution of the Holliday junction [50**], and finally exports one resolved chromosome into the appropriate daughter cell [15**]. FtsK is a key component of the division machinery, localizing to the septum before division and recruiting other division proteins to the septum [51]. The FtsK–XerCD–TopoIV interaction likely serves to ensure that these enzymes act only on the appropriate substrates (dimeric or catenated chromosomes), as only these will remain within the plane of the invaginating septum.

Plasmid and chromosomal Par proteins

The first partitioning determinants to be characterized were the par loci found on the low copy plasmids F and P1. These loci consist of a two-gene operon and a cis-acting centromere-like region (parAB and parS respectively, using the nomenclature of P1), and are sufficient to stabilize plasmids and mediate their appropriate localization. Most bacterial chromosomes also have par loci near their origins of replication, although E. coli and its close relatives apparently encode only the more distant ParA homolog MinD, which spatially regulates cell division. The ParA proteins are ATPases that interact with ParB, which binds to the parS site adjacent to the operon [52–54]. The mechanisms by which the ParAB proteins contribute to DNA segregation remains unclear, however, it has recently been demonstrated that the analogous but unrelated ATPase of R1 plasmid (ParM) forms extended, actin-like helical filaments within cells [20**,55,56,57**]. These dynamic structures could generate the force needed for movement of plasmids to opposite cell poles, if polymerization occurred between plasmid molecules, or they could provide a linear scaffold along which the plasmids could move. It is possible that the function of ParA proteins is mechanistically similar to ParM, as the E. coli ParA homolog, MinD, also forms extended filaments in E. coli that are likely to mediate the pole to pole oscillation of MinC [19**,58]. Other oscillating ParA homologs include the E. coli plasmid pB171 [59] and the B. subtilis chromosomal ParA protein SpoJ, the oscillation of which also depends on MinD [60*].

The chromosomally encoded Par proteins are in some ways similar to the plasmid proteins, as the chromosomal par loci are sufficient to stabilize plasmids and direct their correct positioning within the cell [61,62]. In addition, mutations in the chromosomal par genes increase the proportion of anucleate cells, albeit often only during specific phases of growth [63–66]. However, there are striking differences between plasmid and chromosomal Par proteins. First, in B. subtilis, localization of the ParB homolog Spo0J depends on the chromosomal position of...
its binding site; moving this site away from the origin is not sufficient to target this new region to the location normally occupied by the origin, indicating the existence of a residual origin-targeting mechanism [67**]. Second, *B. subtilis* mutants lacking Spo0J/Soj have an increased chromosomal copy number, most likely owing to increased and asynchronous chromosome replication, suggesting that these proteins regulate chromosome replication [67**,68]. The relatively benign effects of mutations on chromosome partitioning during growth suggests the existence of additional mechanisms by which chromosomes are positioned within the cell, as is supported by recent studies of chromosome positioning during *B. subtilis* sporulation.

**B. subtilis** sporulation and the identification of origin anchoring determinants

The asymmetric cell division event that marks the onset of sporulation in *B. subtilis* has provided an ideal system for studying chromosome dynamics, and has allowed the identification of proteins required for polar chromosome anchoring (Figure 2). At the onset of *B. subtilis* sporulation, the chromosome is reorganized from the bi-lobed structure typical of growing cells to an elongated axial filament [69] in which the origin-proximal 30% of the future forespore chromosome is condensed near one cell pole [70,71]. Polar septation thereby traps the forespore chromosome in the septum, and the remaining portion is translocated across the septum by the SpoIIIE DNA translocase, an FtsK homolog that exports DNA from the cell in which it is synthesized [72,73]. The orientation of the chromosome and targeting of the chromosomal origin region to the extreme cell poles depends on specific regions within the chromosome and on specific DNA binding proteins. A large chromosomal region adjacent to, but not including the replicative origin, was shown to be necessary for polar origin positioning during sporulation [74] and two DNA sequences within and downstream

Figure 2

Chromosome segregation during *B. subtilis* sporulation. (a-c) Micrograph showing *B. subtilis* cells early in sporulation, following staining with FM 4-64 to visualize cell membranes (red) and DAPI to show DNA (blue). Prior to polar septation, the origin-proximal 30% of the future forespore chromosome condenses near one cell pole (arrow 1); the asymmetrically-positioned sporulation septum is synthesized between these two chromosome domains (arrow 2). (d) Localization of the SpoIIIE DNA translocase (green) together with cell membranes (red). The protein first forms a ring at the site of cell division, then assembles a focus at the septal midpoint, and finally it relocates to the cell pole, where it participates in the final stages of the phagocytosis-like process of engulfment [82]. Scale bars = 1 μm. (e) Model for chromosome segregation during sporulation. At the onset of sporulation, the origins (yellow-green circles) are brought to the cell poles by an interaction with RacA (purple stars), while the termini remain near mid-cell (red circles). During septation, the SpoIIIE DNA translocase (green circles) assembles at the septal midpoint, and translocates the forespore chromosome across the septum (yellow arrow).
Recent studies have demonstrated that the sporulation-specific anchoring of the origin to the extreme cell pole depends on RacA, a polarly localized DNA binding protein [76**,77**]. RacA is transiently expressed early in sporulation, and in racA mutants, the sporulation septum often fails to trap DNA, producing anucleate forespores. A mutant lacking both RacA and the Spo0J-Soj (Par) system has unexpectedly severe effects on chromosome orientation during sporulation, suggesting that the RacA protein acts together with the chromosomal par systems to mediate polar chromosome positioning during sporulation [77**]. However, because RacA is not produced during growth, it is likely that an as yet unidentified protein contributes to origin positioning during growth.

**Morphological checkpoints**

Clearly, in bacteria, as in eukaryotes, morphological checkpoints must serve to coordinate cell division with chromosome replication and segregation. One such checkpoint is likely to be conferred by *B. subtilis* Soj, which in addition to contributing to chromosome orientation and replication, acts as a transcription factor governing production of proteins required for synthesis of the sporulation septum [78]. Similarly, in *C. crescentus*, the CtrA transcription factor directly regulates chromosome replication and the production of cell division proteins (as well as proteins involved in morphogenesis), whereas the ParAB proteins couple chromosome replication and cell division [79]. In *B. subtilis* additional links between chromosome segregation and cell division include the DivIVA cell division protein, which is necessary for the polar localization of both RacA and MinCD; indeed, *divIVA* mutants produce a high frequency of anucleate forespores [80]. The polar localization of MinD is in turn required for both the assembly of Soj onto the chromosome [60*] and to regulate the polarity of SpoIIIIE DNA translocase assembly [81*]. No doubt the future will see the identification of additional proteins involved in both chromosome dynamics and septation, as well as the molecular elucidation of additional regulatory checkpoints governing the bacterial cell cycle.

**Conclusions**

The past two years have seen tremendous advances in our understanding of how chromosomal organization is achieved inside of bacterial cells. Proteins specifically involved in anchoring chromosomal origins near the cell pole have been identified. The bacterial SMC chromosome condensation apparatus has been more fully described, opening the way to address how it functions mechanistically. Some of the questions that remain to be addressed include: how do chromosomal origins find their proper positions during exponential growth? Which proteins are responsible for the rapid and directed movement of chromosomal DNA? And how is the timing of chromosome segregation regulated and coordinated with chromosome replication and cell division?

**Acknowledgements**

The author's research is supported by grants from the National Institutes of Health (KP, GM57045) and from the National Science Foundation (KP, NSF 0135955; JP, NSF 0215752). EB is supported by a National Institutes of Health postdoctoral fellowship (GM63692).

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- **of outstanding interest


References [4*-6*] provide excellent comprehensive reviews on bacterial chromosome segregation.


15. Lau IF, Filipe SR, Soballe B, Okstad OA, Barre FX, Sherratt DJ: Spatial and temporal organization of replicating Escherichia coli chromosomes. Mol Microbiol 2003, 49:731-743. This comprehensive paper reports the co-localization of distinct chromosomal regions, as well as the co-localization of these regions with the cell division protein FtsZ, and the RasA protein, which is likely to be a component of the replisome. Surprisingly, separation of the termini frequently occurs after septation, implying that FtsK must serve to translocate one terminus into the nascent daughter cell. This asymmetric DNA translocate activity is strikingly similar to that of B. subtilis SpoIIIE, which acts in the mother cell to translocate DNA into the forespore.


17. Kahng LS, Shapiro L: Polar localization of replicon origins in the multipartite genomes of Agrobacterium tumefaciens and Sinorhizobium meliloti. J Bacteriol 2003, 185:3384-3391. Not all bacteria contain a single circular chromosome, and the mechanism by which a bacterium coordinates the replication and segregation of multiple chromosomes remains unclear. Here the authors report the first study of chromosome segregation in a bacterium with two chromosomes, one of which is circular. The results indicate that independent episomes are anchored to unique positions within the cell.


19. Shih YL, Le T, Rothfield L: 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 2003, 100:7865-7870. This paper demonstrates that Min proteins produce coiled structures extending from one cell pole to another, providing new insight into the mechanism by which Min proteins function to measure the length of the bacterial cell.


28. Hirano M, Hirano T: Hinge-mediated dimerization of SMC protein is essential for its dynamic interaction with DNA. Embo J 2002, 21:5733-5744. Mutations in the hinge domain of Bacillus subtilis SMC are shown to disrupt both dimerization and DNA binding, suggesting that the hinge region might play an important role in modulating SMC-DNA interactions.


40. Soppa J, Kobayashi K, Noirot-Gros MF, Oesterhelt D, Ehrlich SD, Dervyn E, Ogasawara N, Moriya S: Discovery of two novel families of proteins that are proposed to interact with prokaryotic SMC proteins, and characterization of the Bacillus subtilis family members ScpA and ScpB. Mol Microbiol 2002, 45:59-71. The identification and characterization of a pair of conserved proteins (SCP A and Scp B) that interact with Bacillus subtilis SMC and function in chromosome condensation are described [39†,40†].


42. Lindow JC, Kuwano M, Moriya S, Grossman AD: Subcellular localization of the Bacillus subtilis structural maintenance of chromosomes (SMC) protein. Mol Microbiol 2002, 46:997-1009. This paper shows that Bacillus subtilis SMC localizes in foci at positions similar to that of the cellular replisome and that localization depends on ScpA and ScpB but not on ongoing replication. SMC was also shown to frequently, but not always, co-localize with the replisome.


592 Growth and development


A beautiful study which demonstrates that like SpoIIIE, FtsK is capable of translocating along DNA in an ATP-dependent manner, and which also elucidates the biochemical mechanism by which FtsK participates in the resolution of chromosome dimers.


This paper reports the crystal structure of ParM and describes the striking structural similarities between ParM and actin.


Here the authors clarify the controversial localization pattern of Soj (chromosomal versus polar). They also identify a novel role for MinD, a protein that spatially regulates cell division, in regulating the dynamical localization of Soj, demonstrating an intriguing link between the cell division and chromosome segregation machineries.


This paper suggests a role for Spo0J in the regulation of chromosome replication, and also demonstrates that the Spo0J binding site is not sufficient to convey positional information on new regions of the chromosome.


See annotation for [77**].


Papers [76**,77**] report the identification of a sporulation-specific protein necessary for the dramatic change in chromosome architecture which occurs at the initiation of sporulation, when the chromosome becomes anchored to both cell poles, forming an elongated structure known as the axial filament. Importantly, localization of this protein (RacA) requires DivIVA, which could explain why the latter protein has two separable roles in cell division and in chromosome segregation [80]. In addition, evidence is provided [77**,77**] that the DivIVA/RacA and Soj–Spo0j systems act in a concerted fashion to target the chromosomal origin to the cell pole.


This paper reveals an additional role for the MinCD heterodimer, which spatially regulates cell division during vegetative growth, in determining the polarity of chromosome segregation during sporulation.