Published in final edited form as:
Cell Microbiol. 2013 January ; 15(1): 45–52. doi:10.1111/cmi.12049.

Chromosome architecture is a key element of bacterial cellular organization

Jerod L. Ptacin[1] and **Lucy Shapiro**[1]

[1]Department of Developmental Biology, Stanford University School of Medicine,Beckman Center B300, Stanford, CA 94305, USA

Summary

The bacterial chromosome encodes information at multiple levels. Beyond direct protein coding, genomes encode regulatory information required to orchestrate the proper timing and levels of gene expression and protein synthesis, and contain binding sites and regulatory sequences to coordinate the activities of proteins involved in chromosome repair and maintenance. In addition, it is becoming increasingly clear that yet another level of information is encoded by the bacterial chromosome- the three-dimensional packaging of the chromosomal DNA molecule itself and its positioning relative to the cell. This vast structural blueprint of specific positional information is manifested in various ways, directing chromosome compaction, accessibility, attachment to the cell envelope, supercoiling, and general architecture and arrangement of the chromosome relative to the cell body. Recent studies have begun to identify and characterize novel systems that utilize the three dimensional spatial information encoded by chromosomal architecture to coordinate and direct fundamental cellular processes within the cytoplasm, providing large-scale order within the complex clutter of the cytoplasmic compartment.

Introduction

Bacteria are considered to be the most basal of life forms. Despite the implied simplicity of the bacterial cell, unexpected levels of organization of the molecular components within these tiny organisms exist. For instance, a recent study discovered that at least 10% of all proteins in the bacterium *Caulobacter crescentus* (*Caulobacter*) display specific and reproducible sub-cellular localizations (Werner *et al.*, 2009). In addition to protein localization, the chromosomal molecule and its cadre of bound proteins, or nucleoid, is highly compacted to fit into the relatively small cytoplasmic space, but surprisingly displays an elegant and reproducible large-scale organization. In *Caulobacter*, loci along the chromosome occupy specific and reproducible spatial coordinates within the cytoplasm that are arrayed linearly within the cell volume with respect to their genomic position (Fig. 1) (Viollier *et al.*, 2004, Umbarger *et al.*2011). This organization is maintained over successive cycles of replication, segregation, and cell division, and may therefore be considered a dynamic and heritable epigenetic feature of the species. Other bacteria display drastically distinct chromosome organizational schemes. The *Escherichia coli (E. coli)* chromosome, for example, adopts a transversally folded structure that positions the right and left arms of the chromosome toward the cell poles, and origin and terminus of replication near the cell center (Nielsen *et al.*, 2006, Wang *et al.*, 2006)(Fig. 1).

address correspondence to: shapiro@stanford.edu, jptacin@stanford.edu, phone (650) 725-7657.

The authors declare no conflict of interest.

Recent studies have revealed unprecedented insight into the structural organization of bacterial chromosomes and have characterized fundamental cellular processes that require the dynamic spatial organizational information coded in the chromosome for their function. Together, these results are beginning to reveal that three-dimensional chromosome structure is a key architectural feature that is central to bacterial cellular organization and functional physiology. Here we will discuss several recently described bacterial systems that utilize the architectural arrangement of the chromosome within the cytoplasm to facilitate the spatial regulation of key developmental events.

Chromosome architecture can facilitate the regulation of chromosome replication initiation

Although the reproducible subcellular positioning of specific chromosomal loci has been described in various bacteria, the effect of this large-scale chromosomal organization on the bacterial cell cycle for the most part is unclear. One notable exception is the dimorphic alpha-proteobacterium *Caulobacter crescentus* (*Caulobacter)*, which has integrated large-scale chromosomal organization into the basic workings of its cell cycle regulatory system. In the pre-replicative swarmer cell, the chromosomal origin of replication (*Cori*) is positioned near the old cell pole (Jensen *et al.*, 2001, Mohl *et al.*, 1997), where it is physically tethered *via* interactions between a site-specific DNA binding protein ParB, bound to *Cori*-proximal DNA sequences (*parS)*, and the polar scaffolding protein PopZ (Bowman *et al.*, 2008, Ebersbach *et al.*, 2008) (Fig.2). The master cell cycle regulator CtrA is bound to specific sequences in *Cori*, ensuring that the initiator protein DnaA cannot initiate replication (Marczynski *et al.*, 2002, Quon *et al.*, 1998) (Fig.2). To initiate the cell cycle, a phospho-relay signal causes the ClpXP protein degradation machinery to accumulate at the old cell pole where it rapidly and specifically degrades the CtrA master regulator, licensing initiation (McGrath *et al.*, 2006). Meanwhile, an unknown signal stimulates the separation of the ParB/*parS* complex from the polar PopZ network, releasing the licensed origin from its tether and allowing DNA replication to initiate (Fig. 2)(Bowman *et al.* 2010). Thus, *Caulobacter* utilizes the subcellular positioning of the chromosome origin region to efficiently and robustly regulate cell cycle progression.

Nucleoid architecture is a structural scaffold for molecular partitioning machines, including the origin segregation apparatus

To ensure organizational inheritance over generations, many bacteria have evolved dedicated mechanisms to initiate chromosome segregation at specific loci, and to actively transport these segments to specific and reproducible subcellular addresses. Many bacteria utilize DNA partitioning systems (Par systems) related to those found on plasmids to actively segregate a chromosomal centromere-like sequence, *parS*, which in *Caulobacter* has been shown to be both the site of force generation during initial chromosome segregation and the first chromosomal locus to be partitioned (Toro *et al.*, 2008). In addition to the *parS* centromere, bacterial partitioning systems consist of two additional core components: a site-specific DNA binding protein ParB, which binds to *parS*, and an ATPase ParA (Fig. 2). Seminal studies in *Vibrio cholerae* and in the *E. coli* plasmid pB171 showed that actively segregating *parS* loci follow a retracting cloud-like structure of the ATPase ParA, suggesting a conserved mitotic “pulling” mechanism for segregation (Fogel *et al.*, 2006, Ringgaard *et al.*, 2009). Further investigations revealed that ParB/*parS* complexes interact with ParA structures, stimulating ATP hydrolysis in ParA and release of ADP-containing subunits. In this way, multimeric ParB compexes follow along a shortening ParA structure *via* a burnt bridge Brownian ratchet-like mechanism (Fig. 2)(Fogel *et al.*, 2006, Ptacin *et al.* 2010*,* Ringgaard *et al.*, 2009, Vecchiarelli *et al.* 2010*,* Schofield *et al.* 2010*,* Leonard *et al.*, 2005).

Importantly, several studies demonstrate an essential role for cooperative DNA binding by ParA in formation and function of the ParA mitotic structure (Ringgaard *et al.*, 2009, Ptacin *et al.* 2010, Vecchiarelli *et al.* 2010, Leonard *et al.*, 2005, Hui *et al.* 2010), suggesting that the nucleoid itself forms a structural matrix for the assembly of a track-like structure of ParA that guides ParB movement. In organisms such as *Caulobacter*, which partition centromeres unidirectionally from one pole to the other, in addition to polarity determinants such as TipN (Ptacin *et al.* 2010, Schofield *et al.* 2010), the initial asymmetric positioning of the ParB/*parS* complex relative to the ParA/nucleoid structure may facilitate the establishment of unidirectionality in segregation (Fig. 2).

In addition to chromosomal centromere and plasmid partitioning, recent studies have identified other ParA-based molecular machines that partition diverse cargoes throughout the cytoplasm, such as carbon-fixing carboxysome organelles in *Synechococcus elongatus* (Savage *et al.* 2010) and cytoplasmic chemosensory complexes in *Rhodobacter sphaeroides* (Roberts *et al.* 2012). The latter study demonstrated a requirement for chromosomal DNA binding by the ParA component, similar to ParA-mediated DNA partitioning machines, suggesting a common mechanism that utilizes the nucleoid DNA as a structural matrix for ParA-mediated molecular partitioning systems. How the global packaging of the chromosome affects ParA-mediated cargo segregation remains an open and intriguing question.

Dynamic chromosome architecture couples chromosome replication and segregation to the spatial and temporal initiation of cell division

Thus far, we have discussed mechanisms by which chromosome architecture facilitates the initiation of chromosome replication as well as the subsequent segregation of chromosomal origin regions. To ensure proper cell cycle progression, most bacteria also contain systems that couple chromosome replication and segregation to the initiation of cell division. Several spatial sensory systems have been described that sense changing chromosome architecture during segregation and couple these signals to the regulation of the cell division machinery (Fig. 3). During chromosome segregation in *Caulobacter*, the centromere-bound ParB complex also recruits the cell-division inhibitor MipZ, a ParA/MinD-like ATPase that specifically inhibits FtsZ polymerization (Fig. 3A) (Thanbichler *et al.*, 2006). ParB binding to MipZ activates MipZ DNA binding activity, creating a nucleoid-bound gradient of this FtsZ polymerization inhibitor near the ParB/*parS* complex (Fig. 3A)(Kiekebusch *et al.* 2012). After ParB/*parS* segregation to opposite poles, the bipolar positioning of MipZ gradients along the nucleoid prevents FtsZ from initiating cell division near the cell poles and neighboring nucleoid and drives FtsZ assembly near midcell (Fig. 3A). Therefore, in *Caulobacter*, the spatio-temporal signal of dynamically changing chromosome organization coordinates chromosome segregation with the proper positioning of the cell division machinery.

E. coli and B. subtilis have evolved distinct systems to coordinate changing chromosome architecture during segregation with the positioning of the cell division apparatus, but which function using similar principles to the *Caulobacter* MipZ system. One such system is the three-component Min system that prevents FtsZ from forming pole-proximal division septa (reviewed in Bramkamp *et al.*, 2009). Min systems work in concert with another mechanism, termed nucleoid occlusion (NO), characterized thus far in *B. subtilis*, *E. coli*, and *S. aureus* (Bernhardt *et al.*, 2005, Wu *et al.*, 2004, Veiga *et al.* 2011). NO systems utilize spatial information about nucleoid organization to position an inhibitor of divisome formation over the chromosome, driving the assembly of the division apparatus toward nucleoid-free regions near midcell (Fig. 3B). Decoration of the nucleoid by NO proteins, however, is not random. The *B. subtilis* and *S. aureus* NO proteins (termed Noc) are site-specific DNA

binding proteins related to ParB ((Wu *et al.*, 2009, Cho *et al.* 2011, Tonthat *et al.* 2011)), whereas the *E. coli* NO protein (called SlmA) is predicted to adopt a fold related to TetR (Bernhardt *et al.*, 2005). These factors bind specific DNA sequences and spread to neighboring DNA to generate large nucleoprotein clusters (Wu *et al.*, 2009, Cho *et al.* 2011, Tonthat *et al.* 2011). NO protein binding sites are distributed across the origin-proximal 2/3 of the chromosome, but are conspicuously absent from the terminus region that is positioned at midcell (Wu *et al.*, 2009, Cho *et al.* 2011, Tonthat *et al.* 2011). DNA replication and segregation therefore create a post-replicative chromosomal architecture that organizes NO nucleoprotein complexes away from the cell center, and in concert with the polar Min system shepherd divisome formation to midcell between the newly segregated nucleoids as DNA replication is completed (Fig. 3B).

Structural changes in nucleoid architecture and positioning are required for proper sporulation in *B. subtilis*

In addition to regulating proper cell division during vegetative growth in most bacteria, the architectural remodeling of nucleoid structure is required for the complex chromosomal gymnastics during sporulation in *Bacillus* species. The common theme is that the remodeling process involves the coordinated activities of site-specific DNA binding proteins that are recruited to repeated non-coding DNA sequences specifically arrayed within the nucleoid structure, marking distinct 3D positions in the cytoplasmic space.

During sporulation in *B. subtilis*, the newly replicated chromosomes undergo a large-scale rearrangement into an elongated and compacted structure called the axial filament (Ryter *et al.*, 1966), in which the origin regions of each chromosome form the ends of the rod-like structure and are tethered to opposite cell poles (Fig. 3C) (Ben-Yehuda *et al.*, 2003b). Recent studies have identified repeated origin-proximal non-coding DNA sequences that are involved in the compaction of the origin-region of the axial filament and tethering of this complex to the cell pole. One important factor is the ParB homolog Spo0J, which binds to repeated origin-proximal *parS* sequences and forms a large and compact oligomeric complex (Breier *et al.*, 2007, Lin *et al.*, 1998, Murray *et al.*, 2006, Rodionov *et al.*, 2004, Leonard *et al.*, 2004). Spo0J specifically recruits the bacterial condensin complex SMC to these regions (Sullivan *et al.*, 2009, Gruber *et al.*, 2009), likely facilitating the compaction of the ends of the axial filament near the cell poles. Another site-specific DNA binding protein, RacA, binds to a different subset of repeated DNA sequences arrayed along this region of the chromosome and tethers this compacted region to the pole *via* interactions with the polar DivIVA complex (Fig. 3C)(Ben-Yehuda *et al.*, 2003b, Wu *et al.*, 2003, Lenarcic *et al.*, 2009, Ben-Yehuda *et al.*, 2005). Finally, the recently identified RefZ protein binds to yet another repeated non-coding DNA sequence near the origin and terminus regions of the chromosome (Wagner-Herman *et al.* 2012). Although the molecular mechanism has not been established, RefZ complexes have been postulated to direct FtsZ ring formation over a neighboring chromosomal segment to facilitate proper chromosome trapping during prespore development (Fig. 3C)(Wagner-Herman *et al.* 2012). Thus, these systems collaborate to organize the architecture of the sporulating chromosome and couple this organization to proper asymmetric division site placement. This specific nucleoid architecture during division traps important origin-proximal developmental genes within the early prespore and allows the establishment of compartment-specific gene expression programs required for spore development (Losick *et al.*, 1992).

Remarkably, the large-scale architectural rearrangements of the chromosome that facilitate entry into sporulation are transient and rapidly undone during the packaging of the chromosome into the developing pre-spore compartment. The organization of the chromosomal axial filament structure during asymmetric division, in addition to facilitating

compartmentalized gene expression, also presents a specific region of the chromosome to be trapped by the closing septum (Ben-Yehuda *et al.*, 2003a). As the septum encircles this trapped DNA region, the septal DNA-translocating ATPase SpoIIIE recognizes specific non-coding DNA sequence elements to export the chromosome into the pre-spore compartment (Ptacin *et al.*, 2008, Becker *et al.*, 2007). SpoIIIE acts like a molecular wire stripper, transferring naked chromosomal DNA into the developing spore while excluding DNA-bound proteins in the mother cell (Marquis *et al.*, 2008). Finally, during spore germination, the DNA is transiently packaged *de novo* into a ring-shaped structure that may protect the chromosomal DNA from damage before transitioning back into the vegetative organization to begin growth anew (Ragkousi *et al.*, 2000). These intricate rearrangements and *de novo* organizational events illustrate that the chromosomal molecule contains all of the necessary information to orchestrate these highly dynamic and reversible architectural changes. The multitude of systems that have evolved to utilize non-coding information in the chromosome architecture to generate overall morphological and developmental changes in the cell underline the importance and utility of a reproducible nucleoid organization.

Chromosome architecture may organize the cytoplasm by spatially regulating gene expression and protein synthesis

By definition, a reproducible chromosome architecture places specific chromosomal loci into discrete regions within the cytoplasm, and genes themselves are no exception. Until recently, it was believed that after transcription, mRNAs were released from transcription complexes to diffuse throughout the cytoplasm. However, recent work from the Jacobs-Wagner lab has discovered that mRNA molecules in both *Caulobacter* and *E. coli* remain constrained close to their template locations on the nucleoid (Montero Llopis *et al.* 2010). These authors further reported that localized mRNAs prevent the diffusion of active ribosomes, thereby spatially constraining the translation of the coded polypeptide (Montero Llopis *et al.* 2010). These results suggest that the spatial clustering of related genes may facilitate the formation of the protein complexes for which they code. On a larger scale, it remains unclear but tempting to speculate whether the chromosomal location of a gene may bias the final destination of its coded product. However, a concurrent study identified a contrasting phenomenon in *E. coli* that appears to target certain mRNAs to the final location of the polypeptides for which they code in a translation-independent manner (Nevo-Dinur *et al.* 2011), indicating that various mechanisms may impact the spatial ordering of newly-synthesized protein products.

Interplay between chromosome immobilizaton and organization

The reproducible organization of the bacterial chromosome is faithfully maintained across generations of dynamically orchestrated cycles of replication and segregation. Surprisingly, however, the mechanisms that guide and shape this process remain poorly understood. The myriad of nucleoid-associated proteins (NAPs) involved in nucleoid landscaping and their proposed mechanisms of action are outside the scope of this review, and have been recently reviewed elsewhere (Rimsky *et al.* 2011, Browning *et al.* 2010). However, one compelling new paradigm for large-scale chromosome organization appears to be the specific immobilization of certain chromosome segments to cellular landmarks, and subsequent compaction and organization of the rest of the chromosome around these points. Studies on the organization of the *Caulobacter* chromosome identified an interaction between the origin-proximal centromere *parS* region and the new cell pole *via* interactions of ParB with the polar polymeric network of PopZ (described above, Bowman *et al.*, 2008, Ebersbach *et al.*, 2008). Strikingly, when the *parS* region of the chromosome is relocated relative to the rest of the chromosome, the chromosome is concomitantly reorganized relative to the new

parS location (Umbarger *et al.* 2011), implying that the organization of chromosomal loci is "clocked" relative to this tethering point.

E. coli appears to have taken a distinct strategy to coupling chromosome locus tethering and large-scale organization. As mentioned above, the *E. coli* chromosome is packaged into a dramatically different arrangement than that of *Caulobacter*, placing the origin region at midcell, the terminus region generally within this area, and the left and right chromosome arms splayed out toward opposite cell poles (Fig. 1)(Nielsen *et al.*, 2006, Wang *et al.*, 2006). Work by the Boccard and Espeli labs has characterized a novel system that organizes the terminus region of the *E. coli* chromosome into a micron-scale organizational domain, or "macrodomain." This system utilizes a repeated palindromic binding site (*matS*) found only in the terminus (*ter*) macrodomain of the chromosome to recruit a site-specific DNA binding protein, MatP, required for the structural organization of the region (Mercier *et al.*, 2008). In addition to organizing the ter macrodomain, MatP interacts with ZapB, a component of the cell division apparatus, facilitating the maintenance of the *ter* region at the cell center during cell division (Espeli *et al.* 2012*,* Thiel *et al.* 2012). Therefore the *E. coli* MatP/*matS* system and *Caulobacter*'s ParB/PopZ system may promote chromosome organization relative to a reproducible cellular landmark such as the cell pole or developing cell division apparatus. Intriguingly, the deletion of the bacterial condensin subunit MukB in *E. coli* produces cells that place replication origins adjacent to the cell pole (Danilova *et al.*, 2007), suggesting a possible link between origin-proximal condensin complexes and division site placement as well.

Outlook

As we've seen, bacteria maintain specific, reproducible, and genetically encoded links between chromosome organization and the processes of DNA replication, DNA segregation, cell division, and sporulation. While the importance of nucleoid structure as an architectural feature in bacteria has become increasingly clear, the actual underlying structure of bacterial nucleoids is a puzzle that is only beginning to be pieced together. Novel technical approaches are uncovering surprising results. Recent studies using fluorescence deconvolution and high-throughput interaction mapping and localization have described the *Bacillus* and *Caulobacter* chromosomes as ellipsoidal with two compacted arms gently twisted upon one other (Fig. 1) (Umbarger *et al.* 2011*,* Berlatzky *et al.*, 2008). Electron microscopy studies in other organisms have reported similar structures (Butan *et al.* 2011), as well as bizarre and astonishing chromatin configurations such as whirled nucleoid arrangements (Robinow *et al.*, 1994) and nucleoids contained within membrane-bound compartments (Fuerst, 2005). Similar studies in other organisms will undoubtedly yield both similarities and surprising differences in chromosome structures across bacteria, and facilitate the discovery of novel systems that utilize nucleoid architecture to organize and orchestrate cellular development.

Acknowledgments

We thank Grant Bowman, Esteban Toro, and Seth Childers for critical reading of the manuscript and thoughtful suggestions. This work is supported in part by National Institutes of Health grants R01 GM51426 R24 and GM073011-04d to L.S., and NIH/NIGMS fellowship F32GM088966-3 to J.P.

References

Becker EC, Pogliano K. Cell-specific SpoIIIE assembly and DNA translocation polarity are dictated by chromosome orientation. Mol Microbiol. 2007; 66:1066–1079. [PubMed: 18001347]

Ben-Yehuda S, Fujita M, Liu XS, Gorbatyuk B, Skoko D, Yan J, et al. Defining a centromere-like element in *Bacillus subtilis* by Identifying the binding sites for the chromosome-anchoring protein RacA. Mol Cell. 2005; 17:773–782. [PubMed: 15780934]

Ben-Yehuda S, Rudner DZ, Losick R. Assembly of the SpoIIIE DNA translocase depends on chromosome trapping in *Bacillus subtilis*. Curr Biol. 2003a; 13:2196–2200. [PubMed: 14680637]

Ben-Yehuda S, Rudner DZ, Losick R. RacA, a bacterial protein that anchors chromosomes to the cell poles. Science. 2003b; 299:532–536. [PubMed: 12493822]

Berlatzky IA, Rouvinski A, Ben-Yehuda S. Spatial organization of a replicating bacterial chromosome. Proc Natl Acad Sci U S A. 2008; 105:14136–14140. [PubMed: 18779567]

Bernhardt TG, de Boer PA. SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over chromosomes in *E. coli*. Mol Cell. 2005; 18:555–564. [PubMed: 15916962]

Bowman GR, Comolli LR, Gaietta GM, Fero M, Hong SH, Jones Y, et al. *Caulobacter* PopZ forms a polar subdomain dictating sequential changes in pole composition and function. Mol Microbiol. 2010; 76:173–189. [PubMed: 20149103]

Bowman GR, Comolli LR, Zhu J, Eckart M, Koenig M, Downing KH, et al. A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. Cell. 2008; 134:945–955. [PubMed: 18805088]

Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria. Curr Opin Microbiol. 2009; 12:683–688. [PubMed: 19884039]

Breier AM, Grossman AD. Whole-genome analysis of the chromosome partitioning and sporulation protein Spo0J (ParB) reveals spreading and origin-distal sites on the *Bacillus subtilis* chromosome. Mol Microbiol. 2007; 64:703–718. [PubMed: 17462018]

Browning DF, Grainger DC, Busby SJ. Effects of nucleoid-associated proteins on bacterial chromosome structure and gene expression. Curr Opin Microbiol. 2010; 13:773–780. [PubMed: 20951079]

Butan C, Hartnell LM, Fenton AK, Bliss D, Sockett RE, Subramaniam S, Milne JL. Spiral architecture of the nucleoid in *Bdellovibrio bacteriovorus*. J Bacteriol. 2011; 193:1341–1350. [PubMed: 21148724]

Cho H, McManus HR, Dove SL, Bernhardt TG. Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist. Proc Natl Acad Sci U S A. 2011; 108:3773–3778. [PubMed: 21321206]

Danilova O, Reyes-Lamothe R, Pinskaya M, Sherratt D, Possoz C. MukB colocalizes with the *oriC* region and is required for organization of the two *Escherichia coli* chromosome arms into separate cell halves. Mol Microbiol. 2007; 65:1485–1492. [PubMed: 17824928]

Ebersbach G, Briegel A, Jensen GJ, Jacobs-Wagner C. A self-associating protein critical for chromosome attachment, division, and polar organization in *Caulobacter*. Cell. 2008; 134:956–968. [PubMed: 18805089]

Espeli O, Borne R, Dupaigne P, Thiel A, Gigant E, Mercier R, Boccard FA. MatP-divisome interaction coordinates chromosome segregation with cell division in *E. coli*. EMBO J. 2012; 31:3198–3211. [PubMed: 22580828]

Fogel MA, Waldor MK. A dynamic, mitotic-like mechanism for bacterial chromosome segregation. Genes Dev. 2006; 20:3269–3282. [PubMed: 17158745]

Fuerst JA. Intracellular compartmentation in *planctomycetes*. Annu Rev Microbiol. 2005; 59:299–328. [PubMed: 15910279]

Gruber S, Errington J. Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. Cell. 2009; 137:685–696. [PubMed: 19450516]

Hui MP, Galkin VE, Yu X, Stasiak AZ, Stasiak A, Waldor MK, Egelman EH. ParA2, a *Vibrio cholerae* chromosome partitioning protein, forms left-handed helical filaments on DNA. Proc Natl Acad Sci U S A. 2010; 107:4590–4595. [PubMed: 20176965]

Jensen RB, Wang SC, Shapiro L. A moving DNA replication factory in *Caulobacter crescentus*. EMBO J. 2001; 20:4952–4963. [PubMed: 11532959]

Kiekebusch D, Michie KA, Essen LO, Lowe J, Thanbichler M. Localized dimerization and nucleoid binding drive gradient formation by the bacterial cell division inhibitor MipZ. Mol Cell. 2012; 46:245–259. [PubMed: 22483621]

Lenarcic R, Halbedel S, Visser L, Shaw M, Wu LJ, Errington J, et al. Localisation of DivIVA by targeting to negatively curved membranes. EMBO J. 2009; 28:2272–2282. [PubMed: 19478798]

Leonard TA, Butler PJ, Lowe J. Structural analysis of the chromosome segregation protein Spo0J from *Thermus thermophilus*. Mol Microbiol. 2004; 53:419–432. [PubMed: 15228524]

Leonard TA, Butler PJ, Lowe J. Bacterial chromosome segregation: structure and DNA binding of the Soj dimer--a conserved biological switch. EMBO J. 2005; 24:270–282. [PubMed: 15635448]

Lin DC, Grossman AD. Identification and characterization of a bacterial chromosome partitioning site. Cell. 1998; 92:675–685. [PubMed: 9506522]

Losick R, Stragier P. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. Nature. 1992; 355:601–604. [PubMed: 1538747]

Marczynski GT, Shapiro L. Control of chromosome replication in *Caulobacter crescentus*. Annu Rev Microbiol. 2002; 56:625–656. [PubMed: 12142494]

Marquis KA, Burton BM, Nollmann M, Ptacin JL, Bustamante C, Ben-Yehuda S, Rudner DZ. SpoIIIE strips proteins off the DNA during chromosome translocation. Genes Dev. 2008; 22:1786–1795. [PubMed: 18593879]

McGrath PT, Iniesta AA, Ryan KR, Shapiro L, McAdams HH. A dynamically localized protease complex and a polar specificity factor control a cell cycle master regulator. Cell. 2006; 124:535–547. [PubMed: 16469700]

Mercier R, Petit MA, Schbath S, Robin S, El Karoui M, Boccard F, Espeli O. The MatP/*matS* site-specific system organizes the terminus region of the *E. coli* chromosome into a macrodomain. Cell. 2008; 135:475–485. [PubMed: 18984159]

Mohl DA, Gober JW. Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. Cell. 1997; 88:675–684. [PubMed: 9054507]

Montero Llopis P, Jackson AF, Sliusarenko O, Surovtsev I, Heinritz J, Emonet T, Jacobs-Wagner C. Spatial organization of the flow of genetic information in bacteria. Nature. 2010; 466:77–81. [PubMed: 20562858]

Murray H, Ferreira H, Errington J. The bacterial chromosome segregation protein Spo0J spreads along DNA from *parS* nucleation sites. Mol Microbiol. 2006; 61:1352–1361. [PubMed: 16925562]

Nevo-Dinur K, Nussbaum-Shochat A, Ben-Yehuda S, Amster-Choder O. Translation-independent localization of mRNA in *E. coli*. Science. 2011; 331:1081–1084. [PubMed: 21350180]

Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG. The *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. Mol Microbiol. 2006; 62:331–338. [PubMed: 17020576]

Ptacin JL, Lee SF, Garner EC, Toro E, Eckart M, Comolli LR, et al. A spindle-like apparatus guides bacterial chromosome segregation. Nat Cell Biol. 2011; 12:791–798. [PubMed: 20657594]

Ptacin JL, Nollmann M, Becker EC, Cozzarelli NR, Pogliano K, Bustamante C. Sequence-directed DNA export guides chromosome translocation during sporulation in *Bacillus subtilis*. Nat Struct Mol Biol. 2008; 15:485–493. [PubMed: 18391964]

Quon KC, Yang B, Domian IJ, Shapiro L, Marczynski GT. Negative control of bacterial DNA replication by a cell cycle regulatory protein that binds at the chromosome origin. Proc Natl Acad Sci U S A. 1998; 95:120–125. [PubMed: 9419339]

Ragkousi K, Cowan AE, Ross MA, Setlow P. Analysis of nucleoid morphology during germination and outgrowth of spores of *Bacillus species*. J Bacteriol. 2000; 182:5556–5562. [PubMed: 10986261]

Rimsky S, Travers A. Pervasive regulation of nucleoid structure and function by nucleoid-associated proteins. Curr Opin Microbiol. 2010; 14:136–141. [PubMed: 21288763]

Ringgaard S, van Zon J, Howard M, Gerdes K. Movement and equipositioning of plasmids by ParA filament disassembly. Proc Natl Acad Sci U S A. 2009; 106:19369–19374. [PubMed: 19906997]

Roberts MA, Wadhams GH, Hadfield KA, Tickner S, Armitage JP. ParA-like protein uses nonspecific chromosomal DNA binding to partition protein complexes. Proc Natl Acad Sci U S A. 2012; 109:6698–6703. [PubMed: 22496588]

Robinow C, Kellenberger E. The bacterial nucleoid revisited. Microbiol Rev. 1994; 58:211–232. [PubMed: 7521510]

Rodionov O, Yarmolinsky M. Plasmid partitioning and the spreading of P1 partition protein ParB. Mol Microbiol. 2004; 52:1215–1223. [PubMed: 15130136]

Ryter A, Schaeffer P, Ionesco H. Cytologic classification, by their blockage stage, of sporulation mutants of *Bacillus subtilis* Marburg. Ann Inst Pasteur (Paris). 1966; 110:305–315. [PubMed: 4955547]

Savage DF, Afonso B, Chen AH, Silver PA. Spatially ordered dynamics of the bacterial carbon fixation machinery. Science. 2010; 327:1258–1261. [PubMed: 20203050]

Schofield WB, Lim HC, Jacobs-Wagner C. Cell cycle coordination and regulation of bacterial chromosome segregation dynamics by polarly localized proteins. EMBO J. 2010; 29:3068–3081. [PubMed: 20802464]

Sullivan NL, Marquis KA, Rudner DZ. Recruitment of SMC by ParB-*parS* organizes the origin region and promotes efficient chromosome segregation. Cell. 2009; 137:697–707. [PubMed: 19450517]

Thanbichler M, Shapiro L. MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. Cell. 2006; 126:147–162. [PubMed: 16839883]

Thiel A, Valens M, Vallet-Gely I, Espeli O, Boccard F. Long-range chromosome organization in *E. coli*: a site-specific system isolates the Ter macrodomain. PLoS Genet. 2012; 8:e1002672. [PubMed: 22532809]

Tonthat NK, Arold ST, Pickering BF, Van Dyke MW, Liang S, Lu Y, et al. Molecular mechanism by which the nucleoid occlusion factor, SlmA, keeps cytokinesis in check. EMBO J. 2011; 30:154–164. [PubMed: 21113127]

Toro E, Hong SH, McAdams HH, Shapiro L. *Caulobacter* requires a dedicated mechanism to initiate chromosome segregation. Proc Natl Acad Sci U S A. 2008; 105:15435–15440. [PubMed: 18824683]

Umbarger MA, Toro E, Wright MA, Porreca GJ, Bau D, Hong SH, et al. The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation. Mol Cell. 2011; 44:252–264. [PubMed: 22017872]

Vecchiarelli AG, Han YW, Tan X, Mizuuchi M, Ghirlando R, Biertumpfel C, et al. ATP control of dynamic P1 ParA-DNA interactions: a key role for the nucleoid in plasmid partition. Mol Microbiol. 2010; 78:78–91. [PubMed: 20659294]

Veiga H, Jorge AM, Pinho MG. Absence of nucleoid occlusion effector Noc impairs formation of orthogonal FtsZ rings during *Staphylococcus aureus* cell division. Mol Microbiol. 2012; 80:1366–1380. [PubMed: 21477126]

Viollier PH, Thanbichler M, McGrath PT, West L, Meewan M, McAdams HH, Shapiro L. Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. Proc Natl Acad Sci U S A. 2004; 101:9257–9262. [PubMed: 15178755]

Wagner-Herman JK, Bernard R, Dunne R, Bisson-Filho AW, Kumar K, Nguyen T, et al. RefZ Facilitates the Switch from Medial to Polar Division during Spore Formation in *Bacillus subtilis*. J Bacteriol. 2012; 194:4608–4618. [PubMed: 22730127]

Wang X, Liu X, Possoz C, Sherratt DJ. The two *Escherichia coli* chromosome arms locate to separate cell halves. Genes Dev. 2006; 20:1727–1731. [PubMed: 16818605]

Werner JN, Chen EY, Guberman JM, Zippilli AR, Irgon JJ, Gitai Z. Quantitative genome-scale analysis of protein localization in an asymmetric bacterium. Proc Natl Acad Sci U S A. 2009; 106:7858–7863. [PubMed: 19416866]

Wu LJ, Errington J. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating *Bacillus subtilis*. Mol Microbiol. 2003; 49:1463–1475. [PubMed: 12950914]

Wu LJ, Errington J. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. Cell. 2004; 117:915–925. [PubMed: 15210112]

Wu LJ, Ishikawa S, Kawai Y, Oshima T, Ogasawara N, Errington J. Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation. EMBO J. 2009; 28:1940–1952. [PubMed: 19494834]

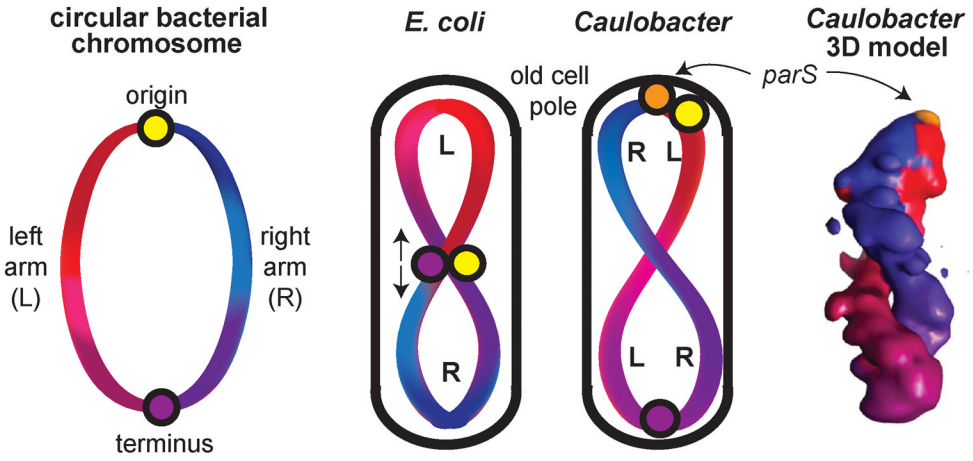

Figure 1.

Schematic of chromosome arrangements in *E. coli* and *Caulobacter* cells

(left) Diagram depicting a circular bacterial chromosome showing origin (yellow) and terminus region (purple), and left (L) and right (R) chromosome arms as red and blue gradients, respectively. (center) Cartoon representation showing the general positioning of the chromosomal molecule in *E. coli* (left center) and *Caulobacter* (right center) cells. In *E. coli*, the origin is located near the cell center, the terminus is broadly localized within this region (arrows), and left and right arms are splayed toward opposite cell poles. *E. coli* does not contain a *parS* locus. In *Caulobacter*, the origin-proximal *parS* region (orange) is tethered to the old cell pole in a newborn *Caulobacter* swarmer cell and the terminus at the opposite cell pole. Loci along each chromosome arm are positioned approximately linearly between. (right) A 3D model of the *Caulobacter* swarmer cell chromosome generated by molecular dynamics simulations based on locus-specific DNA interaction sites, showing chromosomal arms twisted upon one another (adapted from Umbarger et al. 2011).

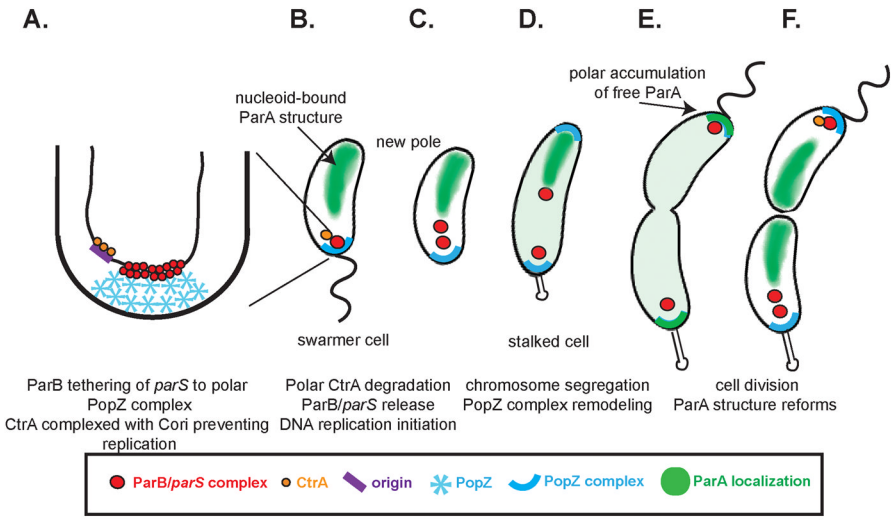

Figure 2.

Chromosome arrangement affects cell cycle regulation and chromosome segregation in *Caulobacter crescentus*

Diagram depicting the subcellular localization of factors that mediate chromosome segregation during the *Caulobacter* cell cycle. (A) A zoomed-in cartoon showing the polar PopZ network (blue stars) tethering the *parS* region of the chromosome (wavy black line) to the cell pole *via* interactions of the ParB complex (red) with PopZ. Nearby, the chromosomal origin region (purple line) is bound by the master cell cycle regulator CtrA (orange), preventing replication initiation. (B) A *Caulobacter* swarmer cell showing the ParB/*parS* complex and the CtrA-bound *Cori* positioned proximally to the old cell pole, and the nucleoid-bound ParA structure (green) extending from the new pole toward the cell center. (C) To initiate the cell cycle, the ParB/*parS* complex is released from the cell pole, and the *Cori*-bound CtrA is degraded, licensing replication initiation. (D) One of the duplicated ParB complexes encounters the nucleoid-bound ParA structure and is moved to the opposite cell pole, shortening the ParA structure and releasing free ParA into solution (light green background). The initial asymmetric localization of the ParB/*parS* complexes relative to the ParA-bound nucleoid structure may facilitate unidirectional transfer towards the new pole. (E) The ParB-bound centromere is tethered to the new cell pole, free ParA is recruited to the cell poles (green curve), and cell division occurs. (F) The ParA structure reforms and replication begins immediately in the stalked cell.

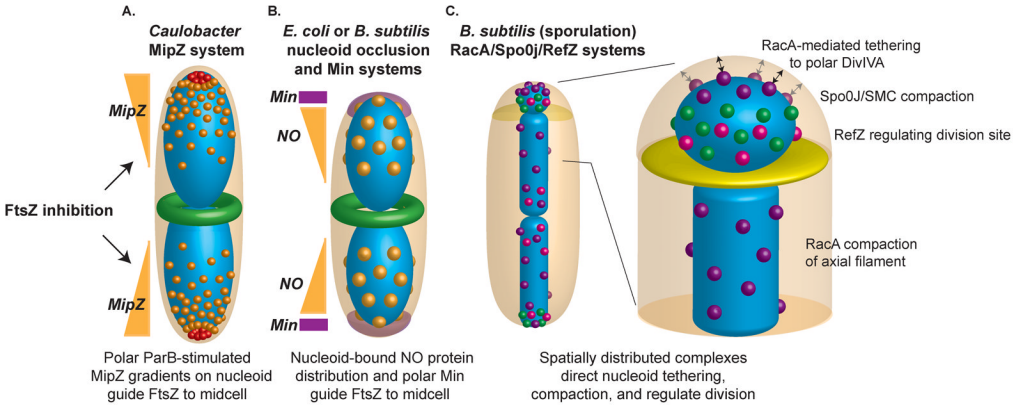

Figure 3.

Cell division site selection is facilitated by chromosome architecture

A. The *Caulobacter* MipZ system utilizes polar ParB complexes to assemble MipZ gradients on the nucleoid that regulate the positioning and timing of FtsZ assembly. Shown is a diagram depicting a pre-divisional *Caulobacter* cell, with newly replicated and segregated nucleoids (blue ellipsoids). Polar ParB complexes (red spheres) recruit MipZ (orange spheres) and stimulate MipZ DNA binding activity, facilitating the formation of a MipZ gradient from poles to midcell that drives FtsZ (green annulus) assembly between newly replicated chromosomes. B. The nucleoid occlusion (NO) systems of *E. coli* and *B. subtilis* vegetative cells are specifically arrayed within the nucleoid structure and, along with the polar Min systems, shepherd divisiome formation to internucleoid regions away from cell poles. Shown is a diagram of an *E. coli* or vegetative *B. subtilis* cell depicting newly replicated nucleoids (blue ellipsoids) and NO complexes (orange spheres) specifically arrayed throughout the polar 2/3 of the chromosome structure. NO complexes and the polar Min system (lavender polar caps) together direct FtsZ (green ring) assembly between the newly replicated nucleoids away from the poles.

C. During sporulation in *B. subtilis*, the spatial distributions of RacA/DivIVA, Spo0J/SMC, and RefZ complexes facilitate proper chromosome arrangement and segregation into the prespore compartment. Diagram depicting a sporulating *B. subtilis* cell. The chromosomes (blue cylinders) are compacted and organized into an axial filament structure by RacA (purple spheres). Specific non-coding DNA sequence repeats in the origin-proximal region of the axial filament are bound by RacA, which tethers this region to the cell pole (arrows) *via* interactions with polar DivIVA (not shown). Distinct sequences recruit Spo0J/SMC complexes (green spheres), which enable compaction of the chromosome origin region. Finally, another family of specifically arrayed DNA sequences recruit RefZ (pink spheres) to origin and termini regions and may regulate FtsZ assembly to ensure proper chromosome trapping by the closing division septum (yellow disc).