Bacterial Chromosome Organization and Segregation

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Abstract

If fully stretched out, a typical bacterial chromosome would be nearly 1 mm long, approximately 1,000 times the length of a cell. Not only must cells massively compact their genetic material, but they must also organize their DNA in a manner that is compatible with a range of cellular processes, including DNA replication, DNA repair, homologous recombination, and horizontal gene transfer. Recent work, driven in part by technological advances, has begun to reveal the general principles of chromosome organization in bacteria. Here, drawing on studies of many different organisms, we review the emerging picture of how bacterial chromosomes are structured at multiple length scales, highlighting the functions of various DNA-binding proteins and the impact of physical forces. Additionally, we discuss the spatial dynamics of chromosomes, particularly during their segregation to daughter cells. Although there has been tremendous progress, we also highlight gaps that remain in understanding chromosome organization and segregation.

Contents

INTRODUCTION

The chromosomes of all organisms must be compacted nearly three orders of magnitude to fit within cells. Moreover, DNA must be packaged in a way that is compatible with a myriad of DNA-based processes, including replication, transcription, repair, recombination, and integration. This challenge is particularly acute in bacteria, as chromosome segregation occurs concomitantly with DNA replication rather than being separated temporally, as in eukaryotes. Efforts to understand the structure and organization of bacterial chromosomes have been greatly enhanced in recent years with major technical developments and innovations, including microscopy-based methods for accurately probing the spatial and temporal dynamics of individual DNA loci and genomic methods for investigating the global conformation and folding properties of chromosomes. These new techniques, in combination with the tried-and-true approaches of genetics, biochemistry, biophysics, and cell biology, have begun to reveal the remarkable mechanisms used by bacterial cells to compact, organize, and segregate their chromosomes. Here, we review these mechanisms and the organizing principles of chromosomes in a top-down manner, moving from the micrometer to the nanometer scale, before discussing recent work on understanding chromosome segregation.

BACTERIAL CHROMOSOME COMPACTION AND ORGANIZATION

Global Organization

Bacterial chromosomes were originally thought to fit randomly within cells, with no stereotypical or reproducible organization. This assumption was initially dispelled when light microscopy studies of *Escherichia coli* cells stained with DNA-specific dyes revealed a discrete body of DNA named the nucleoid (reviewed in Robinow & Kellenberger 1994). Early electron microscopy (EM) and subsequent cryo-EM images of vitreous sections of *E. coli* suggested that the nucleoid has an irregular structure with extensions projecting into the cytoplasm. In rich growth media, the *E. coli* nucleoid occupies about half of the cytoplasmic area and seems to exclude most ribosomes.

This overall arrangement was seen in living *E. coli* and *B. subtilis* cells using fluorescently tagged nucleoid-associated proteins (NAPs), which bind nonspecifically to DNA, and ribosome subunits (Azam et al. 2000, Bakshi et al. 2012, Lewis et al. 2000). The separation of chromosomes from the bulk ribosomes was subsequently observed in other organisms, including *Myxococcus xanthus* and *Streptomyces coelicolor* (Dyson 2011, Harms et al. 2013).

Imaging of fluorescently tagged NAPs in *E. coli* has also suggested that the nucleoid assumes a loosely twisted overall conformation, with no particular handedness (Fisher et al. 2013, Hadizadeh Yazdi et al. 2012). Using fluorescent deoxynucleotide derivatives incorporated into DNA as replication proceeds, a helix-like conformation has also been observed in replicating *B. subtilis* (Berlatzky et al. 2008). In addition, whole cell cryo-tomography has revealed a helix-like structure of the *Bdellovibrio bacteriovorus* chromosome (Butan et al. 2011). The biological significance of a helical fold is unknown but may represent an energy-minimal configuration for fitting chromosomes within rod-shaped cells (Fisher et al. 2013).

Time-lapse microscopy using fluorescently tagged NAPs has also revealed the temporal dynamics of chromosomes. In *E. coli*, waves of nucleoid density flux along the long axis of the cell; the function of this nucleoid mobility is not clear, but it may impact chromosome segregation (as discussed in the section titled Bulk Chromosome Segregation and Structural Maintenance of Chromosomes Proteins) (Fisher et al. 2013).

The spatial arrangement of chromosomes has been inferred by tracking the subcellular positions of individual loci using fluorescence in situ hybridization (FISH), fluorescent repressor operator systems (FROS), and ParB/*parS* systems (Le & Laub 2014). In *Caulobacter crescentus*, 112 loci were examined by FROS in cells containing a single chromosome. The spatial positions of loci within the cell recapitulated the genetic map, with the origin of replication (*oriC*) at one cell pole, the replication terminus (*ter*) at the opposite pole, and the left and right chromosomal arms likely running in parallel down the long axis of the cell, a pattern referred to as the *ori-ter* configuration (Viollier et al. 2004) (**Figure 1*a***). Recent data from large-scale chromosome conformation capture assays (5C and Hi-C) performed on *C. crescentus* are consistent with this pattern (Le et al. 2013, Umbarger et al. 2011). Those studies have revealed high frequency interactions between loci near each other on the same chromosomal arm and slightly lower frequency interactions between loci at similar positions on opposite arms (also see **Figure 3*b***). In replicating *C. crescentus* cells, one new copy of *oriC* is rapidly segregated to the opposite pole. As replication proceeds, the newly generated DNA moves to its respective position, again with loci arranged relative to the origin in a manner that reflects the genetic map (Viollier et al. 2004). Ultimately, the two termini end up midcell, thereby recreating the *ori-ter* pattern in each daughter cell.

The chromosome configuration is substantially different in slow-growing *E. coli*. The origin resides near midcell, with the two chromosomal arms on opposite sides of the cell and the terminus typically near midcell, in a so-called left-*ori*-right configuration (Nielsen et al. 2006b, Wang et al. 2006) (**Figure 1*b***). DNA replication and segregation of the origins to cell quarter positions regenerates a left-*ori*-right organization for each chromosome. By contrast, fast-growing *E. coli* cells adopt an *ori-ter* configuration of the chromosome with polarly localized origins (Youngren et al. 2014) (**Figure 1*c***). Cross sections of fast-growing *E. coli* cells show that the chromosomal arms occupy the outer shell, with the origin and terminus regions within the nucleoid core (Youngren et al. 2014).

In *B. subtilis*, global chromosome organization depends on cell cycle phase and developmental stage. In sporulating *B. subtilis*, the two chromosomes adopt *ori-ter/ter-ori* configurations, with an asymmetric septum trapping a quarter of one chromosome in the prespore compartment (Wang et al. 2014a, Wang & Rudner 2014) (**Figure 1*d***). During vegetative growth, the chromosome alternates between an *ori-ter* and *E. coli*–like left-*ori*-right pattern (Wang et al. 2014a, Wang &

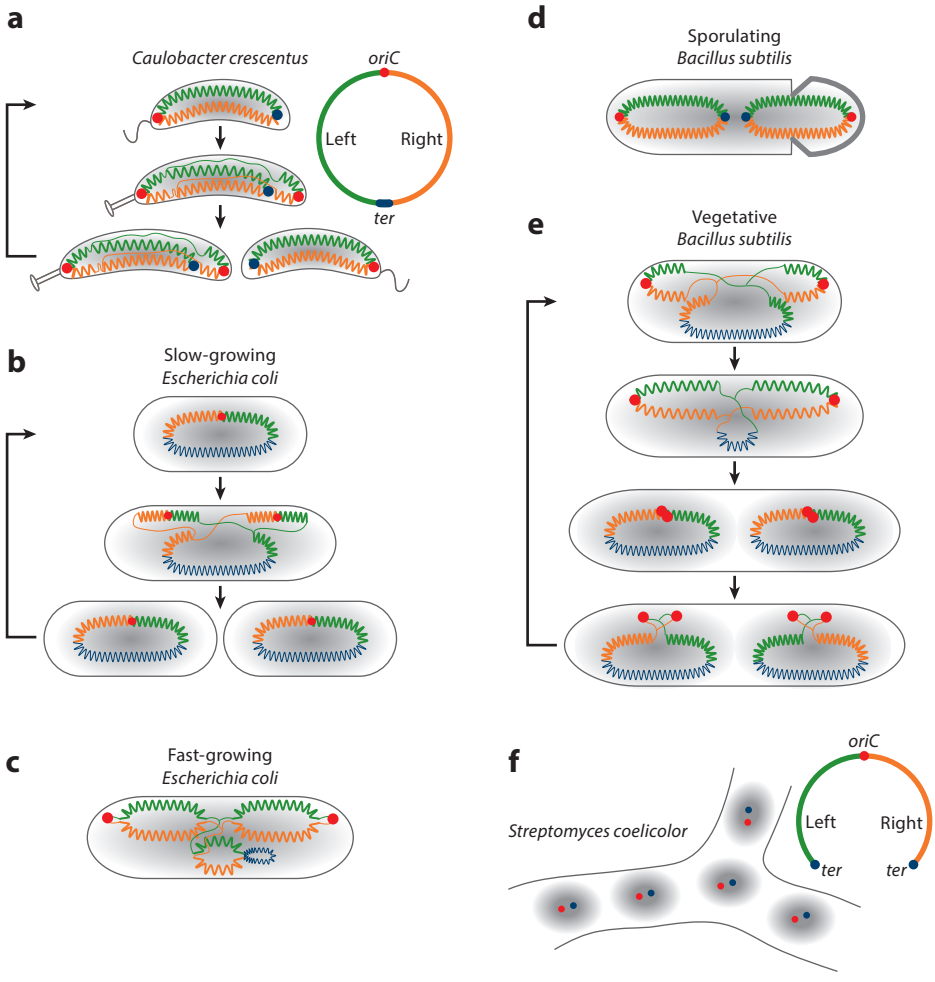


Figure 1

The global organization of chromosomes in (*a*) *Caulobacter crescentus*, (*b*) slow-growing *Escherichia coli*, (*c*) fast-growing *E. coli*, (*d*) sporulating *Bacillus subtilis*, (*e*) vegetative *B. subtilis*, and (*f*) *Streptomyces coelicolor*. For the organism indicated in each panel, the schematics represent the origin of replication (*oriC*) as a red dot and the terminus (*ter*) as a blue dot or line. The left and right arms of the chromosome are colored green and orange, respectively. Thick zigzag lines denote compacted parts of the chromosome, whereas newly synthesized DNA and hypothetically less-organized DNA are illustrated as thin lines. Overall nucleoid distribution is illustrated by gray shading. Black arrows indicate the progression of the global chromosome organization through a cell cycle.

Rudner 2014) (**Figure 1*e***). Template DNA initially adopts a left-*ori*-right configuration, with replicated chromosomes then adopting an *ori-ter* pattern prior to cell division. Why *B. subtilis* employs both configurations is not clear, but it may allow replisomes to move independently on the two arms while also ensuring segregation of newly replicated chromosomes to opposite sides of the cell (Wang & Rudner 2014).

Although most bacterial chromosomes are circular, some are linear, including the multiple ~1 Mb chromosomes in *Borrelia* species and the ~8 Mb chromosomes in *Streptomyces* species

(Chaconas & Kobryn 2010, Dyson 2011). *Streptomyces oriC* is flanked by two chromosomal arms whose ends are spatially close, suggesting that the linear chromosome folds back on itself (Yang & Losick 2001) (**Figure 1*f***). Telomere-binding proteins that cap the chromosome ends may interact, effectively forming a topologically closed chromosome (Tsai et al. 2011), although it remains unknown whether the *Streptomyces* chromosome adopts an *ori-ter* or left-*ori*-right configuration.

There is still relatively little known about chromosome organization in coccoid or other non-rod shaped bacteria. It is also important to emphasize that the spatial positioning of a given locus typically varies up to 10% of the cell length in a population of cells and within a cell over time (Viollier et al. 2004, Wang et al. 2006, Wiggins et al. 2010). DNA typically moves in a subdiffusive manner: It is more constrained than would be expected as a result of Brownian motion. This tight positional variation may result from the crowded, viscoelastic environment of the nucleoid, intranucleoid linkages that restrict DNA movement, supercoiling, or protein binding. The movement of DNA likely also depends on ATP-dependent mechanical processes, as inhibiting ATP synthesis significantly reduces the diffusion coefficient of individual loci (Weber et al. 2012). Nevertheless, certain DNA loci occasionally exhibit superdiffusive or near-ballistic motion, implying that active segregation or relaxation mechanisms deliver them back to a home position (Bates & Kleckner 2005, Javer et al. 2014, Joshi et al. 2011). Advances in time-lapse fluorescence microscopy promise to reveal much more about DNA dynamics in the coming years.

Proteins That Anchor Specific DNA Regions

In general, the *ori-ter* chromosome pattern appears most common in rod-shaped bacterial species. Whether this configuration affords an advantage is unclear, but the polar anchoring of origins, which likely enforces the *ori-ter* pattern, may help ensure that each daughter cell inherits a full copy of the genome. Studies in several organisms have identified proteins that localize to the cell poles and bind *oriC*-proximal regions.

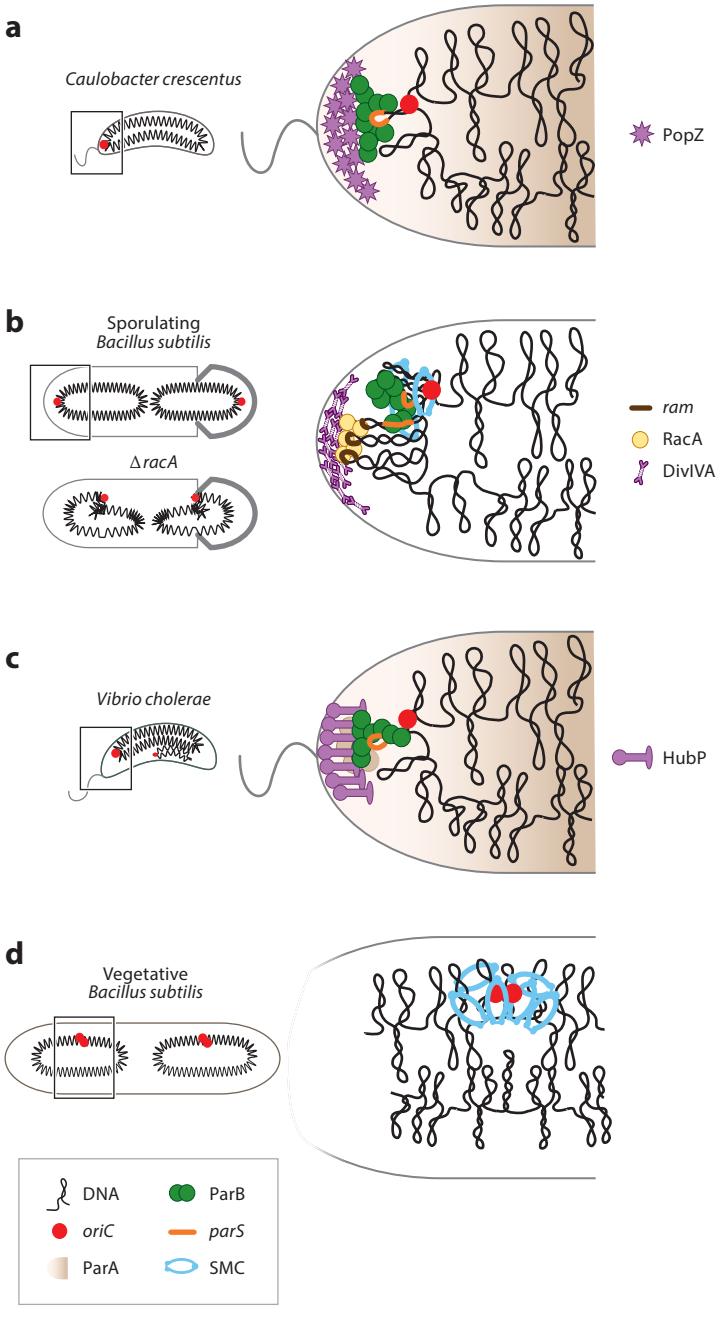
In *C. crescentus*, a *parS* site that is critical for chromosome segregation (discussed in the section titled The ParAB System for Origin Segregation) is located ~13 kb from the origin and is bound by ParB (Mohl et al. 2001, Toro et al. 2008), which also binds PopZ, a cytoplasmic protein that self-aggregates into a proteinaceous matrix at cell poles (Bowman et al. 2008, Ebersbach et al. 2008) (**Figure 2*a***). Moving *parS* away from the origin leads to a global rotation of the chromosome such that the relocated *parS* sites are still polar but the origins are not (Umbarger et al. 2011). This implies that most loci are not actively positioned and instead fall into place based on the position of *parS* and the lengthwise compaction of the nucleoid.

In *B. subtilis*, a protein called RacA accumulates prior to sporulation and concentrates near the cell pole (Ben-Yehuda et al. 2003, Wu & Errington 2003). RacA binds 25 RacA-binding motif (*ram*) sites near *ori*C, helping tether *ori*-proximal regions of the chromosome to the pole (Ben-Yehuda et al. 2005) (**Figure 2*b***). Polar localization of RacA requires a small peripheral membrane protein called DivIVA, which recognizes the concave curvature of the polar membrane (Lenarcic et al. 2009, Oliva et al. 2010, Ramamurthi & Losick 2009). Cells lacking either RacA or DivIVA have disoriented chromosomes with *ori*C positioned near midcell rather than at the poles, and they frequently form empty prespore compartments (Ben-Yehuda et al. 2003).

In *Vibrio cholerae*, a membrane-associated protein called HubP anchors the origin of the large chromosome, ChrI, to the pole (Yamaichi et al. 2012) (**Figure 2*c***). HubP interacts with ParAI, which likely interacts with ParBI, which in turn binds a *parS* site near the ChrI origin. HubP has a peptidoglycan-binding LysM domain, which is required for polar localization.

Although PopZ, RacA, and HubP each anchor chromosomes to a cell pole, these proteins bear no sequence similarity, suggesting they arose independently; this supports the notion that an *ori-ter*

chromosome configuration may be selectively advantageous. However, pole-anchoring proteins may not be strictly necessary for the *ori-ter* arrangement. Some organisms, such as *M. xanthus* and *Pseudomonas aeruginosa*, adopt an *ori-ter* pattern but have a large cytoplasmic gap between *oriC* and the cell pole, suggesting that the origin is not anchored (Harms et al. 2013, Vallet-Gely & Boccard 2013).



In *E. coli*, no polar anchoring complex has been identified. If one exists, it may function only during fast growth, when chromosomes exhibit an *ori-ter* pattern. However, the structural maintenance of chromosomes (SMC) complex (discussed in the section titled Nucleoid-Associated Proteins), called MukBEF in *E. coli*, is required to maintain the left-*ori*-right configuration; cells lacking *mukB* adopt an *ori-ter* configuration even in slow-growth conditions (Danilova et al. 2007). Similarly, in vegetative *B. subtilis* cells, in which the chromosome alternates between left-*ori*-right and *ori-ter* configurations, SMC is required for the left-*ori*-right pattern (Wang et al. 2014a) (**Figure 2*d***). Whether SMC promotes a transverse left-*ori*-right pattern by anchoring origin-proximal regions to midcell or nonpolar regions is unclear. *B. subtilis* SMC and *E. coli* MukBEF do associate with origin-proximal regions (Danilova et al. 2007, Gruber & Errington 2009, Sullivan et al. 2009), but there is no evidence that SMC associates with the cell membrane.

Macrodomains and Chromosomal Interaction Domains

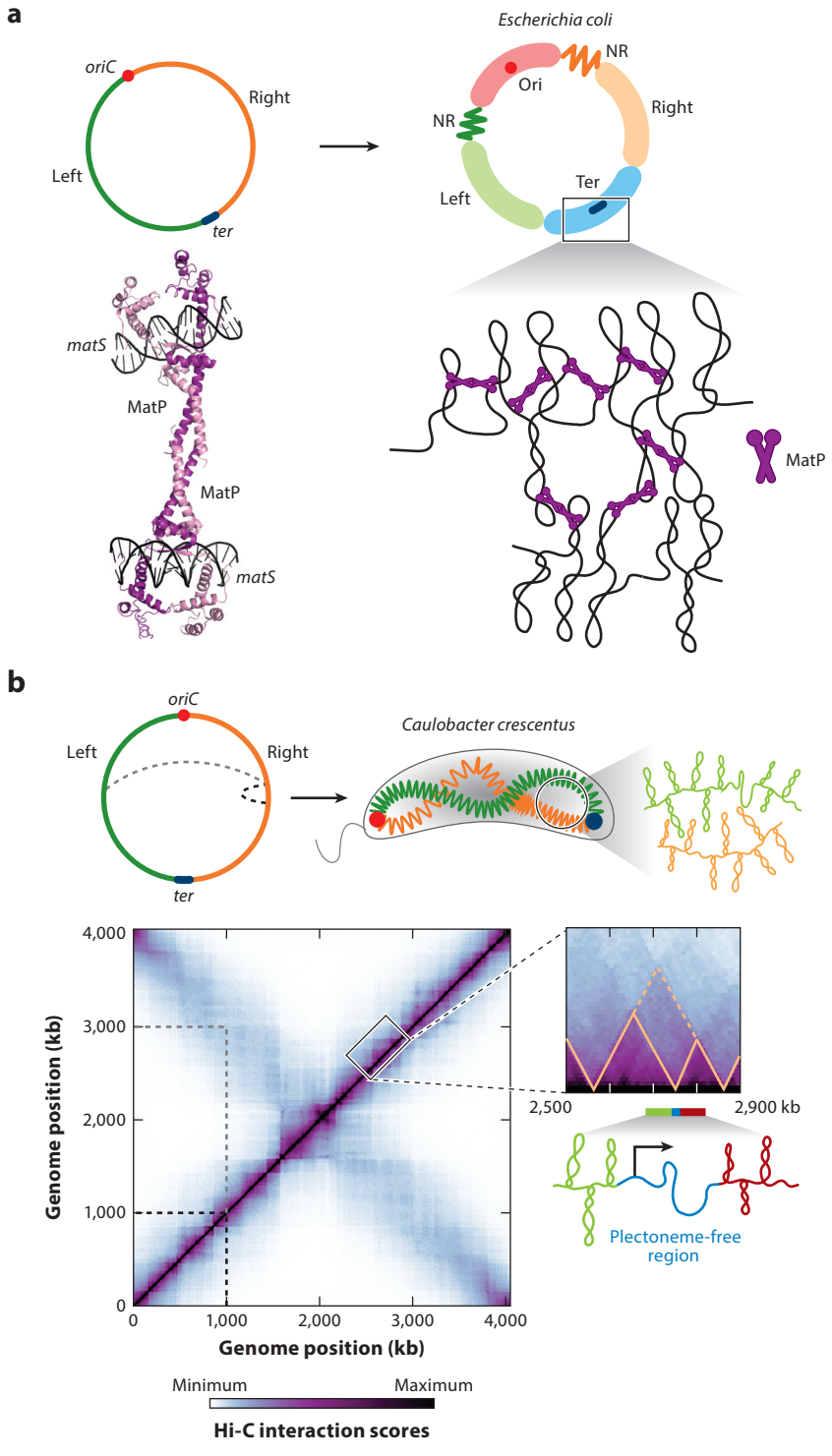
Bacterial chromosomes are further organized into Mb-sized domains called macrodomains, which were first suggested by FISH studies in *E. coli* that demonstrated certain loci frequently co-occupy the same restricted cytoplasmic space (Niki et al. 2000). Subsequently, a λ recombination-based assay found that loci within a given macrodomain interact, and hence recombine, more frequently than loci in different macrodomains (Valens et al. 2004). Collectively, *E. coli* has four macrodomains, Ori, Ter, Left, and Right, with two less-structured DNA regions flanking the Ori macrodomain (**Figure 3*a***). DNA movement within macrodomains is more restricted than in unstructured regions (Espeli et al. 2008). DNA inversions are also more easily tolerated within a macrodomain, suggesting that macrodomains represent a critical level of chromosome organization (Thiel et al. 2012).

A breakthrough in understanding macrodomain organization came from the discovery of *E. coli* MatP, which binds to 13-bp *matS* sites present exclusively in the ~800-kb Ter macrodomain (Mercier et al. 2008). A MatP dimer bound to one *matS* site can form a tetramer with a MatP dimer bound at another site, bringing distal MatP-*matS* complexes together and helping to compact the Ter macrodomain by looping the intervening DNA (Dupaigne et al. 2012) (**Figure 3*a***). Cells lacking MatP exhibit chromosome segregation and terminus resolution problems (Dupaigne et al. 2012, Mercier et al. 2008). Similar but as-yet-unidentified proteins may structure the other *E. coli* macrodomains; MatP homologs appear restricted to enteric bacteria.

Macrodomains per se have not been documented in *C. crescentus*. However, recent Hi-C analyses have revealed that the *C. crescentus* chromosome is divided into approximately 23 chromosomal interaction domains (CIDs), each ~166 kb (Le et al. 2013). Loci within a domain interact preferentially with other loci in the same domain (Le et al. 2013) (**Figure 3*b***). Notably,

Figure 2

Protein-based systems that anchor specific DNA regions. Schematics of polar anchoring complexes are shown for (*a*) *Caulobacter crescentus*, (*b*) sporulating *Bacillus subtilis*, (*c*) *Vibrio cholerae*, and (*d*) vegetative *B. subtilis*. The likely global chromosome organization defect of a *B. subtilis* strain lacking RacA is shown in panel *b*. Specific DNA elements and proteins common to each organism are represented as shown in the legend (*bottom*), with species-specific factors indicated adjacent to each panel. ParA/Soj is not represented in *B. subtilis*; although ParA/Soj is required for the bipolar localization of origins, its own localization is complex (Murray & Errington 2008), and precisely how localization impacts function is unclear. Schematics are adapted from similar drawings in Wang & Rudner (2014). Abbreviations: *oriC*, origin of replication; *ram*, RacA binding motif; SMC, structural maintenance of chromosomes protein.



these domains are often nested, with several adjacent domains forming larger entities potentially similar to macrodomains. Whether chromosomal domains akin to those in *Caulobacter* are also present within *E. coli* macrodomains cannot be resolved using FROS and will instead require high-resolution Hi-C studies.

The CIDs in *Caulobacter* are created in part by highly expressed genes (Le et al. 2013) (**Figure 3*b***). Domain boundaries often coincide with the most highly expressed genes, such as those encoding ribosomal proteins. Inhibiting transcription elongation by adding rifampicin to cells causes an almost complete loss of domains (Le et al. 2013). Additionally, relocating a highly expressed gene, *rsaA*, to an ectopic location is sufficient to induce a new domain boundary (Le et al. 2013). High rates of transcription and the frequent unwinding of DNA likely create regions in the chromosome free of plectonemes, which form when supercoiled DNA twists around itself. These plectoneme-free regions may prevent the diffusion of supercoils and physically separate the flanking domains, thereby decreasing the probability of contact between loci in neighboring domains. The boundaries between chromosomal domains vary in sharpness, which may reflect variation in the rate of transcription of highly expressed genes and differences in transcript length, as well as variability in the local density of DNA-binding proteins. Supercoiling (discussed below) is also important for domain formation and/or maintenance, as the addition of novobiocin largely eliminates domains; negative supercoils may offset positive supercoils, which can impede RNA polymerase during transcription. Finally, because Hi-C reflects DNA-DNA interactions in a population of cells, the domains it identifies are present in most cells. Individual cells, however, may have additional, transient domain boundaries.

Supercoil Domains

Within macrodomains and CIDs, loops of genomic DNA are supercoiled, likely forming plectonemes that coil up around themselves while attached at the base to proteins that help topologically isolate the looped DNA. These plectonemic loops, also called supercoil domains or topological domains, were first seen in electron micrographs of gently lysed *E. coli* cells (Kavenoff & Ryder 1976). Subsequent studies have tried to estimate the number of supercoil domains in *E. coli* by assessing the number of nicks required to fully relax the chromosome, assuming that an individual nick relaxes only the DNA within a given supercoil domain. Initially, relaxation was assessed by measuring the incorporation of trimethylpsoralen, an intercalating dye that preferentially interacts with negatively supercoiled DNA; these assays suggested that *E. coli* harbors approximately 40 topologically isolated domains during exponential growth (Sinden &

Figure 3

Macrodomains and chromosomal interaction domains. (*a*) Macrodomain organization of the *Escherichia coli* chromosome, shown as in **Figure 1** (*left*) or with the four macrodomains, Ori, Ter, Left, and Right, and the two nonstructured regions (NRs) (*right*). MatP (*purple*) organizes the Ter macrodomain. The crystal structure of two MatP dimers, each bound to a *matS* recognition site, is shown (protein data bank ID: 4D8J). (*b*) Chromosome conformation capture assay (5C and Hi-C) and computational modeling have revealed the organization of the *Caulobacter crescentus* chromosome. The Hi-C heat map (Le et al. 2013) indicates the frequency of DNA-DNA interactions across the genome using the color scale shown. The most prominent diagonal indicates frequent interactions within a chromosomal arm (*black dotted lines*), and the other, less prominent diagonal shows interactions between the two arms (*gray dotted lines*). Orange triangles in the inset (*right*) indicate chromosomal interaction domains (CIDs). Highly transcribed genes are thought to create a less compact, plectoneme-free region (*blue*) that serves to spatially insulate DNA (*green* and *red*) in adjacent domains, creating a CID boundary.

Pettijohn 1981). A subsequent study examined supercoil domains by assessing the transcriptional response to double-strand breaks (Postow et al. 2004). The idea was that only DNA within a single, topologically isolated domain would be relaxed after a double-strand break, leading to changes in the transcription of supercoiling-sensitive genes only within that domain. This method estimated the average supercoil domain at ~10 kb, implying the existence of approximately 400 domains in the *E. coli* chromosome, a number that agrees well with the number of loops in chromosomes from lysed cells imaged by EM (Postow et al. 2004).

Supercoil domains have also been probed using recombination as an indirect readout. Unlike λ-integrase, γδ and Tn3 resolvases recombine only if two resolution sites are brought into precise alignment through the slithering of plectonemic DNA. The rate of recombination thus depends on the genomic distance between resolution sites; it is almost undetectable if sites are separated by ~100 kb, suggesting an upper limit on the size of plectonemes in vivo (Higgins et al. 1996). Studies with γδ and Tn3 resolvases indicate that the average plectoneme size in wild-type *Salmonella typhimurium* is ~10 kb, again implying approximately 400 supercoil domains per chromosome (Stein et al. 2005). Certain gyrase mutants, however, can harbor double the number of supercoil domains.

Hi-C data have also contributed to our understanding of plectonemes in vivo. A polymer model of the *Caulobacter* chromosome was recently constructed with parameters corresponding to plectoneme length, width, diameter, flexibility, and radius of collisions (Le et al. 2013). A search for parameter values that reproduced Hi-C data suggested an average plectoneme length of ~8 kb, similar to that measured by recombination and relaxation assays (Stein et al. 2005).

The boundaries between supercoil domains are often dynamic and may depend on both DNA-binding proteins and gene expression. DNA-binding proteins (discussed below) can bridge distant loci, topologically isolating the intervening DNA and preventing the spread of supercoils between adjacent domains. Gene expression also plays a major role in establishing supercoil domains. As noted for CIDs, loci undergoing high rates of transcription can be boundary elements that prevent plectoneme diffusion, although the precise underlying mechanism is not clear. Additionally, transcription contributes to the supercoiling structure of the genome, as RNA polymerase introduces negative supercoils behind itself and positive supercoils in front.

Some supercoil domains likely vary significantly among cells in a population and within a given cell over time. This variability in supercoil location may, in turn, impact the expression of genes whose promoters are sensitive to supercoiling status. However, the domain boundaries associated with very highly expressed genes and observed by Hi-C are static. These domain boundaries are relatively well-distributed across the genome, and bioinformatic analyses indicate that such a pattern of highly expressed genes is common (Wright et al. 2007). The advantage, if any, of distributing domains across a genome is not known, but domain boundaries could help periodically pause DNA replication to promote compaction of recently replicated domains and the decatenation of sister chromosomes. Alternatively, or in addition, dividing the genome into domains may help limit how much of the chromosome relaxes following a nick or double-strand break.

In sum, the relationship between various domains—macrodomains, CIDs, and supercoil domains—is not fully clear yet, but we envision a hierarchical organization. Megabase-sized macrodomains are likely composed of multiple CIDs, each ~100–200 kb and containing multiple, diffusible supercoil domains ~10 kb in size. Very highly expressed genes appear to play a critical role in establishing CID boundaries, which are relatively fixed in a population of cells. The expression of other genes may form transient domains and transient boundaries. The position of genes within domains (at every level) may influence their expression, but the precise relationship between chromosome structure and gene expression remains to be defined.

Nucleoid-Associated Proteins

The organization of bacterial chromosomes is profoundly influenced by DNA-binding proteins and in particular by a heterogeneous class of abundant proteins called NAPs. NAPs typically bind relatively nonspecifically across bacterial genomes, wrapping, bending, or bridging DNA (**Figure 4**). The local action of NAPs ultimately influences global chromosome organization and, in many cases, transcriptional patterns (reviewed in Dillon & Dorman 2010).

E. coli H-NS is a small (15.5 kDa) protein that can bridge DNA, bringing loci separated on the primary sequence level into close physical proximity (**Figure 4**). H-NS has an N-terminal domain, which drives oligomerization, connected by a flexible linker to a C-terminal DNA-binding domain. Chromatin immunoprecipitation (ChIP) studies indicate that *E. coli* H-NS binds hundreds of sites in the genome, with a preference for AT-rich or curved DNA (Grainger et al. 2006, Kahramanoglou et al. 2011). The ability of H-NS to bridge different segments of DNA has been directly demonstrated by both single-molecule and atomic force microscopy (Dame et al. 2000, 2006).

DNA bridging by H-NS likely enables it to constrain negative supercoils by isolating the intervening, looped region of the chromosome. H-NS binding sites also often coincide with supercoiling-sensitive promoters, further suggesting a tight relationship between H-NS and supercoiling (Higgins et al. 1988); in fact, H-NS was originally discovered in a screen for *E. coli* mutants with reduced negative supercoiling (Hardy & Cozzarelli 2005). H-NS has also been examined in vivo by super-resolution microscopy. It forms two discrete foci within the cytoplasm (Wang et al. 2011), although the functional significance of these foci and the DNA loci associated with them are unknown. In addition to bridging distant DNA segments, H-NS can also oligomerize and spread along DNA. Such oligomers can occlude binding sites for RNA polymerase or transcription activators, thereby enabling H-NS to regulate gene expression. This oligomerization of H-NS also enables it to silence spurious transcriptional promoters and horizontally acquired DNA, which is often more AT-rich than host chromosomal DNA (Lucchini et al. 2006, Navarre et al. 2006, Singh et al. 2014). H-NS orthologs, and paralogs known as StpA, are found in many species, although H-NS is not universal. However, other, unrelated proteins, such as Rok in *B. subtilis*, may similarly bridge DNA or oligomerize along AT-rich DNA (Smits & Grossman 2010).

HU is another small (18 kDa), abundant (~30,000 copies/cell) NAP found in many bacteria that coats and wraps chromosomal DNA around itself in a fashion grossly similar to that of histones (Azam et al. 1999) (**Figure 4**). There are two HU subunits, alpha and beta, and both homo- and heterodimers exist, depending on the growth phase in *E. coli* (Claret & Rouviere-Yaniv 1997). HU inserts conserved proline residues into the minor groove of DNA, inducing a sharp bend in the molecule (Swinger et al. 2003). Structural studies also suggest that HU can form an octameric structure with DNA coiled around it (Guo & Adhya 2007, Swinger et al. 2003).

ChIP coupled with deep sequencing, or ChIP-Seq, indicates that HU has little or no DNA-binding specificity, and given its abundance in *E. coli*, HU may coat ~10% of the chromosome (Prieto et al. 2012). Consistent with widespread genomic binding, strains lacking HU often produce anucleate cells, which are suggestive of a general chromosome compaction or segregation defect (Huisman et al. 1989), and strains harboring HU variants with higher DNA-binding affinity have overcompacted nucleoids (Kar et al. 2005). Additionally, Hi-C studies of an HU mutant in *Caulobacter* have revealed a significant decrease in short-range interactions, supporting the notion that HU helps broadly compact the chromosome, possibly by stabilizing plectonemes (Le et al. 2013). HU binding to DNA may also affect the supercoiling status of the chromosome, as HU mutants in *E. coli* show decreased supercoiling, are rescued by mutations in gyrase, and are synthetically lethal when paired with mutations in topoisomerase I (Bensaid et al. 1996, Malik et al.

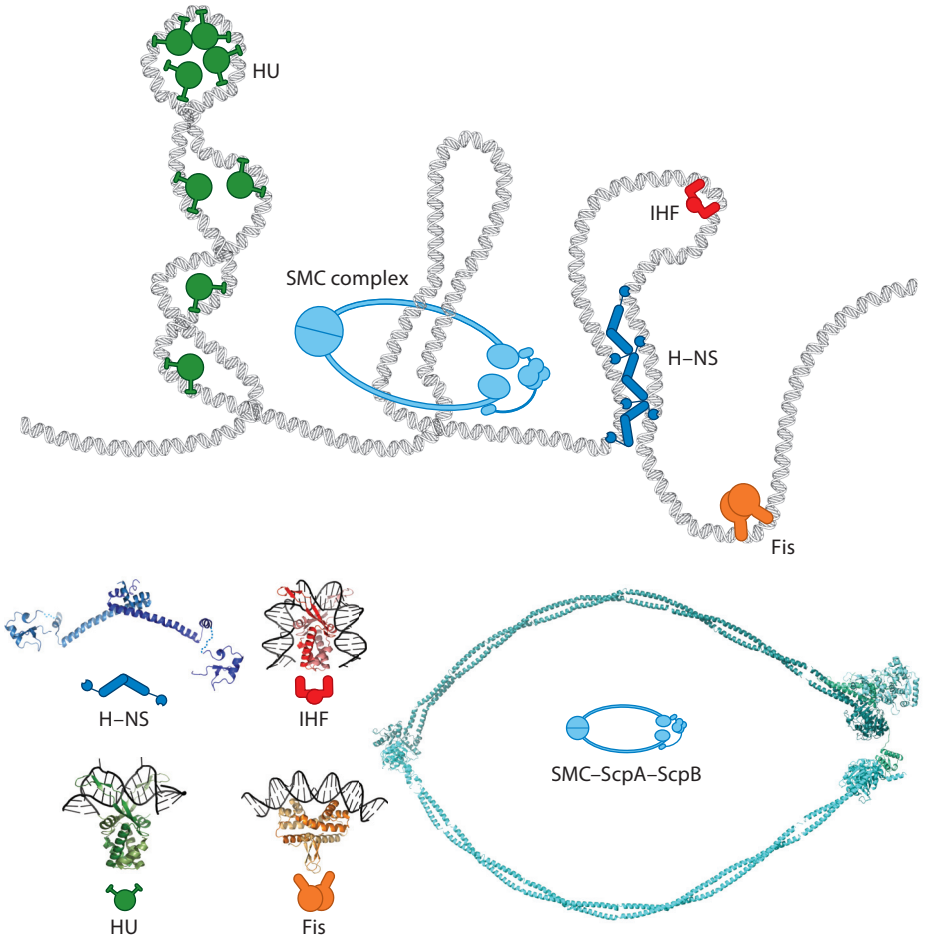


Figure 4

Nucleoid-associated proteins (NAPs) with DNA bridging, wrapping, or bending activities contribute to the organization of the chromosome. The functions of well-studied NAPs are schematized at the top, with the corresponding crystal structures below. H-NS dimers of dimers (*blue*) bridge DNA. Abundant HU (*green*) introduces ~90° bending to DNA and may wrap DNA around itself, thereby promoting short-range DNA interactions. When integration host factor (IHF, *red*) binds to DNA, it induces DNA to form a dramatic U-turn, drastically changing the trajectory of the DNA backbone. Factor for inversion stimulation (Fis, *orange*) is another NAP with DNA-bending activity. Structural maintenance of chromosomes (SMC) complexes (*cyan*) likely form a ring structure that can bring together and handcuff loci that are distal in the primary sequence. The protein data bank (PDB) IDs for the protein and protein:DNA complexes shown are 1P78, 1IHF, and 3JRA for HU, IHF, and Fis, respectively. A hypothetical model of H-NS was constructed from PDB structures 3NR7 and 1HNR. A model of SMC-ScpA-ScpB was derived from PDB structures 4I98, 4I99, and 3ZGX.

1996). Variants of HU with increased affinity for DNA also increase global supercoiling levels (Kar et al. 2005).

Some organisms encode divergent HU paralogs. For example, *S. coelicolor* and *Mycobacteria* encode an HU paralog, HupS/Hlp, that has an extensive C-terminal extension with homology to eukaryotic histone H1 (Mukherjee et al. 2009, Salerno et al. 2009). In *Mycobacterium tuberculosis*,

phosphorylation of HupS decreases its interaction with DNA, highlighting the possibility of post-translational regulation of DNA compaction (Gupta et al. 2014).

Two proteins that can sharply bend DNA are integration host factor (IHF) and factor for inversion stimulation (Fis). IHF bears some sequence similarity to HU and is similarly composed of two subunits, although IHF binds DNA more specifically and introduces dramatic ~160° bends (Rice et al. 1996) (**Figure 4**). Consequently, IHF can dramatically alter DNA shape and facilitate the formation of loops, frequently bringing RNA polymerase together with distant regulatory proteins. IHF also impacts a range of other DNA-based processes, including replication initiation and recombination (Leonard & Grimwade 2005, Mumm et al. 2006).

Like IHF, Fis can bend DNA. It is among the most highly expressed genes during fast growth in *E. coli*, especially following nutrient upshifts (Azam et al. 1999). Fis homodimers bind to AT-rich DNA sequences with narrow minor grooves, bending the DNA by ~50–90° and forming very stable, long-lived nucleoprotein complexes (Stella et al. 2010) (**Figure 4**). Fis binds throughout the genome (Kahramanoglou et al. 2011), impacting transcription, replication, and recombination. Given its genome-wide distribution, Fis probably also influences chromosome compaction and organization in significant ways. Fis-mediated bending of DNA can displace nearby supercoils or preserve the writhe, or coiling of the double helix axis, of DNA, potentially maintaining supercoiled plectonemic loops (Auner et al. 2003). Fis also influences global supercoiling levels indirectly by modulating the expression of gyrase (Schneider et al. 1999).

Although NAPs are generally small proteins, some large proteins also stably associate with and influence the structure of chromosomes. Most prominent in this category is the widely conserved protein SMC, which is homologous to eukaryotic condensin and >125 kDa (reviewed in Nolivos & Sherratt 2014). SMC forms an extended, antiparallel coiled coil with a hinge domain at one end and an ATPase domain at the other (**Figure 4**). Homodimerization via the hinge domains creates a ring-like structure that may encircle DNA. SMC associates with two regulatory proteins, ScpA and ScpB, which likely modulate its ATPase activity, thereby affecting the opening and closing of the homodimeric ring. *E. coli* and other γ-proteobacteria do not encode SMC/ScpA/ScpB and instead produce an analogous complex, MukB/MukE/MukF (Nolivos & Sherratt 2014).

Mutations in SMC produce a range of chromosomal defects in different bacteria, often including an increase in anucleate cells. SMC likely contributes to both chromosome segregation (discussed in detail below) and chromosome compaction. In *B. subtilis* and *E. coli*, mutations in *smc* and *mukB*, respectively, lead to chromosome decondensation visible by DAPI staining (Tadesse et al. 2005, Weitao et al. 1999). Additionally, in *E. coli*, *mukB* mutants display altered supercoiling levels, and these mutants can be partially rescued by other mutations that increase DNA gyrase activity and negative supercoiling (Sawitzke & Austin 2000).

Precisely how SMC proteins affect chromosome compaction is not yet clear. By virtue of its extended, ring-like structure, SMC may bridge different loci in the chromosome. This bridging could help compact the DNA and may also constrain supercoils by producing topologically isolated DNA loops. Notably, in both *E. coli* and *B. subtilis*, SMC proteins associate with origin-proximal regions and are required for the proper positioning of origins (Danilova et al. 2007, Gruber & Errington 2009, Sullivan et al. 2009). Whether origin-proximal regions are preferentially compacted by the associated SMC proteins is not yet clear. ChIP coupled with microarrays, or ChIP–chip, in *B. subtilis* also indicates enrichment of SMC in regions of high transcription, a pattern also seen for eukaryotic SMC, but the functional significance of this localization is unknown (Gruber & Errington 2009).

In *Caulobacter*, cells lacking SMC do not exhibit major defects in chromosome organization (Le et al. 2013) as originally suggested (Jensen & Shapiro 1999), although an ATPase-defective mutant shows a severe defect in sister chromosome separation (Schwartz & Shapiro 2011).

Additionally, Hi-C studies of a Δ*smc* strain have indicated less frequent interactions between loci at approximately equivalent positions on opposite arms of the chromosome, down nearly the entire long axis of the cell (Le et al. 2013). This could indicate that SMC tethers the arms together. Alternatively, SMC could promote the colinearity of the two chromosomal arms by promoting the compaction of each arm along the long axis of the cell. The relative positions of loci in each chromosomal arm may be irregular in cells lacking SMC, explaining the disrupted colinearity of loci observed by Hi-C.

Some traditional transcription factors also have NAP-like properties. The cyclic AMP regulatory protein CRP, which can bend DNA ~90°, binds several hundred sites in the *E. coli* chromosome (Grainger et al. 2005). The leucine-responsive regulatory protein (Lrp), which may influence the expression of ~10% of *E. coli* genes, can form a dimer, octamer, or hexadecamer with an exposed DNA-binding domain, potentially enabling it to bend or wrap DNA (Chen & Calvo 2002).

The abundance of many NAPs varies significantly depending on growth phase and environmental conditions. Indeed, NAPs likely play a critical role in shaping or adjusting chromosome organization in response to different growth conditions. For example, in stationary phase, the *E. coli* nucleoid contains fewer loops than in exponential phase and each loop has more relaxed DNA; however, on the cellular level, the nucleoid becomes significantly more compact in stationary phase. These changes in structure result in part from the elimination of Fis as cells enter stationary phase and from the massive upregulation of Dps (DNA-binding protein from starved cells). Dps binds throughout the chromosome, inducing a stable, crystalline state in the DNA that persists even if cells are lysed (Wolf et al. 1999). Dps physically protects the chromosome from damage during stationary phase. Additionally, Dps chelates Fe^{2+}, helping to prevent it from producing hydroxyl radicals, which could damage the DNA, via a Fenton reaction (Frenkiel-Krispin & Minsky 2006). How Dps is released from DNA as cells exit stationary phase is unknown. SASP (small acid soluble protein) plays a similar role in protecting the chromosomes of *B. subtilis* and *Clostridium difficile* during sporulation (Nicholson et al. 2000). SASP nonspecifically coats the chromosome, inducing a ring-like structure that likely physically shields it and may promote non-homologous end joining repair following double-strand breaks by preventing the cut ends from diffusing apart (Frenkiel-Krispin et al. 2004).

In sum, NAPs and other chromosome-associated proteins are clearly central players in chromosome organization. Although the local, biophysical properties of many of these proteins have been well studied, much remains to be learned about their in vivo functions and how, on a global level, they combine to compact, shape, and organize the genome, and, in turn, affect DNA-based transactions within cells.

Nonproteinaceous Factors that Contribute to Chromosome Organization

Factors other than DNA-binding proteins also contribute significantly to chromosome organization. Macromolecular crowding in the viscous bacterial cytoplasm may aid compaction (De Vries 2010). As noted, the movement of chromosomal loci is generally subdiffusive, implying that the viscoelastic cellular environment influences motion and compaction. Occasional superdiffusive motions, which may reflect stress relaxation mechanisms, further suggest that the chromosome is subject to strong mechanical forces that ultimately impact its compaction and organization.

The physical properties of the chromosome as a large polymer may also influence its organization. One model suggests that the chromosome is a self-avoiding polymer and argues that entropic forces may significantly influence chromosome organization, favoring the separation of supercoil domains (Jun & Mulder 2006). Indeed, in some bacteria, such as *Caulobacter*, the

chromosome occupies nearly the entire cytoplasmic space, suggesting that the inner membrane influences chromosome organization through physical confinement. Additionally, Hi-C studies of *Caulobacter* chromosomes during DNA replication indicate little interaction between sister chromosomes (Le et al. 2013), possibly consistent with DNA supercoiling loops repelling each other. However, in many species, the chromosome does not fill the entire cell, and the NAPs that decorate bacterial DNA likely render chromosomes self-adherent filaments (Hadizadeh Yazdi et al. 2012).

Another factor that may affect chromosome organization is transertion, the coupled translation and insertion of proteins into the membrane by the signal recognition particle (SRP) and the Sec translocase. Transertion may tether DNA to the cell membrane, pulling some DNA out of the nucleoid, which could affect chromosome compaction and segregation. Evidence for transertion has been scant, but a recent FROS study tracking the intracellular position of *tetA*, which encodes a membrane efflux pump, showed that this locus, and nearly 90 kb of DNA around it, moves toward the membrane shortly after inducing *tetA* expression (Libby et al. 2012). Additionally, treating cells with either a transcription inhibitor (rifampicin) or translation inhibitor (chloramphenicol) causes radial shrinkage of the *E. coli* nucleoid, further supporting the notion that transertion represents an expansion force for the chromosome (Bakshi et al. 2012).

BACTERIAL CHROMOSOME SEGREGATION

Chromosome segregation is essential if daughter cells are each to inherit a full copy of the genome. Unlike in eukaryotes, chromosome replication and segregation occur concomitantly in bacteria, apparently without a dedicated, spindle-like apparatus (Nielsen et al. 2006a, Viollier et al. 2004, Wang et al. 2006). The molecular mechanisms responsible for bacterial chromosome segregation are only just beginning to emerge and involve both specific protein components and nonprotein, mechanical-based mechanisms.

One of the earliest models for bacterial chromosome segregation was proposed by François Jacob and colleagues (1963), who suggested that newly replicated origins may get anchored to the cell membrane and segregated passively by cell growth/elongation. However, subsequent studies tracking origins have shown that they segregate much faster than the rate of cell elongation (Fiebig et al. 2006, Viollier et al. 2004, Wang & Sherratt 2010).

DNA replication has also been implicated in chromosome segregation. Early work in *B. subtilis* suggested that the replisome forms a factory at midcell, pulling DNA toward it for replication and extruding replicated DNA to either side of it (Lemon & Grossman 1998). Although this capture-extrusion model can explain bulk, symmetric segregation of chromosomal regions after replication, it cannot apply to bacteria in which the chromosome is asymmetrically replicated and segregated. Additionally, recent studies using fluorescence time-lapse microscopy in *E. coli*, *C. crescentus*, and *B. subtilis* indicate that replisomes are mobile, tracking independently along the chromosome (Bates & Kleckner 2005, Jensen et al. 2001, Reyes-Lamothe et al. 2008, Wang et al. 2014a). Thus, although the replisome and the act of DNA replication could aid chromosome segregation, they likely cannot provide all of the force necessary to carry it out.

The ParAB System for Origin Segregation

The first section of the chromosome segregated is usually the origin-proximal region. In many bacteria, origins of replication are segregated actively via the *parABS* partitioning system (Fogel & Waldor 2006, Ireton et al. 1994, Lin & Grossman 1998, Mohl et al. 2001), first discovered in plasmids because it is often essential for plasmid maintenance (Austin et al. 1985, Gerdes et al. 2010). Homologs of *parABS* were subsequently found to facilitate chromosome segregation in

some bacteria: Nearly 65% harbor this system (Livny et al. 2007). In some cases, the *parABS* system is essential for viability; even when not formally essential, deletion of the system often leads to a significant increase in anucleate cells, demonstrating its importance in chromosome segregation.

In most bacteria, *parS* sites are located near the origin. ParB specifically recognizes and binds to these *parS* sites, often spreading in that region and perhaps bridging more distant DNA to form a large nucleoprotein complex (Graham et al. 2014, Lin & Grossman 1998, Murray et al. 2006). On its own, ParA has weak ATPase activity and binds DNA nonspecifically; its ATPase activity is directly stimulated by ParB (Easter & Gober 2002, Leonard et al. 2005). It was originally proposed, based on studies of plasmids (Ebersbach et al. 2006, Gerdes et al. 2010, Ringgaard et al. 2009), that ParA forms dynamic filaments and, by either a pulling or pushing mechanism, segregates the ParB:*parS* complexes that form on sister chromosomes after replication. According to the pulling model, a ParA filament forms away from the partition complex; the edge of this filament captures a ParB:*parS* complex and the filament retracts, pulling the DNA with it. According to the pushing model, a ParA filament forms between duplicated ParB:*parS* complexes and grows between them, thus pushing them apart.

Early evidence for a pulling mechanism came from studies of origin segregation in *V. cholerae*, which encodes two *par* systems, one for each chromosome (Fogel & Waldor 2006). ParAI was proposed to segregate origins by pulling, as ParAI-YFP (yellow fluorescent protein) does not localize between ParBI:*parSI* complexes and instead localizes between the new cell pole and the segregating ParBI:*parSI* complex. ParAI appears, based on epi-fluorescence microscopy, to form dynamic filaments that retract toward the cell pole in concert with the movement of the ParBI:*parSI* complex, implying a pulling mechanism. However, the precise mechanism of pulling and whether ParAI forms a continuous filament are not clear.

ParAB-dependent origin segregation has also been studied in *C. crescentus*, and initial studies in this system also proposed a pulling mechanism (Ptacin et al. 2010, Shebelut et al. 2010). Origin segregation in *C. crescentus* is a two-step process. After replication, the duplicated origins are released from the pole and separate slightly from one another before one of the origins is translocated unidirectionally to the opposite cell pole (Shebelut et al. 2010). The initial separation does not require ParA, but the subsequent step does (Shebelut et al. 2010, Toro et al. 2008). ATP-bound ParA was postulated to form a filamentous structure across the cell (Ptacin et al. 2010). ParB bound to *parS* sites would contact the edge of this filament and stimulate ParA ATPase activity, resulting in dissociation of ParA molecules from the edge of the filament and a net retraction of the filament away from ParB. Brownian movement of the ParB:*parS* complex would then renew contact with the ParA filament, and the ATP hydrolysis and dissociation cycle would repeat. The higher affinity of ParB for ATP-bound ParA would ensure that a ParB:*parS* complex moves with the retracting ATP-bound ParA filament toward the opposite cell pole.

Although attractive, this pulling model initially assumed that ParA forms a single, continuous filament. Whether such filaments actually occur in vivo is uncertain, and recent studies suggest that an extended filament is not necessary for directional movement; instead, the Mizuuchi group has proposed a diffusion-ratchet model (Hwang et al. 2013; Vecchiarelli et al. 2010, 2012, 2014). By reconstituting a plasmid *parABS* system in vitro, they have shown that ParA-ATP binds DNA nonspecifically. ParB bound to *parS* on a plasmid stimulates ParA ATPase activity, resulting in the release of ParA and local depletion of ATP-bound ParA. The ParB:*parS* complex then diffuses up the ParA-ATP gradient, resulting in net directional movement of the ParB-bound plasmid.

The diffusion-ratchet model is derived from studies of plasmid partitioning, and a subsequent study has suggested that it may also apply to chromosome partitioning in *Caulobacter* (Lim et al. 2014). However, that study argued, based on mathematical modeling, that the diffusion of

ParB:*parS* up short-range ParA-ATP gradients was insufficient to provide the observed directionality of chromosome segregation. Instead, it was suggested that elasticity and dynamic motion help relay, or drive translocation of, the chromosome over short distances. This DNA relay model essentially extends the diffusion-ratchet model, providing a plausible mechanism for the directional segregation of chromosomal loci via the *parABS* system without invoking or requiring large ParA filaments (**Figure 5*a***).

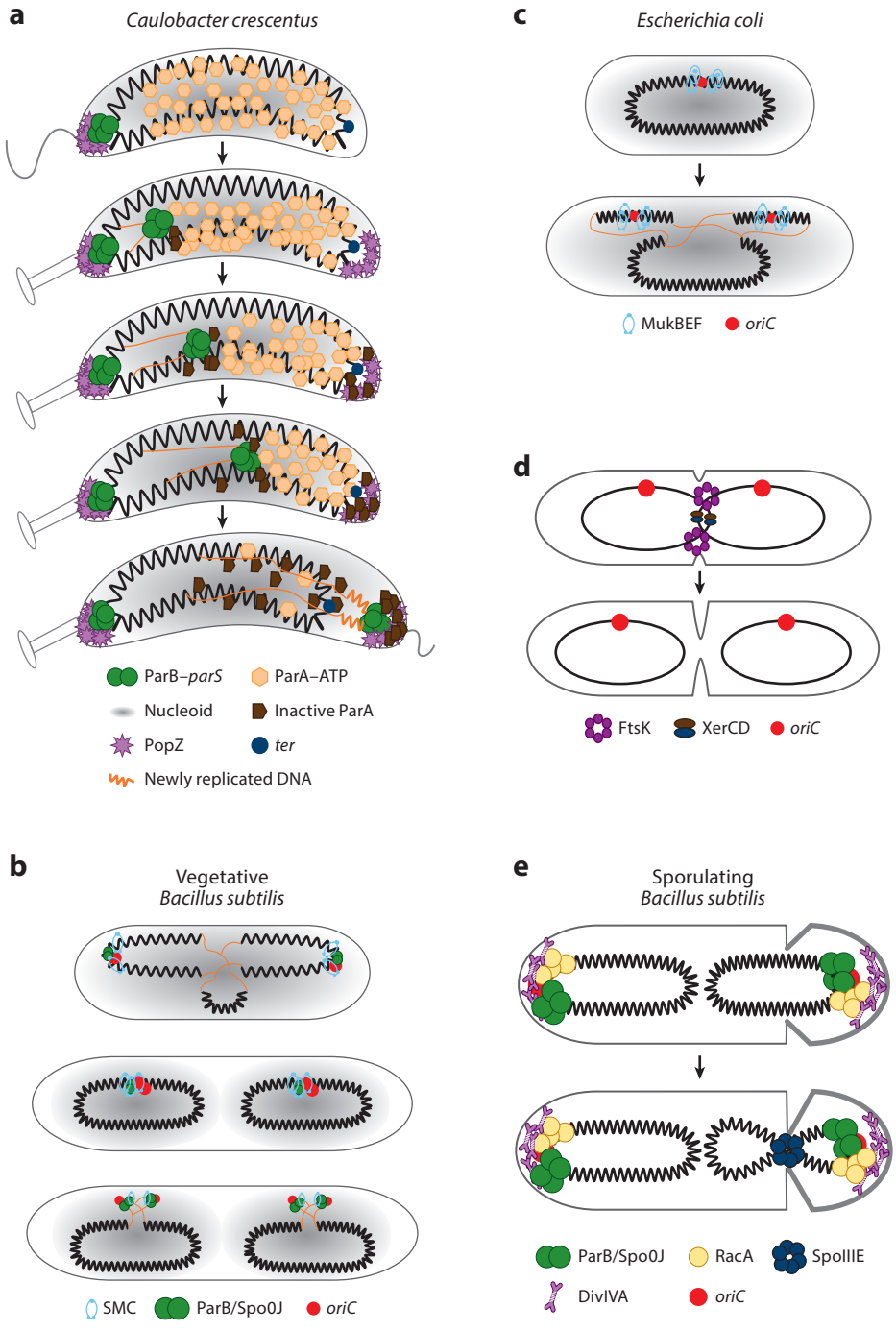
The polar anchoring protein PopZ, discussed above, may also help ensure directional movement of one origin toward the new cell pole in *Caulobacter* by anchoring the origin region to the cell pole (Bowman et al. 2008, Ebersbach et al. 2008, Laloux & Jacobs-Wagner 2013). PopZ may also regulate ParA activity (Ptacin et al. 2014, Schofield et al. 2010), possibly by sequestering ATP-hydrolyzed ParA generated near the translocating origin away from the nucleoid and by regenerating ParA-ATP that can bind the nucleoid again near the pole (Ptacin et al. 2014). This PopZ-dependent regulation of ParA may help ensure unidirectional movement of the translocating origin.

Although many organisms segregate replicated origins to opposite cell poles, some species produce multiple chromosomes that must be spaced evenly across the cell; these include *Synechococcus elongatus* and *S. coelicolor* during sporulation (Jain et al. 2012, Jakimowicz et al. 2007). In the latter case, ParAB is required to space out chromosomes and ParB itself is regularly distributed across the cell. ParA ATPase activity is essential for segregation, and ParA forms an apparent filament during segregation that disassembles prior to septation (Jakimowicz et al. 2007).

Although widespread, *parABS* is absent from some species, including *E. coli*. However, *E. coli* does harbor a *parS*-like site called *migS* that helps promote the bipolar segregation of origins, although it is not formally essential for successful chromosome segregation (Fekete & Chattoraj 2005, Wang & Sherratt 2010, Yamaichi & Niki 2004). A recent study in *E. coli* suggests that the MinDE system, which directly regulates cell division, could also promote chromosome segregation, albeit not by binding a specific site as ParAB does (Di Ventura et al. 2013). MinD and MinE normally oscillate back and forth across cells, inhibiting polymerization of the cytokinetic ring protein FtsZ at the poles and thereby helping force cell division to occur midcell. MinD is proposed to simultaneously bind the membrane and DNA nonspecifically, with MinDE oscillations biasing the movement of replicated DNA regions toward cell poles. However, the role of the Min system in chromosome segregation is difficult to discern, given that MinD and MinE may indirectly affect chromosome organization and segregation through their effect on cell division.

In addition to promoting the segregation of origins, ParA, ParB, and *parS* sometimes have additional functions and interaction partners. In *B. subtilis*, ParA (Soj) can regulate DNA replication initiation by interacting with DnaA (Murray & Errington 2008, Scholefield et al. 2011). Monomeric ParA/Soj inhibits DnaA from forming an oligomeric helix on DNA, thereby preventing replication initiation. By contrast, dimeric ParA/Soj, which binds DNA, appears to promote replication initiation through DnaA, although the precise mechanism of activation is still unclear. Other work in *B. subtilis* has shown that ParB (Spo0J) interacts with SMC, recruiting it to the origin region (Gruber & Errington 2009, Sullivan et al. 2009). This origin-localized SMC helps promote chromosome segregation (see next section) and somehow promotes the transient left-*ori*-right configuration of *B. subtilis* chromosomes noted above (Wang et al. 2014a) (**Figure 5*b***).

In *Caulobacter*, ParB interacts directly with MipZ, a protein similar to MinD that helps determine the midcell placement of FtsZ (Thanbichler & Shapiro 2006). MipZ inhibits FtsZ polymerization; hence, by associating with ParB, MipZ is primarily localized to the polar regions of the *Caulobacter* cell, leaving the midcell region free for FtsZ polymerization. A recent study suggests that the *parS* site in *Caulobacter* interacts not only with ParB, but potentially also with DnaA, providing a link between DNA replication initiation and origin segregation (Mera et al. 2014).



This model posits that DnaA binds *parS* sites, somehow altering the structure of the DNA around it in a manner that promotes ParB binding and, consequently, proper segregation.

Bulk Chromosome Segregation and Structural Maintenance of Chromosomes Proteins

The faithful segregation of two recently replicated origins by *parABS*, and the subsequent anchoring of these origins to opposite cell poles, may dictate the organization and segregation of the rest of the replicated chromosome (Umbarger et al. 2011). In other words, once the global orientation of chromosomes is set by the polar anchoring of the origins, purely physical forces may then drive the rest of segregation. As noted, the extrusion of DNA from replication forks may help push it toward opposite poles. Two studies suggest that segregation may result largely from entropic forces, arguing that, if the chromosome is a self-avoiding polymer, the maximization of entropy will intrinsically separate two chromosomes (Jun & Mulder 2006, Jun & Wright 2010). However, entropy alone may not explain the speed of bulk chromosome segregation in *E. coli*. Additionally, chromosomes are thought to be self-adherent rather than self-avoiding polymers (Fisher et al. 2013, Hadizadeh Yazdi et al. 2012, Kleckner et al. 2014).

Studies in *E. coli* have shown that replicated sister loci are initially cohesed together for roughly 7 to 10 min before being rapidly segregated apart, with some origin-proximal loci remaining cohesed even longer (Joshi et al. 2011). In *E. coli*, the cohesion of DNA is modulated at least in part by SeqA, which binds recently duplicated, hemimethylated DNA (Joshi et al. 2013, Sánchez-Romero et al. 2010). Additionally, sister chromosomes likely form precatenanes, structures in which the DNA from sister chromosomes becomes topologically entangled (Joshi et al. 2011, 2013; Wang et al. 2008). Thus, recently replicated regions cannot separate until sisters are disentangled via topoisomerase IV. Once free of topological constraints and protein-based tethers, duplicated DNA moves bidirectionally, likely producing a more relaxed state in the nascent, sister

Figure 5

Chromosome segregation. (*a*) Origin segregation in *Caulobacter crescentus* relies on the *parABS* system. ParB (*green*) binds *parS* sites located near the origin. Shortly after replication, one ParB:*parS* complex remains polarly localized while the second complex comes in contact with ATP-bound ParA (*light brown*). ParB stimulates ParA ATPase activity, resulting in the release of ParA from DNA (*dark brown*) and contraction of the cloud of ParA-ATP. The migrating ParB:*parS* complex then moves toward the retracting ParA-ATP cloud and thus toward the opposite pole, eventually resulting in full segregation of the origin. PopZ (*purple*) influences ParAB activity directly or indirectly to promote origin segregation. The terminus (*ter*) is denoted as a blue dot. (*b*) In vegetatively growing *Bacillus subtilis*, chromosome organization oscillates between *ori-ter* and left-*ori*-right patterns. Whereas ParA/Soj and ParB/Spo0J (*green*) ensure origin (*oriC*) movement toward opposite poles, the structural maintenance of chromosomes (SMC) complex (*cyan*) relocates the origins to midcell during the initial phase of DNA replication. This oscillation in chromosome organization may promote segregation by preventing entanglement of the chromosomes. (*c*) Origin segregation in *Escherichia coli*. Unlike *Caulobacter* and *B. subtilis*, *E. coli* does not have a ParAB-like system for origin segregation. A distant relative of the SMC complex, MukBEF (*cyan*), localizes around the origin region and is thought to promote origin segregation and origin-proximal chromosome organization. MukBEF may also promote bulk chromosome segregation. (*d*) Circular chromosome replication can result in dimeric or catenated chromosomes, whose resolution requires the action of the DNA translocase FtsK (*purple*) and the tyrosine recombinase XerCD (*blue-brown*). The schematic shown is for *E. coli*. (*e*) Chromosome segregation in sporulating *B. subtilis*. Segregation of the origin region depends on RacA (*yellow*) and Spo0J (*green*), with the rest of the chromosome pumped into the forespore by the DNA translocase SpoIIIE (*blue*). The origin is anchored to the cell pole by RacA and DivIVA (*purple sticks*).

chromosomes. In this way, the periodic buildup and release of mechanical stress may ultimately drive bulk chromosome segregation (Fisher et al. 2013, Kleckner et al. 2014). Some sister loci with particularly long periods of cohesion separate very rapidly and abruptly, consistent with the study noted earlier in which certain regions of the nucleoid displayed near-ballistic movement, sometimes during chromosome segregation (Javer et al. 2014). Notably, Hi-C analysis of *Caulobacter* cells progressing synchronously through the cell cycle has shown that CIDs get reestablished coincident with or shortly after replication, which may help prevent the two newly synthesized chromosomes from becoming entangled, aiding chromosome segregation (Le et al. 2013).

Although purely physical forces play a major, and perhaps dominant, role in bulk chromosome segregation, NAPs and other chromosome-associated proteins likely contribute as well (Junier et al. 2014). For instance, NAPs that compact DNA, such as HU and IHF, probably facilitate the segregation of recently duplicated DNA to opposite sides of cells (Hong & McAdams 2011, Swiercz et al. 2013). Indeed, strains with various NAP deletions often exhibit increased production of anucleate cells, an indicator of defective chromosome segregation (Huisman et al. 1989, Kaidow et al. 1995).

Similarly, supercoiling likely promotes bulk chromosome segregation by compacting DNA, and mutations in the gyrase, Topo IV, and Topo I genes can lead to defects in chromosome segregation (reviewed in Vos et al. 2011). Topo IV, which resolves precatenanes between sister chromosomes, may be particularly critical. When Topo IV activity is disrupted, cells can complete chromosome replication but sisters often remain colocalized (Wang et al. 2008).

Another key player in chromosome segregation is the SMC/ScpA/ScpB complex, or the related MukB/MukE/MukF complex found in *E. coli* and other γ-proteobacteria (**Figure 5*b*,*c***) (Britton et al. 1998, Danilova et al. 2007, Jensen & Shapiro 1999, Niki et al. 1991). In *E. coli*, the absence of MukB prevents the formation of the usual left-*ori*-right chromosome organization pattern and leads to an increase in anucleate cell formation, indicating that MukB/E/F may promote proper chromosome segregation. MukBEF complexes cluster around the origin region, although the mechanism of recruitment is unknown (Danilova et al. 2007) (**Figure 5*c***). It also remains unclear precisely how MukBEF contributes to chromosome segregation and whether it primarily affects the origin or also contributes to bulk chromosome segregation. Additionally, a major challenge is to determine whether MukBEF promotes chromosome segregation indirectly by condensing DNA, which may make other mechanisms of segregation operate more efficiently, or whether the complex plays a more active role in directly partitioning sister chromosomes. Recent studies have demonstrated that MukBEF directly stimulates Topo IV, implying that MukBEF contributes to the disentangling of sister chromosomes (Hayama & Marians 2010, Li et al. 2010, Nicolas et al. 2014).

In *B. subtilis*, SMC proteins are also thought to promote chromosome segregation, as cells lacking SMC exhibit a range of chromosome partitioning defects (Britton et al. 1998, Gruber & Errington 2009, Sullivan et al. 2009). In particular, rapid depletion of SMC reveals that it is required for origin segregation (Gruber et al. 2014; Wang et al. 2014a,b). As in *E. coli*, SMC is recruited to the origin-proximal regions of the chromosome via direct interaction with ParB. However, in contrast to *E. coli* MukB, SMC does not appear to function by promoting Topo IV activity (Wang et al. 2014b). Instead, SMC may primarily condense chromosomal DNA, helping sister chromosomes avoid becoming entangled and increasing the overall efficiency of chromosome segregation. ChIP studies in *B. subtilis* have shown that SMC proteins are also found in regions of high transcription. Thus, in addition to origin condensation, SMC may also aid in bulk chromosome condensation, ensuring fast and efficient segregation (Gruber & Errington 2009).

Terminus Segregation

Although much of chromosome segregation is accomplished by partitioning the origins and the ensuing bulk segregation of DNA, the final segregation of chromosome termini, the *ter* regions, requires dedicated machinery, in part because replication of circular chromosomes can result in catenated or dimeric chromosomes if sister chromosomes recombine (Adams et al. 1992, Peter et al. 1998, Steiner & Kuempel 1998). One major component of the *ter* segregation apparatus is the DNA translocase FtsK, which localizes with cell division proteins to midcell (Bigot et al. 2007, Lesterlin et al. 2004). There, FtsK binds and may stimulate Topo IV to decatenate chromosomes (Espeli et al. 2003). Additionally, FtsK can pump chromosomal DNA to opposite sides of the cell (Lesterlin et al. 2008). This pumping also brings together, near FtsK, the *ter*-proximal *dif* loci from sister chromosomes. FtsK directly activates the tyrosine recombinase XerCD, which can resolve dimeric chromosomes by catalyzing site-specific recombination between two *dif* loci (**Figure 5*d***) (Grainge et al. 2007, Steiner et al. 1999).

Bringing the *dif* loci together requires that FtsK-dependent pumping be directional. In *E. coli*, FtsK recognizes short motifs called KOPS (FtsK orienting polar sequences) that are overrepresented in the chromosome and heavily biased in their orientation toward *dif* (Bigot et al. 2005, Löwe et al. 2008, Sivanathan et al. 2006). Although FtsK translocase activity is not essential in *E. coli*, presumably because dimeric chromosomes are produced in only ~15% of cells per replication cycle, *ftsK* is essential for viability in the absence of MukBEF. This latter finding underscores the idea that FtsK contributes to bulk chromosome segregation in addition to specifically promoting the decatenation and resolution of sister chromosomes. Moreover, FtsK is present in bacteria with linear chromosomes, in which decatenation and dimer resolution are not essential for terminus segregation (Chaconas & Kobryn 2010, Flärdh & Buttner 2009); in these organisms, FtsK probably functions mainly to pump DNA from sister chromosomes to opposite sides of the division plane. The FtsK homolog SpoIIIE in *B. subtilis* also pumps DNA, localizing to the septum formed during sporulation between a mother cell and a forespore compartment. As discussed above, the polarly localized protein RacA helps anchor one origin inside the forespore, with SpoIIIE then pumping most of the rest of the chromosome into that compartment (**Figure 5*e***) (Ben-Yehuda et al. 2003; Wu & Errington 1994, 1998).

In most bacteria, cytokinesis is actively delayed until sister chromosomes are fully segregated to opposite sides of the cell, preventing the guillotining of DNA. In *E. coli*, the mechanism responsible, called nucleoid occlusion, involves a protein called SlmA that binds to specific DNA sites that are enriched in the terminus-proximal region of the chromosome. SlmA also binds to and blocks FtsZ polymerization (Bernhardt & de Boer 2005). Hence, SlmA blocks cell division until the terminus-proximal regions of the chromosome have been segregated away from midcell. In *B. subtilis*, a similar mechanism occurs, involving the unrelated protein Noc, which binds to DNA sequences across the chromosome. Noc does not specifically target FtsZ or other cell division proteins; instead, it appears to form large nucleoprotein complexes that physically occlude the division apparatus (Adams et al. 2015, Wu & Errington 2004, Wu et al. 2009).

Chromosome Segregation in the Absence of Replication

Although chromosome segregation is usually concomitant with, and linked to, DNA replication, cells may sometimes need to segregate regions of their chromosomes independent of replication. For example, DNA damage can require major movements of chromosomal DNA if homologous chromosomes must pair to promote recombination-based repair. Recent work in *E. coli* has shown that DNA near the site of a double-strand break can move, pair with its homologous partner, and

then be resegregated to its approximate original position (Lesterlin et al. 2014). This movement appears to involve large RecA filaments, but the nature of these filaments, how they drive homolog pairing, and whether they also participate in locus resegregation are unknown as yet. Nevertheless, this initial work suggests that bacteria have mechanisms to move and resegregate portions of their chromosomes; it will be critical to determine whether the mechanisms responsible overlap with or are different from those used to drive the segregation that occurs concomitantly with DNA replication.

FUTURE PERSPECTIVES

There are many outstanding questions and challenges in understanding the principles and mechanisms of chromosome organization and segregation in bacteria. New, powerful tools have been developed, including Hi-C and super-resolution fluorescence microscopy, which are enabling unprecedented investigations at many different spatial scales. Studies of model organisms continue to provide new insights, with work on other species helping to reveal the general, conserved properties of bacterial chromosomes and the idiosyncrasies of specific bacteria. Future work will undoubtedly continue to provide important new insights into the fundamental organization and functioning of bacterial chromosomes. Because of the central importance of chromosomes, this work promises to impact our understanding of nearly every physiological function of bacteria. Some of the immediate questions for future studies are:

- How does chromosome organization influence gene expression and vice versa?
- How does chromosome organization, including domain structure, influence DNA-based transactions such as DNA replication, DNA repair, and recombination?
- Many NAPs are individually dispensable but display synthetic effects when deleted in combination; how do NAPs work together to organize the chromosome, support chromosome segregation, and regulate transcription?
- How do the biochemical and biophysical properties of SMC and NAPs ultimately enable the cellular-level phenomena of chromosome compaction and segregation?
- How do chromosomes successfully segregate in species that do not have the ParAB-*parS* system?
- What drives bulk chromosome segregation, and what are the relative contributions of purely physical forces and protein-based systems?

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