Organization and segregation of bacterial chromosomes

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Abstract | The bacterial chromosome must be compacted more than 1,000-fold to fit into the compartment in which it resides. How it is condensed, organized and ultimately segregated has been a puzzle for over half a century. Recent advances in live-cell imaging and genome-scale analyses have led to new insights into these problems. We argue that the key feature of compaction is the orderly folding of DNA along adjacent segments and that this organization provides easy and efficient access for protein–DNA transactions and has a central role in driving segregation. Similar principles and common proteins are used in eukaryotes to condense and to resolve sister chromatids at metaphase.

DNA transactions
Processes in the cell that act on DNA: for example, transcription, replication, recombination and repair.

Plectonemic loops
Also known as interwound loops, these are loops of DNA that are twisted together as a result of under- or over-winding the DNA duplex.

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The visualization and characterization of the genetic material in bacteria has had a bumpy and controversial history. In eukaryotes, the orderly segregation of sister chromatids in mitosis was described in awe-inspiring detail in the 1880s[1]; by contrast, the bacterial chromosome, which tends to stain uniformly with basic dyes, was for many years believed to be unstructured. It was not until the 1930s that light microscopists using DNA dyes with acid-treated cells convincingly demonstrated that the bacterial chromosome was concentrated in discrete bodies with soft irregular outlines[2,3] (FIG. 1A). These images changed the view of the bacterial chromosome from a formless material to a defined structure that hinted at orderly and predictable behaviour[4]. These cloud-like nuclear bodies were named nucleoids.

Cryoelectron microscopy of vitreous sections of nucleoids revealed structures with features similar to those observed using DNA dyes (FIG. 1B) and showed irregular and dispersed morphologies that occupy about half of the intracellular space. Two striking features of these images were the presence of many coral-like projections that extended into the cytoplasm and the exclusion of the ribosomes from the nucleoid[4]. Similar compartmentalization has since been observed using fluorescence microscopy[5] (FIG. 1C). These images still provoke our thinking about the bacterial chromosome. We envision a dynamic DNA surface that interacts with proteins in the cytoplasm. Although proteins can penetrate into and reside within the interior of the nucleoid, most DNA transactions are thought to occur at its periphery.

In the early 1970s, Pettijohn and colleagues[6–9] developed methods to lyse *Escherichia coli* gently and to obtain nucleoids for direct electron microscopy visualization, providing an enduring image of the bacterial chromosome as a collection of plectonemic loops (that is, interwound loops) emanating from a dense core (FIG. 1D) that is suggested to be organized by proteins and RNA[6–8,10]. The composition, organization and function (and even existence) of the core remain important and outstanding issues in the field. These studies led to the rosette model of the bacterial chromosome in which interwound loops are organized by a nucleoid scaffold (FIGS 1D,2a), creating a structure that resembles a bottlebrush. However, the molecular nature of this compact aggregate of DNA, its cellular localization and organization, and its local and global dynamics in living bacteria remained elusive.

Various technical advances (BOX 1) are providing new and exciting insights into bacterial chromosome organization and dynamics. These include fluorescence-microscopy-based live-cell imaging to track multiple chromosomal loci in real time during cell division cycles, along with the development of genome-wide molecular and analytical approaches to study the conformations of chromosomes and the patterns of chromosome-associated proteins. In this Review, we draw on these recent studies to discuss our current understanding of two problems: how the chromosome is organized and compacted in the bacterial cell and how the replicated chromosomes are disentangled and segregated. We discuss these topics separately but, as you will see, they are intimately connected. Our guiding premise is that the orderly folding of the chromosome, which occurs along adjacent DNA segments (called lengthwise condensation) in lockstep (that is, in synchrony) with its replication, generates its higher-order organization and functions as the driving force for bulk chromosome segregation. Throughout,

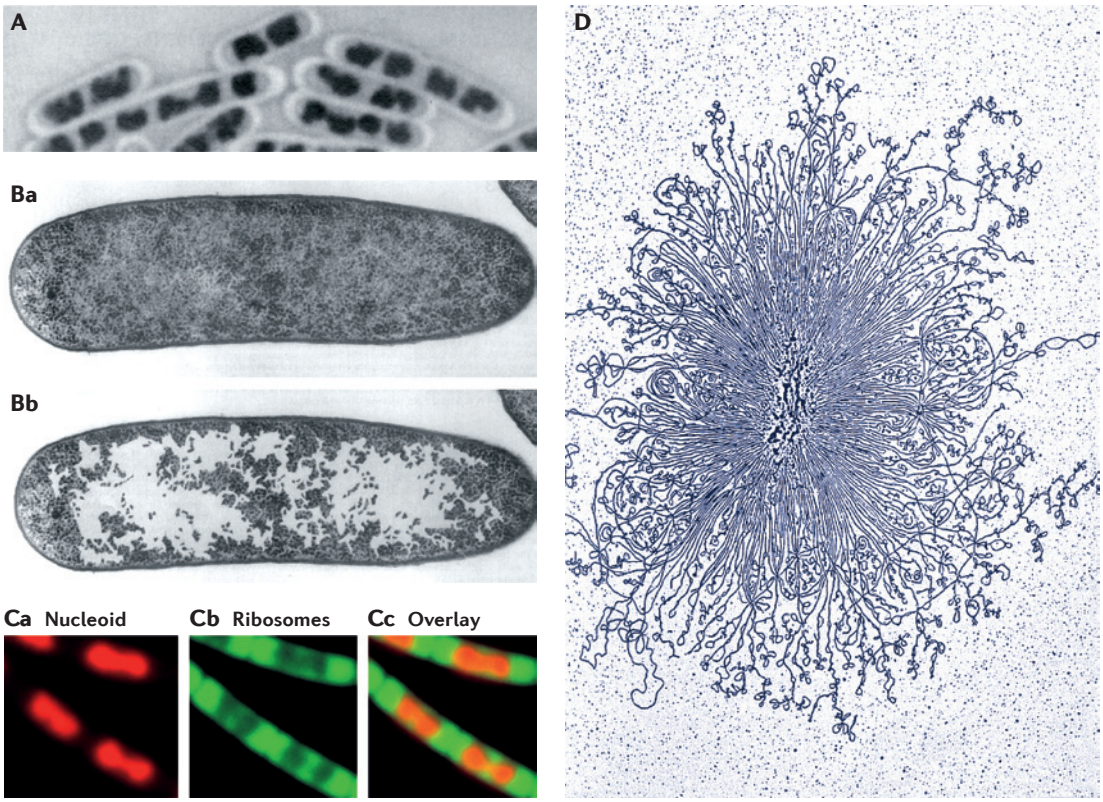


Figure 1 | **The bacterial nucleoid.** **A** | *Bacillus subtilis* nucleoid stained with Giemsa using acid-treated cells. **B** | The nucleoid of growing *Escherichia coli* in thin section after cryofixation followed by freeze substitution. Panels **a** and **b** show the same section; in the panel **b**, the ribosome-free spaces were enhanced by colouring by hand. **C** | Nucleoid (**a**; stained with 4′,6-diamidino-2-phenylindole (DAPI), coloured red) and ribosomes (**b**; labelled with RplA–GFP, coloured green) in live *B. subtilis* cells growing in rich media. Part **c** shows the overlay of the two images. Despite this commonly depicted cloud-like appearance of the bacterial chromosome, the morphology of the nucleoid varies among bacteria and is influenced by growth rate and environmental conditions. For example, the nucleoid in *Caulobacter crescentus*, and in slow-growing *E. coli* and *B. subtilis*, appears to be more diffuse and occupies a greater proportion of the cell cytoplasm (not shown). **D** | A gently isolated *E. coli* nucleoid bound by cytochrome C, spread on an electron microscope grid, stained with uranyl acetate and visualized by transmission electron microscopy. The length of *E. coli* and *B. subtilis* cells is 2–5 μm, whereas the nucleoid in panel **D** is approximately 20 μm in length. Parts **A** and **B** are reproduced, with permission, from REF. 4 © (1994) American Society of Microbiology. Part **D** is reproduced, with permission, from © Designergenes Posters Ltd; in memory of Dr Ruth Kavenoff 1944–1999.

we highlight which principles and molecular mechanisms are shared with eukaryotes and which aspects are specific to the chromosomal dynamics of bacteria.

Chromosome compaction and organization

Most bacteria contain a single circular chromosome of 2–8 Mb in size that replicates bidirectionally from a unique origin (*oriC*). If stretched out, this DNA molecule would be >1 mm in length, whereas the space occupied by the nucleoid is <1 μm in diameter. Accordingly, the chromosome must be linearly compacted more than 1,000-fold to fit inside the bacterial cell[11,12]. The DNA is condensed in an orderly and hierarchical fashion, and we describe this compaction and organization from its smallest unit to its largest domain.

Topological domains. The principal mechanism by which the bacterial chromosome is compacted is by negative DNA supercoiling. This under-winding of the DNA duplex generates plectonemic loops and branches like the ones observed by electron microscopy in the nucleoid spreads. Supercoiling condenses the chromosome, but it also draws DNA in on itself, pulling it away from non-contiguous DNA (such as replicated sister DNA). Unlike plasmids, which can be relaxed by one single-strand break, numerous nicks are required to relax the chromosome completely, suggesting that chromosomal DNA is organized into supercoiled domains that are topologically insulated from each other[8]. Elegant molecular experiments that exploit supercoiling-sensitive activities (such as transcription and recombination) suggest that independent topological domains vary in size but are on average 10 kb[13–15]. Thus, a 4 Mb genome would have approximately 400 topologically isolated domains. In the context of the highly schematized bottlebrush model for the nucleoid, these domains are the branched plectonemic loops that make up the bristles (FIGS 1D,2a). Although this model

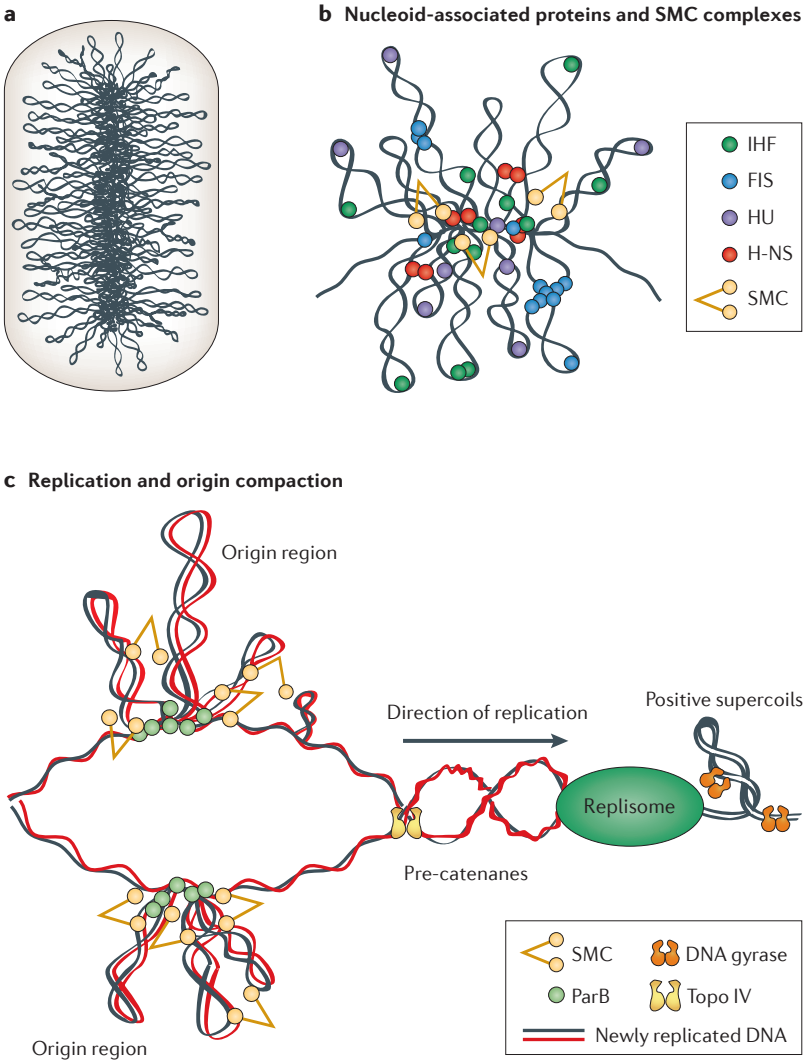


Figure 2 | **Topological organization of the bacterial chromosome.** **a** | Schematic representation of the bottlebrush model of the nucleoid. This diagram depicts the interwound supercoiled loops emanating from a dense core. The topologically isolated domains are on average 10 kb and therefore are likely to encompass several branched plectonemic loops. The macrodomains discussed in the main text are not depicted in this model. **b** | Schematic representation of the small nucleoid-associated proteins and the structural maintenance of chromosome (SMC) complexes. These proteins introduce DNA bends and also function in bridging chromosomal loci. **c** | The diagram depicts replication fork progression and compaction of the origin region. Replication generates positive supercoils ahead of the fork, which can diffuse behind the replisome, producing pre-catenanes. Positive supercoils are removed by DNA gyrase and topoisomerase IV (Topo IV), and pre-catenanes are unlinked by Topo IV. Newly replicated origin regions are thought to be compacted by the SMC complexes that are recruited to the origin by ParB and by the action of small nucleoid-associated proteins (not shown).

of radial loops constitutes the simplest configuration, more complicated inter-segmented linkages are also possible. In addition to their role in condensing the chromosome, these topological domains protect the chromosome from DNA relaxation, assist in decatenation of chromosomal links and have been proposed to aid in the repair of double-strand breaks by maintaining broken ends in close proximity[14].

Decatenation
The act of unlinking interlocked (or catenated) rings.

For these interwound loops to be topologically insulated, they need boundary elements (so-called domainins) that restrict the free rotation of DNA. Domainins are thought to function by constraining loops and are likely to be concentrated at the nucleoid core. Many factors have been proposed to serve as domainins, including abundant, small nucleoid-associated proteins, structural maintenance of chromosome (SMC) condensin complexes, topoisomerases, RNA polymerase and even RNA[14,16,17] (FIG. 2b). Most of these factors and their roles in chromosome organization and compaction are discussed in greater detail below. Despite the image of a rosette with a static core that the nucleoid spreads evokes, the current view is that the interwound loops and their boundaries (and, by extension, the domainins that define them) are highly dynamic, changing in response to DNA transactions that occur within and between them[13,14,16]. Consistent with this idea, chromosomal loci display remarkable, albeit constrained, mobility within the nucleoid[18–20]. Thus, we envision a dynamic nucleoid core or scaffold composed of a loose assemblage of domainins. This fluid scaffold provides structure and organization to the supercoiled loops without imposing rigidity.

Supercoiling homeostasis is principally governed by the opposing actions of DNA gyrase, which introduces negative supercoils, and topoisomerase I (Topo I), which relaxes them[21–23]. Gyrase and Topo I localize and act throughout the chromosome[24,25]. However, gyrase and another topoisomerase (namely, Topo IV) are also enriched ahead of replication forks and transcription bubbles, where they have key roles in alleviating the positive supercoils introduced by DNA unwinding[26,27]. Positive supercoils ahead of a replication fork that are not attended to can diffuse backwards, generating entangled sister strands (called pre-catenanes)[28,29] (FIG. 2c). Topo IV is the principal enzyme responsible for removing these entanglements[30–32] and, as such, it plays a central part in segregating the replicated chromosomes. In eukaryotes, Topo II has an analogous function to Topo IV, removing entanglements generated by DNA replication to resolve sister chromatids during the early stages of mitosis[33–35].

Unconstrained supercoils alone cannot account for the degree of compaction exhibited by the bacterial chromosome. Approximately half of the chromosome is thought to be constrained by small, abundant DNA-binding proteins[36] (FIG. 2b). These proteins are the bacterial equivalent of eukaryotic histones. Instead of wrapping DNA into nucleosomes, they bind specifically and nonspecifically throughout the genome and facilitate chromosome compaction and organization by introducing bends in the DNA and by bridging chromosomal loci. Bending facilitates condensation of adjacent DNA segments, whereas bridging stabilizes DNA loops[37–45]. This bridging activity suggests that these proteins function as domainins[36,41]. In *Escherichia coli*, the principal histone-like proteins are HU, IHF, Fis and H-NS. Other bacteria (such as *Bacillus subtilis* and *Caulobacter crescentus*) have a subset of this class of proteins. Cells that lack these factors have defects in chromosome segregation. However, the nucleoid does not appear to be

dramatically decondensed in their absence, raising the possibility that other factors may have a more important role in compaction and constraining supercoiled domains. Interestingly, as cells enter the stationary phase, a different set of nucleoid-associated proteins is induced[45]. These proteins remodel the bacterial chromosome into a strikingly compact structure[46]. Similarly, during *B. subtilis* sporulation, a separate set of proteins remodels the spore chromosome into a compact toroidal structure in preparation for dormancy[47].

Box 1 | Technical advances for studying bacterial chromosomes

Localizing individual loci

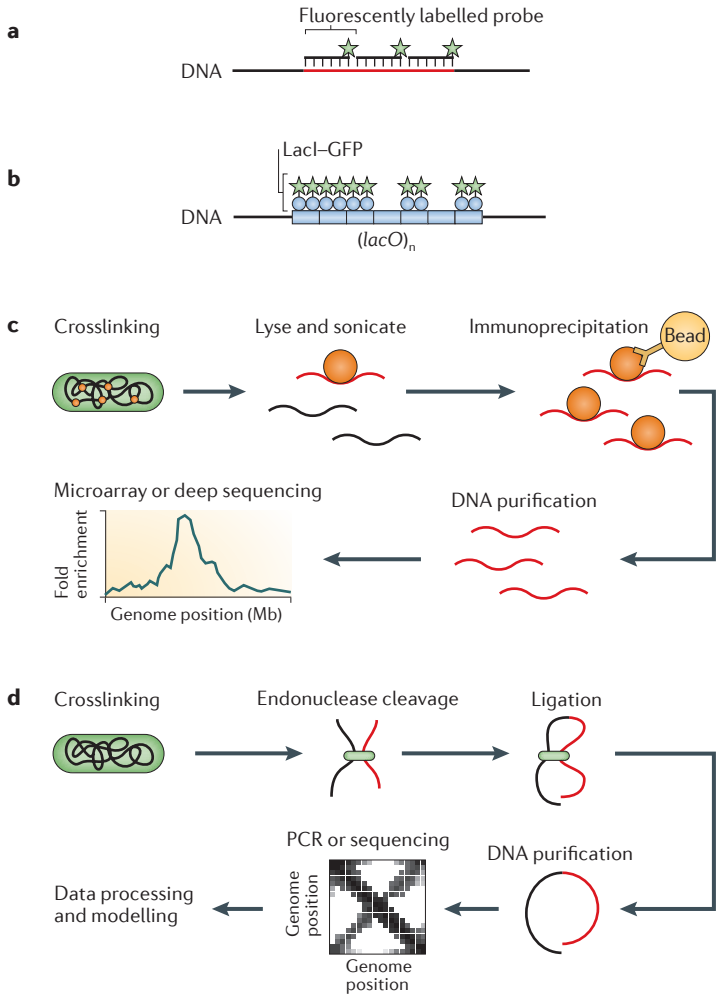
Fluorescence* in situ *hybridization (FISH). Visualization of individual genetic loci using fluorescently labelled locus-specific DNA probes in fixed and permeabilized cells (see part **a** of the figure).

Fluorescently tagged DNA-binding proteins, including the fluorescence repressor–operator system (FROS). Visualization of the localization of individual genetic loci in live cells can be achieved using fluorescently labelled DNA-binding proteins (see part **b** of the figure). For FROS, this involves fusions between fluorescent proteins and repressor proteins (namely, LacI, TetR or lambdaCI), which are used to track engineered tandem arrays of cognate operator sequences (such as *lacO*, *tetO* or λO_L1) in the chromosome[69,70,75,154]. Alternatively, fluorescent fusions to plasmid-encoded ParB proteins can be used to track bound *parS* sites[85]. As plasmid *parS* sites do not resemble chromosomal *parS* sites, the fluorescent ParB fusion protein does not bind to or interfere with the native partitioning system.

Genome-wide methods

Chromatin immunoprecipitation (ChIP)-based methods. These approaches allow the genome-wide identification of binding sites for DNA-binding proteins (see part **c** of the figure). Protein–DNA complexes are crosslinked and isolated by immunoprecipitation. The DNA region bound by the protein of interest is then identified by hybridizing to a microarray (for ChIP–chip) or by high-throughput sequencing (for ChIP–seq).

Chromosome conformation capture methods. These examine global conformation of the chromosome by assessing the frequency that any two DNA loci are in close proximity and thus can be crosslinked. The strategies include the original chromosome conformation capture (3C) method and its higher-throughput derivatives chromosome conformation capture carbon copy (5C) and Hi-C[81,155,156] (see part **d** of the figure).



Method	Advantages	Disadvantages
FISH	• Direct and highly specific detection of chromosomal loci • Analysis can be carried out in wild-type strains without the need to modify the genome	• Requires fixed and permeabilized cells, which can alter the conformation of the chromosome and produce artefacts • Provides only a static picture of the chromosome • Technically challenging: labelling yield is less than 100%
Fluorescent DNA-binding proteins	• Live-cell method • Provides dynamics of individual loci on short and long timescales • Labelling efficiency near 100%	• Requires insertion of exogenous sequences into the genome • Tight binding of proteins to their target sequences can produce replication roadblocks • Interactions between the DNA-binding proteins or fluorescent tags can artificially increase cohesion of genetic loci
ChIP-based methods	• Provides genome-wide information • Reports on sequence-specific and nonspecific protein–DNA interactions	• Population-based assay provides ensemble information only • Crosslinking efficiency and the quality of antibodies can result in false-positive and false-negative results
3C, 5C and Hi-C	• Provides a global three-dimensional conformation of the chromosome inside the cell • Reports on short-range and long-range interactions	• Population-based assay provides only ensemble information; asynchronous cultures will complicate interactions and compromise the quality of the model • Technically challenging: optimization of several steps can be difficult and time-consuming

The highly conserved SMC condensin complex is perhaps the best candidate to constrain plectonemic loops and to function as a dynamic nucleoid scaffold[48,49] (FIG. 2b). In eukaryotes, SMC complexes function in chromosome condensation, sister chromatid cohesion, recombination and X-chromosome dosage compensation[50,51]. The SMC complex in most bacteria is composed of the SMC protein, a kleisin (closure) subunit called ScpA and a third protein called ScpB[52–55]. Structural and functional analogues of this complex (called MukB, MukF and MukE) are found in *E. coli*[56–58]. Cells that lack any of the proteins in the condensin complex are inviable at 37 °C. At lower temperatures, bacteria survive without this complex, but they have decondensed nucleoids and severe defects in chromosome segregation[52,54,55,59]. The mechanism by which these large complexes organize and compact DNA has remained enigmatic and is the subject of intense research. Biochemical studies indicate that SMC complexes or higher-order multimers can bridge and constrain DNA loops[17,48,60,61]. Our view is that this bridging activity works hand-in-hand with supercoiling and the small nucleoid-associated proteins to fold the chromosome along adjacent segments. If correct, then these complexes are likely to act locally on neighbouring stretches of DNA, despite their large size. Understanding how the condensin complex functions *in vitro* and *in vivo* lies at the heart of understanding how the bacterial chromosome is organized and compacted and how sisters are segregated.

Macrodomains. The nucleoid is further organized into higher-order structures called macrodomains. These large regions (800 kb–1 Mb in size) have been identified in *E. coli*, but we suspect that they are a common feature of many bacterial chromosomes. The higher-order organization that defines a macrodomain does not appear to have a central role in the process of chromosome segregation but refines it and increases its fidelity[62] (see below). Organization of the chromosome into macrodomains was first recognized using fluorescence *in situ* hybridization (FISH)[63] (BOX 1). Large regions of the genome spanning the origin (*ori*) and terminus (*ter*) exhibited spatially restricted localization patterns that were distinct from the rest of the chromosome, suggesting that loci in these regions cluster. Interestingly, a genetic assay based on recombination to assess the frequency of random collisions between different sites on the chromosome identified the same ori and ter macrodomains. This assay further delineated two additional insulated domains flanking the ter macrodomain (called the left and right macrodomains) and two flexible or unstructured regions lying on either side of the ori macrodomain[64]. Consistent with the idea of structured macrodomains and unstructured flexible regions, time-lapse imaging revealed that chromosomal loci have different dynamic behaviours depending on their positions in the chromosome[65]. Loci in the unstructured regions displayed greater mobility than those within macrodomains.

The molecular mechanism underlying macrodomain organization is still unknown. However, recent evidence suggests that sequence-specific DNA-binding proteins participate in this higher-order organization. Bioinformatics analysis identified the sequence motif *matS*, which was highly over-represented in the *E. coli* ter macrodomain and almost absent from the rest of the genome[62]. This sequence element facilitated the discovery of the DNA-binding protein MatP, which binds all 23 *matS* sites *in vivo*. Interestingly, MatP localizes as a focus that overlaps loci that are present in the ter macrodomain, suggesting that it gathers or organizes *matS* sites. In this capacity, it could act as a site-specific domainin; alternatively, it could function in bundling interwound loops in the terminus region. In support of the idea that MatP is the ter macrodomain organizer, loci in this domain become more mobile in cells that lack MatP and more frequently recombine with neighbouring domains. Recent structural determination of MatP bound to *matS* revealed a long tetrameric linkage that would allow two MatP dimers to bridge two distant *matS* sites[66]. *In vivo* analysis of mutants that are unable to tetramerize suggests that DNA bridging has a crucial role in condensation of the ter macrodomain but is not required to define the terminus region as a macrodomain. How MatP defines the ter macrodomain, the identity of the proteins that specify the other macrodomains in *E. coli* and whether similar domains exist in other bacteria are all active areas of investigation.

Recent work on the localization of nucleoid-associated protein H-NS and the DNA loci it interacts with suggests that this DNA bridging protein could impose a different type of organization on the *E. coli* chromosome. This study revealed that H-NS forms 1–4 stable complexes at or near the nucleoid centre. Genes regulated by H-NS that are located in all four macrodomains and in the two unstructured regions all appear to be present in these central H-NS complexes[67,68]. How these H-NS interaction centres influence and are influenced by the macrodomains described above is not yet clear.

Cellular organization of the chromosome. Thus far, we have considered the organization and compaction of the chromosome without the spatial reference of the bacterial cell in which it resides. The development of methods to visualize individual chromosomal loci in live cells using fluorescence microscopy[69–72] (BOX 1) revealed a degree of spatial organization that had not previously been appreciated[72–76]. This robust spatial organization reinforces our thinking about how the chromosome is compacted and informs our models for how newly replicated DNA is segregated.

The first cytological studies aimed at defining the subcellular localization of chromosomal loci were done in *B. subtilis*[74]. Analysis of a locus adjacent to the origin revealed that replication initiates at or near mid-cell and that newly replicated origins rapidly segregate to the outer edges of the nucleoid. The subcellular localization of four chromosomal positions suggests that, on completion of replication, the nucleoid adopts an organization in which the origins are present near opposite cell poles, the termini are at mid-cell, and the left and right chromosome arms lie between them[73] (FIG. 3A). How and when the origin moves to mid-cell to initiate a new

Topoisomerases
Enzymes that modify DNA topology. Some of these enzymes affect supercoiling by under- or over-winding the DNA, whereas others decatenate interlocked rings.

DNA gyrase
A topoisomerase that introduces negative supercoils into DNA. Often referred to as gyrase. This enzyme functions by cutting both strands of the DNA, passing a looped strand of DNA through the cut site followed by resealing.

Pre-catenanes
Interlocked rings that are generated during replication. Many of these rings are unlinked before the completion of replication. Those that remain after replication is complete are called catenanes.

Sporulation
The process by which a bacterial cell differentiates into a dormant and stress-resistant cell type called a spore.

Chromosome arms
The origin and terminus of replication divide the genome into separate replicated halves. Each half is referred to as a replichore or a chromosome arm.

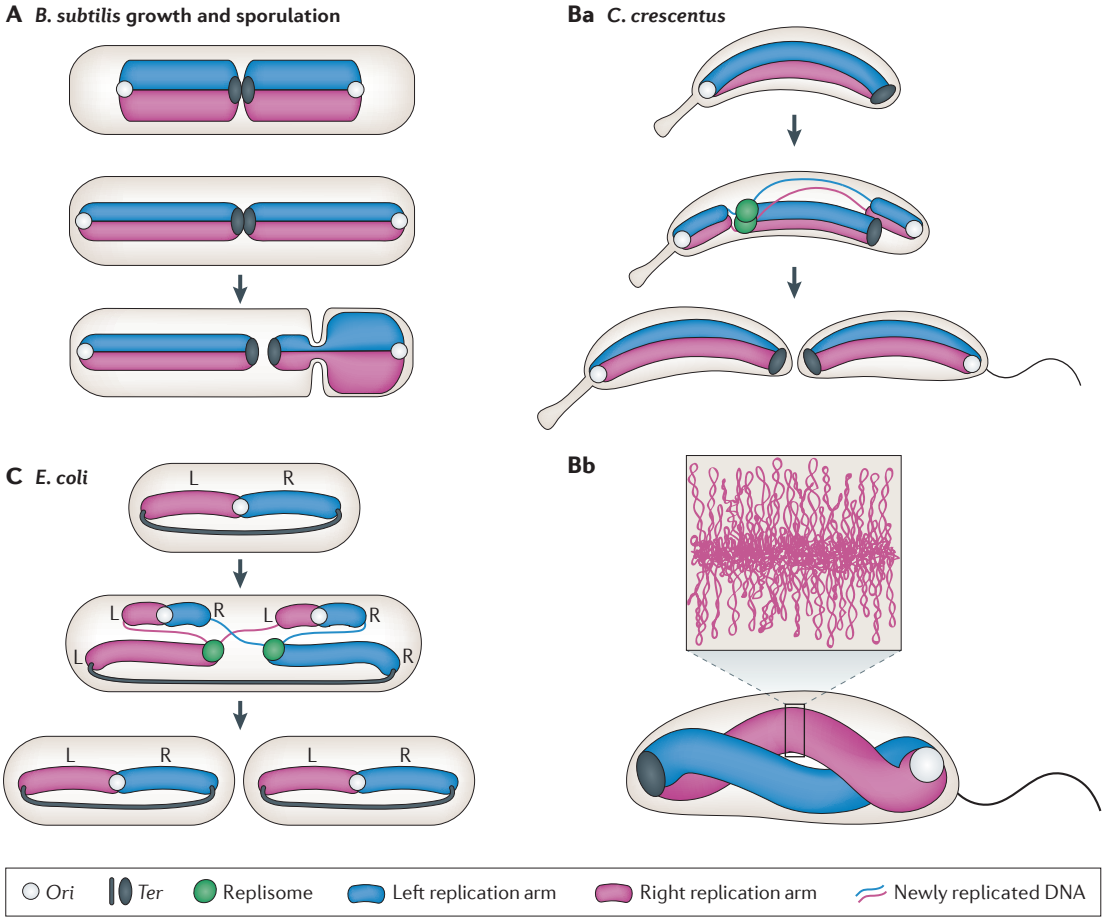


Figure 3 | **Spatial organization of bacterial chromosomes.** Cellular organization of the chromosome in *Bacillus subtilis* during growth and spore formation (**A**); slow-growing *Caulobacter crescentus* (**B**) and *Escherichia coli* (**C**) during vegetative cell cycles. During sporulation in *B. subtilis*, an asymmetric division traps ~25% of the chromosome in the smaller spore compartment[77,129]. A DNA translocase (not shown) pumps the remaining 75% of the chromosome into the spore after cytokinesis[140]. **Bb** shows a model for the spatially separate but gently twisted arms of the *C. crescentus* chromosome on the basis of chromosome conformation capture[81]. The inset shows the plectonemic loops within one of the arms. This figure is adapted, with permission, from REF. 157 © (2012) US National Academy of Science.

round of replication and whether this movement affects the overall organization of the chromosome are questions that remain to be addressed. Interestingly, using an elegant genetic assay[77], a similar '*ori–ter ter–ori*' nucleoid layout was identified in sporulating *B. subtilis* cells (FIG. 3A). During sporulation, the replicated chromosomes assemble into an elongated structure that extends from one cell pole to the other[74,78,79]. Within this serpentine-shaped structure, the replicated origins are located at opposite cell poles, and the termini reside at mid-cell.

Analysis of chromosome organization in *C. crescentus* and *E. coli* followed the early cytological studies in *B. subtilis* and benefited from improved fluorescent proteins, methods to track chromosomal loci and system-wide approaches. In *C. crescentus*, analysis of >100 separate loci revealed that the physical location of loci inside the cell recapitulates the genetic map[76]. In cells that have not yet initiated replication, the origin and terminus are present at opposite poles, and all other loci are organized along the long axis of the cell in an order that directly correlates with their position in the genome (FIG. 3B). Unlike the mid-cell initiation of replication in *B. subtilis*, replication initiates in *C. crescentus* at the origin-containing cell pole, and then one of the replicated origins rapidly moves to the opposite pole. Replicated loci on the left and right arms follow suit. Thus, when replication is complete, the sister chromosomes have an *ori–ter*, *ter–ori* organization (FIG. 3B). The linear organization of the chromosome arms suggests an orderly folding of adjacent DNA segments. However, the resolution of this cytological approach is not sufficient to assess whether or not the two chromosome arms are spatially resolved or entangled[80].

Recent experiments in *C. crescentus* have used a high-throughput chromosome conformation capture assay (called 5C; BOX 1) combined with computational modelling to characterize long-range chromosomal interactions. These studies have addressed the disposition of the two arms and have provided the first three-dimensional

model of a bacterial chromosome[81] (FIG. 3B). Consistent with the fluorescence microscopy studies, the model suggests that in *C. crescentus* the right and left arms of the chromosome are symmetric and linearly organized along the *ori–ter* axis (FIG. 3Ba). Importantly, the two arms are spatially separated, although they gently twist around each other approximately one and a half times (FIG. 3Bb). Thus, it appears that this bacterium condenses its chromosome along its length, generating two bottlebrushes: one for each chromosome arm. This three-dimensional rendering of the *C. crescentus* chromosome is reminiscent of the twisted nucleoid structures observed by fluorescence microscopy in *B. subtilis*[82] and *E. coli*[83].

Similar systematic and genome-wide cytological analyses were carried out in *E. coli* using slow-growing cells with a eukaryote-like cell cycle, such that newborn progeny have a single copy of the chromosome (FIG. 3C). These studies revealed that the organization of the *E. coli* chromosome is strikingly different from *C. crescentus*. At birth, the origin localizes near mid-cell with the left and right chromosome arms in opposite cell halves[84–86]. To complete the circle, the terminus region spans the length of the cell to bridge the two arms. During the process of replication (and after its completion), the sister chromosomes are organized into a left–*ori*–right, left–*ori*–right conformation (FIG. 3C), such that cell division recapitulates the original organization in the daughter cells. Despite the difference in global chromosome organization, much like the situation in *C. crescentus* the left and right chromosome arms are linearly organized and have approximately constant packing density[84–86]. The observed left–*ori*–right organization is consistent with the low frequency of recombination between loci in the left and right macrodomains. However, the role of the *ter* region as an extended connector of the left and right arms appears to be at odds with a structured and compact ter macrodomain[65]. How this macrodomain fits in the context of the cellular organization of the chromosome remains to be discovered. In summary, although there are fundamental differences in the arrangement of the chromosome in different bacteria, the emergent and unifying theme is that the DNA is linearly organized and condensed along its length with approximately constant packing density.

Chromosome segregation

The past two decades have revealed an amazing degree of spatial organization of the bacterial chromosome. Importantly, as we have discussed, this organization is generated in lockstep with replication and as a part of the segregation process. With this backdrop, we now turn our attention to how the replicated sisters are segregated. Segregation of most bacterial chromosomes can be broken down into three discrete steps: separation of the newly replicated origins; bulk chromosome segregation; and resolution and transport of the replication termini at the division septum. A surprisingly small set of highly conserved proteins has been implicated in these steps. We discuss each step separately and in the context of the patterns of nucleoid organization described above and their recreation in the next generation.

Septum
The structure generated during the division process that compartmentalizes a cell into two daughter cells.

Plasmid maintenance
The processes that ensure faithful inheritance of a plasmid in daughter cells.

Origin segregation

Unlike eukaryotic cells, which have temporally distinct phases for DNA replication, chromosome condensation and sister chromatid segregation, bacteria organize, compact and segregate their chromosomes progressively as the sister chromosomes are generated[76,87,88] (with exceptions described below). Accordingly, much attention has been focused on how the origins are segregated, as origin repositioning provides a path and a destination for the rest of the chromosome.

The mechanism by which the newly replicated origins are segregated has been the subject of speculation and investigation for more than half a century. The origin attachment model proposed by Jacob, Brenner and Cuzin[89] in 1963 was among the first and endured for more than three decades. This model posits that the two newly replicated origins are tethered to the cell envelope close to mid-cell and are separated by cell growth between them. It is now clear that cell elongation in rod-shaped bacteria is not restricted to zonal growth at mid-cell but occurs throughout the cell cylinder. Furthermore, the movement of the origins away from mid-cell is much faster than the rate of cell growth[18,76,90,91]. Thus, this attractively simple model cannot account for origin segregation.

As opposed to passive segregation embodied in the origin attachment model, active partitioning systems were first identified on plasmids in the 1980s[92]. These partitioning systems are essential for stable plasmid maintenance of low-copy-number plasmids[93–97], and their molecular characterization continues to have an important role in our understanding of how chromosomal origins are segregated. Remarkably, over 65% of all sequenced bacterial genomes contain a chromosomally encoded partitioning (*par*) locus[98]. These species include *B. subtilis*[99,100], *C. crescentus*[101] and *Vibrio cholerae*[102]. By contrast, *E. coli* and its close relatives do not possess this system. Chromosomal *par* loci (like their plasmid counterparts) consist of two genes, *parA* and *parB*, as well as a *cis*-acting DNA site called *parS*. This centromere-like DNA element is frequently present in multiple copies and is almost always located in close proximity to the replication origin[98]. Insertion of this three-component partitioning module onto an unstable plasmid improves plasmid maintenance even in unrelated host bacteria (including *E. coli*)[95,103,104]. Thus, this locus has all the information required to partition DNA harbouring the *parS* sequence.

For years, bacterial cell biologists have searched for a mitotic apparatus akin to the machinery used by eukaryotes to segregate sister chromatids. The Par system is likely to be the closest bacterial counterpart. However, instead of segregating fully replicated sister chromatids, this system helps to separate newly replicated origins (FIG. 4a). ParB is a DNA-binding protein that site-specifically binds to the *parS* sites, generating a large nucleoprotein complex adjacent to the origin[100,105,106]. ParA is an ATPase with nonspecific DNA-binding activity that acts on this centromeric complex[96,107]. Instead of microtubules and motor proteins, the ParA motor uses the nucleoid (a veritable sea of nonspecific DNA) to pull

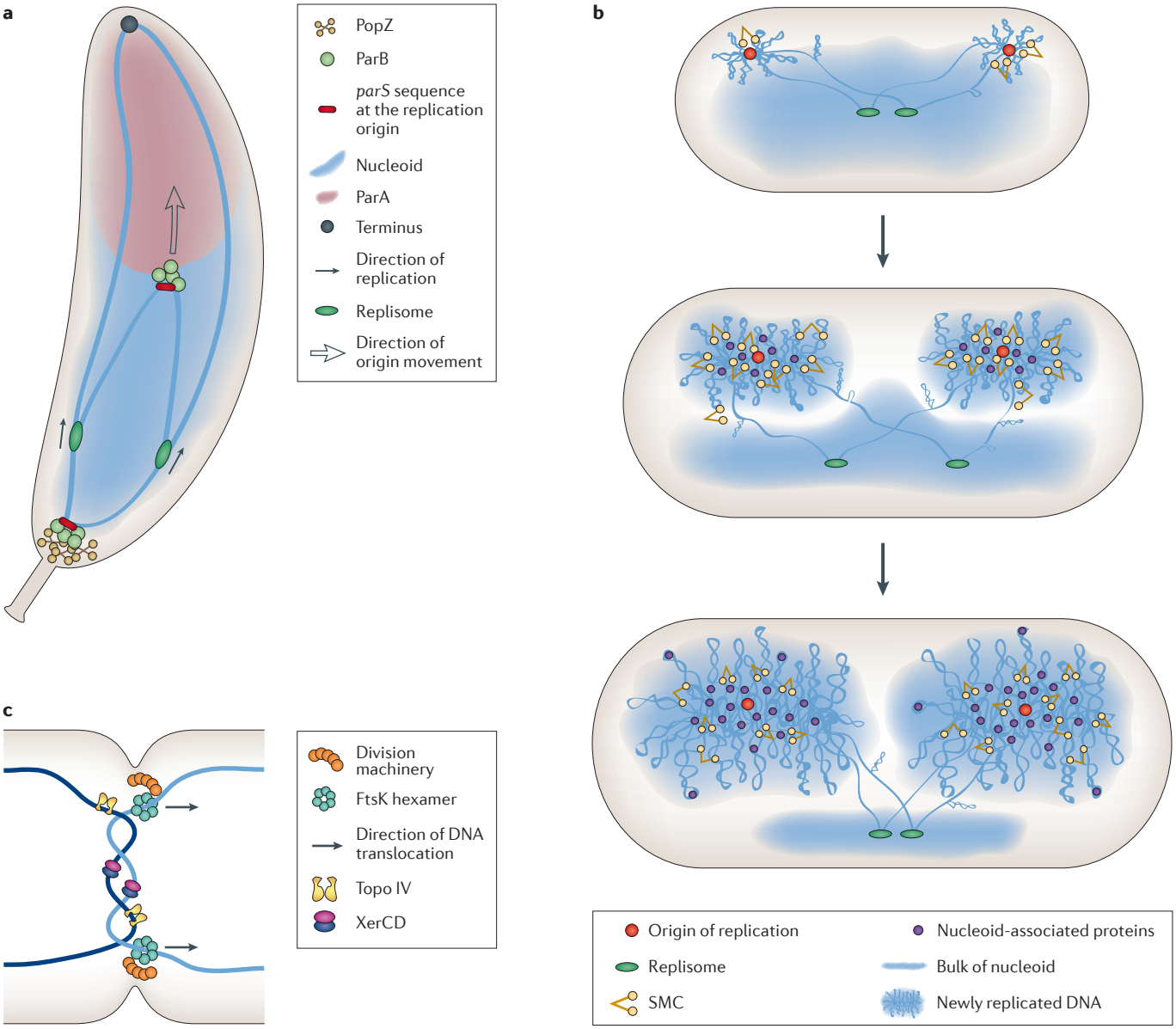


Figure 4 | **Chromosome segregation viewed in three steps.** **a** | Schematic model of partitioning (Par)-system-mediated origin segregation in *Caulobacter crescentus*[112–114]. The origin region is tethered to the cell pole through interactions of *parS*-bound ParB with the polar anchor PopZ. After initiation of replication at the cell pole with a stalk, one of the sister origins is pulled towards the opposite pole through interactions between *parS*-bound ParB and ParA(ATP), which is bound nonspecifically to the nucleoid. These interactions trigger hydrolysis of ParA(ATP) and release of ParA(ADP) from the nucleoid. The ParB–*parS* complex then binds to neighbouring ParA(ATP) on the nucleoid (alternatively, another ParB in the nucleoprotein complex engages a nearby ParA(ATP) before release of the first). Repeated cycles of binding, hydrolysis and release results in movement of the ParB–*parS* complex towards the cell pole and a ParA-free nucleoid in its wake. This so-called diffusion–ratchet mechanism allows the ParB–*parS* complex to 'surf' on top of the nucleoid towards the pole[108,110]. In *C. crescentus*, an additional protein (called TipN) located at the cell pole is required for Par-mediated segregation[112,113] (not shown). TipN localizes to the new cell pole, where it probably functions to regenerate ParA(ATP), helping to set up a ParA(ATP) gradient on the nucleoid. In *par*-containing bacteria that do not anchor their origins at the cell pole, the partitioning system helps to reposition the newly replicated origins at the polar edges of the nucleoid[79]. **b** | A schematic model of bulk chromosome segregation that may be conserved among various bacterial species. After newly replicated origins are separated, lengthwise condensation mediated by supercoiling, small nucleoid-associated proteins and the structural maintenance of chromosome (SMC) complexes, in lockstep with replication, drive disentanglement and segregation of the sister chromosomes. **c** | A schematic of terminus segregation in *Escherichia coli*. The replicated terminus is translocated to appropriate daughter cell by the FtsK DNA translocase, whereas topoisomerase IV (Topo IV) and XerCD resolve catenanes and chromosome dimers, respectively. FtsK specifically localizes at the division septum, where it participates in cytokinesis and DNA segregation.

the newly replicated origins towards opposite cell poles[108] (FIG. 4a). Elegant biochemical and cytological analyses have begun to uncover the mechanistic underpinnings of this simple segregation system (FIG. 4a). We refer the interested reader to recent reviews on this topic[96,109–111].

Interestingly, in some bacteria the Par system has a role in both establishing and maintaining the cellular organization of the chromosome. For example, in *C. crescentus*, after the origin is replicated at the cell pole, one of the ParB–*parS*–origin complexes is pulled to the opposite cell pole in a ParA-dependent manner[112–115]. When it gets there, ParB-bound *parS* interacts with a polar anchoring protein called PopZ[116,117]. Accordingly, the partitioning system together with PopZ helps to regenerate the *ori*–*ter* linear organization of the chromosome.

***Par*-independent origin segregation.** Despite the high degree of conservation among *par* loci, many bacteria, including *E. coli*, lack these partitioning modules. Nonetheless, the replicated origins in *E. coli* rapidly move away from mid-cell[72,75,88]. Moreover, when this partitioning locus is deleted from *par*-containing species, in most cases there are only modest defects in chromosome segregation, and the separation of replicated origins is impaired but not eliminated[90,99,118–120]. Accordingly, these modules function to refine origin segregation and to improve its efficiency but in most cases may not be the driver of it.

What then is the underlying mechanism by which origins are segregated? Orderly lengthwise condensation and resolution of the replicated origins (discussed below) could explain their separation, but it does not account for the faster rate of origin movement compared to more distal chromosomal loci[121]. It is possible that factors that have yet to be discovered are responsible for origin repositioning. However, an intriguing alternative model proposed by Kleckner and colleagues[122] posits that origin regions are extruded towards the cell poles as a result of intranucleoid pushing forces. In this model, the replicated origins undergo condensation and resolution from each other but remain cohesed at specific origin-proximal sites (called snaps). Meanwhile, DNA replication and compaction continue unabated. The accumulation of these DNA bodies in the confined space of the bacterial cell generates internal pushing forces. When these forces exceed the strength of the snaps, cohesion is lost, resulting in the abrupt and rapid extrusion of the condensed origin regions towards opposite poles. Because replication probably initiates at the nucleoid periphery, the newly replicated DNA is naturally compartmentalized from the unreplicated chromosome. This helps to prevent entanglements and provides an unimpeded path for the extruded origins. The molecular basis of the snaps is currently unknown. However, two closely spaced origin-proximal regions on the *E. coli* chromosome with snap-like properties have recently been described[87,88,122]. This model requires further investigation and refinement but, if correct, could be broadly relevant both in bacteria that possess and in those that lack partitioning loci.

Anucleate cells
For bacteria, refers to cells lacking a chromosome.

Origin segregation must be more highly orchestrated and nuanced than this compelling model suggests. One observation that highlights this is the strong positional bias in the segregation of the leading and lagging strands of newly replicated DNA in *E. coli*[123]. The chromosome arms generated by leading-strand synthesis are more frequently located at the outer edges of the nucleoid, whereas the lagging-strand-synthesized arms are present on the opposite side of the origins close to mid-cell. How the leading and lagging strands are positioned on a particular side of the origin and whether this orientation is established before or after origin segregation is not known.

Bulk chromosome segregation

As highlighted throughout this Review, our guiding premise is that the orderly folding of the replicated sisters along adjacent DNA segments serves as the principal driver of bulk chromosome segregation. This lengthwise condensation is mediated by the concerted action of supercoiling, small nucleoid-associated proteins and SMC condensin complexes (FIG. 4b). Compaction of neighbouring DNA segments draws replicated sisters away from each other, makes the newly generated DNA stiffer and thicker and, with the help of Topo IV, eliminates pre-catenated entanglements[32,124,125].

This model is consistent with the linear organization of the bacterial chromosome within the cell and its almost uniform packing density[76,86]. It is also in line with time-lapse imaging of chromosomal loci during replication in *E. coli*, which shows that newly replicated sister loci are sequentially segregated and colocalize with neighbouring genetic loci, thus suggesting that condensation and segregation proceed in a coupled manner. Finally, consistent with this condensation resolution scheme, bulk chromosome segregation is impaired when the proteins and processes that function in chromosome compaction are compromised. Specifically, cells with defects in supercoiling or that lack the small nucleoid-associated proteins or components of the SMC complex have segregation defects that are characterized by the formation of anucleate cells[52,57,59,126–129]. This model intuitively makes sense but is also supported by mathematical modelling of two flexible polymer rings: compaction of catenated rings in an orderly and locally controlled manner along their lengths is sufficient to eliminate entanglements between them, providing that a mechanism (such as Topo IV-mediated decatenation) exists to unlink the rings[124].

The folding of the chromosome in on itself probably initiates at the origin and is propagated outwards. Replicated DNA is then sequentially and progressively gathered into these condensed structures (FIG. 4b). Intriguingly, SMC and MukB complexes are enriched at the origin of replication in *E. coli*, *C. crescentus* and *B. subtilis*[58,129–131]. The mechanism by which they are concentrated at this site in *E. coli* and *C. crescentus* is unknown. However, in *B. subtilis*, the SMC complex is recruited to the origin by ParB bound to origin-proximal *parS* sites[129,131] and, like its eukaryotic counterpart, is enriched at the highly transcribed ribosomal RNA

(rRNA) genes, most of which reside in close proximity to the origin[132]. Interestingly, the *parS* sites are principally clustered to the left of the origin in *B. subtilis*, whereas the rRNA operons are present on the right arm. We imagine that origin-localized SMC has an important role in 'seeding' independent lengthwise condensation of the left and right chromosome arms.

A corollary to this model is that condensation seeded at the origin could also function to dictate the overall organization of the bacterial chromosome. We imagine that recruitment of SMC to the left and right side of the origin leads to local lengthwise condensation along the two arms, generating more rigidified structures into which newly synthesized DNA is folded. In support of this idea, Danilova and colleagues[58] found that *E. coli* MukB mutants that successfully inherit a chromosome switch from a left–*ori*–right organization to an *ori*–*ter* organization. The absence of origin-localized condensin complexes is thought to be responsible for this switch[58,133]. We suspect that SMC complexes work similarly in *C. crescentus* and sporulating *B. subtilis*. However, in these cases, the origin is anchored at the cell pole. Thus, as a result of this constraint on the origin, the left and right arms lie side-by-side in separately condensed bodies rather than on opposite sides of the origin.

Recently, a model for bulk chromosome segregation based on conformational entropy was proposed[134,135]. In this model, segregation is driven by the tendency of confined polymers to separate from each other in a cylindrical container. We favour a model in which lengthwise folding of the replicated sisters drives their separation because it is consistent with the linear organization of the chromosome and the segregation defects observed in cells lacking compaction proteins, and it is applicable to all bacterial cells, regardless of their shape. However, we suspect that rod-shaped bacteria take advantage of their carefully constructed geometry, and in these organisms entropic sorting forces could facilitate the condensation resolution process discussed here.

Segregation of the terminus region

In principle, condensation resolution should be sufficient to segregate replicated sister chromosomes before cytokinesis. However, bacteria have evolved a septum-localized DNA translocase to ensure efficient segregation of the terminus and to attend to the particular challenges of replicating a circular chromosome (FIG. 4c).

Replicating a circular chromosome generates two topological challenges: catenanes and dimeric chromosomes. When pre-catenanes are not removed by Topo IV during replication, the replicated sisters remain linked to each other, forming interlocked rings (catenanes)[28,29]. In addition, as a result of homologous recombination between sisters during replication repair and an uneven number of crossovers, ~15% of the population ends up with conjoined sister chromosomes as a single circular dimeric chromosome[136]. Thus, to complete sister chromosome segregation, Topo IV must remove the catenanes, and a recombinase (called XerCD in *E. coli*, and RipX and CodV in *B. subtilis*) must convert the chromosome dimers into monomers[137–139]. Decatenation and dimer resolution are coordinated and facilitated by a DNA translocase (called FtsK in *E. coli* and *C. crescentus*, and SpoIIIE in *B. subtilis*). These membrane-anchored ATPases associate with the cell division apparatus at mid-cell and take advantage of strand-specific base composition skew in the DNA to translocate the chromosome arms towards the replication termini and the site of dimer resolution (FIG. 4c). These translocases are used when DNA is present at the septum as a result of missegregation, chromosome dimers or catenanes. Interestingly, the *B. subtilis* DNA translocase is also used during sporulation to pump ~75% of the chromosome into the developing spore[77,129,140] (FIG. 3A). Translocation during vegetative growth and sporulation brings the termini to mid-cell, where XerCD and Topo IV can catalyse their unlinking and complete the segregation process[141–145] (FIG. 4c).

Concluding remarks and future directions

Live-cell imaging and genome-scale molecular approaches have taken the disembodied image of the bacterial nucleoid — plectonemes emanating from a central core — and provided a context in which to interpret it. The linear organization of the chromosome with its uniform packing density and the ordered layering of chromosomal loci during replication provide a clearer picture of the nucleoid and suggest a plausible and compelling mechanism for its segregation.

Defining how origins are segregated with and without a Par system and the mechanism by which SMC compacts DNA are outstanding issues that will be addressed in the near future. Defining SMC action will inform (and be informed by) studies on eukaryotic SMC complexes. However, an equally important and challenging question is how the different compaction and segregation factors interface during the replication–segregation cycle. Intriguing hints of interconnections have been described over the past decade. In *B. subtilis*, the ParA protein appears to regulate replication initiation[146], whereas ParB bound to *parS* recruits SMC to the origin to facilitate compaction and segregation[129,131]. Moreover, the MukB component of the condensin complex in *E. coli* has been found to interact with Topo IV and to stimulate its activity *in vitro*[147]. To round out this picture, the FtsK translocase also interacts with Topo IV[148], facilitating terminus separation and cell division, and the ter macrodomain protein MatP has recently been shown to interact with cell-division protein ZapB[149]. Understanding how these factors (and others) work together will provide a more complete picture of how the chromosome is organized and accurately segregated with such high fidelity.

The other major challenge for the future is to understand how chromosome condensation and segregation are influenced by the physicochemical properties of the cell and basic cellular processes. We have touched on a possible role for confinement but have not mentioned the crowded and metabolically active cytoplasm in which the nucleoid resides. Molecular crowding can contribute to chromosome compaction directly by

Catenanes
Interlocked rings (of circular chromosomes or plasmids) that cannot be separated without breaking the covalent bonds in DNA.

Dimeric chromosomes
Sister chromosomes conjoined into a single circle. Dimeric chromosomes result from an uneven number of homologous recombination events between sisters during replication.

creating a phase separation between the DNA and the rest of the cytoplasm and indirectly by enhancing the interactions between the chromosome and DNA-binding proteins[150,151]. How crowding and confinement influence organization and segregation remain to be elucidated. Additionally, the chromosome is constantly being pushed and bullied by the replication and transcription machineries as well as recombination and repair proteins. These activities clearly influence chromosome dynamics. Interestingly, inhibition of transcription leads to a dramatic decondensation of the nucleoid, the molecular basis of which remains unknown[152,153]. Understanding the interplay between the condensation and segregation machineries in the context of the crowded and metabolically active cell is, of course, a long way off but is a goal that is worthy of our efforts.

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