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Multilayer chromosome organization through DNA bending, bridging and extrusion

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All living cells have to master the extraordinarily extended and tangly nature of genomic DNA molecules — in particular during cell division when sister chromosomes are resolved from one another and confined to opposite halves of a cell. Bacteria have evolved diverse sets of proteins, which collectively ensure the formation of compact and yet highly dynamic nucleoids. Some of these players act locally by changing the path of DNA through the bending of its double helical backbone. Other proteins have wider or even global impact on chromosome organization, for example by interconnecting two distant segments of chromosomal DNA or by actively relocating DNA within a cell. Here, I highlight different modes of chromosome organization in bacteria and on this basis consider models for the function of SMC protein complexes, whose mechanism of action is only poorly understood so far.

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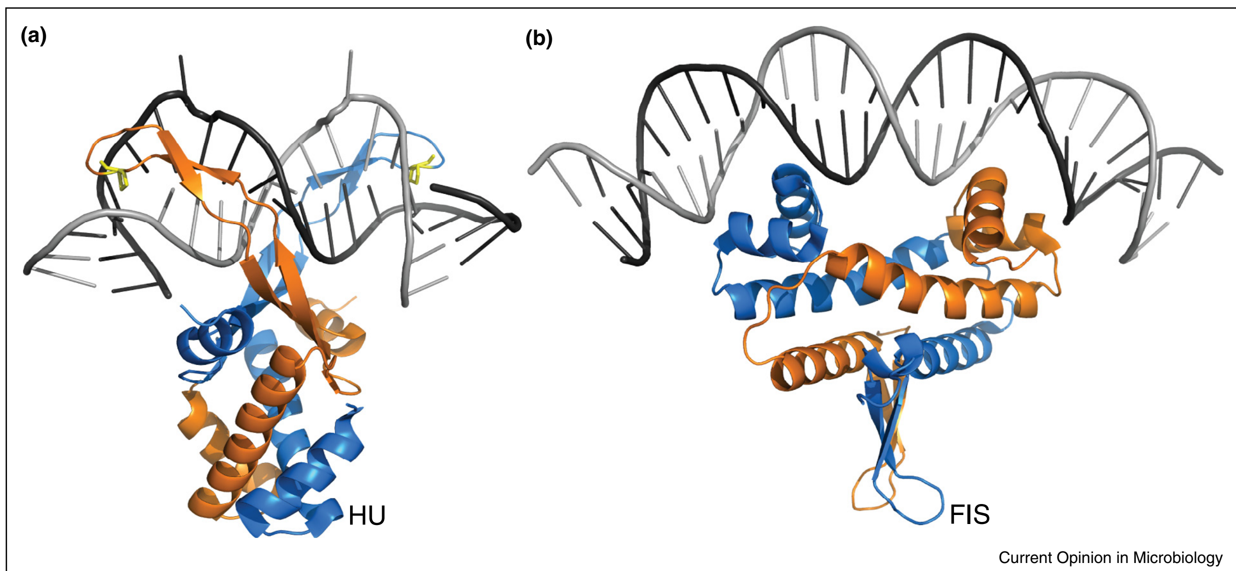
Introduction

Chromosomal DNA, the carrier of genetic information, constitutes a biomolecule with an utmost unusual aspect ratio. Its contour length amounts to several millimeters in bacteria, whereas the diameter of the DNA double helix is only about 2 nm. The former extends roughly three orders of magnitude beyond the dimensions of a typical bacterial cell. In this length scale the DNA double helix is essentially flexible. Chromosomal DNA in pure solution thus adopts an extended and soft random coil configuration [1]. Within the cell however, genomic DNA exists in a much more compact state, called the nucleoid, generally occupying only a fraction of the total cell volume thereby enabling the spatial segregation of sister chromosomes during cell division. At the same time, nucleoids are highly dynamic entities providing apparently unrestricted access to the DNA during replication, repair and transcription. Nucleoids are also internally organized in ways that position parts of the circular chromosome at defined locations within the cell [2]. In turn, this spatial arrangement of the chromosome impinges upon the cellular localization of the cell division machinery by a process called nucleoid occlusion [3–5]. Several factors contribute to the formation of compact, organized and yet dynamic nucleoids. (I) Negative DNA supercoiling — *i.e.* the underwinding of the two strands of DNA through the action of DNA gyrase supported by RNA polymerase — leads to the local folding of DNA into structured superhelices, also called plectonemes [6•,7]. (II) The packed cytoplasmic environment itself limits the boundaries of nucleoids *via* a phenomenon known as macromolecular crowding [1]. (III) In addition to these physical aspects of chromosome compaction, a number of DNA binding proteins play critical roles as chromosomal architects. Here, I highlight different mechanisms by which proteins organize chromosomes at local and global levels in bacteria.

Abundant DNA bender

A number of small but highly abundant DNA binding proteins, designated as NAPs (nucleoid associated proteins), associate with chromosomes in bacteria. Many NAPs bind to DNA with low sequence specificity and cause local deformation, or bending, of the backbone of B-form DNA. A nearly ubiquitous representative of NAPs in bacteria is the HU protein. It binds as a homo- or heterodimer to the minor groove of an ~36 bp stretch of DNA. Two proline residues on a HU dimer intercalate into DNA, leading to sharp kinks in the DNA backbone (in total ~140°) (Figure 1a) [8]. HU is present in high numbers per chromosome allowing it to coat and bend about 10% of chromosomal DNA in *Escherichia coli* (~30,000 HU proteins per cell) [9]. Factor for inversion stimulation (FIS), a NAP conserved in most gram-negative bacteria, is expressed at similar or even higher levels in *E. coli* [9]. Its dimers bind to the major groove of DNA and bend the DNA backbone with angles varying between 50° and 90° (Figure 1b) [10]. Besides serving architectural roles, NAPs can regulate the transcription of a large set of genes, produce diffusion barriers for DNA supercoils and support other chromosomal processes such as site-specific DNA recombination and DNA replication [11,13,14]. This multitude of functions makes it difficult to establish the precise contribution of individual NAPs to genome folding as null mutant phenotypes are highly pleiotropic. An estimate of the combined

Figure 1



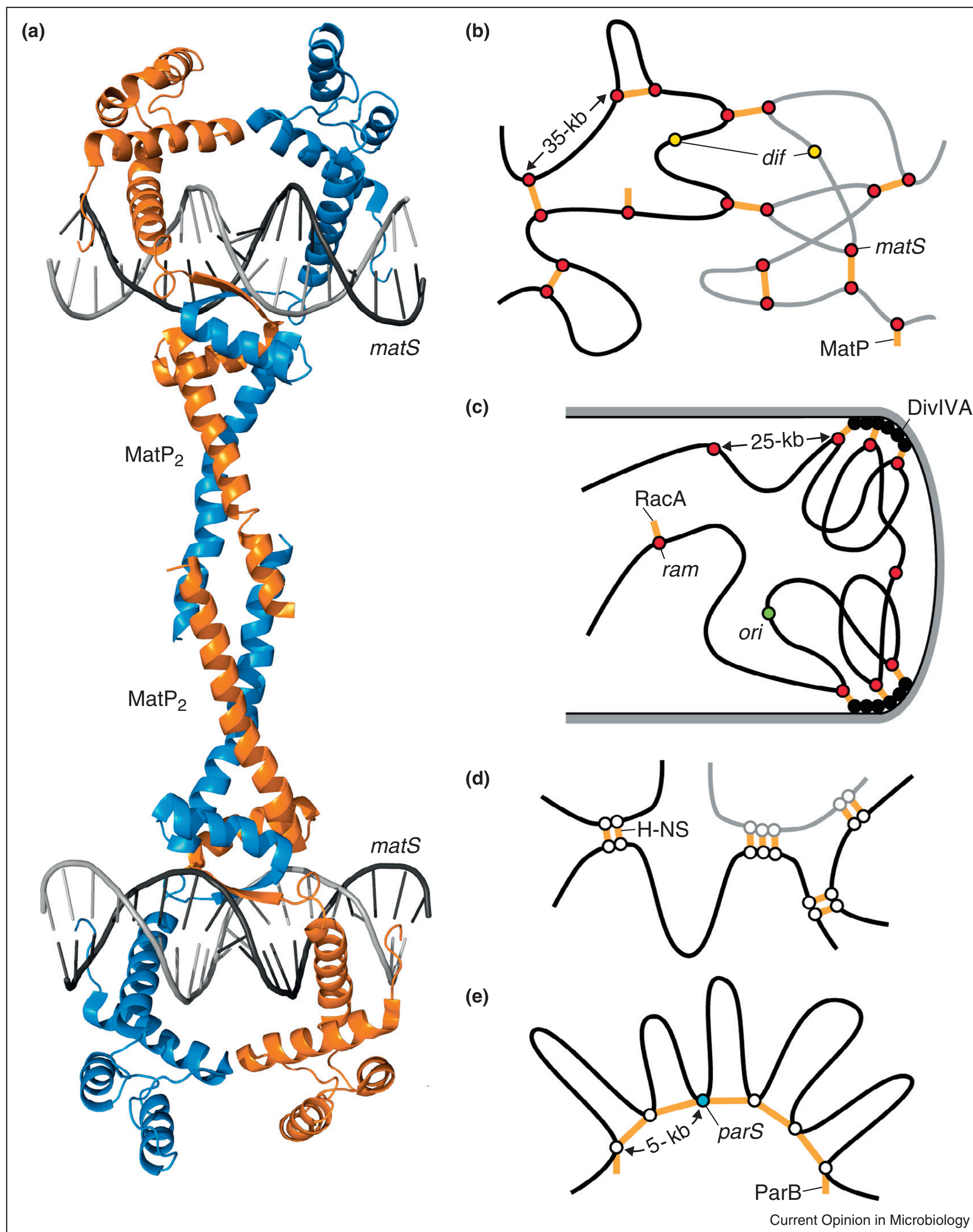
Structural basis for DNA bending by HU **(a)** and FIS **(b)** protein. Cartoon representation of crystal structures of HU and FIS protein bound to short pieces of DNA (PDB: 1P78 and 3JRA). Structures are shown in the plane of bent DNA. Monomers are colored in orange and blue colors. Intercalating proline residues in HU are shown as sticks in yellow color.

influence of local changes in DNA topology on the compactness of nucleoids is thus not attainable by current experimental approaches. HU proteins are known to be phosphorylated in *Bacillus subtilis* and *Mycobacterium tuberculosis*. In the latter organism phosphorylation of HupB eliminates its ability to bind to DNA [15,16]. Moreover, the expression levels of several NAPs change drastically at transitions between growth phases, likely explaining, at least in parts, the distinct appearance of nucleoids at different stages of growth [9,17]. Whether DNA bending NAPs, like FIS, HU or its paralogue IHF, organize chromosomes at levels beyond local DNA distortion *in vivo* is not fully clear. In principle protein–protein contacts or regular spacing of their binding sites on DNA, could give rise to DNA super-helices, loops or coils, analogous to eukaryotic chromatin fibers. Certainly, at promoters the interplay between several NAPs and the transcription initiation complex can have a strong influence on gene expression [11,12].

DNA bridges

Recent advances have disclosed an unexpectedly intricate internal organization of bacterial nucleoids. Parts of chromosomes are folded into isolated substructures called macrodomains, first identified in *E. coli*. These chromosomal domains are precisely arranged within the nucleoid and the bacterial cell [2,18,19•,20,21]. Simple bending of DNA cannot explain the formation of large chromosomal subdomains or their cellular positioning. Instead, several examples — discussed below — indicate that interconnection, or bridging, of distant stretches of DNA may be a general principle underlying the formation of isolated domains in bacterial chromosomes. These domains are implicated in conferring robust chromosome segregation, proper cellular organization and possibly gene regulation. The formation of the *ter* macrodomain in *E. coli* represents one of the earliest and best studied examples [22]. An 800-kb-long region around the replication terminus in the *E. coli* genome comprises about 20 scattered *matS* sites (Figure 3a) [23], which are recognized by dimers of MatP protein. Upon binding to *matS* sites MatP dimers undergo a conformational change promoting the formation of dimer-of-dimers. Distantly located *matS* sites thus get physically interlinked by MatP tetramers and as a consequence the *ter* region is folded and condensed into a macrodomain (Figure 2a, b) [24••]. MatP also holds sister chromosomes together, presumably by bridging *matS* sites located on opposite sister DNA molecules. Resolution of MatP mediated sister cohesion appears to rely on the severing of MatP tetramers through competitive binding of the cell division protein ZapB to MatP dimers [25•]. In *B. subtilis* DNA sequences near the replication origin (*ori*) are targeted to the cell pole during sporulation. About 25 copies of *ori* proximal *ram* sites are anchored to the cell membrane possibly *via* a direct interaction between the *ram* binding protein RacA and membrane associated DivIVA (Figure 2c) [26,27]. *ram* sites are thus indirectly kept in proximity through their localization to the polar cell envelope. This clustering of *ram* sites promotes the initial entrapment of *ori* proximal DNA

Figure 2



Domain formation by DNA bridging. **(a)** Crystal structure of MatP tetramers bound to two *matS* DNA molecules in cartoon representation (PDB: 4D8J). Chains in the two MatP dimers are shown in orange and blue colors. **(b)**–**(e)** Models for the formation of chromosomal domains by different DNA bridging proteins. Sister DNA molecules are shown in black and gray colors. Specific DNA sequences are indicated by red (*matS* and *ram* in (b) and (c), respectively) and blue circles (*parS* in (e)). Random DNA sequences and DivIVA protein are shown in white and black circles, respectively. All protein bridges are represented by orange bars. Arrows indicate average (b, c) or estimated (d) distances between binding sites.

in the pre-spore compartment [28]. However, not all chromosomal domains are governed by well-defined and precisely positioned DNA sequence motifs. The γ-proteobacterial NAP H-NS features only a mild preference for AT-rich or curved DNA and binds to several hundred apparently randomly distributed genomic positions in *E. coli* [29,30,31]. *In vitro* H-NS is able to interconnect DNA molecules as shown by

single-molecule manipulation experiments using magnetic tweezers and atomic force microscopy [32,33]. The molecular basis for DNA binding and bridging by H-NS however is still poorly understood. *In vivo* H-NS proteins are localized into one or two narrow foci at the center of nucleoids in super-resolution microscopic images. Several tested H-NS bound positions on the chromosome co-localize with these H-NS foci, suggesting that many genomic loci are recruited into H-NS clusters, presumably through a mechanism of DNA-protein-DNA bridging (Figure 2d) [34•]. Sequestration of DNA could help to repress the transcription of the large number of H-NS target genes in *E. coli*. In *B. subtilis*, the unrelated Rok protein might fulfill a similar role [35]. Another example of a DNA bridge, the DNA partitioning protein ParB, displays both sequence-specific as well as non-specific DNA binding properties, possibly governed by two independent binding sites for DNA [36–38]. Together, the two modes of DNA binding organize small domains in chromosomal DNA (~10 kb) presumably by interconnecting *parS* sites with neighboring stretches of DNA [39,40] (Figure 2e). DNA bridging activity of any protein in general holds the risk of interfering with chromosome segregation through the creation of protein-linked sister chromosomes. As is the case with MatP, dedicated mechanisms might exist that prevent or overcome any sister chromosome interconnections formed by DNA bridging proteins such as ParB or H-NS. Intriguingly, an artificial roadblock engineered close to *parS* hinders the spreading of ParB toward downstream sequences [41,42]. A possible explanation for this conserved feature of ParB is that it diffuses along DNA *via* one of its DNA binding domains while at the same time holding onto *parS*, thus forming an expanding loop of DNA. Accordingly, ParB might constitute a DNA looping rather than a simple DNA bridging protein (Figure 2e). These examples demonstrate that DNA bridging proteins can have profound effects on nucleoid architecture *via* formation of isolated chromosomal domains.

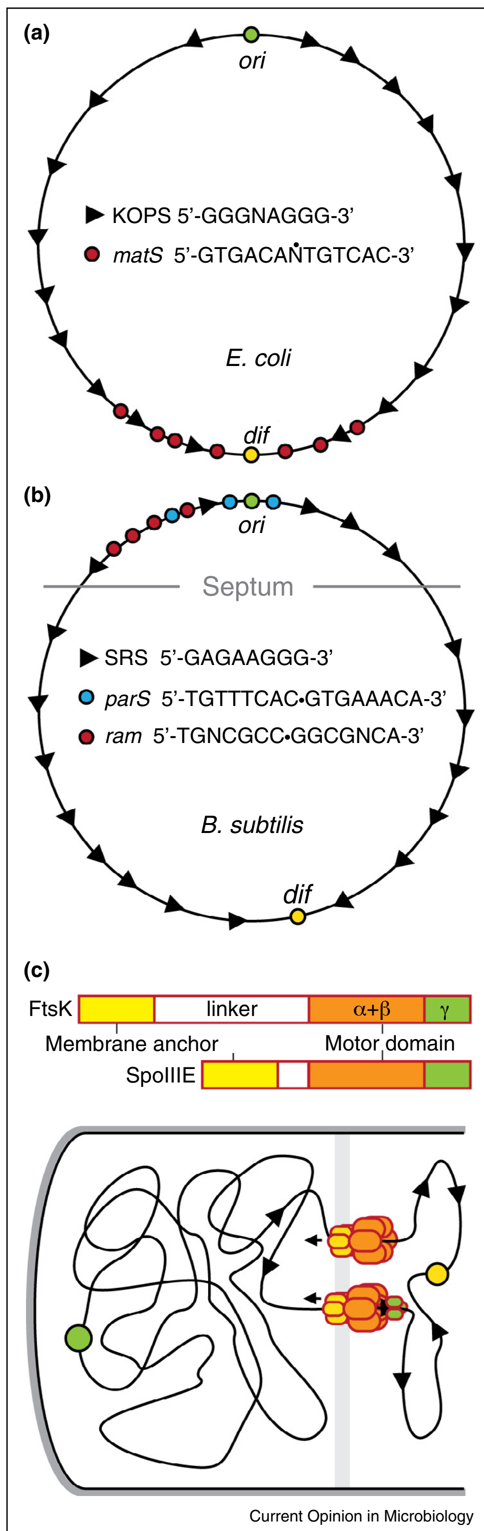
DNA extruder

The above mechanisms of chromosome organization are passive and largely driven by high affinity protein-DNA contacts. There is also active, ATP hydrolyzing processes that support chromosome segregation by repositioning certain parts of the chromosome within a cell. The ParABS system for example relies on a diffusion-capture mechanism driven by a propagating ParA ATPase gradient to segregate replication origins toward opposite poles of a cell [43]. DNA extruders, such as FtsK and SpoIIIE proteins, form AAA$^+$ ATPase channels or pores at the cell division site through which they actively pump DNA from one daughter cell compartment to the other (Figure 3) [44]. *B. subtilis* SpoIIIE is especially important during asymmetric cell division in sporulating cells when it delivers a large fraction of the chromosome into the small pre-spore compartment [45]. Directionality during DNA translocation is provided by the presence of asymmetric, non-palindromic KOPS and SRS sequences in *E. coli* and *B. subtilis*, respectively [46–48]. These sequences are inversely oriented on the left and right arms of the chromosome and guide the loading or activation of the hexameric DNA translocase (Figure 3) [49–51]. Translocation of DNA through FtsK not only clears DNA from the septum but also serves to position *dif* sequences at the cell division site. The XerCD recombinase then resolves chromosome dimers — occasionally created during DNA replication — by site-directed recombination of aligned *dif* sites [52]. In principle, any DNA tracking protein that is anchored onto a cellular structure might bring about DNA translocation. Accordingly, ''replication factories'' have been hypothesized to promote chromosome segregation through extrusion of newly replicated DNA in *B. subtilis*. However, direct evidence is still lacking possibly due of difficulties in separating the essential DNA replication activity of the replisome from any potential role in chromosome segregation. DNA tracking proteins could also promote DNA loop formation if they held onto one stretch of DNA while moving along another.

SMC: DNA bridges or framework structures for DNA loop extrusion?

SMC protein complexes are highly conserved and nearly ubiquitously present in bacteria, as well as in archaea and eukaryotes. They are crucial for chromosome segregation in a number of bacteria and feature a highly unusual architecture (Figure 4a) [53]. They consist of a long intramolecular, antiparallel coiled-coil that is linked to a hinge domain at one end and an ABC-type ATPase head domain at the other. Homodimerization of Smc occurs at the hinge domain and also in an ATP-dependent manner at the head domain [54,55]. In addition, the ScpAB subcomplex connects the head domain of one Smc protein with the coiled-coil of the other. Overall, Smc_2-$ScpA_1B_2$ comprises an elongated ring-shaped structure (Figure 4a) [56••,57]. Smc-ScpAB and MukBEF, a structurally deviant version present mainly in γ-proteobacteria, do not display obvious DNA sequence specificity, however they are localizing in foci near DNA replication origins [58–63]. In *B. subtilis* and *Streptococcus pneumoniae* formation of these ''condensation centers'' is mediated by the ParB/*parS* complex and is crucial for efficient chromosome segregation [59–61]. Destruction, or depletion, of components of Smc-ScpAB in *B. subtilis* leads to interlinked sister replication origins, implying that Smc-ScpAB promotes the initial stages of chromosome partitioning (Figure 4b) [64•,65•]. In *E. coli*, mutations in the *mukBEF* operon result in polarly rather than centrally positioned replication origins and — possibly as a consequence — longitudinal rather than transversal chromosome organization [58]. How Smc-ScpAB and MukBEF

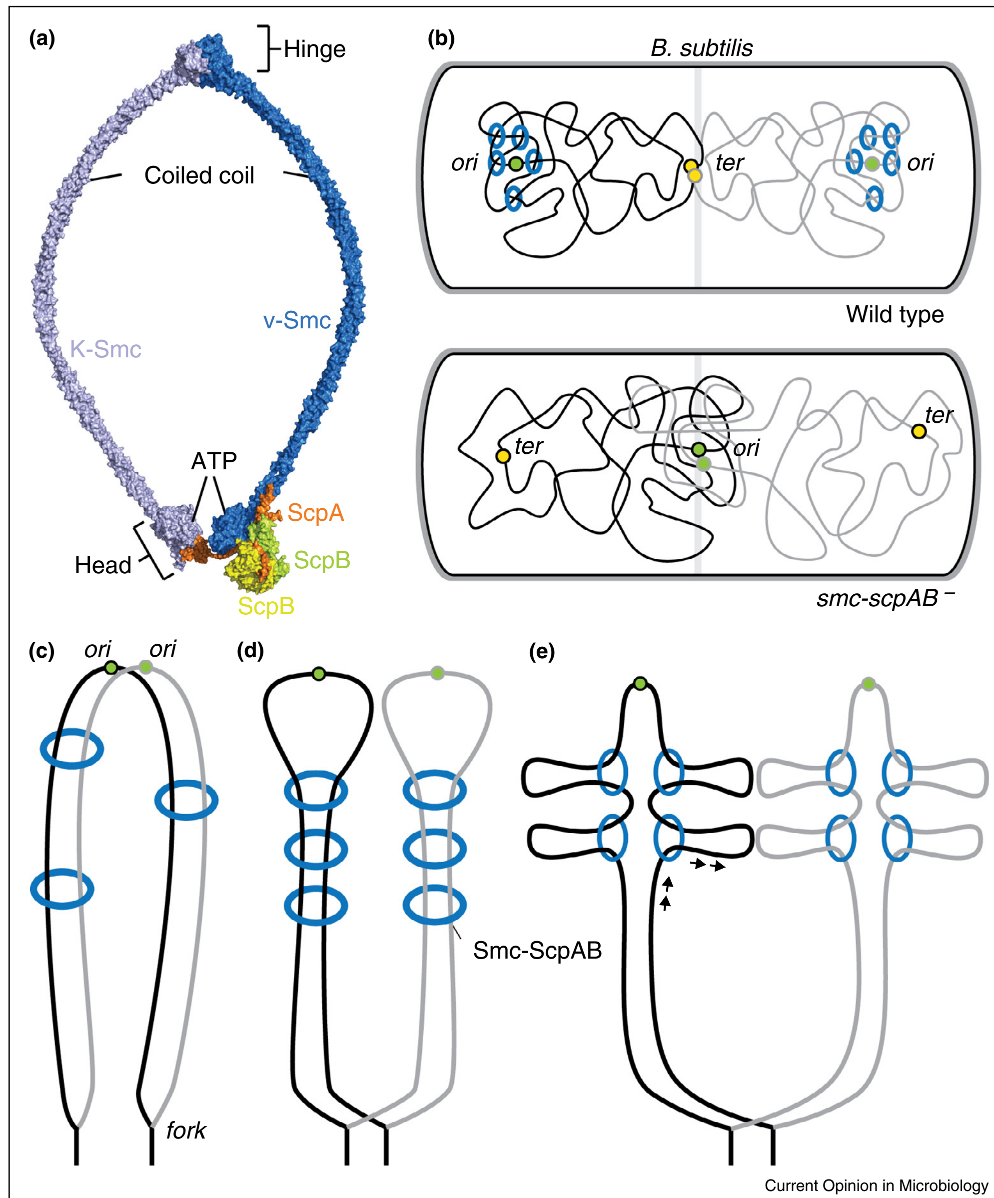
Figure 3



DNA extrusion by FtsK/SpoIIIE. Distribution of selected DNA sequence elements on the circular genomes of *E. coli* **(a)** and *B. subtilis* **(b)**. Black dots mark symmetry points in palindromic DNA sequences. Color coding as in Figure 2. **(c)** Domain organization of FtsK and SpoIIIE and their cellular mechanism of action. For simplicity the γ-domains for recognition of KOPS/SRS by FtsK/SpoIIIE are only shown in the bottom model representation.

engage with chromosomes and how they promote segregation and positioning of replication origins however is largely unclear. Due to the low estimated numbers of MukBEF (<100 complexes) per chromosome, DNA bending seems to be a highly unlikely mechanism [66••]. DNA bridging on the other hand is the mode of action of the related eukaryotic cohesin complex [67]. Cohesin holds sister chromatids together by embracing sister DNA within its ring [68,69]. Accordingly, other SMC protein complexes have been proposed to support DNA linkage [67,70]. Three possibilities could be envisaged for DNA bridging by Smc-ScpAB: cohesion of sister DNA (Figure 4c), linkage of left and right chromosome arms (d) and bridging of DNA from within a chromosome arm (e). The latter two models provide intuitive explanations for how Smc-ScpAB might support separation of sister DNA: by drawing individual DNA molecules onto themselves and thus minimizing spatial overlap between chromosomes. However, sophisticated mechanisms are needed to allow bridging of inter- or intra-arm DNA by Smc-ScpAB while avoiding the formation of sister DNA linkages. Intriguingly, chromosome arms are aligned along the long axis of the cell in *Caulobacter crescentus* and *Pseudomonas aeruginosa* [21,71]. In *smc* mutants this chromosomal organization is less pronounced as judged by 3C-type experiments, indicating that Smc directly or indirectly helps aligning chromosome arms within the cell, conceivably by holding them together (Figure 4d) [19•]. It is not obvious, however, how Smc-ScpAB could specifically recognize left and right arms of a chromosome and distinguish them from the two arms of the sister chromosome. On the other hand, a mechanism of DNA extrusion could create intra-chromosome loops of DNA and by doing so *per se* prevent the formation of any sister or arm interconnections (Figure 4e) [72]. Such a mechanism would require processive movement and consume energy. It is unclear what process might drive DNA translocation through the Smc ring? The intrinsic ATPase activity of Smc-ScpAB is quite low ($<1\ s^{-1}$) [57]. Thus, huge step sizes would be necessary to achieve sufficient levels of DNA compaction within a cell division cycle. Instead, the tracking of DNA by another motor protein could be physically linked to the Smc-ScpAB ring. Consistent with such a model, the cohesin complex is pushed along chromosomal DNA by RNA polymerase [73]. Moreover, eukaryotic condensin, as well as Smc-ScpAB, colocalize with highly transcribed genes in ChIP experiments, possibly indicating a connection between active transcription and SMC [59,74]. Alternatively, Smc-ScpAB and MukBEF might simply stabilize pre-formed loops of DNA. Obviously, many questions remain to be addressed to reveal SMC's basic mechanism of action. Whatever the underlying mechanism might be, its understanding will surely provide fruitful insights into the landscaping of genomes in all domains of life.

Figure 4



Bacterial SMC proteins. Hypothetical model **(a)** for the overall architecture of pentameric Smc_2-$ScpA_1B_2$ based on several separate crystal structures [56••]. **(b)** Cellular arrangement of chromosomes in wild-type cells (top panel) and of interlinked sister chromosome arising in the absence of Smc-ScpAB activity (bottom panel). Color coding as in Figure 2. Models for the bridging and looping of chromosomal DNA by Smc-ScpAB rings: specific for sister DNA **(c)**, for left and right arm of the chromosome **(d)** and intra-arm DNA **(e)**. Arrows indicate how DNA loops might be extruded from the Smc-ScpAB ring (e).

Conclusions

The examples discussed here highlight the variety of mechanisms that bacterial cells utilize to deal with the tangly nature of their genetic material. Surprisingly, only a small subset of proteins (*i.e.* HU, FtsK and Smc-ScpAB) are present throughout all major branches of bacteria, demonstrating the high adaptability of chromosome organization in bacteria. In contrast, the main players in chromosome segregation in eukaryotes are ubiquitous. At least in parts the former is likely due to the diversity in bacterial cell shapes and life styles, and their repercussions on chromosome partitioning. Future studies on more diverse bacterial organisms will likely reveal many more players — including DNA benders, bridges and extruders — and corroborate the notion that synergistic action of exchangeable parts governs robust chromosome segregation in bacteria.

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