Chromosome organization in bacteria: mechanistic insights into genome structure and function

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Abstract | Bacterial chromosomes are folded to compact DNA and facilitate cellular processes. Studying model bacteria has revealed aspects of chromosome folding that are applicable to many species. Primarily controlled by nucleoid-associated proteins, chromosome folding is hierarchical, from large-scale macrodomains to smaller-scale structures that influence DNA transactions, including replication and transcription. Here we review the environmentally regulated, architectural and regulatory roles of nucleoid-associated proteins and the implications for bacterial cell biology. We also highlight similarities and differences in the chromosome folding mechanisms of bacteria and eukaryotes.

In all organisms, DNA is folded to fit inside the cell or its compartments. This is necessary because an organism's chromosome exceeds the cell's length by several orders of magnitude. Since the 1950s, 'spreads' of liberated intracellular macromolecules, visualized by electron microscopy, have demonstrated the difficulty of this task. Genetic material readily spills out of lysed cells or nuclei to fill a volume many times larger than originally occupied[1]. The precise mechanisms of DNA folding were first understood for eukaryotes. Hence, the basic structural units of eukaryotic folded DNA (nucleosomes; FIG. 1a) were identified as 'beads on a string'[2]. The discovery of higher-order structures (chromatin) was facilitated by the large size of eukaryotic cells, which makes them more amenable to light microscopy. Indeed, the basic dynamics of eukaryotic chromosomes during cell division were evident even before the genetic code was understood[3]. It has taken much longer to understand chromosome organization in bacteria. Repeating structural units have never been identified, and early visualizations showed little more than a tangled mess[1]. In retrospect, this is unsurprising. Bacteria lack most DNA-folding factors present in eukaryotes, so few cues can be taken. Furthermore, bacterial nucleoids undergo large changes in organization at different growth phases.

When we previously reviewed this topic in 2011 (REF.[4]), evidence was emerging that bacterial chromosomes are not merely unstructured bodies of DNA but instead fold into independent domains finely structured at the nanoscale. Advances in microscopy, structural biology and genome-scale approaches (BOX 1) have revealed many of the underlying molecular mechanisms. In this Review, we discuss these mechanisms and their impact on wider cell biology. Beginning at the level of individual DNA-folding proteins, we explain how DNA in bacteria is folded into myriad structures by looping, bending and twisting of the DNA. Subsequently, we explain how these DNA contortions can influence not only nucleic acid compaction but also gene expression and DNA replication. On a whole-chromosome scale, we describe the characteristics of individual domains and discuss the possibility that this organization concept is conserved across bacterial species. Throughout, we highlight similarities and differences in the DNA-folding mechanisms used by bacteria and eukaryotes.

The nucleoid-associated proteins

Unlike eukaryotes, chromosomes of bacteria are not usually folded into regularly repeating structural units (FIG. 1a). Instead, the chromosome is folded into a range of different conformations by nucleoid-associated proteins (NAPs) (FIG. 1b–e). These are described further in the following sections.

Loop and filament formation by H-NS. The histone-like nucleoid structuring protein (H-NS) is a small (137 amino acids in *Escherichia coli*) polypeptide that binds the DNA minor groove using a carboxy-terminal arginine hook motif[5]. This is favoured for DNA with elevated AT content containing a TpA dinucleotide or 'step'[6]. Hence, H-NS-bound genomic segments are AT rich and have often been acquired by horizontal gene transfer[7–11]. The amino-terminal domain of H-NS contains two sites that facilitate 'daisy chaining' of H-NS via head-to-head and tail-to-tail contacts[12] (FIG. 1b). This drives the formation of lateral nucleoprotein filaments (FIG. 1b, top) or loops between DNA segments bridged by H-NS[13,14] (FIG. 1b, bottom).

Chromosome
An essential molecule containing some or all of the genes required by an organism to survive and reproduce. Whereas chromosomes are made of DNA, not all DNA is chromosomal. Extrachromosomal DNA molecules such as plasmids also encode genes, although these genes are not absolutely required for an organism's survival and reproduction.

Chromatin
A compact macromolecular complex of DNA and structuring proteins.

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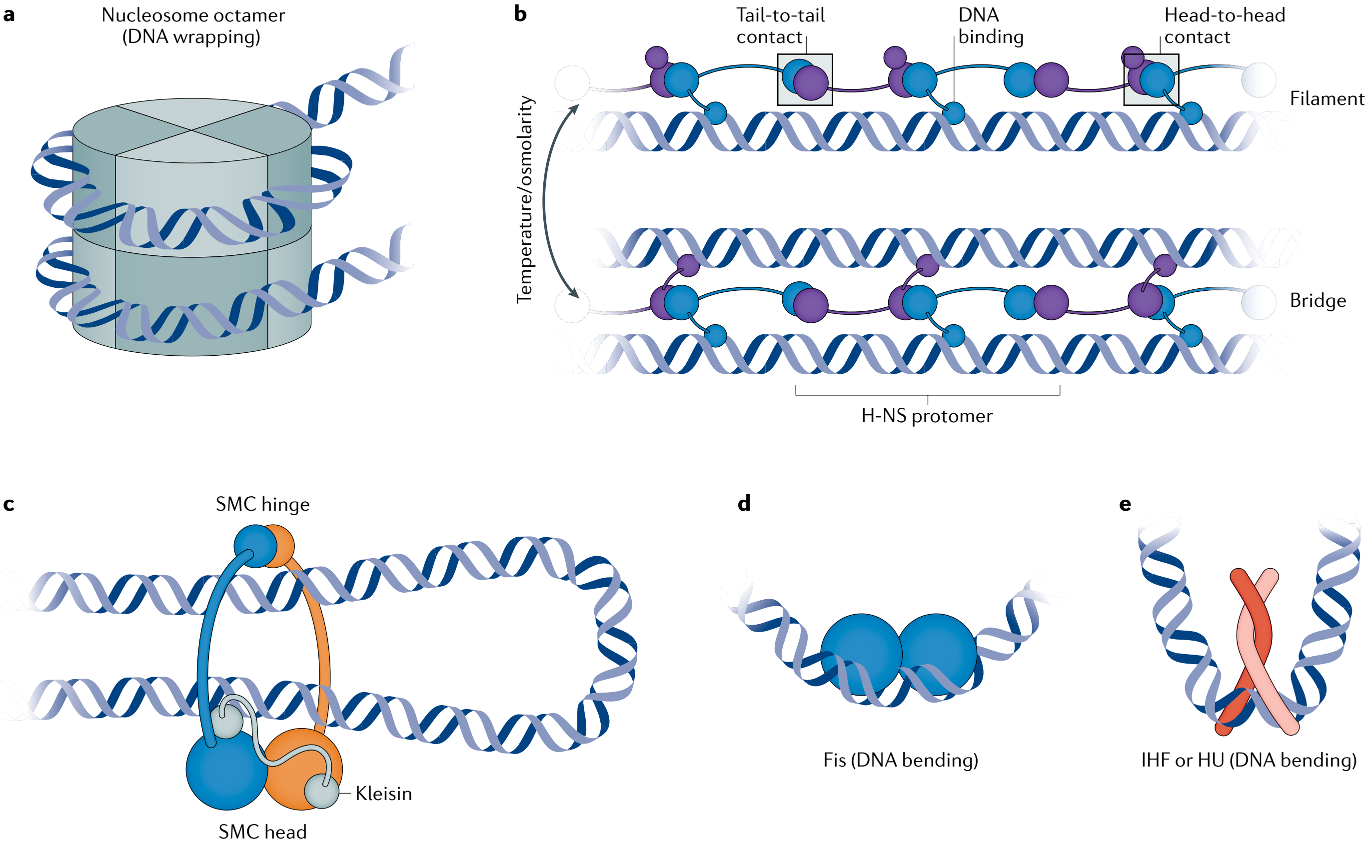


Fig. 1 | **DNA is locally folded by nucleoid-associated proteins in bacteria, histones in eukaryotes and the evolutionarily conserved SMC complex. a** | Eukaryotic chromosomes are folded into regularly repeating structural units known as nucleosomes. Typically, a nucleosome consists of 147 bp of DNA wrapped around an octameric histone core. **b** | The 'daisy chaining' of histone-like nucleoid structuring protein (H-NS) along the DNA by head-to-head and tail-to-tail contacts forms H-NS–DNA filaments (top) and DNA–H-NS–DNA bridges (bottom), the latter of which results in the formation of DNA loops[12–14]. In vitro studies indicate that the switch between the two modes of DNA binding by H-NS is mediated by changes in temperature and osmolarity[63,123–126]. **c** | Structural maintenance of chromosomes (SMC) complexes are DNA-looping proteins comprising a pair of SMC monomers, kleisin and the kite (kleisin-interacting winged-helix tandem elements) or hawk (HEAT repeat subunits containing proteins associated with kleisins) accessory/regulatory proteins. Each SMC monomer consists of a 'hinge' dimerization domain, an ATPase 'head' domain and an antiparallel coiled-coil 'arm' extending between the hinge and head domains. SMC complexes form DNA loops either by embracing a pair of DNA segments in a single ring or by the dimerization of two rings that each trap a DNA segment[19–24] (also see FIG. 2). **d** | Factor for inversion stimulation (Fis) binds its target sequences as a dimer and induces a 50–90° bend in the DNA[47,48]. **e** | Integration host factor (IHF) and heat-stable protein from *E. coli* strain U93 (HU) also function as DNA-bending proteins. IHF generates sharp 160° hairpin bends in the DNA, whereas HU functions as a flexible hinge[51] — it bends DNA less sharply but over a range of different angles[55,56].

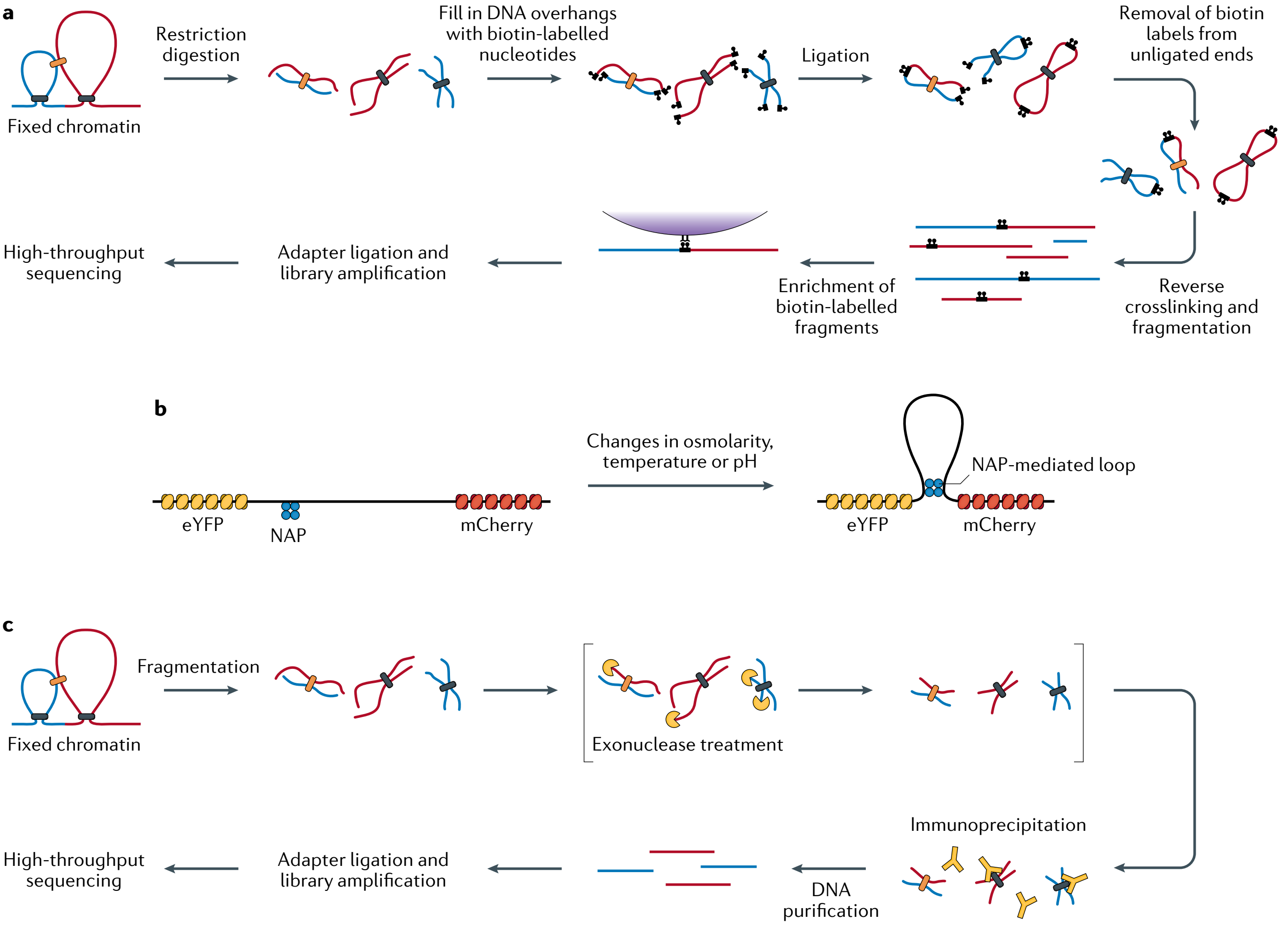
Proteins functionally similar to H-NS are found in diverse bacteria. Often these have arisen via convergent evolution: that is, the independent evolution of the same function. For example, in *Burkholderia* spp. and *Mycobacterium tuberculosis*, the functional equivalents of *E. coli* H-NS are Bv3f and Lsr2, respectively. The proteins share structural similarity only in the arginine hook motif responsible for DNA binding[6,15]. Furthermore, *Bacillus subtilis* Rok shares no structural similarity with H-NS, Bv3f or Lsr2 yet fulfils the same physiological role[16], by binding AT-rich DNA and having a strong preference for sequences containing a TpA step[17]. The interaction is mediated by lysine side chains in a winged helix that make contacts with the DNA backbone[17]. The MvaT protein of *Pseudomonas* species uses lysine residues in an AT-pincer motif to make similar contacts[18].

DNA looping by SMC proteins. Structural maintenance of chromosomes (SMC) complexes are tripartite rings composed of a pair of SMC monomers, kleisin and the accessory/regulatory kite (kleisin-interacting winged-helix tandem elements) or hawk (HEAT repeat subunits containing proteins associated with kleisins) proteins[19–22]. Each SMC monomer consists of a 'hinge' dimerization domain involved in the formation of a V-shaped SMC dimer, an ATPase 'head' domain and an antiparallel coiled-coil 'arm' extending between the hinge and head domains. The SMC dimer is bound to a kleisin complex to form a ring that captures DNA[19,21,23,24] and, by encompassing two DNA segments, forms a loop[25] (FIG. 1c, FIG. 2, top). Such a loop may also form by the dimerization of SMC dimers that each embrace a DNA segment[26] (FIG. 2, bottom). Kleisins also recruit the

Nucleoids
Structures found in prokaryotic cells that contain chromosomes, bound proteins and other associated molecules (for example, RNAs). Nucleoids are functionally similar to the nuclei of eukaryotic cells but are not enclosed within a membrane. Nucleoids can be found in eukaryotic organelles believed to be bacterial in origin.

Genome
The complete set of genes encoded by the DNA content of a given organism. The genome includes genes encoded by chromosomal and extrachromosomal DNA, and intervening non-coding regions.

Box 1 | Techniques used to study chromosome structure and organization



Hi-C

Hi-C is a chromosome conformation capture (3C)-based method used to study the three-dimensional organization of the chromosome[176–178]. It is a high-throughput technique that determines the probability of interaction between pairs of genomic loci in an unbiased manner at resolutions of up to 1 kb (REFS[90,177]). Hi-C allows the study of chromosome organization in situ in the nucleus or nucleoid, and changes therein in response to environmental stimuli, and — by alignment with genome-wide protein-occupancy profiles such as chromatin immunoprecipitation (ChIP) data sets — identification of proteins involved in chromosome structuring[38,44,80,81,85,89–91,177]. Hi-C is generally an ensemble technique providing an averaged chromosome interaction profile. Single-cell Hi-C is gaining momentum in the field of chromosome biology, yet has still to be applied to bacterial organisms[179–181].

The technique involves treating cells in culture with formaldehyde to chemically crosslink all DNA–protein and protein–protein interactions within the cell, hence fixing the structure of the chromosome (see the figure, part **a**). Of the remaining steps outlined in the figure part **a**, the key principle is that loci that are close to each other in three-dimensional space are ligated into individual DNA-ligation products regardless of their position along the primary genome sequence. Ligation products are read out using high-throughput sequencing to identify interacting pairs of genomic loci en masse. The sequencing data are represented as a heatmap of genome-wide interaction probabilities.

Fluorescent repressor operator system

The fluorescent repressor operator system (FROS) is a microscopy-based technique used to determine the position and track the dynamics of specific loci in living cells. Loci of interest are marked with an array of ‘operator’ sequences that can be recognized and bound by ectopically expressed ‘repressor’ proteins that are translationally fused to fluorescent protein labels (see the figure, part **b**). Different loci can be tagged and independently tracked in live cells to determine the spatial position and inter-relationship of positions, establishing changes in structure in response to environmental stimuli. Changes in chromosome structure are evident as changes in the distance between pairs of loci. FROS is a single-cell technique that reveals non-averaged chromosome dynamics. Despite limitations in its throughput, it is more powerful in establishing direct structure–function relations than Hi-C.

Chromatin immunoprecipitation

ChIP uses antibodies that target a DNA-binding protein of interest to isolate the factor and the chromosomal regions associated with it following enzymatic, chemical or physical genome fragmentation. Often combined with high-throughput sequencing (ChIP–seq), the technique measures genome-wide patterns of DNA binding at single-base-pair resolution.

In this method, the chromosome of cells in culture is fixed with formaldehyde to crosslink all protein–protein and protein–DNA interactions (see the figure, part **c**). The chromosome is fragmented and antibodies are used to immunoprecipitate any DNA segments that are bound by the protein of interest. In ChIP–seq, the immunoprecipitated library is then purified and sequenced to determine the genome-wide DNA-binding profile of the protein of interest.

eYFP, enhanced yellow fluorescent protein; NAP, nucleoid-associated protein.

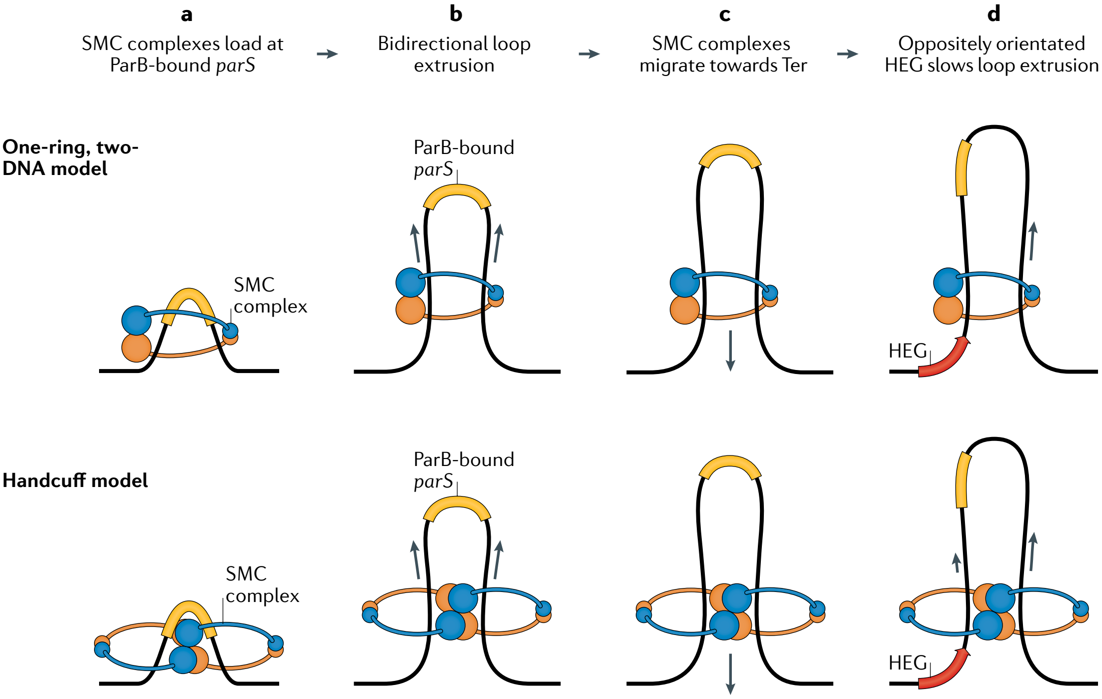


Fig. 2 | **SMC proteins function as loop-extruding factors. a** | The structural maintenance of chromosomes (SMC) complex is loaded onto the DNA at ParB-bound *parS* sites in bacteria[37–39]. **b** | The SMC complex then bidirectionally 'pulls' the DNA through its ring to extrude a growing, unknotted loop of DNA[40,42,43]. The loop may be formed by an SMC complex entrapping two DNA strands within a single ring (one-ring, two-DNA model (top)) or by a dimer of two SMC rings that each trap one DNA segment (handcuff model (bottom)). **c** | Loop extrusion allows bacterial SMC complexes to progressively move from the ParB-bound *parS* sites that are positioned close to the origin of replication towards the terminus (Ter) region[38,44]. **d** | The progression of SMC along the DNA is slowed by convergent transcription[38,44] and may be slowed by up to 80% on encountering an oppositely oriented highly expressed gene (HEG)[44].

kite and hawk proteins. Bacterial and archaeal SMC–kleisin complexes, and the eukaryotic Smc5–Smc6 complex, recruit the kite proteins. The condensin and cohesin SMC complexes of eukaryotes recruit the hawk proteins[20,22].

In bacteria, three classes of SMC family proteins have been identified: SMC–ScpAB in *B. subtilis* and *Caulobacter crescentus*, SMC-like MukBEF in *E. coli* and other gammaproteobacteria and deltaproteobacteria, and the MukBEF-like MksBEF, which has been detected in a wider range of bacterial species. These SMC family proteins are involved in segregation of newly replicated sister chromosomes[27–36]. SMC–kleisin complexes are loaded onto the chromosome at the centromere-like *parS* sequences, positioned close to the origin of replication, by the *parS*-binding protein, ParB[37–39]. Loading factors for the SMC-like proteins MukBEF and MksBEF are currently unknown. Once associated with DNA, SMC complexes generate and maintain DNA loops, and are mechanistically characterized as loop-extruding factors[40,41] (FIG. 2). By contrast, there is currently no evidence to suggest that SMC-like MukBEF and MksBEF play the same role. First proposed in 2001 (REF.[42]), and formalized theoretically in 2012 (REF.[43]), loop extrusion involves the clamping of the protein complex around contiguous DNA sequences[42] (FIG. 2a). The factor then 'pulls' the DNA through the clamp to produce a growing, unknotted loop of DNA[40] (FIG. 2b). SMC proteins bidirectionally extrude DNA and progressively move along the chromosome[43] towards the terminus (FIG. 2b,c). The SMC extrusion complex may consist of a pair of DNA molecules pulled through one SMC ring, referred to as the 'one-ring, two-DNA model'[25] (FIG. 2, top), or one DNA molecule pulled through each ring of a 'handcuffed' pair[26] (FIG. 2, bottom). The rate of loop extrusion by SMC is affected by transcription. Oppositely oriented highly expressed genes (HEGs) attenuate the progression of SMC[38,44] (FIG. 2d). For instance, in *B. subtilis*, SMC progression can be slowed by more than 80% due to an oppositely oriented HEG[44]. As with other DNA-looping proteins, SMC proteins have been suggested to function by static loop formation — stably anchoring a pair of DNA loci to form a loop[45]. However, as supporting evidence that SMC proteins largely function by active loop extrusion in bacteria, chromosome arms progressively align from the origin to the terminus on replenishment of the SMC loading factor ParB in *B. subtilis*[44], but they aberrantly align upon repositioning *parS* sites in *C. crescentus*[38].

DNA bending by IHF, HU and Fis. Whereas H-NS and SMC proteins manage DNA loops[46], other NAPs primarily bend the DNA. For example, the factor for inversion stimulation (Fis) binds DNA as a dimer (FIG. 1d) by virtue of a helix–turn–helix motif[47]. Fis recognizes a 15-bp degenerate DNA palindrome characterized by a G at position 1

Nucleoid-associated proteins
(NAPs). A broad term to describe any proteins implicated in organizing bacterial chromosomes. Here we consider structural maintenance of chromosomes (SMC) proteins as NAPs due to their association with the nucleoid and their role in shaping nucleoid structure. SMC proteins — discovered later than other NAPs and initially studied primarily in the context of chromosome segregation — have historically (and in our view unjustly) not been classified as NAPs.

Nucleoprotein
A generic term, applicable to prokaryotes and eukaryotes, to describe DNA in complex with bound proteins.

and a C at position 15 (5′-GNNVRWWWWWYVNNC-3′). Target recognition is driven by the shape of the minor groove resulting from the binding site sequence rather than the sequence itself[47]. The degree of DNA bending induced by Fis binding can vary between 50° and 90° (FIG. 1d) depending on the flanking DNA sequence[48]. Fis is often found at points where DNA duplexes cross[49,50]. This may stabilize plectonemes in supercoiled DNA.

DNA bending by integration host factor (IHF) is more severe, generating 160° bends[51] (FIG. 1e). IHF binds its consensus sequence (5′-WATCAANNNNTTR-3′)[52] as a heterodimer, composed of α-subunits and β-subunits. The minor groove is contacted by a β-ribbon arm that protrudes from each subunit of the heterodimer (FIG. 1e). This interaction is favoured by A-tracts. The insertion of a proline residue at the tip of each β-arm into the DNA base stack induces a hairpin bend by kinking the DNA on either side of the hairpin apex[51]. High intracellular concentrations of IHF permit non-specific interactions with many non-specific DNA targets, probably in a manner similar to heat-stable protein from *E. coli* strain U93 (HU) (see below). The protein IHF has been identified only in Gram-negative bacteria.

HU shares 40% sequence identity with IHF subunits[53]. Unlike IHF, HU is found widely distributed among bacteria[54]. In *E. coli*, HU forms α-subunit and β-subunit heterodimers. However, HU homodimers predominate across other bacteria, in which a single gene encoding HU is often present. HU has no sequence specificity, but its mode of target recognition is similar to that of IHF. DNA is bent to a lesser extent by HU than IHF and over a range of different angles — akin to a flexible hinge[55,56]. Binding of HU also occurs preferentially at naturally bent or distorted DNA[57]. The bends induced by each HU β-arm force the DNA out of a single angular plane[55,58]. Consequently, sequential binding of HU dimers induces coiling of the DNA around the bound proteins to form filaments. This means that HU can restrain negative supercoils in DNA and, alongside topoisomerase I, introduce negative supercoils in circular DNA[59–61]. Generally, DNA is negatively supercoiled in bacteria to facilitate DNA transactions that require DNA melting[62].

Plectonemes
DNA loops in which the double-stranded DNA is wrapped around itself as a result of supercoiling.

Supercoiled
Pertains to supercoiling, which is underwinding or overwinding of the double helix that causes the double-stranded DNA to fold into higher-order structures: plectonemes and toroids. To alter DNA supercoiling levels, enzymatic breaking and rejoining of DNA strands is required.

Topoisomerase
An enzyme that alters DNA supercoiling by breaking and rejoining DNA strands. Mechanistically, topoisomerases are distinguished by whether they break and rejoin either a single strand (type I) or both strands (type II).

Modulation of NAP function by other proteins. The architectural properties of NAPs may be regulated by paralogues and NAP modulators. For instance, DNA binding by H-NS is regulated by its paralogue StpA and by Hha, a NAP modulator that belongs to the YmoA family of proteins[63,64]. StpA shares 58% sequence identity with H-NS and forms homodimers, and heterodimers with H-NS, in vitro. Heterodimers are likely to predominate in vivo as the StpA homodimer is susceptible to proteolysis[65,66]. Therefore, H-NS-bound regions of the chromosome also contain StpA[67]. StpA stimulates DNA bridging by H-NS and stabilizes the structure against changes in temperature and Mg^{2+} or K^{+} concentration[64]. Hha is an 8-kDa protein involved in the regulation of H-NS-like proteins. Factors such as Hha lack a DNA-binding domain and interact with the N-terminal domain of H-NS to enhance DNA bridging[63,64,68,69]. Disruption of the H-NS–Hha interaction relieves the repression of H-NS–Hha co-regulated operons, such as *hilA*, with minimal disruption of the H-NS binding profile at the operon[69].

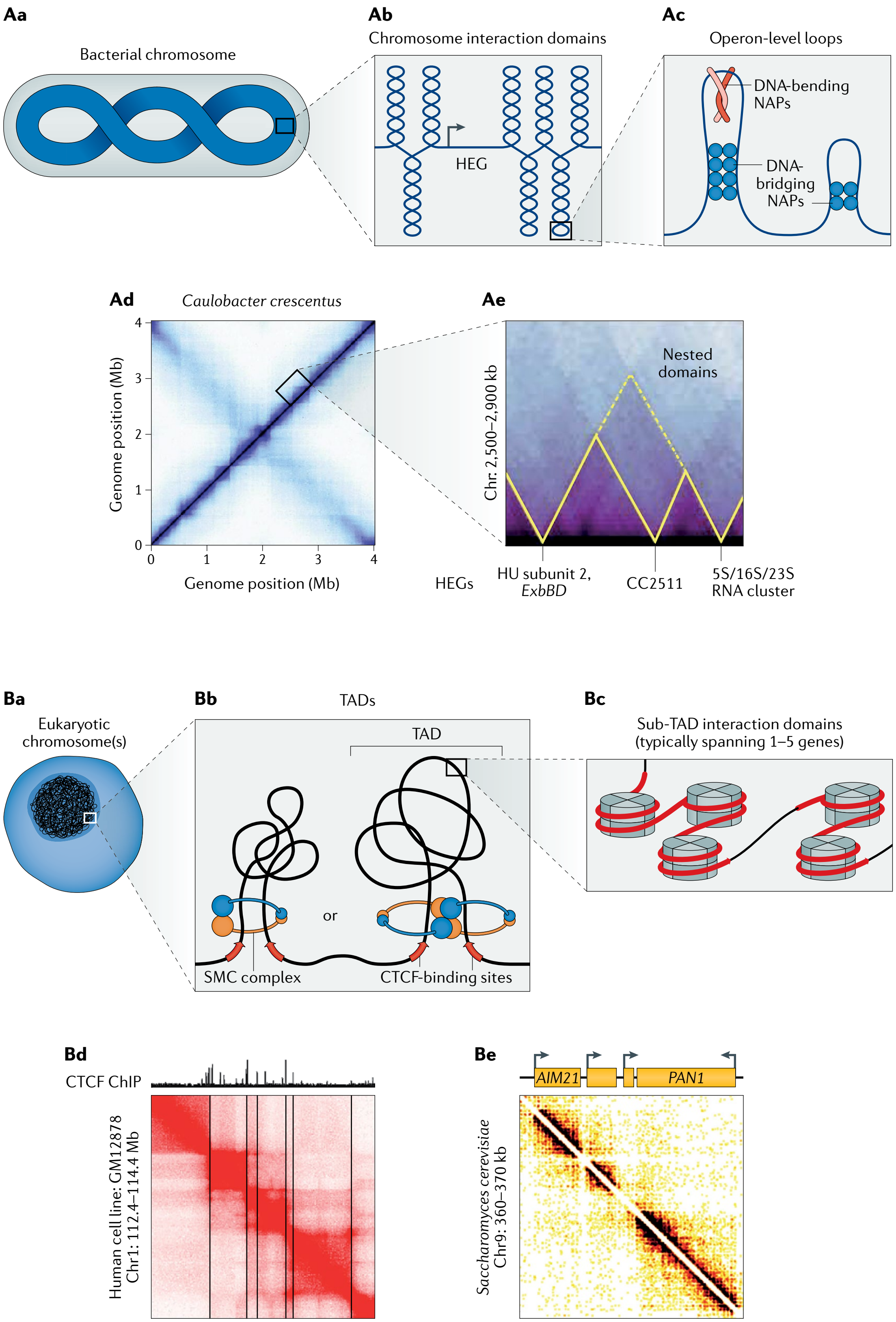
Comparison with eukaryotic and archaeal DNA-folding proteins. The nomenclature for NAPs (for instance, ‘histone-like nucleoid structuring protein’ for H-NS) can imply a relationship to eukaryotic histones. However, there are few similarities at the protein level. Most notably, H-NS is histone-like in only one regard: it is an abundant DNA-binding protein. Even so, there are many examples of eukaryotic DNA-binding proteins that utilize arginine hooks to bind AT-rich DNA[70]. These can have global DNA-folding properties. For example, the metazoan special AT-rich sequence-binding protein 1 (SATB1) has genome-wide roles in DNA folding and, like H-NS, might link higher-order nucleoprotein structures and gene regulation[71–73]. HU, like histones, is able to induce DNA supercoiling[59–61]. Indeed, the ability of HU to wrap DNA in filaments hints that the protein has the capacity to form structures similar to hypernucleosomes in archaea[74,75]. However, although the protein–DNA co-crystal structures are comparable for archaeal histones and HU, results from solution studies do not support this model[56]. Structurally and functionally, SMC complexes in bacteria and eukaryotes have similar functions in managing DNA loops (see later).

Analogously to eukaryotic histones, bacterial NAPs also undergo post-translational modifications. To date, 29 post-translational modifications have been identified for *E. coli* H-NS that may fine-tune its properties. Acetylation neutralizes charges of Lys83, Lys87, Lys96, Lys120 and Lys121, which are known to facilitate DNA binding. Succinylation of Lys96, Lys120 and Lys121 may also interfere with DNA binding due to steric hindrance[76–79]. Some HU proteins have terminal extensions enriched in lysine, proline or alanine repeats, reminiscent of the (S/T)PKK motifs found in eukaryotic histone H1 that are subject to post-translational modification[54].

Local patterns of DNA folding

Chromosome interaction domains. At a scale of tens to hundreds of kilobases, the bacterial chromosome (FIG. 3Aa) is partitioned into chromosome interaction domains (CIDs)[80,81] (FIG. 3Ab), which are analogous to the topologically associating domains (TADs) in eukaryotes[82–84] (compare FIGS 3Ab and 3Bb). CIDs and TADs exhibit a high degree of self-interaction and are insulated from flanking regions.

Hi-C in *C. crescentus* indicates that the chromosome is organized into 23 CIDs during exponential growth in rich medium and 29 CIDs in starvation conditions, with the length of these domains ranging between 30 and 420 kb (REFS[80,85]). The boundaries between CIDs correspond to positions of HEGs that are more than 2 kb in length[80,85] (FIG. 3Ab). In *C. crescentus*, these include, for instance, genes within the ATP synthase and NADH–quinone oxidoreductase gene clusters during exponential growth in rich medium, and starvation-induced genes such as *CCNA_03169* (which encodes an Lrp-like asnC family transcription regulator) and *CCNA_03327* (which encodes a histidine kinase involved in signal transduction) during periods



of starvation. Housekeeping genes such as those within the ribosomal protein gene cluster form CID boundaries during both conditions, albeit of different strength. The ribosomal protein gene cluster forms a sharp CID boundary in exponential phase; at this stage of growth, the genes in the cluster exhibit a high transcription rate. During starvation, in accordance with the decreased rate of gene expression within the cluster, the sharpness of the boundary diminishes[80]. Recombination-based experiments indicate that while HEGs generally form

◀ Fig. 3 | **Chromosomes are hierarchically organized in bacteria and eukaryotes.** **A** | Bacterial chromosome organization. At a global scale, the bacterial chromosome is spirally folded to fit within the bacterial cell (part **Aa**). Regions of the chromosome sequentially close to each other interact in three-dimensional space as evidenced by the presence of a primary diagonal of high interaction frequency in a Hi-C contact map (part **Ad**). Except for *Escherichia coli*, all reported bacterial chromosome interaction profiles also show a secondary diagonal of low interaction frequency that lies perpendicular to the primary diagonal (part **Ad**). This feature indicates interaction between the chromosome arms that run alongside each other in the spirally organized chromosome (interarm interaction)[80,81,86,101]. At the scale of tens to hundreds of kilobases, the chromosome is subdivided into chromosome interaction domains (CIDs) (part **Ab**)[80,81]. CIDs exhibit self-interaction and are insulated from flanking chromatin. These structures are observed as squares along the primary diagonal of a Hi-C map (part **Ad**) or as triangles when observing one half of the symmetric Hi-C map (part **Ae**). Bacterial CIDs are nested[80,81,86]: larger domains (broken yellow line) are organized into smaller subdomains (solid yellow line) (part **Ae**)[80]. The boundaries between the domains are typically formed by highly expressed genes (HEGs) more than 2 kb in length that physically separate the flanking chromatin (part **Ab**)[80,85]. The smallest structural unit of organization of the bacterial chromosome may correspond to loops formed at the level of individual operons by nucleoid-associated proteins (NAPs) (part **Ac**). **B** | Eukaryotic chromosome organization. The eukaryotic chromosome, localized inside the nucleus (part **Ba**), is organized into topologically associating domains (TADs) (part **Bb**), which are analogous to the bacterial CIDs. TADs are formed by loop extrusion[43,88,89]. Eukaryotic structural maintenance of chromosomes (SMC) proteins load onto the chromosome and extrude DNA loops (FIG. 2) until the complexes collide with inward-facing CCCTC-binding factor (CTCF)-binding sites (part **Bb**)[43,88,89]. TAD boundaries — identified as the region between two squares along the diagonal of a Hi-C matrix — occur at genomic regions enriched in CTCF (part **Bd**)[90–92]. Eukaryotic TADs are nested, with the smallest sub-TAD interaction domains typically comprising up to five genes (parts **Bc,Be**)[96]. ChIP, chromatin immunoprecipitation; HU, heat-stable protein from *E. coli* strain U93. Hi-C contact maps in parts **Ad** and **Ae** are adapted with permission from REF.[80], AAAS. GM12878 Hi-C map in part **Bd** is adapted with permission from REF.[91], Elsevier. *Saccharomyces cerevisiae* Micro-C map in part **Be** is adapted with permission from REF.[96], Elsevier.

plectoneme-free regions that act as supercoil diffusion barriers, only long HEGs can generate extended supercoil diffusion barriers that insulate CIDs by physically separating flanking chromatin[85]. Indeed, the ectopic insertion of a long HEG is sufficient to establish a CID boundary in the chromosome[80].

The *B. subtilis* chromosome is organized into 20 CIDs 50–300 kb in length. While 60% of the CID boundaries coincide with HEGs, ~30% overlap with sections of the genome bound by the protein Rok[81]. This observation implies that Rok (and, by extrapolation, other bacterial NAPs) could function as domain barriers. The *E. coli* chromosome appears to be organized into 31 CIDs between 40 kb and ~300 kb in size. Twenty-two of the CID boundaries correspond to the positions of HEGs, and nine boundaries coincide with positions of genes that code for proteins with an export signal sequence[86]. This may be relevant in light of the hypothesis that chromosomes are membrane appended by coupled transcription–translation–translocation[87]. While multiple systems contribute to the formation of CID boundaries in bacterial chromosomes, the hierarchical structural organization that they contribute to is conserved. Bacterial CIDs exhibit a nested domain organization with each domain composed of smaller subdomains[80,81,86] (FIG. 3Ad,Ae). The smallest units of this organization may correspond to individual operons (FIG. 3Ac)

In eukaryotic chromosomes (FIG. 3B), TADs are typically formed by loop extrusion[43,88,89]. Several lines of evidence suggest that SMC complexes including cohesin, condensin and Smc5/6 function as loop-extruding factors. Unlike their bacterial counterparts, eukaryotic SMC complexes do not appear to be loaded onto the chromosome/chromatin at a specific DNA sequence. On clamping around the DNA, eukaryotic SMC complexes continue to extrude loops until the complexes spontaneously dissociate from the DNA, collide with another factor or encounter an appropriately oriented TAD boundary element[43,88,89]. TAD boundaries are encoded in the genome as CCCTC-binding factor (CTCF)-binding sites. These sites exhibit a directionality, such that a TAD forms only between a pair of inward-facing CTCF sites[90–92] (FIG. 3Bb). Indeed, deletion or inversion of CTCF-binding sites disrupts TAD boundaries in vivo[89]. Curiously, in *Drosophila melanogaster*, SMC complexes and CTCF are not markedly enriched at TAD boundaries. In flies, this role is played by the insulator complexes BEAF32–CP190 and BEAF32–Chromator[93–95].

As in bacteria, the TADs of eukaryotic genomes are nested domains[90,94,96]. The smallest organizational units of TADs correspond to individual genes in *Saccharomyces cerevisiae*[96] (FIG. 3Be). The functional relevance of this organization and the molecular determinants of the boundaries are still unclear.

The impact of DNA supercoiling. Local patterns of DNA supercoiling influence DNA folding within topologically isolated regions of the *E. coli* chromosome (FIG. 3Ab). However, tools to measure chromosome-wide patterns of DNA folding have become accessible only in recent years[97,98]. As noted already, DNA is in an average state of negative supercoiling. However, supercoiling density is unevenly distributed and varies across phases of growth. In particular, a gradient of increased negative supercoiling runs from the origin of replication to the terminus, along each arm of the chromosome, only in starved cells[97]. This gradient requires the protein HU. The wrapping of DNA around HU and the change in twist of the double helix mediated by the protein are consistent with effects of HU on global DNA supercoiling[97]. The expression levels of HU also vary greatly across different phases of growth[99], potentially explaining effects on DNA topology. Collectively, this may also explain why loss of HU has different effects on intrachromosome interactions in different bacteria[80,86] with different levels of DNA supercoiling[100].

Interactions between chromosome arms. In bacteria, progression of SMC from the origin to the terminus mediates contacts between the right and left replichores, resulting in their parallel alignment. This manifests itself as a characteristic ‘secondary diagonal’, perpendicular to the main diagonal in Hi-C matrices of bacterial chromosomes[80,81,101] (FIG. 3Ad). Curiously, this secondary diagonal is absent in the contact maps of the *E. coli* chromosome[86] despite the presence of the SMC-like MukBEF system. The MukBEF complex, in the absence of ATP, consists of a V-shaped MukB dimer, the MukF kleisin, which extends between the pair of MukB head domains, and four MukE kite proteins. Unlike other characterized kleisins, MukF is not monomeric[19,20,102]. Instead, MukF forms a dimer via its N-terminal winged-helix domain

Replichores
The sections of a chromosome between the origin and the terminus of replication. Circular chromosomes are usually divided into a left replichore and a right replichore.

and binds MukB via its C-terminal domain[103,104]. On binding ATP, the MukB ATPase heads dimerize and sterically displace one of the two MukF monomers, rendering the N terminus of the MukB-bound MukF available for dimerization with another ATP-bound MukBEF complex[104]. The subsequently formed dimer is the minimal functional unit of MukBEF[105]. ATP-bound MukBEF stably associates with the chromosome and is involved in its condensation, while ATP hydrolysis results in MukBEF dissociation[105,106]. The hydrolysis of both ATP molecules contained within the dimerized head domains is required for a single MukBEF unit to release the DNA[104–106]. The MukBEF complex has been proposed to move along the chromosome as a 'rock climber'. In this model, ATP hydrolysis in a single unit of a MukBEF dimer releases it from the chromosome, while the other unit remains bound. ATP binding then allows the released MukBEF unit to capture a different segment of the DNA and hence move along the chromosome[105] (FIG. 4A). The release–capture cycles of this model imply that the minimal functional unit of MukBEF in *E. coli* cannot promote and maintain interarm interactions as observed for loop-extruding SMCs such as SMC–ScpAB in *B. subtilis*. Other models that consider that a MukBEF dimer is not completely released from DNA on ATP hydrolysis speculate that MukBEF may still carry a loop extrusion functionality[107] (FIG. 4B).

Global chromosome organization

Over the past 10 years, advances in genome-scale approaches have improved our understanding of bacterial DNA folding at the micrometre scale (BOX 1). Most notably, chromosomal patterns of NAP binding and physical interaction frequencies have revealed independently organized macrodomains with distinct properties[4]. Such structures are best defined for *E. coli*, where the chromosome is divided into four macrodomains and two non-structured regions. All macrodomains exhibit reduced intracellular mobility compared with the non-structured chromosomal regions. Thus, macrodomains tend to interact with the non-structured regions but not with other macrodomains[108].

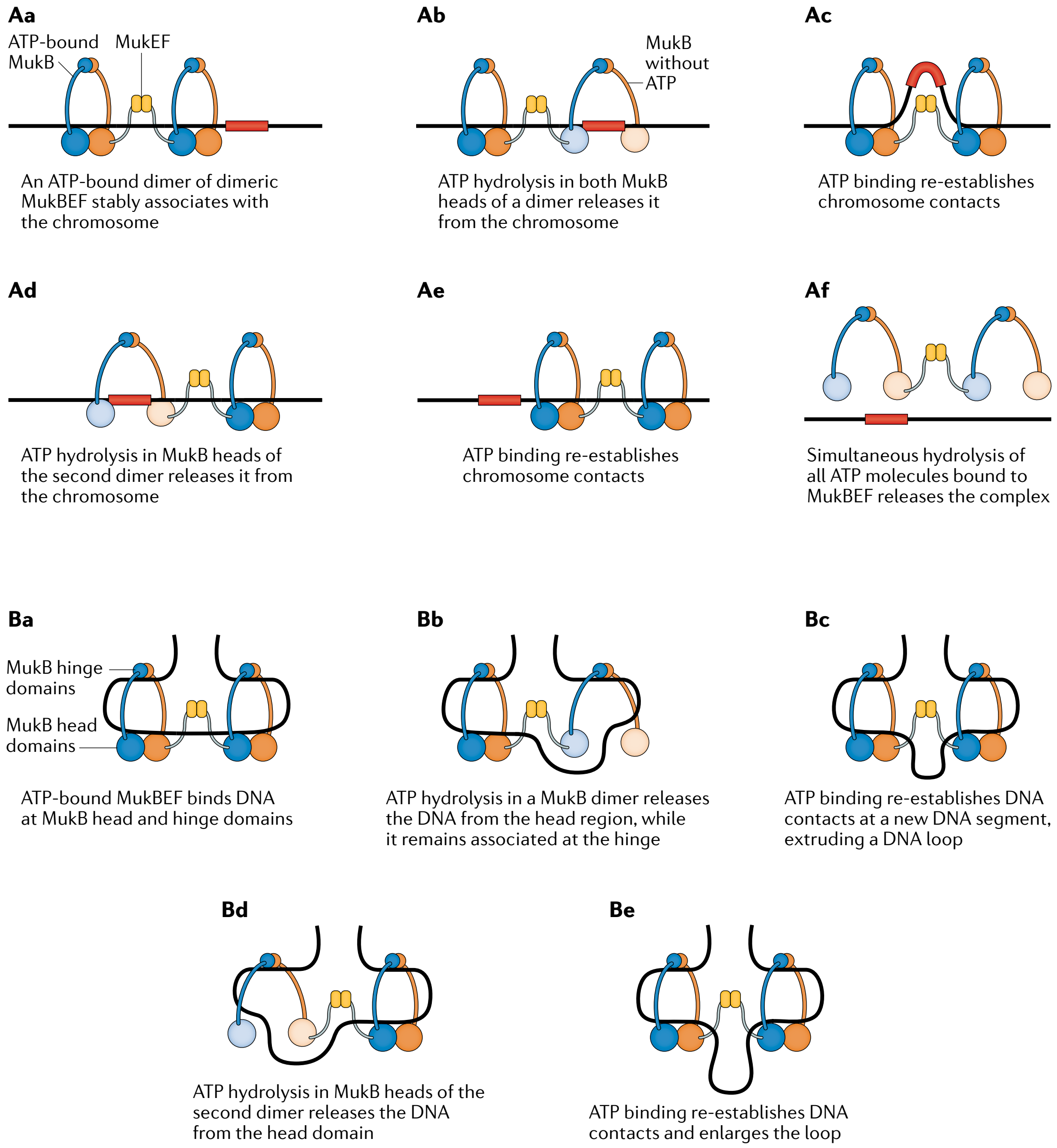
Constraint of Ori macrodomain mobility by MaoP. The Ori macrodomain contains the origin of chromosome replication *oriC*[109,110]. The constrained mobility of Ori requires the *yifE* gene product, MaoP (macrodomain Ori protein), and a 17-bp motif in the upstream intergenic region (5′-CTAATACTCCGCGCCAT-3′) named *maoS* (macrodomain Ori sequence)[109]. In otherwise wild-type cells, inactivation of *maoS*/MaoP specifically increased the mobility of Ori[109]. It is not known how MaoP acts over long distances to constrain DNA mobility.

Condensation of Ter macrodomain structure by MatP. The terminus (Ter) macrodomain is diametrically opposed to Ori (FIG. 5Aa) and encompasses the replication terminus. A major breakthrough for understanding Ter was the identification of a sequence repeated 23 times in Ter but not elsewhere in the *E. coli* chromosome (5′-GTGACRNYGTCAC-3′)[111]. The same sequence uniquely occurs in equivalent parts of many bacterial chromosomes[111]. This DNA site, named 'macrodomain Ter sequence' (*matS*) is the target of the macrodomain Ter protein (MatP). This interaction is highly specific, as shown by MatP exclusively binding these DNA targets in chromatin immunoprecipitation experiments[111]. Loss of MatP activity leads to decondensation of the Ter macrodomain[111]. MatP consists of three domains: an N-terminal four-helix bundle, a central β-strand–helix–helix and a C-terminal coiled coil[112]. Interaction of MatP with DNA is mediated by the β-strand–helix–helix, which resembles ribbon–helix–helix structures found in other DNA-binding proteins[112]. MatP binds DNA as a dimer mediated by interactions involving both the N-terminal domain and the central domain. The C-terminal coiled coil is required for tetramerization of MatP. Such tetramers can generate bridges between distal *matS* sites on the chromosome, effectively condensing the Ter macrodomain[112,113]. This is evident in Hi-C experiments: deletion of *matP* specifically restructures Ter, with reduced intradomain interactions being observed[86]. Loss of MatP also prevents correct positioning of the DNA replication at midcell, and this depends on an interaction between MatP and division apparatus-associated protein ZapB[114].

Other proteins with macrodomain-specific DNA-binding properties. In *E. coli* at least two additional proteins, SeqA and SlmA, have macrodomain-specific DNA-binding properties[115,116]. However, unlike MaoP and MatP, there is no evidence that SeqA and SlmA contribute to the overall folding of these domains. Briefly, SeqA is involved in sequestration of the DNA replication origin after a new round of DNA replication has been initiated[117]. This is permitted because newly replicated DNA is hemimethylated at 5′-GATC-3′ motifs targeted by DNA adenine methylase (Dam) and SeqA[118]. These 5′-GATC-3′ motifs are under-represented in the Ter macrodomain and over-represented elsewhere, particularly near the origin of replication[116]. Similarly, SlmA binds throughout the *E. coli* chromosome, except in the Ter macrodomain[115]. SlmA recognizes the sequence 5′-GTGAGTACTCAC-3′ and is required for correct cell division[115]. SlmA, SeqA, MatP and MaoP are co-conserved in bacteria encoding Dam methylase, suggesting that these bacteria use similar strategies to organize their chromosomes. Indeed, even in bacteria lacking Dam, proteins with similar patterns of chromosome-wide DNA binding have been identified. For instance, in *B. subtilis* the nucleoid occlusion (Noc) protein appears to be the functional equivalent of SlmA[119]. Similarly, in *C. crescentus*, GapR targets a large region surrounding the origin of replication[120,121].

Environmental regulation

The structure of the bacterial chromosome changes in response to the environment (FIG. 5Ab). In part, this is because a small number of NAPs (most notably H-NS and MvaT) can undergo conformational changes in response to specific ligands[63,122]. More commonly, the intracellular concentration of NAPs alters in response to environmental triggers[99]. These two scenarios are discussed in more detail below.



An ATP-bound dimer of dimeric MukBEF stably associates with the chromosome

ATP hydrolysis in both MukB heads of a dimer releases it from the chromosome

ATP binding re-establishes chromosome contacts

ATP hydrolysis in MukB heads of the second dimer releases it from the chromosome

ATP binding re-establishes chromosome contacts

Simultaneous hydrolysis of all ATP molecules bound to MukBEF releases the complex

ATP-bound MukBEF binds DNA at MukB head and hinge domains

ATP hydrolysis in a MukB dimer releases the DNA from the head region, while it remains associated at the hinge

ATP binding re-establishes DNA contacts at a new DNA segment, extruding a DNA loop

ATP hydrolysis in MukB heads of the second dimer releases the DNA from the head domain

ATP binding re-establishes DNA contacts and enlarges the loop

Fig. 4 | **MukBEF moves along the chromosome as a 'rock climber'. A** | MukBEF movement along a single DNA molecule (based on the rock climber model proposed in REF.[105]). **Aa** | The minimal functional unit of the MukBEF complex corresponds to a dimer of dimers — MukB$_4$E$_4$F$_2$. When each of the MukB heads is bound to ATP, the MukBEF complex remains stably associated with the chromosome. **Ab** | Hydrolysis of ATP in both MukB heads of the same dimer disengages the MukB heads, and releases the dimer from the chromosome. The MukBEF complex remains bound to the DNA via the ATP-bound dimer. **Ac** | ATP binding to MukB of the released dimer re-establishes chromosome contacts with a different chromosomal locus. **Ad,Ae** | A DNA segment release and capture cycle in the second MukBEF dimer allows the complex to move along the chromosome as a 'rock climber'. **Af** | Simultaneous hydrolysis of all four ATP molecules bound to a MukBEF complex releases it from the chromosome. This step may involve a MukBEF 'unloading' factor. **B** | MukBEF as a loop-extruding factor[107]. **Ba** | ATP-bound MukBEF binds chromosomal DNA at the MukB head and hinge domains. **Bb** | ATP hydrolysis in a MukBEF dimer releases the chromosomal DNA segment bound at the MukB head domains. **Bc** | ATP binding re-establishes MukB head–DNA contacts at a new DNA segment, thus generating a DNA loop. **Bd,Be** | A release–capture cycle in the second MukBEF dimer results in loop enlargement, and hence loop extrusion.

Regarding environmentally triggered conformational changes of NAPs, helix α3 of H-NS is unstable and frequently buckles. This folds one of the DNA-binding domains of the H-NS dimer onto the body of the protein[63]. A similar conformation is also adopted by the H-NS family protein MvaT under low-osmolarity conditions; electrostatic interactions occur between a positively charged patch at the C-terminal DNA-binding domain and a negatively charged patch at the N-terminal domain[122]. In the folded conformation, one of the two DNA-binding domains of the protein dimer is unavailable for DNA binding, thus favouring the formation of lateral filaments along DNA[63,122] (FIG. 1b, top). Magnesium ions stabilize helix α3 in H-NS to prevent

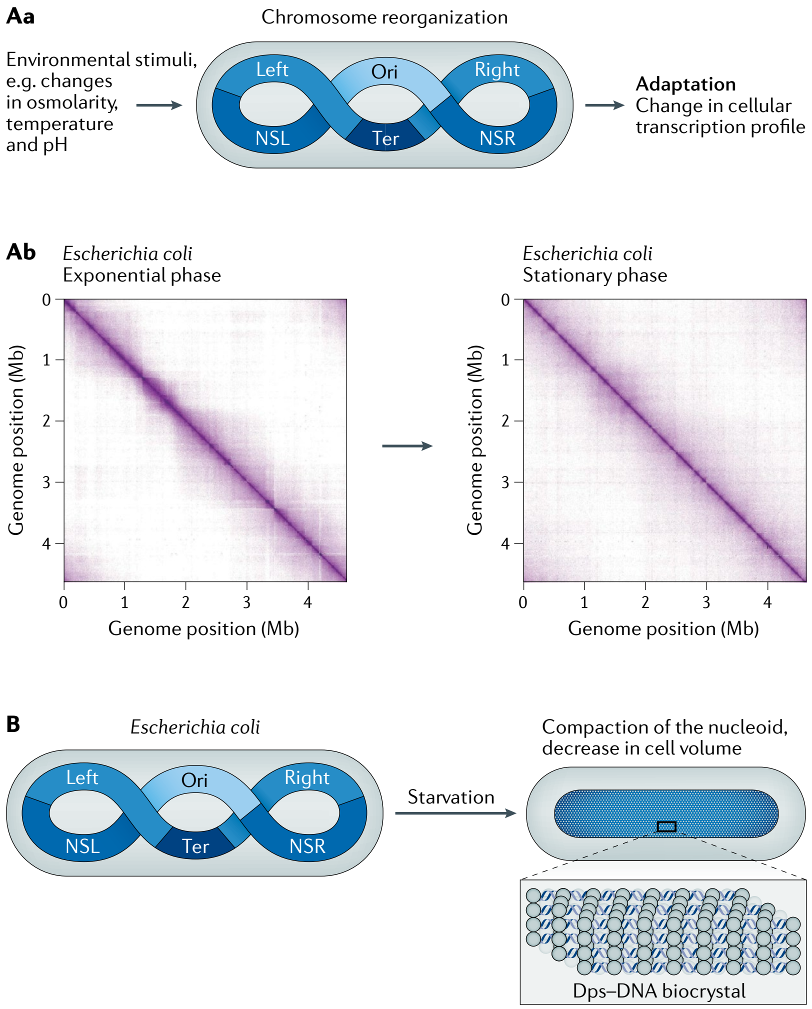


Fig. 5 | **Environmental stimuli induce changes in chromosome organization. A** | DNA reorganization in growing bacteria. **Aa** | Reorganization of the bacterial nucleoid is induced in response to environmental stimuli such as changes in osmolarity, temperature and pH. Consequently, activity of specific sets of genes required for environmental adaptation is altered. **Ab** | The transition between the exponential and stationary phases of growth of *Escherichia coli* is associated with a reorganization of the bacterial chromosome. Specifically, the chromosome exhibits a weakening of compartmentalization into chromosome interaction domains. This is observed as 'blurring' of the squares along the main diagonal[86]. **B** | Chromosome reorganization in starved bacteria. Reorganization of the bacterial nucleoid can be induced by starvation or stress. Such changes are apparent by light microscopy and indicate compaction of the nucleoid, often accompanied by a reduction in the overall cell volume[127]. The inset depicts the nucleoid structure in molecular detail as revealed by electron microscopy. Most notably, DNA-binding protein from starved cells (Dps; pale blue spheres) drives the formation of an ordered crystal lattice that incorporates DNA[131]. These structures are phase separated from other compartments of the cell and resistant to damage[50,133]. NSL, left non-structured region; NSR, right non-structured region; Ori, Ori macrodomain; Ter, Ter macrodomain. *E. coli* contact maps in part **A** are adapted with permission from REF.[86], Elsevier.

buckling[63]. Correspondingly, high-osmolarity conditions destabilize the electrostatic interaction between the N- and C-terminal domains of MvaT[122]. Hence, both DNA-binding domains of the H-NS and MvaT dimers become available for DNA binding and bridged loops can form[63,122] (FIG. 1b, bottom). H-NS is also temperature sensitive. High temperatures reduce the cooperativity of H-NS oligomerization and favour its dissociation from DNA[123–126].

A less subtle mechanism controlling chromosome dynamics is based on levels of NAP expression, which can change substantially. This is most notable during stress and starvation, when the nucleoid is reorganized into a condensed crystalline structure[127] (FIG. 5B). Most NAPs are present at lower levels in starved cells, and Fis, which is among the most abundant DNA-binding proteins during periods of rapid cell division, is undetectable[128]. Conversely, while they are undetectable during rapid growth, DNA-binding protein from starved cells (Dps) and curved DNA-binding protein A (CbpA) accumulate to 175,000 and 14,000 copies per cell in stationary phase[99,129]. Both bind the DNA highly cooperatively, and interactions between DNA-bound protein molecules lead to DNA compaction[50,130]. Electron micrographs of Dps–DNA complexes reveal that they are organized in a crystalline lattice in vitro[131] (FIG. 5B). A similar structure is observed in vivo when Dps is expressed in exponentially growing Δ*fis* strains[131,132]. Complexes of Dps or CbpA with DNA are resistant to damage induced by chemical and biological nucleases[50,133]. Hence, expression of these proteins is thought to protect the integrity of the genetic material in harmful environments. However, how these highly condensed protein–DNA structures coexist with other cellular processes has been a mystery. Recent work has shed light on the puzzle, revealing that supercondensed nucleoids of starved *E. coli* cells are phase-separated organelles[133]. Phase separation is an inherent physical property of macromolecules (such as proteins) to self-organize into condensates or 'droplets' in a crowded environment such as the cell interior. Binding of Dps to DNA in vitro blocks access to nucleases and hydroxyl radicals, but the DNA remains fully permissive to transcription[129,133]. This is because the physical properties of some molecules allows them to move between separated phases, while other molecules are trapped within a specific phase.

Interplay with genome transactions

As alluded to earlier, understanding how chromosome folding impacts other cellular processes has been a long-standing area of interest. Although Dps seems unable to impede transcription, the same is not true of other NAPs, which can have specific effects on gene expression. Furthermore, additional roles have been identified for NAPs in chromosome replication and segregation, as well as cell cycle progression. Here we describe the intricate interplay between NAPs, genome structure and diverse types of genome transaction.

Silencing of horizontally acquired genes by H-NS. H-NS targets DNA sequences that have a high AT content, often acquired by horizontal gene transfer. H-NS binding at these loci represses transcription (known as xenogeneic silencing)[9]. Remarkably, the majority of transcription suppressed by H-NS at such loci is spurious in nature[134], arising from the high probability of sequences that fortuitously resemble promoter elements for RNA polymerase in high-AT-content DNA[135,136]. Left unchecked by H-NS, this transcription imposes a severe fitness defect due to titration of RNA polymerase and a global downshift in transcription of housekeeping genes[134].

Canonical gene regulation by H-NS. Although most promoters repressed by H-NS have spurious output, H-NS also plays a key role in regulating transcription of mRNAs. In these instances, the mechanisms by which H-NS influences promoter activity appear diverse. A common mechanism of repression by H-NS involves blocking the binding of RNA polymerase, or transcriptional activator proteins, completely[137,138]. Alternatively, at the *rrnB* P1 and *hdeAB* promoters, H-NS-induced DNA-looping traps RNA polymerase, interfering with promoter escape[139,140]. Similarly, but not involving loop formation, a direct contact between RNA polymerase and H-NS can interfere with promoter clearance[141]. Because H-NS-controlled looping is mediated by environmental factors, many H-NS-regulated genes are responsive to temperature and osmolarity. For instance, *proVWX* (the *proU* locus) is an H-NS-regulated osmosensitive operon. Its regulation requires two elements, the upstream regulatory element positioned upstream of the transcription start site and the downstream regulatory elements in the coding region that extends across the transcription start site[142–144]. The two elements operate synergistically in H-NS-mediated osmoregulation[145]. Such synergy could imply lateral or bridge-mediated interactions between the elements. Although direct evidence is lacking, in vitro experiments showing that only H-NS-mediated bridging is sensitive to osmolarity lead us to hypothesize that H-NS represses transcription of the *proU* operon by loop formation and that relief of repression involves local restructuring of the chromosome[63,126]. In pathogenic bacteria, H-NS can be utilized to control the expression of virulence factors during host colonization, with contributions from additional proteins that alter the ability of H-NS to multimerize and/or bind DNA[146].

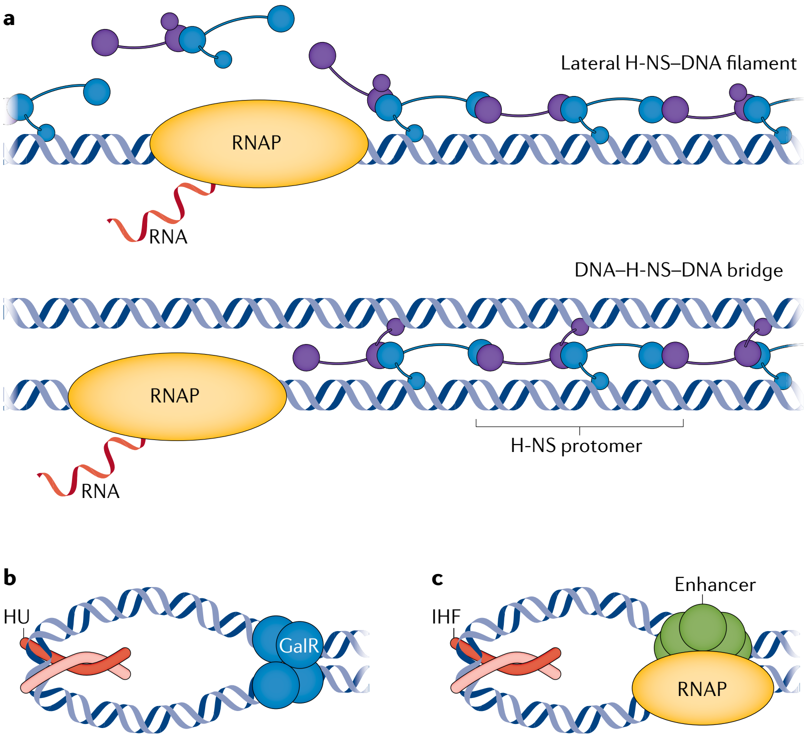


Fig. 6 | **Modulation of transcription by nucleoid-associated proteins. a** | Alternative types of histone-like nucleoid structuring protein (H-NS)–DNA complexes have different effects on transcription elongation. Top panel: lateral H-NS–DNA filaments can be invaded by RNA polymerase (RNAP). These H-NS–DNA complexes are unable to prevent transcription elongation and are either transiently displaced or remodelled as a result[126,147]. Bottom panel: bridged DNA–H-NS–DNA complexes are potent blocks to transcription and result in stalled elongation complexes[126]. **b** | Stabilization of a DNA bend by HU facilitates repression. At the *Escherichia coli gal* operon the GalR repressor protein forms a repressosome that is stabilized by HU binding to the bent DNA[154]. **c** | Activation of transcription by DNA bending. Promoters that are dependent on enhancer-binding proteins for transcription require integration host factor (IHF) as a cofactor. The sharp DNA bend introduced by IHF brings the distally bound enhancer-binding protein into the proximity of RNAP so transcription can be activated[157]. HU, heat-stable protein from *E. coli* strain U93.

Regulation of transcription elongation by H-NS. As well as regulating the initiation of transcription, H-NS can control transcription elongation by impeding the progression of RNA polymerase. The ability of H-NS to hinder transcript extension depends on the type of H-NS–DNA complex. For example, RNA polymerase can transcribe genetic information through lateral H-NS–DNA filaments (FIG. 6a, top panel), whereas H-NS–DNA bridges efficiently block transcript extension and are likely to trap RNA polymerase in the loops formed[126] (FIG. 6a, bottom panel). In both cases, it is not known if RNA polymerase advancement removes H-NS from the DNA or if the nucleoprotein complex is transiently remodelled[147].

Roles of bacterial H-NS in regulating transcription versus eukaryotic nucleosomes. In summary regarding the transcriptional roles of H-NS, loci bound by H-NS are often not permissive to binding of RNA polymerase or regulatory proteins but can be remodelled for transcription to occur. By analogy, in eukaryotes, nucleosomes block transcription initiation, and so promoters are usually nucleosome-free. Histone modifications lead to remodelling of chromatin that impacts transcription[148]. For H-NS, transcription itself could lead to local remodelling of the nucleoprotein complex[147]. Furthermore, 'antisilencing' transcription factors can perturb repressive nucleoprotein filaments or interfere with their formation[5].

Activation and repression of specific promoters by Fis. In general, Fis activates the expression of genes encoding products that are important for rapid cell division[149]. Conversely, Fis is often a repressor of genes that allow utilization of alternative carbon sources or terminal electron acceptors[150,151]. The DNA-folding activity of Fis appears to be important for counteracting the supercondensation of chromosomes mediated by Dps[132]. Taken together with the gene regulatory roles of Fis, this implies the protein is crucial to prepare cells for maximal rates of growth on exiting periods of starvation. This is consistent with observations that Fis is present at detectable levels only when cells are dividing rapidly[128]. The ability of Fis to activate or repress transcription is dependent on the position of binding and on interactions with other regulators at a given promoter. Hence, the mechanisms by which Fis activates and represses transcription are similar to those used by canonical transcription factors. For instance, to activate transcription of ribosomal RNA operons, Fis facilitates the recruitment of the transcriptional apparatus via a specific contact with the C-terminal domain of the RNA polymerase α-subunit[152]. The same contact is made by many canonical transcriptional activators[153]. Similarly, the mechanisms of transcription repression by Fis are not unusual and involve occlusion of RNA polymerase or transcription factors[150,151].

Stabilization of DNA repression loops by HU. Although it is unable to recognize specific DNA sequences, HU can regulate transcription from specific promoters. This results from the ability of HU to bind and stabilize certain deformations in DNA. This behaviour has been described for the *E. coli* galactose operon regulatory region. Two promoters at this locus are repressed by the activity of the repressor protein GalR. Maximal repression by GalR is mediated by interactions between GalR molecules bound at distal sites to create a repression loop. HU binds at the apex of the DNA loop and stabilizes the nucleoprotein complex, thus enhancing repression[154] (FIG. 6b). As HU affects global patterns of DNA supercoiling, genes responsive to DNA topology are part of the HU regulon[155,156].

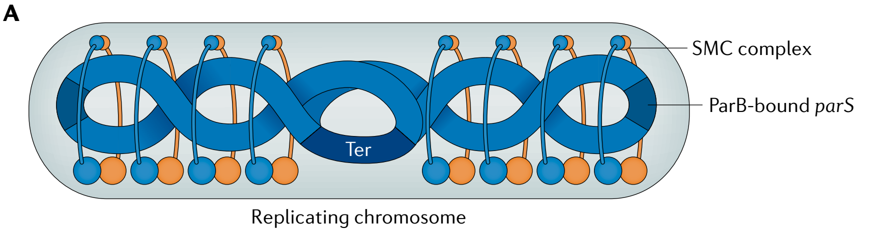
IHF can regulate transcription by bending the DNA. Like Fis, IHF is able to activate and repress transcription by binding to specific sites near promoters. This can be due to the ability of IHF to sharply bend DNA. For instance, IHF binds upstream of many *E. coli* promoters dependent on the alternative σ-factor, σ^{54}. σ-factors are general transcription factors (functionally similar to those found in eukaryotes) that are used by bacterial RNA polymerases to bind selectively to specific promoters. By bending the DNA, IHF facilitates interactions between RNA polymerase and enhancer proteins bound upstream. This stimulates promoter opening to activate transcription[157] (FIG. 6c). The binding and bending of DNA by IHF can also repress transcription. In one example, at the *E. coli nrf* promoter, IHF DNA binding alters interactions with a bound activator to hinder transcription activation[158]. The role of IHF as an activator or repressor depends on local nucleoprotein organization. Hence, there is no universal position upstream of a promoter from which IHF consistently exerts an activating versus repressive effect on transcription.

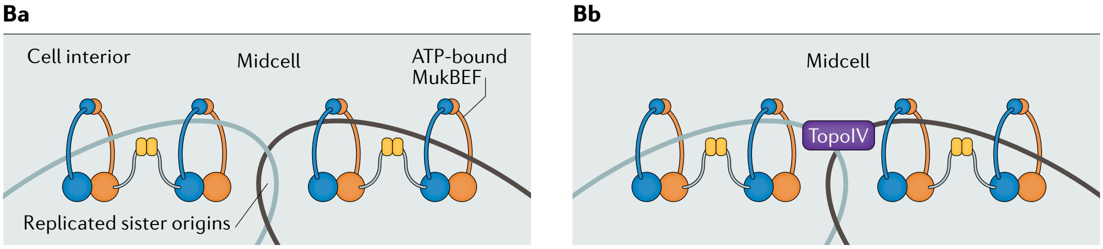
Interplay with replication and chromosome segregation. Chromosome architecture and NAPs also influence chromosome replication and segregation. The bacterial equivalent of the mitotic apparatus, the ParAB–*parS* partitioning system, and SMC proteins that are proposed to regulate origin firing in *B. subtilis*[81] are evidently involved in the segregation of bacterial chromosomes into opposite cell halves concomitantly with replication[159–161] (FIG. 7A). In *C. crescentus* and *B. subtilis*, the ParB partitioning protein binds to the *parS* sequences present close to *oriC* to form a nucleoprotein complex on both sister chromosomes (FIG. 7A). The ParA ATPase is recruited to the complex and generates the free energy required for the resolution of the sister origins and their segregation[162]. The loop-extruding complex SMC–ScpAB is also recruited to the origin of replication by ParB[33,37,39], from where it migrates along the chromosome to the terminus, extruding disentangled DNA of a single chromosome. This structurally separates sister chromosomes and favours their segregation[38,44,81] (FIG. 7A).

The ParAB–*parS* partitioning system is absent in some species of gammaproteobacteria and deltaproteobacteria. In these organisms, the SMC-like MukBEF complex participates in chromosome segregation. In *E. coli*, MukBEF complexes containing an ATP-bound MukB are recruited to the origin[34,163,164]. MukB, in turn, recruits topoisomerase IV, a type II topoisomerase, which decatenates replicated sister origins[165,166]. Immediately after decatenation, the segregated origins, and the associated MukBEF clusters, move towards the quarter positions of the cell[34,105] (FIG. 7B). Computational modelling suggests that segregation is driven by a self-organizing gradient of MukBEF and the origin of replication[167]. MukBEF is also recruited to *matS* sites in the Ter macrodomain. At these sites, MatP and ATP hydrolysis by MukB release MukBEF complexes and associated topoisomerase IV enzymes. In Δ*matP* strains and strains with an ATPase-defective MukB, MukBEF accumulates in the Ter macrodomain, where it recruits topoisomerase IV and promotes early resolution of the chromosome terminus[168].

Interplay with cell cycle progression. GapR, a conserved NAP of alphaproteobacteria, is a master regulator of cell cycle progression. Its binding sites overlap with loci bound by other regulators of cell cycle progression, including CtrA, MucR1, MucR2 and GcrA[169]. GapR-depleted and Δ*gapR* strains of *C. crescentus* are temperature sensitive and exhibit cell division defects, forming filamentous, undivided cells or anucleate cells[120,121,169]. GapR binds to the origin of replication of the *C. crescentus* chromosome, where it is involved in the initiation of replication[121]. GapR also binds DNA ahead of the replication fork[120], where it interacts with DNA gyrase or topoisomerase IV to relieve positive superhelical stress[170]. Indeed, GapR deletion is associated with a lengthened S phase and stalling of the replication fork[120]. Furthermore, GapR binds the *parS* locus, at which it plays a role in the segregation of newly replicated sister origins[121]. Sister chromosome segregation in *C. crescentus* constitutes a ParA-independent slow step that involves the separation of the pair of *parS*–ParB nucleoprotein complexes and a ParA-dependent fast step that localizes one of the sister origins to the opposite pole. GapR regulates the initial slow step of segregation, as evidenced by the remerging of resolved *parS*–ParB complexes in *ΔgapR* cells[121].

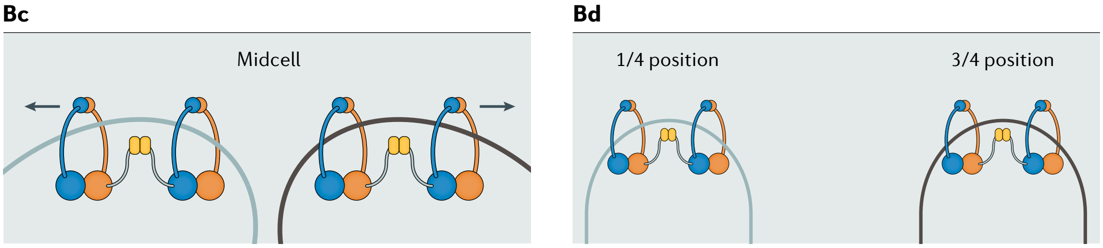
The NAP SlmA also controls cell cycle progression[171]. SlmA bound to SlmA-binding sites (SBSs) on the chromosome is involved in signalling the polymerization of the cytokinetic FtsZ ring[115]. As a nucleoid occlusion factor, SlmA also ensures that the FtsZ ring is precisely positioned around the site of Ter decatenation to prevent the ‘guillotining’ of the chromosome[172].





ATP-bound MukBEF complexes are recruited to the origin of replication

MukB recruits TopoIV, which decatenates replicated sister origins



The decatenated origins, associated with MuKBEF, move away from midcell

Origins localize at quarter positions in the cell

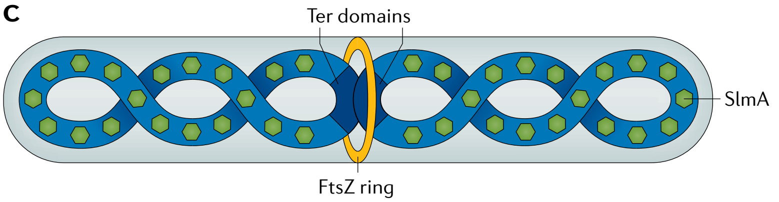


Fig. 7 | **Chromosome organization has an impact on chromosome segregation and cell cycle progression.** **A** | ParAB–*parS*-mediated chromosome segregation. Structural maintenance of chromosomes (SMC) complexes are loaded at ParB-bound *parS* sites in the origin domain[33,37,39]. SMC complexes progressively move along the chromosome (not to scale) towards the terminus (Ter) domain, driving the alignment of chromosome replichores and promoting the segregation of sister chromosomes[38,44,81]. For ease of representation, the handcuffing model (FIG. 2, bottom) is not shown. **B** | MukBEF-mediated origin segregation. **Ba** | ATP-bound MukBEF complexes are recruited to the replicated origins[34,163,164]. **Bb** | MukB recruits topoisomerase IV (TopoIV), a type II topoisomerase, which decatenates the entangled sister origins[165,166]. **Bc,Bd** | Once decatenated, the sister origins, associated with MukBEF, move from the midcell position towards the quarter positions in the cell[34,105]. The segregation is proposed to be driven by a self-organizing gradient of MukBEF and the origin of replication[167]. **C** | Cell cycle progression. Cell division requires FtsZ ring assembly. FtsZ polymerization occurs at midcell, where the segregating Ter domains are located and SlmA is occluded. DNA-bound SlmA promotes the depolymerization of FtsZ at non-Ter regions, preventing 'guillotining' of the chromosome[115,171–173].

SlmA plays its role by regulating the dynamics of FtsZ polymerization within phase-separated FtsZ droplets. In membrane-bound phase-separated systems, FtsZ polymerizes within phase-separated droplets to form filaments at membrane boundaries. The presence of SBS-bound SlmA counteracts this polymerization[171]. This suggests that in FtsZ phase-separated structures in the bacterial cytoplasm, SBS-bound SlmA antagonizes the assembly of the FtsZ ring[171]. In *E. coli*, SBSs occur throughout the chromosome except at the Ter region[115,173]. This way, an SlmA-free region is produced within the cell when replication reaches the chromosome terminus. The FtsZ ring assembles at this site to initiate bacterial cytokinesis[171] (FIG. 7C). The precise positioning of the FtsZ ring is reinforced by MatP-mediated condensation of the Ter macrodomain[111]. MatP also interacts with the septal proteins ZapA and ZapB to position Ter at midcell[114].

Conclusions and future perspectives

The past 10 years has seen the establishment of broadly applicable models for the folding of bacterial chromosomes. DNA-bending and DNA-bridging proteins play a key role in chromosome folding at the level of individual genes and in the formation of CIDs with sizes up to 300 kb. Higher-order chromosome folding leads to the formation of macrodomains. Although these principles have been best studied in *E. coli*, biased binding of proteins across the chromosomes of distantly related bacteria suggests widespread relevance[119–121]. Future challenges include better understanding local changes in DNA folding and how these impact on other nucleic acid transactions within living cells. For example, biophysical techniques have defined the structures that H-NS can form with nucleic acids in vitro but it is still not clear whether or how such structures impact transcription in vivo. Furthermore, although we understand how individual NAPs organize DNA, it is not obvious how the concerted efforts of all NAPs combine within cells. In that light, it is of particular interest that many non-model organisms seem to harbour NAPs with enhanced functionality, for instance combining the ability to bend and bridge in a single protein[174]. While it is expected that general principles of DNA organization are conserved throughout the bacterial domain of life, and in fact all domains of life[175], we speculate that bacteria occupying extreme environmental niches may have fine-tuned the molecular mechanisms to better cope with environmental challenges.

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Author contributions

All authors researched data for the article, substantially contributed to discussion of the content and wrote the article. R.T.D. and D.C.G. reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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