Review

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Building bridges within the bacterial chromosome

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All organisms must dramatically compact their genomes to accommodate DNA within the cell. Bacteria use a set of DNA-binding proteins with low sequence specificity called nucleoid-associated proteins (NAPs) to assist in chromosome condensation and organization. By bending or bridging DNA, NAPs also facilitate chromosome segregation and regulate gene expression. Over the past decade, emerging single-molecule and chromosome conformation capture techniques have investigated the molecular mechanisms by which NAPs remodel and organize the bacterial chromosome. In this review we describe how such approaches reveal the biochemical mechanisms of three NAPs that are believed to facilitate DNA bridging: histone-like nucleoid structuring protein (H-NS), ParB, and structural maintenance of chromosomes (SMC). These three proteins form qualitatively different DNA bridges, leading to varied effects on transcription and chromosome segregation.

Shaping the bacterial chromosome

Both eukaryotic and prokaryotic organisms must dramatically condense their genomic DNA to package it into cells that are only about one thousandth their length. Simultaneously, DNA must remain accessible for essential processes like DNA replication, repair, transcription, and chromosome segregation. In eukaryotes, genomic DNA is wrapped around histone proteins to form nucleosomes that are further organized into higher-order structures. Bacteria lack histones but have evolved other mechanisms to condense and organize their chromosomes. These factors include DNA supercoiling, macromolecular crowding, the highly conserved condensin complexes comprising structural maintenance of chromosomes (SMC) proteins and their subunits, and the small NAPs that are unique to bacteria. SMCs and NAPs play an important role in not only maintaining genome compaction but also dynamically reorganizing the genome to make it accessible for gene expression. By specifically and nonspecifically binding to the genome, NAPs appear to contribute to the formation of isolated topological regions known as microdomains (Box 1) with an average size of 10 kb [1]. These microdomains are further organized into larger regions called macrodomains (Box 1) with a size of approximately 1 Mb [2,3].

For the past two decades, studies on bacterial chromosome condensation and organization have focused on NAPs that can be classified as DNA benders or DNA bridgers (Figure 1). Examples of well-studied DNA benders include HU (a histone-like protein from *Escherichia coli* strain U93) and integration host factor (IHF). These proteins can nonspecifically bind to DNA by inserting two arms into the minor groove, inducing curvature of more than 90° [4]. DNA bridgers form dynamic connections between DNA duplexes resulting in large DNA loops that can span several kilobases. Besides condensing chromosomes, DNA bridgers also function in chromosome segregation and transcription silencing. In this review we focus on how single-molecule and chromosome conformation capture techniques (Box 2 and Table 1) are contributing to our understanding of how bacteria organize and compact their chromosomes. As a case study, we examine three NAPs – H-NS, ParB, and SMC – believed to be DNA bridgers. We describe how these proteins form qualitatively different protein–DNA bridges and speculate on how these differences might be important in determining their unique biological functions. For additional discussion of NAPs and their functions, see the reviews in [5,6].

H-NS

The enterobacterial H-NS is a small and abundant protein that is involved in both chromosome organization and gene regulation [7,8]. Although H-NS lacks a consensus DNA-binding sequence, it binds with high affinity to AT-rich DNA sequences that display sequence-induced curvature [9–11]. Bacterial promoters commonly comprise such motifs and H-NS specifically binds to these sites, influencing the regulation of a wide variety of genes [12,13]. Examples of H-NS-regulated genes include its own gene, *hns* [14], *rrnB*, which is responsible for ribosomal RNA synthesis [15], *proU*, which controls an osmotically inducible transport system [16], and horizontally acquired virulence genes such as *virF* [17]. Mutations in *hns* typically cause an increase in transcription, indicating that H-NS is predominantly a gene silencer [18].

How H-NS negatively regulates transcription remains controversial. For some genes, such as the *Shigella virB*, H-NS binding sites overlap the promoter region; in these cases, H-NS is likely to occlude RNA polymerase binding [19]. For others, such as the *E. coli* and *Salmonella typhimurium proU* operons, H-NS influences transcription

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Box 1. Domains within the bacterial chromosome

The bacterial chromosome is organized into domains of various lengths. On the smallest level, chromosomal DNA is divided into microdomains with an average size of 10 kb [1]. These domains are negatively supercoiled and form plectonemic loops that are topologically insulated from each other [1,91,92]. In the popular bottlebrush model of the bacterial chromosome, microdomains form the bristles of the brush that extend out from a dense DNA core [93]. Microdomains prevent relaxation of the entire chromosome on single-strand nicking; such unraveling would have disastrous consequences given that plectonemic loops substantially compact the chromosome.

The formation of insulated topological domains requires boundary regions that are kept free of plectonemic loops. This can be accomplished by two mechanisms: (i) preventing rotation of the DNA at the domain boundaries (Figure IA); or (ii) keeping the DNA within these regions in an unwound state (Figure IB). NAPs, especially those involved in DNA bridging, have long been assumed to contribute to domain formation (so called domainins) by acting through the first mechanism. Growing evidence, however, suggests that NAPs may instead act by condensing DNA within a microdomain. Chromosome conformation capture coupled with deep sequencing (see Table 1 in main text) in *Caulobacter crescentus* showed that deletion of HU and SMC only weakly affected local domain boundaries [78]. The strongest determinant of boundaries was the presence of highly expressed genes where the DNA is kept free of plectonemic loops by active transcription [i.e., mechanism (ii) above].

On the significantly longer length scale of micrometers, the bacterial chromosome is further organized into macrodomains. Experimental efforts in *Escherichia coli* that measured chromosome dynamics by site-specific labeling [2,94] and recombination frequency along the chromosome [3] identified the Ori, Ter, Left, and Right macrodomains along with two unstructured regions. Each macrodomain is approximately 1 Mb.

The molecular origins of macrodomain organization remain unclear. Potential domainins such as MatP and SeqA have been identified based on both *in vivo* DNA-binding profiles using ChIP [95,96] and genome conformation capture [40]. Unlike NAPs, MatP and SeqA do not have any influence on gene expression and exhibit high sequence specificity, binding either exclusively inside or outside the Ter macrodomain. MatP recognizes 23 specific DNA-binding sites known as *matS* sites within the Ter region [95]. Structural studies demonstrate that MatP tetramers can bridge distant *matS* sites to organize the Ter domain by DNA condensation [97]. SeqA, by contrast, can sequester newly replicated origins by binding to hemimethylated sites [98]. Thus, it was proposed that SeqA can organize the Ori domain by properly orientating the chromosome during replication [99].

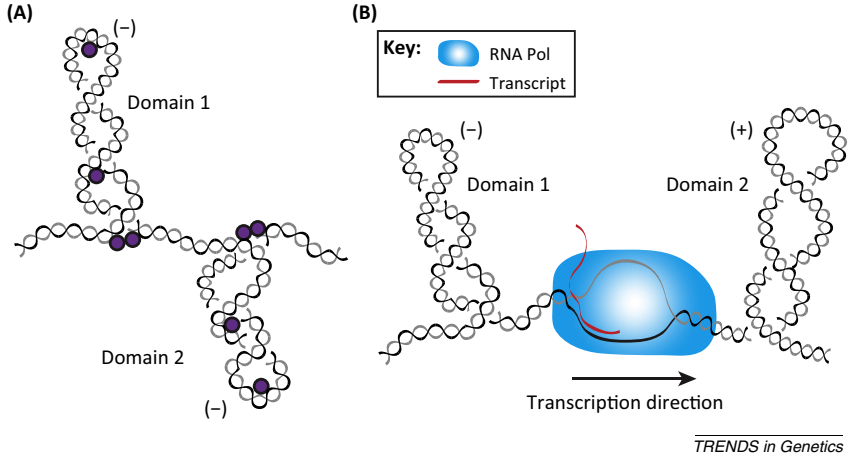

Figure I. Models of DNA topological domain formation. **(A)** By bridging DNA segments, nucleoid-associated proteins (NAPs) (purple circles) can prevent the rotation of negatively supercoiled DNA at domain boundaries. **(B)** During transcription, positive and negative supercoils are generated ahead and behind RNA polymerase. By keeping the intervening DNA in an unwound state, transcription acts to keep the domains separated. Note that the DNA and proteins are not drawn to scale.

despite binding downstream of the promoter [16,20]. Some clues to the functional role of H-NS in transcription can be gained by understanding how H-NS interacts with DNA. Early *in vitro* studies demonstrated that purified H-NS can condense DNA [21] and overexpression of H-NS in *E. coli* was found to compact the nucleoid and cause cell death [22]. Subsequent single-molecule experiments have provided greater insight into how H-NS can condense DNA. Atomic force microscopy (AFM) (Table 1) images indicated that H-NS can bridge adjacent DNA segments or form compact DNA–protein foci [23,24]. DNA loops trapped by H-NS might prevent the loading of RNA polymerase or stall its translocation (Figure 2A). A scanning force microscopy (SFM) (Table 1) study of the reconstituted complex between RNA polymerase and H-NS at the *rrnB* P1 promoter [25] showed that H-NS-mediated bridging of the upstream and downstream regions of the promoter traps RNA polymerase in an open initiation complex, supporting previous biochemical studies [26].

The molecular mechanism by which H-NS bridges DNA is closely related to its structure. H-NS is a 15.6-kDa protein with an N-terminal oligomerization domain and a C-terminal DNA-binding domain connected by a flexible linker (Figure 2A) [27,28]. The N-terminal domain of H-NS is made of a short coiled-coil motif that can interact in an antiparallel manner during dimerization [29–31]. A crystal

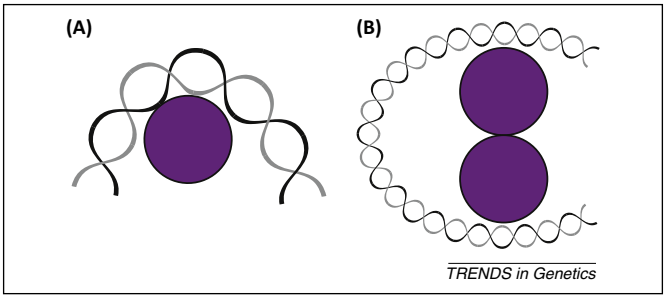

Figure 1. Schematic view of how nucleoid-associated proteins (NAPs) (purple circles) either **(A)** bend a local DNA segment or **(B)** bridge distant loci.

Box 2. Single-molecule force spectroscopy

Various single-molecule manipulation techniques have been used to examine the interaction between NAPs and single DNA molecules. In general these studies apply force to DNA and measure how NAPs change the elastic properties of the DNA on binding. Most of these approaches rely on the attachment of a micron-sized bead onto a DNA end that serves as a handle to apply force. Optical tweezers (Figure IA) are a commonly used technique that provides subnanometer spatial and submillisecond temporal resolution [100]. Briefly, a highly focused laser beam is applied to trap a polystyrene bead attached to one end of a DNA molecule while the other end of the DNA can be tethered to either a glass surface or a second bead held by another optical trap or micropipette. Multiple optical traps can be used to control double-tethered DNA molecules in 3D in a 'dumbbell' [101] or 'quadruple' [32] configuration, enabling the study of intermolecular interactions such as DNA bridging.

The application of force with magnetic tweezers (Figure IB) or buffer flow (Figure IC) results in lower spatial resolution than with optical tweezers but is substantially easier to implement while allowing greater multiplexing. In these experiments, one end of a DNA molecule is attached to the surface of a coverslip and the other end to a paramagnetic bead. In magnetic tweezers techniques (Figure IB), a pair of permanent magnets or electromagnets is used to apply an upward force proportional to the magnetic field gradient. By rotating the magnetic field, a torque can also be applied on the DNA to introduce positive and negative twists, allowing studies of how DNA-binding proteins modulate or recognize DNA topology [102]. In a flow-stretching experiment (Figure IC), laminar buffer flow exerts a constant drag force on the bead that is uniform along the extended DNA molecule. Reactions that convert single-stranded DNA to double-stranded DNA or vice versa, such as in DNA replication [103,104], can be measured with high accuracy by tracking the bead position over time.

DNA can also be stretched in the absence of a bead due to the drag force on the DNA chain (Figure IC). This approach can be readily combined with single-molecule fluorescence imaging, allowing the study of protein translocation on DNA [105,106], DNA replication and repair [107–110] and DNA-binding proteins [64,111]. Laminar flow produces a gradient of tension on the extended DNA that is highest at the tether point and lowest at the free end. By observing the motions of site-specific labels on DNA, DNA motion capture exploits this variation in tension to distinguish between different DNA compaction mechanisms [64].

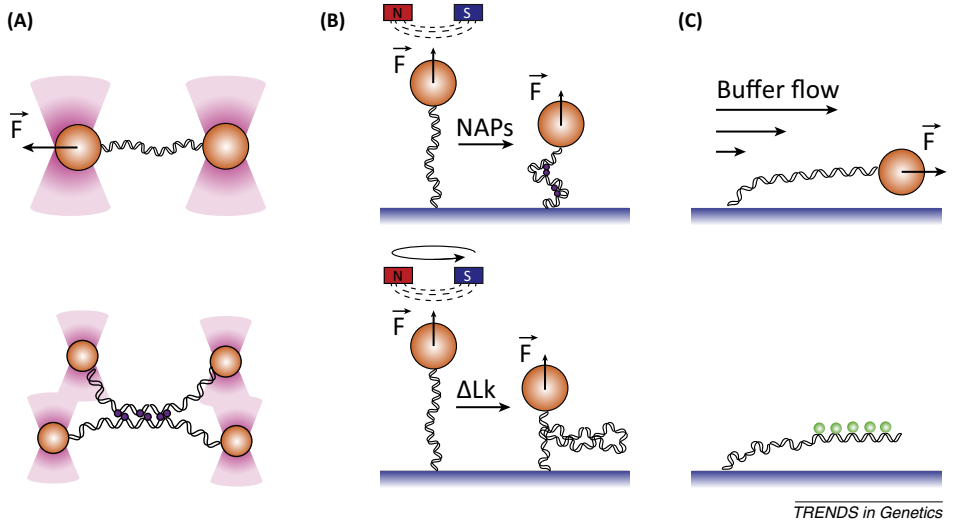

Figure I. Single-molecule techniques to study DNA bridging proteins. **(A)** Optical tweezers. Top: A single DNA molecule is tethered to micron-sized beads (orange spheres) that can be trapped using a highly focused laser beam (pink). Bottom: Multiple traps can be used to study intermolecular interactions such as DNA bridging mediated by nucleoid-associated proteins (NAPs) (purple circles). **(B)** Magnetic tweezers. Top: A single DNA molecule that is immobilized on a glass surface is tethered to a magnetic bead. A pair of permanent magnets can apply a magnetic force that is proportional to the magnetic field. DNA bridging mediated by NAPs can be detected by tracking the distance between the magnetic bead and the surface. Bottom: Twisting the magnets can change the linking number (ΔLk) of the DNA, resulting in the formation of a plectoneme that alters the distance between the magnetic bead and the surface. **(C)** Flow-stretching. Top: Laminar buffer flow exerts a constant drag force on the bead, resulting in uniform tension along the extended DNA molecule. Bottom: Laminar flow exerts drag along the length of a piece of DNA and the tension at any point is equal to the net drag force on all DNA beyond that point. Hence, the tension is highest at the tether point and decreases toward the free end. By site-specifically labeling the DNA with quantum dots (green spheres), DNA motion can be tracked using total internal reflection fluorescence microscopy (TIRFM), providing a simple assay to study the effect of DNA tension on compaction by NAPs. Note that the DNA and proteins are not drawn to scale.

structure of a truncated variant of *S. typhimurium* H-NS lacking the DNA-binding domain further reveals a secondary dimerization site at the C-terminal end of the oligomerization domain that allows H-NS dimers to be linked in a superhelical chain [29]. The helical structure suggests a model in which H-NS dimers can mediate the formation of a DNA plectoneme by stacking between two DNA duplexes with each dimer's C-terminal domain binding to one DNA arm [29]. Such a model is consistent with results from previous optical tweezers experiments (Box 2 and Table 1) in which *E. coli* H-NS was able to bridge two independently trapped DNA molecules when they were crossed [32]. Quantitative measurements of the unzipping of bridged DNA indicated that an average of a single H-NS dimer spans one helical turn of DNA [32]. Precoating the DNA with high concentrations of H-NS before their crossing inhibited bridging, which the authors proposed is due to H-NS dimers spanning both strands as opposed to intermolecular bridging between H-NS dimers [32].

Intriguingly, single-molecule magnetic tweezers studies (Box 2 and Table 1) in combination with AFM imaging have shown that H-NS can also extend DNA by polymerizing along its length [24,33–35]. The extension mode of H-NS is highly sensitive to concentrations of divalent ions: above 5 mM magnesium, the DNA extension mode of H-NS is inhibited and instead H-NS switches to bridging DNA [24]. Increasing pH or temperature results in less DNA extension without detectably impacting DNA bridging

Table 1. Emerging approaches to the study of bacterial chromosomes

	Method	Principle	Strengths	Limitations	Refs
Nanomanipulation of individual DNA molecules	Optical tweezers	A highly focused laser beam is used to trap and move micron-sized beads attached to the ends of DNA molecules in solution	3D manipulation Multiple traps can be used to study interaction between substrates High spatial resolution: 0.1–2 nm	Photodamage Thermal noise Complicated instrumentation	[32,101]
	Magnetic tweezers	A pair of permanent magnets separated by a small gap (~1 mm) is used to extend a surface-tethered DNA by exerting a pulling force on a micron-sized paramagnetic bead	Rotating the magnets can introduce DNA supercoils by changing linking number Multiplexing High spatial resolution: 5–10 nm	Temporal and spatial resolution limited to video detection rate 3D manipulation requires sophisticated electromagnetic feedback control	[34,66,84,86]
	DNA motion capture (flow stretch)	A buffer flow is used to extend a single DNA molecule with one end tethered to the surface of a flow cell; DNA motion is tracked by imaging quantum dots site specifically attached to DNA	Can track DNA motions that are both perpendicular and parallel to the extension direction Relatively high throughput Readily coupled to single-molecule fluorescence imaging of labeled proteins	Limited force manipulation on DNA Low spatial resolution: 100 bp of DNA length	[64]
In vitro *single-molecule imaging*	AFM/SFM	A cantilever with a sharp tip is used to probe the surface of a sample; measurements of cantilever deflection as the tip is scanned over the sample are used to generate a topological map	Provides structural details with subnanometer resolution	Specimen preparation and contact by cantilever tip can introduce artifacts Static image of a protein–DNA complex	[23,25]
	Fluorescence imaging of labeled proteins	Proteins that are either fused to fluorescent proteins or chemically labeled with fluorescent dyes are deposited on a coverslip surface decorated with DNA substrates; dynamic interactions between proteins and DNA are tracked using TIRFM[a] in real time	Direct visualization of protein–DNA complex assembly Provides measurements of protein diffusion and DNA binding/unbinding kinetics	Complications in labeling protein with fluorescent dye Short trajectories (within minutes or less) due to photobleaching	[47–49]
In vivo *fluorescence imaging*	Epifluorescence	Spatial localization of fluorescently fused proteins is tracked in live cells in real time	Live-cell, real-time imaging Commercially available microscope	Diffraction-limited resolution (200–250 nm) Fluorescent fusion proteins can affect protein function or result in localization artifacts due to oligomerization	[46,56,64]
	Super-resolution	Protein of interest is either fused to a photoactivatable fluorescent protein (PALM) or labeled with photoswitchable organic dye via antibodies (STORM[b]); stochastic switching of fluorescent probes between bright and dark states allows determination of the position of molecules with subdiffraction-limited precision	10–55 nm spatial resolution	STORM requires fixation of cells Same limitations with fluorescent fusion proteins as in epifluorescence	[38,52,82]
Genome wide	ChIP	Crosslinked protein–DNA complexes are isolated by immunoprecipitation; DNA-binding sites are identified by reversing the crosslinks and determining the DNA sequence by hybridizing to a microarray (ChIP-chip) or	Provides high-resolution genome-wide specific and nonspecific protein–DNA interaction maps	Information is averaged over a large number of cells Crosslinking efficiency and antibody specificity can lead to enrichment artifacts	[62,69]

Table 1 (*Continued*)

	Method	Principle	Strengths	Limitations	Refs
		high-throughput sequencing (ChIP-seq)			
	Chromosome conformation capture	Chromosomal DNA segments in close proximity are crosslinked in living cells, extracted, digested with restriction enzymes, and intramolecularly ligated; joined junctions are then detected by PCR or deep sequencing allowing the calculation of chromatin contact frequencies	Detects long-range and global 3D genomic interactions in living cells	Population-based method that requires a large number of cells to accumulate enough ligation junctions Resolution is limited by choice of restriction enzyme, detection method, and sequencing depth (>10 kb) Cross-linking efficiency	[38,54,78]

[a]Total internal reflection fluorescence microscopy.

[b]Stochastic optical reconstruction microscopy.

[24,33]. H-NS mutants that are defective in gene silencing fail to extend DNA but remain able to bridge DNA in the presence of 2 mM magnesium [35]. Taken together with the low concentration of magnesium *in vivo* and the fact that gene silencing and DNA binding by H-NS are strongly affected by pH and temperature [36,37], these results have been used to argue that H-NS can locally extend DNA *in vivo* [24,35].

It remains unclear whether H-NS primarily bridges or extends DNA *in vivo* or whether these functions are mutually exclusive. Using super-resolution fluorescence microscopy (Table 1) of a fluorescent fusion to H-NS in *E. coli* cells and a chromosome conformation capture assay (Table 1), it was shown that genes regulated by H-NS interact over long distances to form two compact foci per chromosome [38]. Point mutations in *hns* that disrupt oligomerization or DNA binding abolished silencing of genes regulated by H-NS and the formation of fluorescent H-NS foci *in vivo* [38]. Likewise, interaction frequencies between H-NS-regulated genes decreased significantly in an *hns*-null strain [38]. Based on these findings, it was proposed that H-NS proteins bound at different loci interact over long distances to organize the chromosome [38]. However, subsequent work has challenged these conclusions: H-NS foci were found to form only with fluorescent protein fusions that are susceptible to aggregation [39] and a genome conformation capture study failed to find evidence for clustering of H-NS binding sites [40]. Although these studies cast doubt on whether H-NS plays a role in long-range bridging, they do not address whether H-NS can locally bridge DNA. A recent single-molecule magnetic tweezers study provides some clues on how the dual binding modes of H-NS could affect local DNA topology: H-NS can suppress or trap DNA plectonemes by extending or bridging DNA, respectively [34]. Further work is necessary to understand how H-NS interacts with DNA in cells and how H-NS activity is regulated.

ParB

ParB is part of the highly conserved *parABS* system, which is involved in low-copy-number plasmid partitioning and chromosome segregation. ParB interacts with the other two components of this system by binding to the *parS* DNA element and stimulating the activity of the Walker-type ATPase ParA [41–45]. Two competing models have been proposed to describe how plasmid-encoded ParA can actively segregate DNA loci, based on single-molecule reconstitution experiments (Table 1) using fluorescently labeled

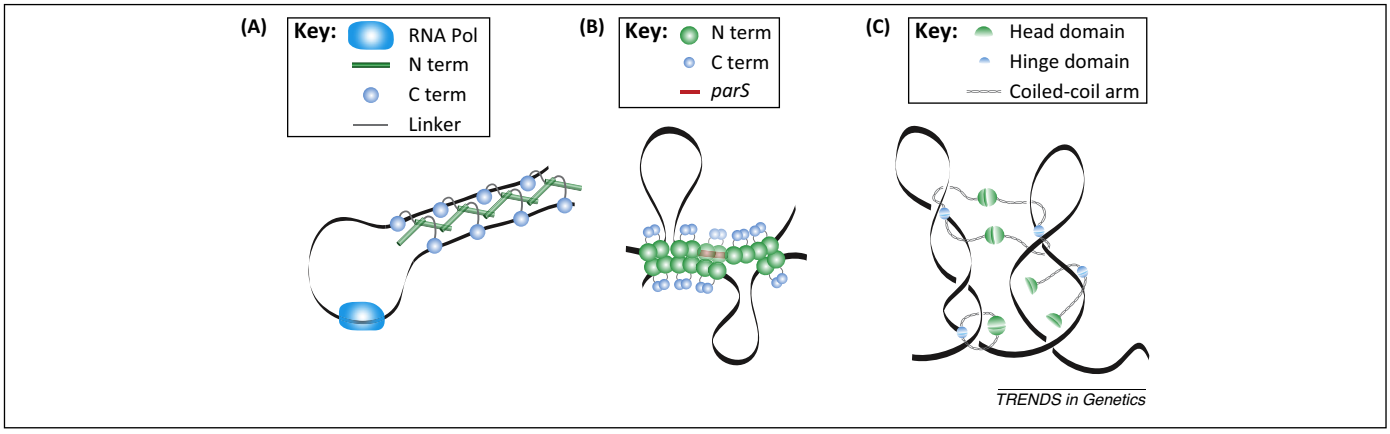

Figure 2. Different ways to build a DNA bridge. **(A)** Histone-like nucleoid structuring protein (H-NS) dimers span between two DNA duplexes (each duplex is represented as a single black line) through C-terminal DNA-binding domains. A secondary N-terminal dimerization site allows H-NS dimers to polymerize along the DNA. By bridging upstream and downstream regions of a promoter, H-NS may trap RNA polymerase inside a DNA loop to repress transcription. **(B)** ParB (Spo0J) binds specifically to a *parS* site as a dimer and can nonspecifically spread over several kilobases through DNA bridging interactions and possibly nearest-neighbor interactions. This ParB–DNA complex may act to compact the origin region or facilitate structural maintenance of chromosomes (SMC) protein loading. **(C)** SMC dimers may act to stabilize or bridge supercoiled DNA loops. It remains unclear whether SMC dimers function in isolation or as part of higher-order complexes, possibly mediated through their ATPase head domains or by non-SMC subunits (not shown). Note that the DNA and proteins are not drawn to scale.

protein components. In the filament-based model, ParA encoded by the *E. coli* plasmid pB171 was proposed to pull plasmids through cycles of ParA polymerization and depolymerization [46]. However, ParA encoded by the P1 and F plasmids was proposed to move ParB–*parS* complexes on plasmids according to a diffusion–ratchet model [47–50]: ParA-ATP, which can nonspecifically bind to a DNA-carpeted flow cell that mimics a nucleoid surface, interacts with ParB bound to a plasmid and anchors the protein–DNA complex to the surface. Simultaneously, ParB can stimulate ParA-ATP hydrolysis; ParA-ADP is released from the vicinity and diffuses away. A ParA depletion zone is thus generated after all anchoring points are broken. A time delay in the recycling of ParA-ATP allows diffusion of ParA and the creation of a ParA concentration gradient. Plasmids loaded with ParB can then ratchet along the nucleoid in chase of a higher local ParA concentration.

In vivo super-resolution fluorescence imaging of the chromosomally encoded *parABS* system in *Caulobacter crescentus* and *Vibrio cholerae* showed that ParA interacts with ParB–*parS* complexes to position newly replicated origins by two possible mechanisms: (i) ParA forms a mitotic-like spindle that dynamically pulls the origin [51,52]; or (ii) nucleoid-associated ParA acts to transiently tether and 'relay' the origin across the cell [53]. Given that the origin-proximal *parS* sites are the first genomic elements transported and anchored at the new cell pole, they establish the global orientation of the entire chromosome during segregation [54]. However, in other bacteria, such as *Bacillus subtilis*, deletion of *parA* (*soj*) does not have a significant effect on chromosome partitioning [55]. It should be noted, however, that the *parABS* system becomes critical in segregating replicated origins in the absence of the condensin complex SMC [56].

The role of ParB in origin positioning remains ill defined but requires its unique ability to bind nonspecific DNA adjacent to *parS* in a phenomenon called 'spreading' (Figure 2B). Spreading is a general property of both plasmid and chromosomal ParB systems; it was first discovered in plasmid ParBs, where it was observed that overexpressing ParB could silence *parS*-proximal genes [57–59]. ChIP (Table 1) experiments demonstrated that endogenous levels of chromosomal ParB are capable of spreading thousands of base pairs surrounding *parS* sites without affecting the expression of nearby genes [60–63]. Furthermore, insertion of a DNA-binding protein 'roadblock' adjacent to a *parS* site attenuates ParB spreading unidirectionally, leading to the proposal that ParB nucleates on *parS* sites and laterally coats the DNA in a 1D filament [57,59,63].

To further examine the mechanism of ParB spreading, Graham *et al.* used single-molecule 'DNA motion capture' experiments (Box 2 and Table 1) to directly visualize how the formation of a higher-order ParB–DNA complex remodeled individual DNAs [64]. In this assay, bacteriophage λ genomic DNA molecules were tethered at one end to a functionalized glass surface and extended under buffer flow (Figure 3). Analogous to motion capture techniques used in computer animation, DNA movement was observed by labeling five specific loci on the DNA with quantum dots conjugated to catalytically inactive EcoRI. Plasmid and chromosomal ParBs were found to dramatically compact the flow-stretched DNA, starting from the free end of the DNA and proceeding to the tether point. Similar end-biased compaction was observed for the *E. coli* DNA-bridging protein H-NS using buffer conditions that favored its bridging activity. The end-biased DNA compaction observed for ParB is most consistent with ParB trapping DNA loops that form on thermal fluctuations; compaction occurs from the free end of the flow-stretched DNA where the tension is lowest and looping is energetically favorable [65]. Subsequent magnetic tweezers experiments have similarly demonstrated that ParB (Spo0J) can condense DNA and stabilize DNA supercoils by bridging [66]. By contrast, DNA motion capture experiments with the DNA-bending protein HBsu (the *B. subtilis* homolog of *E. coli* HU) caused compaction throughout the DNA molecule because the local DNA deformations associated with DNA bending are relatively insensitive to the variation in tension within flow-stretched DNA. Quantification of the kinetics of DNA compaction by *B. subtilis* ParB (Spo0J) showed a nonlinear dependence on protein concentration suggesting that its DNA bridging behavior requires cooperative interactions between ParB dimers.

To determine whether the ability of ParB to trap DNA loops is required for ParB to spread *in vivo*, Graham *et al.* looked for ParB (Spo0J) mutants that eliminated DNA compaction in single-molecule experiments and abolished spreading *in vivo*. Although the family of ParB proteins is structurally diverse, they all comprise a C-terminal dimerization domain and an N-terminal DNA-binding domain

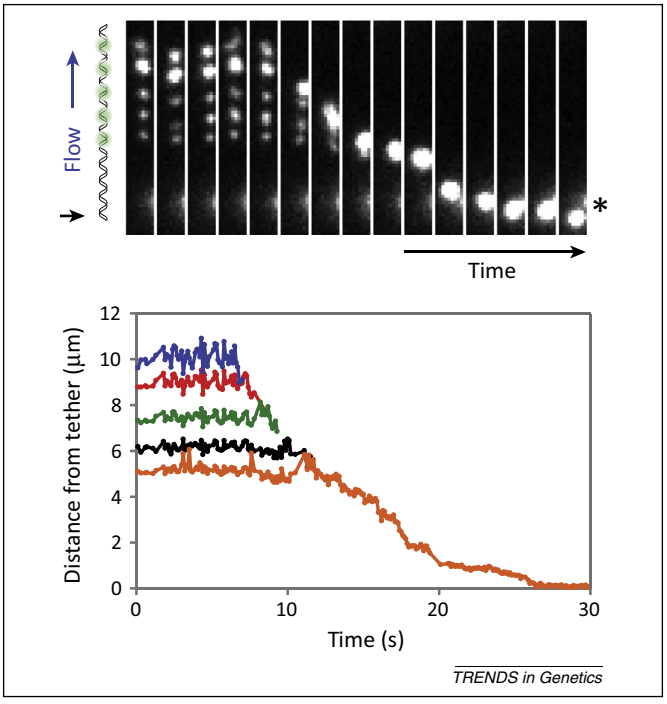

Figure 3. DNA motion capture assay to visualize ParB (Spo0J)-mediated compaction of flow-stretched DNA. Top: Bacteriophage λ genomic DNA (black lines in the cartoon on the left; not drawn to scale) was labeled at EcoRI sites with EcoRIE111Q-conjugated quantum dots (green spheres; not drawn to scale), extended by buffer flow from the tether point (indicated by the small black arrow), and exposed to 100 nM Spo0J protein. Montages of frames from compaction movies (2 s/frame) are shown on the right. The asterisk indicates a quantum dot that is nonspecifically bound to the surface at the edge of the field of view. Bottom: Tracking of individual quantum dots by Gaussian fitting. Adapted from [64].

that contains a helix–turn–helix (HTH) DNA-binding motif [67]. Previous genetic screens identified the ParB (Spo0J) loss-of-function mutants G77S and R80A within the highly conserved ParB Box II of the N-terminal domain [62,68]. Further mutational analysis within ParB Box II revealed a short, arginine-rich patch of residues including R80 that abolished DNA bridging ability yet still bound DNA with wild type affinity *in vitro* [64]. These mutants were also defective in spreading as measured by ChIP and focus formation *in vivo*, indicating that DNA bridging by ParB (Spo0J) is necessary for spreading. By contrast, G77S and the HTH domain mutant R149A could still compact DNA *in vitro* although they exhibited the same defects as the bridging mutants *in vivo*, demonstrating that DNA bridging is not sufficient for spreading and suggesting a role for other types of interactions (e.g., nearest-neighbor interactions).

Taken together, these findings support a model in which ParB (Spo0J) binds *parS* and spreads over many kilobases of DNA by recruiting a small number of other ParB dimers (~20) through DNA bridging and possibly nearest-neighbor interactions (Figure 2B) [64]. This 3D ParB–DNA assembly could provide a platform to load the bacterial condensin SMC, which is thought to promote chromosome segregation [69,70] or potentially provide ParA with a high local concentration of ParB [71]. Such a 3D ParB–DNA complex formation model is in agreement with recent modeling of ParB spreading [72]. Both nearest-neighbor interactions and 3D bridging interactions were found to be necessary to drive the formation of a condensed ParB–DNA complex in computer simulations [72].

SMC

SMC proteins play essential roles in chromosome organization and segregation in all domains of life [73]. In eukaryotes, SMCs condense chromosomes to facilitate chromosome segregation (condensins) and also link newly replicated chromosomes before disjunction and segregation (cohesins). Other cohesin-like SMCs assist in the repair of double-strand breaks, presumably by holding broken DNA strands together [74]. Bacterial SMC complexes are thought to function primarily in chromosome condensation, although a growing body of evidence suggests that SMC-like proteins may also play a role in DNA repair [75]. Cell biological imaging of SMC fusions demonstrate that, for many bacterial species, SMC foci are found within or near the origin of replication [69,70,76,77]. Chromosome conformation capture experiments in *C. crescentus* have recently suggested that these SMC clusters may play a role in aligning chromosome arms [78].

SMCs are large polypeptides, often over 1000 residues in length, characterized by a distinctive V-shaped structure that forms on dimerization (Figure 2C). The N and C termini contain ATP-binding domains known as Walker A and B motifs, respectively. Within SMC proteins, long linker regions on either side of a globular hinge domain fold back to form an antiparallel coiled coil, bringing together the N and C termini that form the ATPase head domain. SMC monomers dimerize via their hinge domains. The distance from the hinge to the head domain is approximately 50 nanometers, implying that an SMC dimer is capable of encircling relatively distant pieces of DNA (Figure 2C), although whether or how this occurs remains an open area of investigation. ATP binding is thought to promote the association of the two head domains, effectively closing the SMC ring. Essential to their biological function *in vivo*, SMCs interact with non-SMC subunits. The bacterial SMC forms an asymmetric 2:1:2 complex with the kleisin subunit ScpA and non-SMC subunit ScpB, analogous to eukaryotic SMC complexes [79].

It remains unclear how SMCs interact with DNA and how this interaction is regulated by non-SMC subunits and by ATP binding and hydrolysis. For recent SMC reviews see [80,81]. Here we highlight how single-molecule studies are helping to uncover the molecular mechanisms of SMCs. Most of this work has focused on a highly divergent SMC homolog from *E. coli* called MukB. MukB interacts with the non-SMC subunits MukE and MukF. Single-molecule imaging in live *E. coli* cells has provided insights into MukBEF composition and dynamics within the bacterial chromosome [82]. Calibrated fluorescence imaging of YPet fusions to MukB, MukE, and MukF revealed broad distributions in the intensity of foci with an apparent stoichiometry of 4:4:2 for MukB:E:F. Foci with higher MukB copy numbers were increasingly elliptical, suggesting that these complexes were more condensed. To probe the dynamics of MukB, Badrinarayanan *et al.* used photoactivated localization microscopy (PALM) (Table 1) to activate individual photoactivatable red fluorescent proteins (PAmCherry) fused to MukBEF [82]. Approximately 20% of MukBEF molecules were found to be statically associated with complexes while the rest were diffusing throughout the cell. Fluorescence recovery after photobleaching (FRAP) experiments revealed that statically bound MukBEF rapidly exchanged with the diffusing fraction with a time constant of 50 s. MukB mutants that were impaired in ATP hydrolysis exchanged at a dramatically slower rate, while mutants that could not bind ATP or engage the head domains failed to form complexes.

Magnetic tweezers experiments demonstrated that MukB can compact DNA stretched by weak forces ($F < 0.5$ pN) [83]. This DNA condensation is performed by multiple MukB molecules in a highly cooperative fashion and is only weakly stimulated by ATP. Studies of the budding yeast cohesin complex heterodimer (SMC1–SMC3) showed a similar ability to condense DNA in the absence of ATP [84]. Somewhat surprisingly given its presumed functional equivalence with the bacterial SMC, Condensin I immunopurified from *Xenopus laevis* egg extracts showed a strict dependence on ATP for DNA condensation [85]. The absence of a strong dependence on ATP for MukB is consistent with a model that the bacterial SMC is not a motor protein fueled by ATP to perform DNA compaction; instead, ATP is likely to bias the formation of SMC conformations that facilitate DNA condensation. Both MukB and Condensin I displayed an average DNA compaction step of around 80 nm with a broad distribution of decompaction steps ranging up to hundreds of nanometers. These large decompaction steps suggest that SMCs are capable of trapping DNA loops, which is also consistent with the observation that SMCs can compact DNA under weak forces only.

The capacity of MukB to bridge DNA was directly probed in magnetic tweezers experiments in which two DNA duplexes were tethered to the same bead [86]. Petrushenko *et al.* found that rotating the bead and thus twisting the two DNA molecules together facilitated DNA bridging in a MukB-dependent manner. As with DNA condensation, ATP stimulated MukB bridging but was not strictly required. SMC family members have been implicated in stabilizing or forming numerous topological structures including supercoiled loops and chiral knots. MukB bridging was greatly favored when DNA was twisted to yield right-handed rather than left-handed crossings. Such topological selection may occur because MukB sharply bends DNA on binding in a manner analogous to type II topoisomerases [86,87].

Collectively, these studies suggest that MukB dimers interact cooperatively to form large complexes within the bacterial chromosome. ATP binding and head engagement are strictly required for cluster formation *in vivo* and stimulate DNA condensation *in vitro*. Stoichiometric measurements in cells show that the building block of MukB clusters is a dimer of MukB dimers. These MukB complexes are likely to act locally to condense neighboring DNA. The mechanism of MukB-mediated DNA compaction remains to be fully elucidated but one possibility is that MukB bridges the plectonemic DNA loops that they help to form by bending or wrapping DNA; such bridging may assist in condensing and organizing the origin region.

Concluding remarks

In organizing and compacting the bacterial chromosome, there are many ways to build a DNA bridge. Here we have examined three DNA-binding proteins – H-NS, ParB, and SMC – that form DNA bridges in qualitatively different manners that have implications for their biological function. Each bridge builder is targeted to the chromosome through different means. By recognizing DNA curvature or an AT-rich nucleation site [88], H-NS is directed to numerous promoter regions where it forms filaments that can bridge DNA duplexes, trapping or blocking the progression of RNA polymerases (Figure 2A). ParB is highly localized to specific loci through sequence-specific interactions with *parS* sites that are typically found near the origin of replication. Bound to *parS*, ParB then interacts with nucleoid-bound ParB through bridging and possibly nearest-neighbor interactions to span thousands of DNA bases (Figure 2B). Given the relatively low density of molecules within each complex, ParB is not a significant block to replication or transcription. How the bacterial SMC is localized on DNA remains an open question. In some species, ParB recruits SMC to DNA [69,70,77], potentially through a direct physical interaction or by binding to DNA structures formed by ParB. The ability of SMC to sense DNA topology may also facilitate its loading onto plectonemic loops. Within clusters, SMC locally condenses DNA, probably by bridging between DNA segments (Figure 2C). How these SMC-mediated bridges contribute to chromosome segregation needs to be further examined.

Single-molecule and chromosome conformation capture techniques have greatly increased our understanding of how NAPs organize DNA, yet many questions remain. The continued development of quantitative single-molecule imaging in cells will enable insights that go beyond protein localization, allowing the examination of how DNA bridges are disassembled and reassembled to allow transcription and DNA replication. Furthermore, these techniques should enable us to more directly probe how the chromosome is reorganized on cellular stress and changes in environmental conditions. More advanced single-molecule approaches *in vitro* will allow the simultaneous observation of protein binding and DNA conformation in more physiologically realistic biochemical systems. Of particular importance is a quantitative understanding of how different DNA-binding proteins work together. For example, DNA-bending proteins have been implicated in both facilitating and antagonizing DNA bridging [89,90]. The continued development of both *in vivo* and *in vitro* single-molecule approaches will undoubtedly provide greater molecular insight into how NAPs organize and condense the bacterial chromosome.

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