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The Major Architects of Chromatin: Architectural Proteins in Bacteria, Archaea and Eukaryotes

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The genomic DNA of all organisms across the three kingdoms of life needs to be compacted and functionally organized. Key players in these processes are DNA supercoiling, macromolecular crowding and architectural proteins that shape DNA by binding to it. The architectural proteins in bacteria, archaea and eukaryotes generally do not exhibit sequence or structural conservation especially across kingdoms. Instead, we propose that they are functionally conserved. Most of these proteins can be classified according to their architectural mode of action: bending, wrapping or bridging DNA. In order for DNA transactions to occur within a compact chromatin context, genome organization cannot be static. Indeed chromosomes are subject to a whole range of remodeling mechanisms. In this review, we discuss the role of (i) DNA supercoiling, (ii) macromolecular crowding and (iii) architectural proteins in genome organization, as well as (iv) mechanisms used to remodel chromosome structure and to modulate genomic activity. We conclude that the underlying mechanisms that shape and remodel genomes are remarkably similar among bacteria, archaea and eukaryotes.

Keywords nucleoid, nucleus, DNA compaction, histones, nucleosomes, histone-like proteins, architectural proteins

INTRODUCTION

The volume of unconstrained prokaryotic and eukaryotic genomes is considerably larger than the space into which they have to fit. For example, the human genome is ~2 m long, has an unconstrained volume of 3×10^7 μm^3 in solution and yet is confined to a cell nucleus that is about 200 μm^3. Similarly, the circular *Escherichia coli* genome is 2 mm in circumference, has a volume of 200 μm^3 and is present in a structure called the nucleoid, which is ~0.5 μm^3. Cells of the archaeal lineage have similar dimensions and genome sizes to those of bacteria, and therefore face the same problem (see Figure 1 for representative images of cells and their genomes from the three kingdoms). The effective volume occupied by a semi-flexible polymer, such as DNA, in the absence of any compacting factors, is determined by its persistence length. This is defined as the length along a polymer composed of many hypothetical segments at which the orientation of one segment still influences that of the next segment. Thus a stiff polymer occupies more space than a flexible one. The persistence length of DNA is 50 nm (Bloomfield *et al*., 2000). It is an intrinsic polymer property that is only to a small extent susceptible to changes in solution conditions. This

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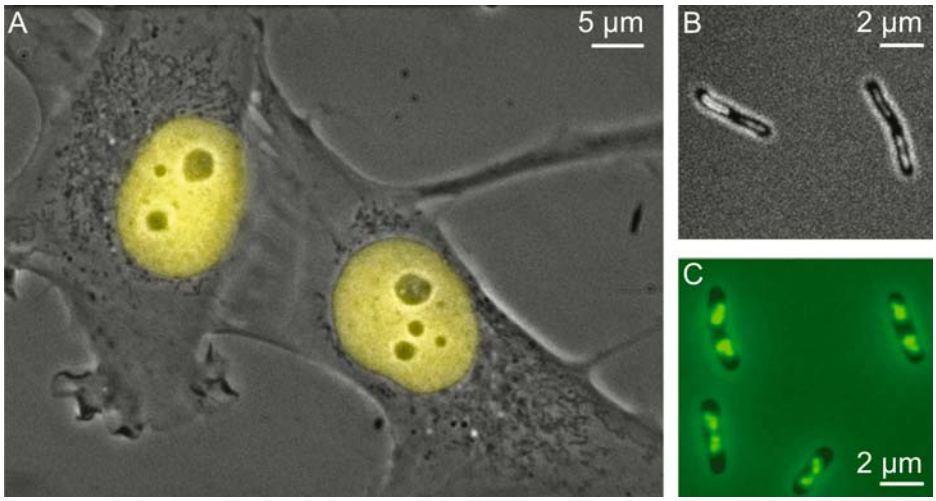

FIG. 1. Cellular localization of the genome in cells from different kingdoms of life. A) Microscopic image of a living human fibroblast (phase contrast) showing the nucleus by expression of a nuclear YFP-tagged DNA repair protein (DDB2). B) Microscopic image of a living archaeal cell (*Methanothermobacter thermautotrophicus*) in late exponential phase of growth, showing the nucleoid by staining with DAPI (courtesy of Dr. J.P. Chong) and C) Microscopic image of a living bacterial cell (*Escherichia coli*) in exponential phase of growth, showing the nucleoid by expression of GFP-tagged H-NS protein (courtesy of Dr. C.L. Woldringh).

implies that cells need dedicated "tools" to reduce the effective volume of their genome sufficiently to fit inside the cell. The key mechanisms employed for this purpose are DNA supercoiling, macromolecular crowding and the association of DNA-binding proteins that fold the genome into a more condensed structure. Due to the combined effects of the above-mentioned DNA compaction mechanisms, the concentration of DNA in prokaryotic nucleoids and in eukaryotic interphase nuclei is high (~30 mg ml^{-1}). Highly concentrated DNA tends to self-assemble and form aggregates, whereas the functionality of the genome depends on accessibility. Architectural proteins are likely to play an important role in counteracting aggregation, thereby making the genome a flexible and accessible structure (Minsky *et al*., 1997). In this review, we discuss the roles of DNA supercoiling and macromolecular crowding in genome compaction and give an overview of the different architectural DNA-binding proteins involved in genome organization that are present in the three kingdoms. We then give an overview of the spatial organization (i.e. higher-order folding) of chromatin in bacteria, archaea and eukaryotes inside the nucleus or nucleoid. The functional implications of crowding and supercoiling are subsequently discussed, in addition to mechanisms employed by the architectural proteins to modulate genome function and remodel chromosomes. Certain combinations of proteins with specific architectural features act in concert while others effectively antagonize each other. Examples of concerted and opposing action of benders, wrappers and bridgers are found throughout the three kingdoms, suggesting that the underlying mechanisms that shape and remodel genomes are remarkably similar throughout the kingdoms of life.

MECHANISMS TO COMPACT CHROMOSOMAL DNA

DNA Supercoiling

An effective way to reduce the volume of a long DNA molecule is to introduce supercoils. These arise when the number of times that the two single strands of a DNA duplex are wound around each other is either reduced or increased. Such activity is exhibited by an extensive repertoire of DNA topoisomerases conserved among all organisms. Supercoils can also arise due to the interaction with DNA binding proteins that locally affect the helical structure of DNA by either reducing or increasing the helical repeat.

The predominant structure of DNA found in nature is a right-handed double-stranded helix with a helical repeat of 10.5 bp, referred to as B-form DNA. As a result of the two single strands being wound around each other, these strands are linked if one assumes their ends as being joint or fixed. The topological state of such a DNA molecule can be described by the following equation:

$$Lk = Tw + Wr, \qquad [1]$$

where *Lk* is the linking number, corresponding to the number of times that the two strands in the DNA duplex are wound around each other. *Lk* always has an integer value and is invariant as long as the two strands in the duplex remain intact. By convention the sign of the value of *Lk* is positive for "standard" right-handed B-DNA. An important consequence of the invariance of *Lk* is that *Tw* and *Wr* can be interconverted. The twist, *Tw*, describes the number of times one DNA strand crosses the other in a duplex. The writhe, *Wr*, is the number of times that the duplex

crosses itself. Finally, a useful length-independent measure of superhelicity is the superhelical density (σ) of DNA, which is defined as the quotient of the linking number of the molecule in the supercoiled state and that in a relaxed state (Vologodskii, 1992; Bloomfield *et al*., 2000).

The effect of supercoiling on the volume occupied by a DNA molecule arises from a combination of factors. First, supercoiling accompanied by plectoneme formation leads to a 10% reduction in the radius of gyration (a measure of the volume) of the DNA (Fishman and Patterson, 1996). Second, long supercoiled molecules become divided in branches, and this further reduces the effective volume they occupy (Cunha *et al*., 2001; Odijk, 2002). Third, supercoiling facilitates the association of architectural proteins (see below).

The DNA of bacteria and eukaryotes is usually negatively supercoiled; locally it either exists in a toroidal (left-handed) or an interwound plectonemic (right-handed) form. The plectonemic state is energetically more favorable. In eukaryotes and some archaea the majority of negative supercoils is constrained within toroidal structures composed of DNA that is wrapped around the surface of histone proteins. It is the wrapping of DNA around a protein core that induces writhing of the DNA helix axis. In bacteria negatively supercoiled DNA is usually found in a plectonemic form (Figure 2) (Travers and Muskhelishvili, 2007). The overall topological state of DNA from thermophilic archaea is not entirely clear and has been proposed to be relaxed or positively supercoiled (Charbonnier *et al*., 1992; Charbonnier and Forterre, 1994; Herzel *et al*., 1999), whereas DNA from mesophilic archaea is reported to be negatively supercoiled (Charbonnier and Forterre, 1994; Musgrave *et al*., 2000).

The topological state of DNA is enzymatically controlled and maintained by the action of DNA topoisomerases. In bacteria, for instance, the superhelical density of DNA is prevented from changing beyond $\pm 15\%$ the average level (Wang, 1985; Drlica, 1992) by the joint action of topoisomerases with opposing activities (Zechiedrich *et al*., 2000). Two mechanistically different approaches to alter DNA topology are employed by these enzymes. Either they pass one strand of the duplex DNA through a break in the opposing strand (type I topoisomerases) or they pass both strands of duplex DNA simultaneously through a double-stranded gap generated in the DNA (type II topoisomerases) (Champoux, 2001; Espeli and Marians, 2004; Luttinger, 1995; Wigley, 1995). There are striking differences in the functionality of topoisomerases within one class. For instance, the bacterial topoisomerase I relaxes only negative supercoils, while eukaryotic type I topoisomerases can relax both negative and positive supercoils. Hyperthermophilic and thermophilic archaea as well as some (hyper)thermophilic bacteria use a type I topoisomerase enzyme, reverse gyrase, to actively induce positive supercoils into DNA (Bouthier de la Tour *et al*., 1990; Tse-Dinh, 1998; Forterre *et al*., 2000; Nadal, 2007). Generally, type II topoisomerase enzymes in eukaryotes and archaea are capable of relaxing supercoils with both positive and negative sign at the expense of ATP. These enzymes are not capable of increasing the degree of supercoiling (Champoux, 2001). A member of this class found in bacteria and mesophilic archaea, DNA gyrase, is however unique in its ability to actively introduce negative supercoils, again at the expense of ATP (Reece and Maxwell, 1991).

In all organisms genomic DNA exhibits a periodicity that is correlated with the bending of DNA and that reflects the way in which DNA is packaged (Herzel *et al*., 1999; Schieg and Herzel, 2004). These periodicities represent the number of base-pairs per helical turn relative to the surface that the DNA helix axis follows in space. In eukaryotes, this surface represents the wrapping of DNA around the histone octamer, whereas for plectonemic DNA this surface is a virtual cylinder in which the DNA coils (Bates and Maxwell, 2005). The apparent periodicity for plectonemic bacterial DNA is about 11.20 bp/turn (Lee and Schleif, 1989; Law *et al*., 1993; Becker *et al*., 2005; Travers, 2006), but this does not mean that the DNA is physically undertwisted to this extent. Intrinsically, however, plectonemic DNA is significantly undertwisted ($\sim$10.65 bp/turn), promoting strand separation. Nucleosomal DNA in eukaryotes has a periodicity of $\sim$10.20 bp/turn, which is due to the compression of DNA grooves facing the nucleosome surface (mostly A–T sequences) and widening of the DNA grooves facing away from the nucleosome surface (mostly C–G sequences). In contrast to bacterial DNA, nucleosomal DNA is intrinsically overtwisted ($\sim$10.35 bp/turn) (Richmond and Davey, 2003; Bates and Maxwell, 2005), possibly due to the slight overstretching of DNA in the nucleosome core particle (Prunell, 1998; Gore *et al*., 2006). Although negatively supercoiled, the slight overtwisting of DNA in the nucleosome does not allow strand separation during DNA-transacting processes. Each nucleosome contributes to the formation of a single negative supercoil (Simpson *et al*., 1985; Prunell, 1998), but these supercoils are completely constrained in nucleosomes. Dissociation of the histone octamer from DNA facilitates untwisting of the DNA (Bates and Maxwell, 2005; Travers and Muskhelishvili, 2007), since the negative supercoiling is no longer constrained (Figure 2). It has been proposed that nucleosomal DNA, upon release of the histone proteins, adopts a topology similar to that of bacterial DNA (Travers and Muskhelishvili, 2007). Adopting such a topology might play a role in transcription initiation in eukaryotes. In contrast, about half of the supercoils in bacteria is at all times present as free (i.e. not fixed) supercoils and the remaining half is constrained by DNA-binding proteins. Unlike the situation in eukaryotes this likely results from a dynamic dissociation/association of architectural proteins, where on average at all times a large number of proteins is bound to the DNA and thus capable of affecting DNA topology.

Macromolecular Crowding

Transcription of genomic DNA and translation of mRNA result in the production of considerable amounts of RNA and protein, respectively. The high concentration of these macromolecules (100–400 mg ml^{-1}) in the cell results in crowding conditions that cause strong depletion/attraction forces (Figure 3) (Hancock, 2004b; Marenduzzo *et al*., 2006). Depletion

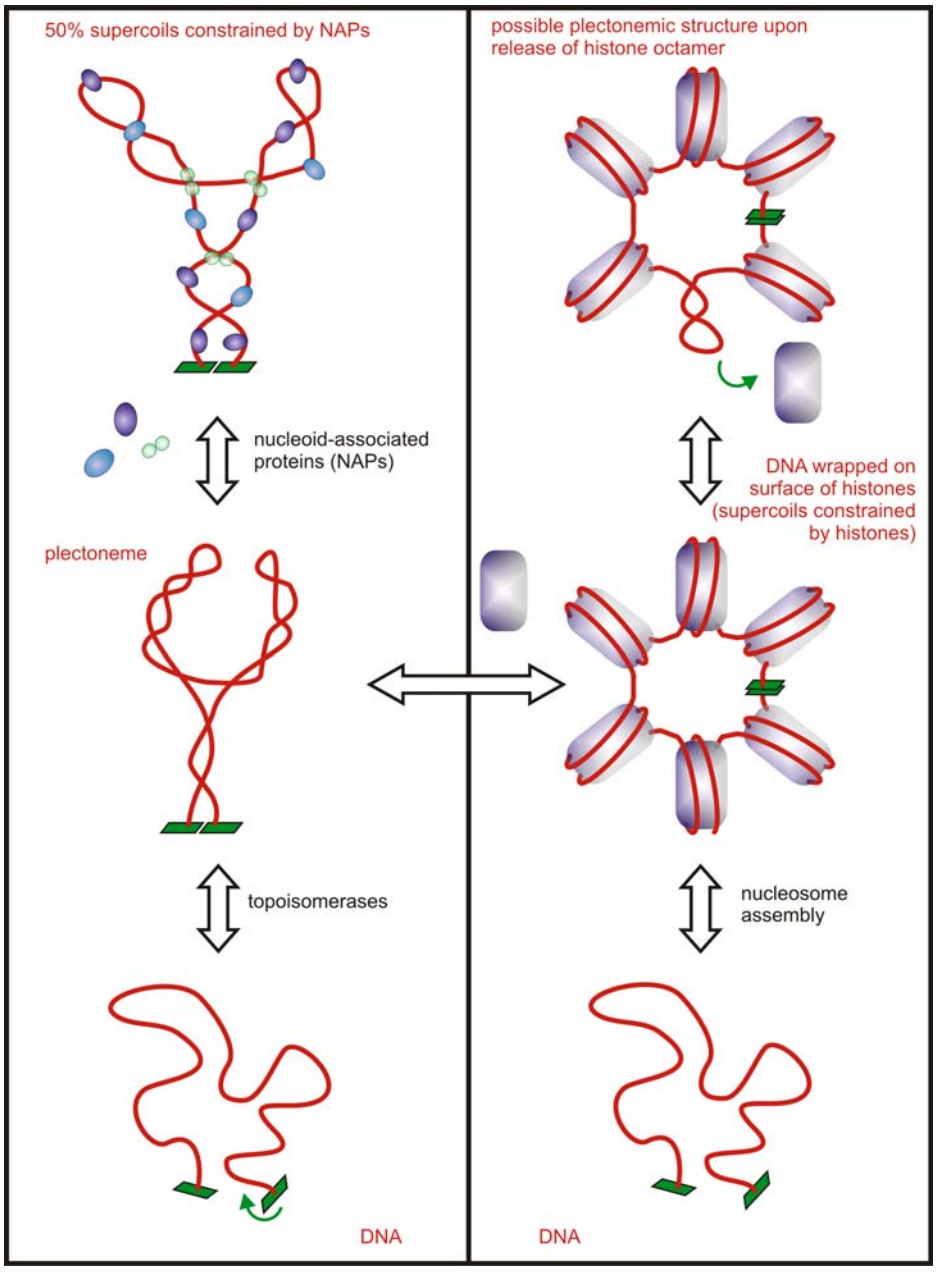

FIG. 2. DNA supercoiling in prokaryotes and eukaryotes. Bacteria and some archaea have enzymes that allow them to introduce supercoils into the DNA at the expense of ATP, which results in the formation of plectonemic structures (left panel). In bacteria, DNA gyrase introduces negative supercoils, while reverse gyrase leads to positive supercoils in some thermophilic organisms. The free superhelicity is partially constrained (about ~50%) by association of nucleoid-associated proteins (NAPs). The loss of the remaining free superhelicity by relaxation is prevented by the formation of topologically isolated domains (see section on loop structures of genomes) (Postow *et al*., 2004). In eukaryotes (and archaea encoding histone proteins), which lack DNA gyrase, supercoils are introduced by wrapping of DNA around the nucleosome surface (right panel). These supercoils are constrained on the surface of histone proteins. Disassembly of a nucleosome (by remodelling) and subsequent unwrapping of the DNA, can release supercoils resulting in a topology similar to that in bacteria (i.e. plectonemic rather than toroidal supercoils)(Travers, 2006). Since free supercoils are only slowly removed by topoisomerases in eukaryotes, this local free superhelicity could contribute to strand separation during transcription or result in recruitment of structure-sensitive regulatory proteins (see main text) (Travers, 2006).

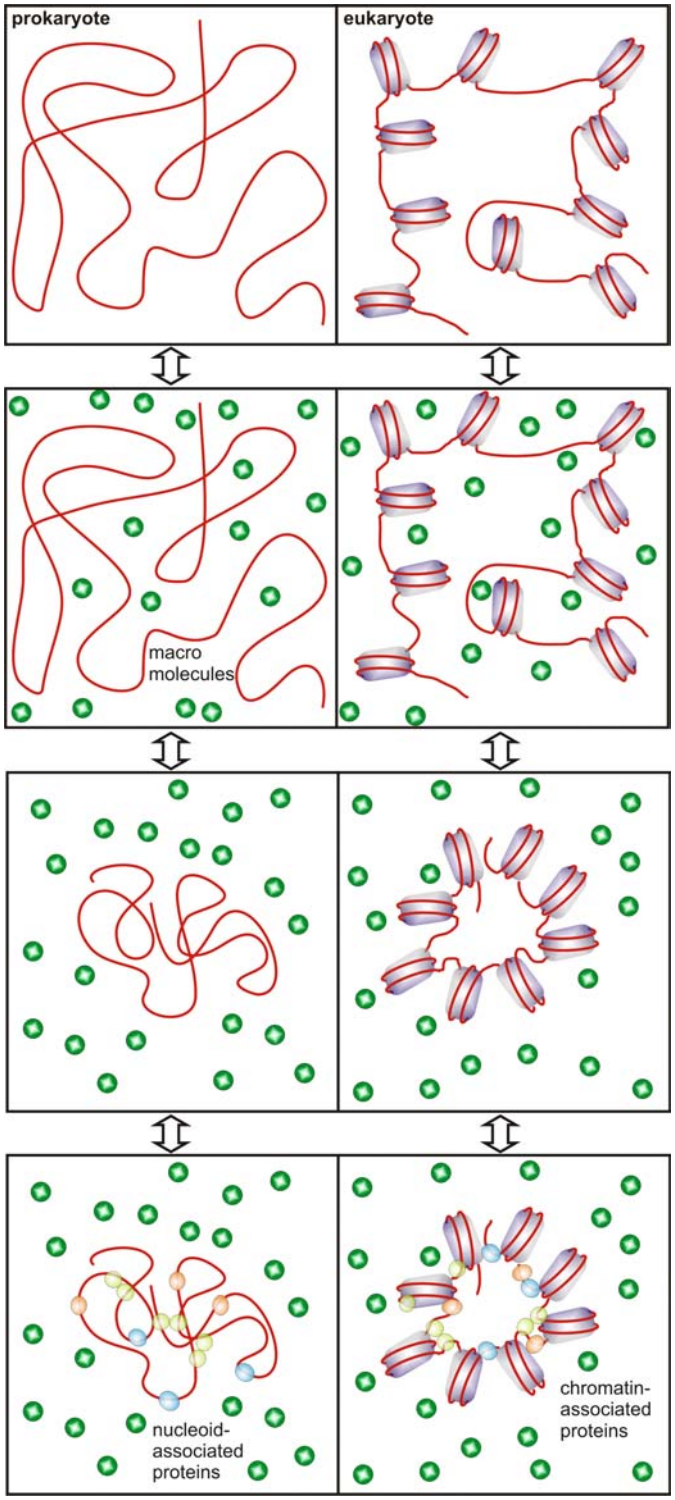

FIG. 3. Macromolecular crowding leads to strong compaction of genomes. The concentration of macromolecules (RNA and proteins) in the nucleus and nucleoid is about 100–400 mg/ml (indicated by the green spheres). Such a high macromolecular concentration results in entropy-driven compaction of genomic DNA (indicated in red, wrapped in nucleosomes depicted in purple) (Hancock 2007, Hancock, 2004) regardless whether DNA is wrapped in nucleosomes and shaped by other architectural proteins. For simplicity, DNA in prokaryotes is drawn as free of architectural proteins. It is important to note that it is not devoid of architectural proteins in general. In addition, such crowding conditions shift the equilibrium constant for many protein-DNA interactions, pushing the equilibrium towards binding of these proteins to DNA (Zimmerman 1993). Since crowding drives compaction of genomes non-specifically, it is expected to play an important role in genome compaction and to have only a minor role in regulation of genome functions by altering crowding conditions.

forces arise in a mixture of differently-sized particles when the interaction between larger particles causes an overlap in the excluded volumes surrounding these particles (Hancock, 2007b). This, in turn, increases the volume accessible to smaller particles causing strong (osmotic) attractive forces that can lead to significant conformational changes such as the spontaneous formation of helical structures (Hancock, 2007b; Snir and Kamien, 2005). Counter-intuitively, aggregation of larger particles in a crowded environment is favored since it increases the entropy of the system (Marenduzzo *et al*., 2006). Entropic depletion/attraction forces significantly influence the interaction between macromolecules in a crowded environment but not in a diluted system. The concentration of macromolecules in nucleoids and nuclei are well within the range where depletion/attraction forces occur and therefore are expected to considerably drive compaction of the genomic DNA (Hancock, 2004b, 2007) (Figure 3). It has been suggested that genome organization is mainly entropy-driven. Accordingly, when bacterial nucleoids are isolated under low-crowding conditions, strong expansion of these structures is observed (Cunha *et al*., 2001), while the nucleoids remain compact under high-crowding conditions (Murphy and Zimmerman, 1997; Cunha *et al*., 2001). Likewise, compartments in the eukaryotic nucleus such as nucleoli disassemble under low-crowding conditions (Hancock, 2004a, 2004b). Taken together, it is likely that the crowded environment within cells causes considerable self-association of chromatin contributing to genome organization. In addition, depletion/attraction forces may favor the association of architectural proteins with genomic DNA (Zimmerman, 1993) that may have a significant effect on genome folding (Figure 3).

INVENTORY OF ARCHITECTURAL PROTEINS ACROSS THE THREE KINGDOMS OF LIFE: THE BOTTOM-UP VIEW ON CHROMATIN ORGANIZATION

Architectural chromosomal proteins are generally small, basic proteins that interact with DNA. These proteins can have different structural effects on DNA, such as bending, bridging or wrapping it (Figure 4A, Table 1).

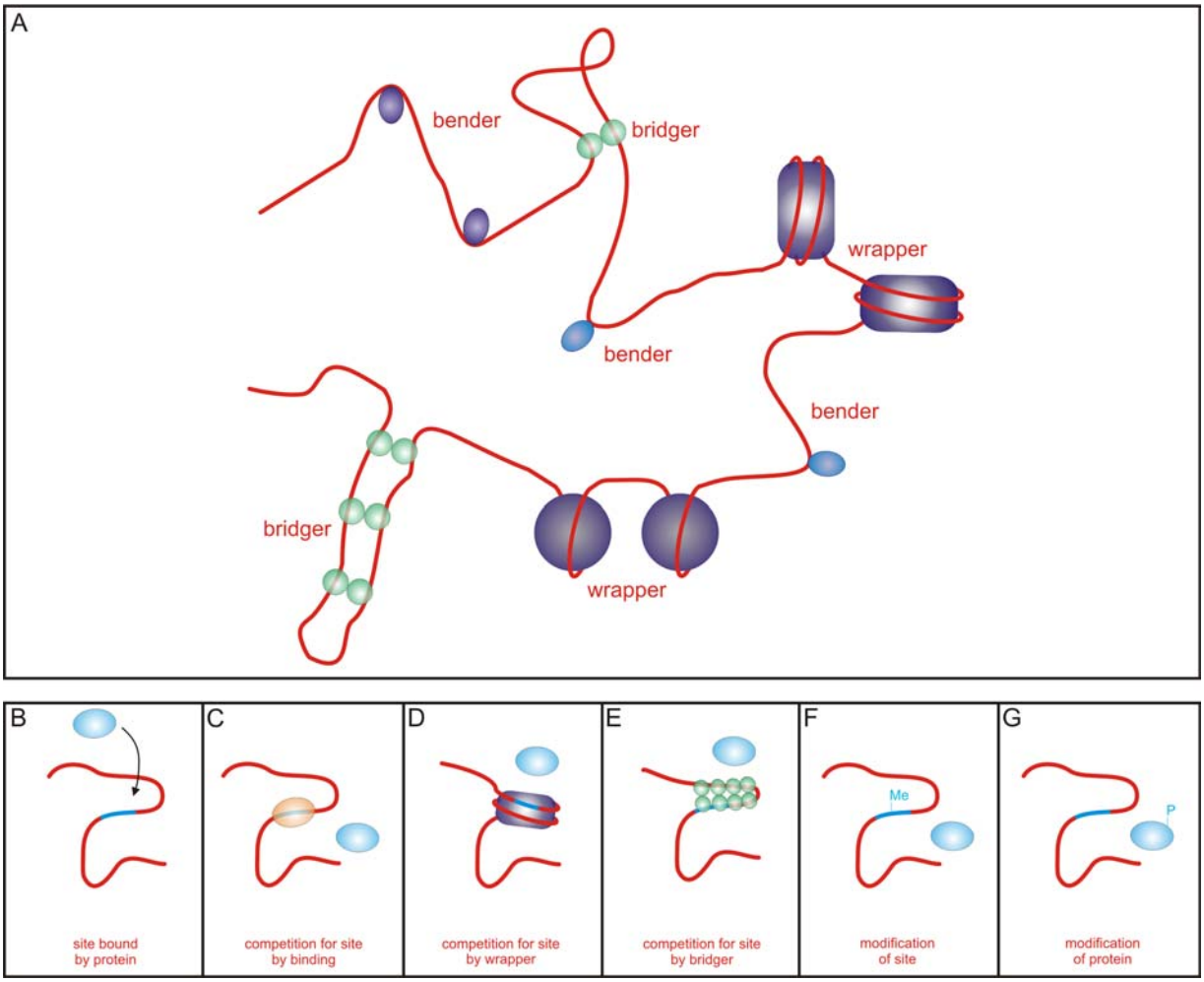

FIG. 4. Bending, wrapping and bridging of DNA by architectural proteins and mechanisms to modulate the occupancy of binding sites on the DNA. A) Chromatin in cells across all kingdoms is shaped by proteins that can be classified according to their architectural mode of action: 1) bending of DNA (depicted by the purple and blue ovals), 2) wrapping of DNA (depicted by the purple circle and rectangle that wrap DNA once or twice around their surface, respectively) and 3) bridging of two DNA duplexes (depicted by the green circles). Representatives of these three classes of architectural proteins are discussed in the main text. B) Schematic representation of mechanisms to modulate binding site occupancy by C) direct competition for a binding site with another protein, D) modulating the binding of a protein by wrapping its target site around a protein surface (such as archaeal or eukaryotic nucleosomes, or Lrp-like proteins in bacteria, or E) modulating the binding of a protein by forming a bridged filament that includes the target site, F) modification of the binding site which alters the affinity of the protein binding to this site. This could be DNA methylation or histone methylation acting to lower or increase the affinity of a protein for such a modified site, G) modification of the DNA-binding protein (for instance by phosphorylation).

DNA Wrappers

These proteins are able to wrap DNA by folding it around their surface, resulting in considerable volume reduction of the DNA. In addition, wrappers induce writhe contributing to DNA supercoiling. The best-known example of DNA wrapping is found in eukaryotes. Essentially all eukaryotes express histone proteins that form nucleosomes with the genomic DNA. Histone proteins have a characteristic histone-fold (3 hydrophobic α-helices) and interact with DNA by inserting arginine residues into the minor groove every helical turn (Luger *et al*., 1997). The protein core of the nucleosome is the histone octamer, which is formed by histones H2A, H2B, H3 and H4. Two H3–H4 dimers form a tetramer through interactions between the histone-folds (between H3–H3′) and two H2A–H2B dimers associate with this tetramer to form the octamer (with interactions between H2B and H4). Nucleosomes occur essentially every 200 bp of DNA, wrapping ~146 bp in a left-handed 1.6 turns around the histone octamer. The DNA in nucleosomes is bent smoothly around

TABLE 1
Overview of different classes of architectural proteins (wrappers, benders or bridgers) and their distribution in bacteria, archaea and eukaryotes. Note that homologues of the eukaryotic histones are found in the other kingdoms. H1 is absent in archaea, but H1 homologues have been identified in Gram negative bacteria, whereas core histone proteins are absent from bacteria, but have been identified among euryararchaea (see main text).

		wrappers	*benders*	*bridgers*
Eukaryotes		H2A, H2B, H3, H4 (core histones)	HMG	H1 (linker histone)
				BAF
				SMC
Archaea	Crenarchaea	*Lrp**	Cren7	Alba
			Sul7	Lrp*
				SMC
	Euryarchaea	HMfA and HMfB (histones)	MC1	Alba
		*Lrp**	HU	Lrp*
				SMC
Bacteria	Gram positive	*Lrp**	HU	Lrp*
				Lsr2 (H-NS-like)
				SMC
	Gram negative	*HU**	HU*	H-NS
		*Lrp**	IHF	Lrp∗
			Fis	*Fis**
				SMC
				H1-like proteins

*these proteins have been proposed to exhibit dual architectural properties, that are likely dependent on protein concentration or DNA binding sequence. The secondary binding mode is indicated in italic.

the surface of the histone proteins although locally the minor groove is kinked where the DNA is in contact with the H2A–H2B dimers (Richmond and Davey, 2003). Nucleosomes are generally considered repressive to DNA-transacting processes, such as transcription. Recently, nucleosomes were shown to undergo spontaneous conformational changes during which the DNA transiently unwraps from the histone surface. Nucleosomal DNA is fully wrapped for ∼250 ms, and then transiently unwraps (10–50 ms), allowing proteins to access the DNA wrapped in nucleosomes (Li *et al*., 2005). However, the main mechanism to access DNA wrapped in nucleosomes is likely through chromatin remodeling (see section on control of genome function mechanisms). The N- and C-terminal tails of histones are subject to extensive post-translational modifications (such as acetylation and methylation). Many chromatin-binding proteins contain motifs that specifically recognize these modifications and modulate chromatin structure and function (Taverna *et al*., 2007).

Archaea from the *Euryarchaeal* lineage contain homologues of histone proteins, suggesting an ancient origin for this protein family (White and Bell, 2002; Sandman and Reeve, 2006) (see Woese *et al*. (1990) for a classification of the kingdoms of life). The *Crenarchaea* do not generally encode histone homologues, although there are exceptions such as *Thermofilum pendens* where histone genes may have been acquired by lateral gene transfer (Sandman and Reeve, 2006). Histone genes are also susceptible to loss during evolution. The euryarchaeal Thermoplasmatales appear to have lost their histone genes (White and Bell, 2002) as have the eukaryotic unicellular dinoflagellates (Moreno Diaz de la Espina *et al*., 2005). The best studied example of an archaeal histone is the HMf protein from the hyperthermophile *Methanothermus fervidus* (Sandman *et al*., 1990). Two HMf polypeptides exist (HMfA and HMfB) that can form homodimers and heterodimers with different DNA binding properties (Sandman *et al*., 1994). The sequence and structure of HMf proteins is related to that of eukaryotic core histones and HMf proteins compact DNA into nucleosome-like structures (Starich *et al*., 1996; Sandman *et al*., 1998). Archaeal nucleosomes consist of tetramers, comparable to the H3–H4 dimer in eukaryotes that wrap ∼60 bp of DNA (Grayling *et al*., 1997). The archaeal histones are considerably smaller than their eukaryotic counterparts and, with some exceptions, lack the C- and N-terminal tails that are the sites of extensive post-translational modification (acetylation, methylation, ubiquitylation, etc.) in eukaryotes (Jenuwein and Allis, 2001; Sandman and Reeve, 2006) (see section on control of genome function mechanisms). The absence of

such tails suggests that archaeal histones are not subject to similar regulatory processes as found in eukaryotes. In line with this finding there is no evidence for post-translational modification of archaeal histone proteins (Sandman and Reeve, 2006).

Although less frequently, architectural proteins that wrap DNA are also present in bacteria. Only one protein containing a structurally conserved histone-fold has so far been reported (in the hyperthermophile *Aquifex aeolicus)*, but it is not clear if this protein binds DNA (Qiu *et al*., 2006). Conserved examples are members of the Lrp/AsnC family (leucine-responsive regulatory protein/asparagine synthase C gene product) that form disc-shaped octameric structures with multiple binding sites that wrap DNA around themselves in a right-handed superhelix (de Los Rios and Perona, 2006; Thaw *et al*., 2006). This mode of wrapping constrains positive supercoils and promotes DNA compaction. Although positive supercoiling upon binding of *B. subtilis* LrpC has indeed been observed, *E. coli* Lrp appears to constrain negative supercoiling (Thaw *et al*., 2006; Pul *et al*., 2007). If two Lrp octamers associate, a hexadecamer is formed that can wrap DNA around itself by almost two full turns, resulting in a nucleosome-like structure (Thaw *et al*., 2006). Recently, Lrp was shown to form a repressive nucleoprotein complex (somehow aided by H-NS) at ribosomal RNA promoters in *E. coli*, which involved extensive DNA wrapping of the promoter region around the Lrp octamer (Pul *et al*., 2007). Based on these results it has been suggested that the mode of binding as well as the mechanism of repression by nucleosomes and by Lrp is similar (Pul *et al*., 2007). Genes that code for Lrp-like proteins are also present in many archaeal genomes, and are thought to mediate gene expression (Peeters *et al*., 2006).

DNA Benders

The dominant mode of DNA packaging in eukaryotes and some archaea appears to be wrapping of DNA. In bacteria and *Crenarchaea*, however, this is not likely to be the main mode of organizing DNA since these organisms lack histone proteins. Even in histone-containing *Euryarchaea*, the chromosomal DNA is not packaged in the higher-order structures seen in eukaryotes, and there are probably not sufficient histone proteins to package the complete archaeal genome (Sandman and Reeve, 2005). Instead, proteins that bend DNA are widespread throughout the prokaryotes and these proteins play an important role in chromatin organization. The best studied examples of DNA benders are members of the HU/IHF family (Swinger and Rice, 2004). HU-like proteins (histone-like protein from E. coli strain U93) are dimeric and consist of a compact core of α-helices from which two flexible β-ribbon arms emanate. The arms are inserted into the minor groove and the DNA is bent around the protein by up to 160°. In the case of IHF (integration host factor) this occurs with sequence specificity (Goodrich *et al*., 1990). HU has to date not been shown to exhibit sequence specificity, but has been shown to bind preferentially to intrinsically flexible DNA (Goshima *et al*., 1994; Tanaka *et al*., 1993). At high concentrations HU dimers can be packed tightly side-by-side in a cooperative manner resulting in effective stiffening of the DNA (Skoko *et al*., 2004; van Noort *et al*., 2004). Based on crystal structures of *E. coli* HU—where a helical filamentous arrangement of HU provides a potential surface for wrapping of DNA—an old model for HU action according to which DNA is wrapped around a multimeric HU core was recently revived (Rouviere-Yaniv *et al*., 1979; Guo and Adhya, 2007). The nature of this secondary binding mode, as well as its physiological relevance, is currently the subject of debate since *in vivo* data in support of such structures is lacking. Moreover, the cellular concentration of HU is by far insufficient to wrap the entire (or a large part of the) genomic DNA in such structures. The major physiological architectural role is thus most likely the bending of DNA. However, it is possible that local stiffening—whether due to wrapping or not—of the genomic DNA occurs *in vivo* and has a regulatory function (Luijsterburg *et al*., 2006). HU-like proteins are conserved and can be found in all bacteria as well as in mitochondria and chloroplasts. Interestingly, HU-like proteins are also found in a branch of *Euryarchaea* that lack histones (i.e. the *Thermoplasmatales*) (DeLange *et al*., 1981), but appear to be absent in most other *Euryarchaea*. Similarly, the eukaryotic dinoflagellates also do not have histone proteins, but encode HCC (histone-like proteins of *Crypthecodinium cohnii*) proteins that share homology with bacterial HU (as well as with eukaryotic H1 proteins) (Wong *et al*., 2003). These isolated examples are probably the result of lateral gene transfer events from bacteria (White and Bell, 2002). Another DNA bender from proteobacteria termed Fis (factor for inversion stimulation), consists of a hydrophobic core from which two DNA-binding helix-turn-helix motifs emanate (Pan *et al*., 1996). These recognition helices are spaced 2.5 nm apart and insertion into two adjacent minor grooves (3.4 nm apart) induces bending of the DNA by up to 50°. Additional curvature of the DNA is generated by interactions between the DNA flanking the Fis binding site and the sides of the Fis dimer, resulting in bending up to 90° (Pan *et al*., 1996). Binding of Fis drives considerable compaction of DNA, presumably by non-specific binding (i.e. not to specific Fis sites along the genome) and concomitant bending (Skoko *et al*., 2005) similar to the mechanism suggested for HU (van Noort *et al*., 2004).

Proteins that bend DNA can also be found in the other kingdoms. An example of a conserved archaeal DNA bender is the small Sul7 protein (also known as Sso7d or Sac7d) from the genus *Sulfolobus* of the *Crenarchaea* (White and Bell, 2002) that has been suggested to play a role in chromatin organization. Sul7 binds non-specifically to DNA and bends it by up to 60° by inserting hydrophobic residues into the minor groove. The DNA-interacting surface is a triple-stranded β-sheet and the DNA is bent away from the protein (Robinson *et al*., 1998), which is opposite to the way DNA is bent by bacterial HU. A protein with similar mode of binding and architectural properties (Cren7) was recently found to be highly conserved amongst hyperthermophilic *Crenarchaea* (Guo *et al*., 2007). Interestingly, a long flexible loop close to the DNA-interacting surface of Cren7 can

be methylated. The N-terminal tail of this protein (opposite to the DNA-binding domain) contains conserved lysine and serine residues (Guo *et al*., 2007) that may be acetylated and methylated/phosphorylated respectively, similar to the N-terminal tails of eukaryotic histones (Jenuwein and Allis, 2001) (see section on control of genome function mechanisms). Finally, another abundant DNA bender in archaea is MC1 (methanogen chromatin protein 1), which is found in the *Euryarchaeal* halophiles and *Methanosarcinales*. MC1 is structurally similar to Sul7 and Cren7 and introduces bends in DNA up to 120° (Le Cam *et al*., 1999; Paquet *et al*., 2004; Guo *et al*., 2007). Although the methanosarcinales do have histone proteins, MC1-like proteins appear to be the predominant nucleoid-associated proteins in these organisms (Reeve, 2003). *In vitro* experiments have shown that MC1 from *Methanosarcina mazei*—like Cren7—is methylated on a single lysine residue. (Manzur and Zhou, 2005). It appears that the two major modes of DNA packaging are wrapping by histone/HMf proteins (in eukaryotes and archaea) or bending by HU/Sul7/Cren7/MC1 proteins (in bacteria and archaea).

While packaging of the genome in eukaryotes is primarily due to the wrapping of DNA around nucleosomes, members of the high mobility group (HMG) family of proteins play a supplementary, possibly modulatory, role in chromatin organization (Bustin and Reeves, 1996; Thomas and Travers, 2001). Three HMG families have been described containing either an AT-hook domain (HMG-A), an HMG-box (HMG-B) or a nucleosome binding domain (HMG-N) (Bustin, 2001). Of these proteins only HMG-B is believed to primarily act as an architectural protein (Thomas and Travers, 2001; Agresti and Bianchi, 2003). Members of the HMGB subfamily typically contain two so-called HMG box domains separated by a small linker. HMG box domains are found in a wide variety of proteins that are capable of bending DNA (Thomas and Travers, 2001). The DNA-binding domain contains three α-helices that fold into a typical wedge shape, the concave surface of which can delve deep into and widen the minor groove of DNA. One of the four residues that usually constitute this wedge partially intercalates between two base pairs and introduces a kink in the DNA (King and Weiss, 1993). At a distance of 2 bp from the first kink, a second kink is induced due to partial intercalation of two additional residues (Murphy *et al*., 1999). This induces a bend into DNA of up to 80° for a single HMG box and up to 130° for tandem boxes. Many HMG box proteins contain a basic extension (either on the C- or N-terminal side) that binds in the compressed major groove on the face of the helix opposite the widened minor groove and stabilizes the intercalation-induced bend (Love *et al*., 1995; Lnenicek-Allen *et al*., 1996; Allain *et al*., 1999). While HMG box proteins can promote DNA compaction by bending (Skoko *et al*., 2004), a picture is emerging in which these proteins function by favoring the partially unwrapped state of nucleosomes by binding at the entry/exit point, which possibly facilitates nucleosome remodeling (Travers, 2003).

DNA Bridgers

Proteins from this group form dynamic cross-links between DNA duplexes or, at a larger scale, complexes between DNA and other architectural proteins, leading to the formation of loops. In this manner, many of the members of this class of proteins are capable of imposing an additional level of higher-order organization to the chromosome. The importance of DNA bridging as a chromatin organizing principle was first recognized in bacteria (Dame *et al*., 2000; .2005, Luijsterburg *et al*., 2006), although a similar mode of binding had been described for conventional bacterial transcription regulators such as Lac repressor (Muller-Hill, 1998). The best characterized representative of an archetypical DNA bridger is H-NS (histone-like nucleoid structuring protein) found in *Escherichia coli* and the H-NS-like proteins found in closely related Gram-negative proteobacteria (Bertin *et al*., 1999). The H-NS protein has a dimeric structure and consists of two α-helical dimerization domains and two independent DNA binding domains that are connected by two unstructured flexible linkers (Dorman *et al*., 1999; Luijsterburg *et al*., 2006). Due to the presence of two DNA binding domains the protein can interact with two DNA duplexes simultaneously (Dame *et al*., 2000, 2006).The protein binds with relatively high affinity to DNA of any sequence (Dorman, 2007), although a well-defined preferential binding site has also been recently reported (Bouffartigues *et al*., 2007; Lang *et al*., 2007). Besides proteins with sequence similarity to H-NS from *E. coli*, functional homologues of H-NS, such as MvaT from the *Pseudomonades*, have been identified based on the ability to complement the phenotype of *hns* knock-out strains (Tendeng and Bertin, 2003) and to bridge DNA duplexes similar to H-NS (Dame *et al*., 2005). A DNA-bridging protein (termed Lsr2) was recently identified in *Mycobacterium tuberculosis* and related Gram-positive actinobacteria. These are phylogenetically distant from Gram-negative bacteria and lack H-NS sequence homologues (Chen *et al*., 2008). Lsr2 complements the phenotype of *E. coli* hns-strains (Gordon *et al*., 2008) and was shown to bridge DNA duplexes in a manner similar to H-NS (Dame *et al*., 2000, 2005; Chen *et al*., 2008). This observation suggests that proteins that bridge DNA are functionally conserved amongst bacteria. An unexpected feature of Fis, which was originally identified as a DNA bender (see above), is its ability to bridge DNA, resulting in the formation of loops (Schneider *et al*., 2001; Skoko *et al*., 2005). This bridging probably involves protein–protein interactions between DNA-bound Fis dimers (Skoko *et al*., 2006).

Members of the Lrp/AsnC family (discussed in the section "wrappers") appear to have a second mode of interaction with DNA that involves duplex bridging (Beloin *et al*., 2003; Tapias *et al*., 2000). It is not fully understood how bridging occurs, but it seems likely that it involves Lrp in a lower oligomeric state (at least a dimer) than the octamer structure resolved by X-ray crystallography (Leonard *et al*., 2001; Thaw *et al*., 2006), or that it involves only a subset of the available DNA binding domains. Both wrapping and bridging by Lrp is likely to contribute to DNA

organization in bacteria and archaea (Peterson *et al*., 2007), but bridging of DNA by Lrp is expected to be a more general phenomenon, since it does not require (a series of correctly spaced) high-affinity binding sites.

DNA bridging proteins are not restricted to bacteria and are found in archaea and eukaryotes as well. Most archaeal genomes encode one or more version of the Alba (Sac10b family) protein (White and Bell, 2002). Alba (acetylation lowers binding affinity) was first observed as an abundant nucleoid-associated protein in the crenarchaeote *Sulfolobus acidocaldarius* (Grote *et al*., 1986). Electron microscopy studies showed that the Alba protein forms regular fibrous structures with plasmid DNA, possibly wrapping two DNA double helices around one another (Lurz *et al*., 1986). These data suggest that the Alba protein can perform a DNA bridging function to help organize archaeal DNA. Alba binds to dsDNA and also interacts with ssDNA and RNA *in vitro* (Guo *et al*., 2003). Alba adopts a dimeric structure in solution, with two β-hairpin arms extending from a highly basic central body. It has been proposed that these two hairpins are inserted into adjacent minor grooves on the same DNA segment (Wardleworth *et al*., 2002). This suggests that the mechanism of bridging is distinct from that of proteins like H-NS, where the two subunits within the dimer individually interact with different DNA segments. The precise molecular details of the interaction of Alba with DNA and its bridging mechanism remain uncertain. Bridging may involve dimmer–dimer interactions (as suggested for Fis) or an as yet unidentified DNA-binding surface on the body of the dimer. Intriguingly, and in contrast to the archaeal histone, reversible modification of the Alba protein by acetylation of a specific lysine side-chain has been observed in *S. solfataricus* (Bell *et al*., 2002). A consequence of acetylation is a modest reduction in affinity of the protein for dsDNA, although the functional significance and generality of this effect remain unclear at present (Jelinska *et al*., 2005). In *S. solfataricus*, a paralog of the Alba protein (henceforth "Alba2"), is expressed at low levels and forms obligate heterodimers with Alba, resulting in a heterodimeric species. Alba2 binds DNA with much lower affinity than Alba. Alba expression is growth phase dependent (Dinger *et al*., 2000) and modulation of the ratios of the two Alba proteins could thus be an efficient means to remodel archaeal chromatin in response to changes in growth conditions (Jelinska *et al*., 2005), with downstream effects on global gene expression. Many other archaeal genomes encode two paralogs of the *alba* gene, although these have not been studied biochemically.

While in eukaryotes the major architectural proteins are histones that wrap DNA to form nucleosomes, folding of nucleosomal arrays into higher-order structures is modulated by histone H1, also referred to as linker histone. Histone H1 lacks a characteristic histone-fold, but contains a globular domain consisting of a winged-helix motif that is flanked by a long C-terminal glycine-rich tail and a shorter N-terminal—often basic—stretch (Allan *et al*., 1980). The globular domain and C-terminal tail of H1 are required for high affinity binding to chromatin and H1 was shown to interact with chromatin transiently in the order of minutes (Lever *et al*., 2000; Misteli *et al*., 2000). The globular H1 domain contains two DNA binding surfaces on opposite sides of the protein, similar to the suggested structure of bacterial H-NS (Goytisolo *et al*., 1996; Luijsterburg *et al*., 2006). Accordingly, the presence of two DNA-binding domains provides H1 with the ability to bridge DNA duplexes (Clark and Thomas, 1988). Analysis of H1 mutants in combination with molecular modeling using available atomic structures suggested that one of the DNA-binding surfaces interacts with the major groove about one helical turn away from the nucleosome dyad (Ramakrishnan *et al*., 1993; Luger *et al*., 1997; Brown *et al*., 2006). Additional residues surrounding this surface interact with the DNA backbone, which is facilitated by the curvature of the DNA due to wrapping around the core histone proteins. The second DNA-binding surface on the opposite site of the globular domain interacts with the minor groove of the entering or exiting linker DNA (Brown *et al*., 2006). Accordingly, mutations in either one of the DNA-binding domain abolished the ability to bridge DNA and to bind to nucleosomes, suggesting that bridging DNA duplexes is required for stable binding of H1 to nucleosomes (Goytisolo *et al*., 1996; Duggan and Thomas, 2000; Brown *et al*., 2006). Cooperative binding of linker histones drives the folding of loosely organized, irregularly shaped arrays of nucleosomes in a highly regular compact chromatin fiber (Kepper *et al*., 2008; Routh *et al*., 2008), suggesting a molecular basis for linker histone-mediated switching between different functional chromatin states. In this respect, it should be noted that the length of the linker region on the DNA determines the degree of compaction induced by the association of H1 to nucleosomal arrays. If the length of this region is below a threshold value the regular compact chromatin fiber is not formed (Routh *et al*., 2008).

The eukaryotic dinoflagellate*Crypthecodinium cohnii* that lacks histone proteins does encode the HCC1, HCC2 and HCC3 proteins that share homology with both eukaryotic H1 proteins and bacterial HU proteins (Wong *et al*., 2003). In a later work by the same group, the homology to the eukaryotic H1 proteins is no longer acknowledged and instead the HCC proteins are presented as DNA bending HU homologues only (Chan *et al*., 2006). However, HCC3 does not complement the phenotype of HU-deficient cells and is thus not functionally equivalent to HU (Chan and Wong, 2007). Instead, scanning force microscopy of HCC3–DNA complexes showed that it acts as a typical DNA bridging protein (Chan and Wong, 2007), which is not surprising considering its homology with H1 proteins.

It is interesting to note that a lot of bacteria contain homologues of the eukaryotic H1. Examples are the Hc1 and Hc2 proteins in *Chlamydia* (Hackstadt *et al*., 1991; Perara *et al*., 1992), in which H-NS-like proteins appear to be absent. When expressed in *E. coli* these proteins induce strong compaction of the nucleoid (Barry *et al*., 1992). Hc1 can be methylated by SET (Su(var), E(z) and Trithorax) domain proteins (Murata *et al*., 2007), which likely modulates its chromatin organizing ability.

In other bacteria the H1 homologue is encoded in addition to H-NS-like proteins. *Escherichia coli* encodes the H1-like TolA protein (Kasinsky *et al*., 2001). *Bordetella pertussis* encodes two H1-like proteins, Bph1 (Zu *et al*., 1996) and Bph2 (Goyard, 1996), and the H-NS-like Bph3 protein (Bertin *et al*., 1999), while *Pseudomonas aeruginosa* encodes the H1-like AlgP protein (alginate regulatory protein) (Deretic and Konyecsni, 1990) in addition to five different H-NS-like proteins (MvaT and paralogs) (Tendeng *et al*., 2003). It should be noted that in most instances the DNA binding properties of bacterial H1-like proteins have not been explicitly investigated. Although the origin of H1-like proteins is not entirely clear, it has been suggested that this class of proteins originated in bacteria, in contrast to the core histones that are believed to originate from archaea (Kasinsky *et al*., 2001; Sandman and Reeve, 2006).

Barrier-to-autointegration factor (BAF) is another conserved small (10 kDa) DNA bridging protein associated with eukaryotic chromatin (Margalit *et al*., 2007). This protein, like most DNA bridging proteins, is dimeric and its association with two duplex DNA segments is mediated through two independent DNA binding domains containing helix-hairpin-helix motifs (Bradley *et al*., 2005; Zheng *et al*., 2000). DNA binding of these motifs involves contact with phosphate groups and not with DNA bases thus ensuring sequence independence (Bradley *et al*., 2005). Little is known about its *in vivo* functionality except that loss of BAF is not compatible with life. However, BAF does seem to have definite contributions to chromatin organization since over-expression leads to highly condensed chromatin in cells (Segura-Totten *et al*., 2002). Compaction is likely due to its direct DNA binding activity, as well as its ability to interact with core histone H3 and linker histone H1 (Montes de Oca *et al*., 2005). Additionally, BAF interacts with proteins in the nuclear lamina and consistent with these interactions is localized in the periphery of nuclei in mammalian cells. This suggests a scenario in which BAF tethers chromatin to the nuclear lamina, which has been suggested as a mechanism to down-regulate the expression of genes (Finlan *et al*., 2008). Finally, the functionality of BAF is modulated by phosphorylation of its N-terminus, which reduces its DNA binding activity and possibly the interaction with other binding partners (Nichols *et al*., 2006).

All of the aforementioned proteins are relatively small in size; generally not larger than ~25 kDa. Structural maintenance of chromosomes (SMC) proteins are at least one order of magnitude larger in size (Huang *et al*., 2005; Hirano, 2006), allowing them to act over large distances. SMC proteins are conserved among members of all three kingdoms, suggesting that they are the most conserved of all architectural proteins and that they were responsible for chromosome organization long before other, sometimes more specialized, proteins emerged (Nasmyth and Haering, 2005; Hirano, 2006). SMC proteins share a common primary structure consisting of five distinct domains i.e. an N-terminal globular domain followed by a very long coiled-coil domain (50–100 nm), a hinge domain of variable length, another long coiled-coil domain and a C-terminal globular domain. The polypeptide folds back onto itself around the hinge, giving rise to a long intra-molecular anti-parallel coiled-coil. SMC monomers associate via hinge–hinge interactions resulting in a V-shaped SMC dimer that is believed to be the functional SMC unit (Hirano, 2006; Strunnikov, 2006). These V-shaped dimers have angular orientations that vary between 0 and 180 degrees, allowing the heads to come together (Melby *et al*., 1998). There are currently several models to explain the DNA bridging activity of SMC complexes, which all incorporate the notion that they can enclose multiple DNA duplexes. According to one of the models, the V-shaped SMC protein embraces two (or more) DNA duplexes simultaneously and ring closure occurs due to intra-molecular interactions between the heads (Volkov *et al*., 2003). An alternative model suggests the formation of clusters of multiple SMCs due to intermolecular head–head interactions embracing multiple DNA molecules (Hirano and Hirano, 2004; Mascarenhas *et al*., 2005). Distinct from smaller architectural proteins, the sheer size of SMC proteins can promote long-range interactions between DNA segments, allowing these proteins to play important architectural roles in essential processes such as genome organization, transcription and DNA repair.

PLASTICITY OF CHROMATIN-PROTEIN DISTRIBUTION IN THE KINGDOMS OF LIFE

Although not universal, the octameric nucleosome is a highly conserved entity in eukaryotes, and its evolution can be traced back from the tetrameric nucleosomes found in many archaea. In prokaryotes, however, a diverse array of chromatin proteins exists with overlapping properties and functions that are susceptible to change by gene loss and lateral gene transfer. Thus, plasticity of chromatin proteins is apparent from the appearance of histone genes in isolated crenarchaeal and bacterial genomes (that generally do not encode histones), loss of histone genes (often replaced by HU-like proteins) in particular euryarchaeal and primitive eukaryotic genomes, and the limited distribution of a variety of small, abundant DNA binding proteins in bacteria and archaea. Nonetheless, although specific types of proteins have a limited distribution in a particular kingdom or even across kingdoms, architectural proteins that wrap, bend or bridge DNA are functionally conserved and widespread in all kingdoms of life. These architects of chromatin organize and regulate folding and functioning of the genome according to principles that are remarkably similar.

HIGHER ORDER FOLDING OF GENOMES IN CELLS: THE TOP-DOWN VIEW ON CHROMATIN ORGANIZATION

Loop Structure of Bacterial Genomes

The bacterial nucleoid consists of topologically isolated domains, which protect the genome from the potentially harmful effects of global relaxation of DNA supercoiling, resulting from the presence of even a single discontinuity in one of the strands of duplex DNA. Early electron microscopy studies suggested

that isolated nucleoids consist of a central core from which supercoiled loops emanate (Kavenoff and Bowen, 1976). The size of such loops ranges from 2 kbp to 65 kbp, with an average value of 10 kbp in gently lysed nucleoids (Postow *et al*., 2004). Additional *in vitro* and *in vivo* approaches corroborate an average size of supercoiled loops in both *E. coli* and *Salmonella typhimurium* of ~10 kbp, yielding a total of about 400 of such loops. Importantly, these studies also revealed that the nucleoid is a dynamic structure and that the boundaries of these topological domains are distributed in a seemingly random manner throughout the genome (Higgins *et al*., 1996; Postow *et al*., 2004; Deng *et al*., 2005; Stein *et al*., 2005).

Factors that set the boundaries of these domains induce (transient) cross-links between genomic loci or between one locus and an immobile structure such as the membrane. These cross-links could be the result of DNA transacting processes such as replication and transcription, or they could result directly from the binding of architectural proteins. Evidence has been put forward that very strong promoters can form domain barriers (Deng *et al*., 2004). It was even suggested that the loop structure of both bacterial and eukaryotic genomes is the result of transcriptional activity (Marenduzzo *et al*., 2007). However, less than, 20 genes in *E. coli* are expressed at a sufficiently high level to form a domain barrier (Deng *et al*., 2004). These genes include the rRNA genes (expressed from the *rrn* operons). The majority of the genes in *E. coli*, however, is expressed at such low levels that their expression has little impact on the domain structure. The domains that are established by transcription persist even without active transcription, indicating that domain formation and termination do not coincide with transcription (Deng *et al*., 2005; Stein *et al*., 2005). As mentioned, domain boundaries in the genome can also arise through attachment of DNA to the membrane. Such attachments occur upon expression of transmembrane proteins or secreted proteins, when transcription, translation and membrane translocation are coupled in a process referred to as transertion (Norris, 1995; Wang and Lynch, 1993; Woldringh, 2002). The number of sites at which transertion occurs has been estimated at about 20 per cell (Woldringh, 2002). Taken together the above processes are thus not likely to account for more than a small fraction (10–20%) of the domain barriers that exist. This suggests the need for additional factors to establish topological domains. In fact, a genetic screen for proteins involved in setting boundaries between domains revealed two known architectural proteins that might play such a role: H-NS and Fis (Hardy and Cozzarelli, 2005).

The possible involvement of these two proteins prompted studies aimed at mapping the distribution of these proteins along the bacterial genome. ChIP-on-chip data for H-NS binding along the *E. coli* and *S. typhimurium* genome (Navarre *et al*., 2006; Oshima *et al*., 2006) revealed that H-NS patches are distributed in a seemingly random (but determined by higher than average AT content) manner along the genome, similar to the distribution of domain barriers identified previously (Higgins *et al*., 1996; Postow *et al*., 2004; Deng *et al*., 2005; Stein *et al*., 2005). About 350 such H-NS patches were identified with an average spacing of 11 kbp, suggesting that H-NS could indeed account directly for the major fraction of all looped domains by bridging adjacent H-NS-bound tracts (Noom *et al*., 2007). Interestingly, mapping of Fis binding sites along the genome revealed an average spacing of 5 kbp between such sites (Cho *et al*., 2008). A fraction of these sites may be involved in DNA loop formation (possibly yielding a domain boundary) due to the reported DNA bridging activity (Schneider *et al*., 2001; Skoko *et al*., 2005). It is important to note that the bridging by these proteins does not impose a fixed configuration on the genome. The domains will be dynamic and not continuously fixed at one location in each cell within a population, as domain barriers may differ in stability (due to smaller bridged regions being less stable than larger ones) (Noom *et al*., 2007). The stated number of 350 should therefore also be considered as an upper limit to the number of H-NS induced domains present under the exponential growth conditions of the experiment. For instance, in stationary phase of growth the overall number of domains is reduced from 400 to 200 (Higgins *et al*., 1996) along with the amount of H-NS expressed being more than halved (Talukder *et al*., 1999). Although not identified as an explicit candidate in the genetic screen (Hardy and Cozzarelli, 2005), it is likely that SMC proteins (MukB in *E. coli*) also play a role in the formation of loop domains. The fact that due to their mode of binding and the lack of high-affinity binding sites they are likely quite loosely associated with DNA could signify that SMC proteins give rise to domain barriers that are both very dynamic and not specifically localized. DNA bending and wrapping proteins are likely predominantly bound within domains to promote compaction.

A recent study in *Caulobacter crescentus* showed that the cellular position of more than 100 genomic loci is correlated with their linear position along the genome (Viollier *et al*., 2004). Thus, each gene occupies a specific sub-cellular position. This suggests a model in which the chromosome is arranged such that the two halves of the chromosome between the origin and the terminus are arranged linearly as a series of loops perpendicular to the cell axis (Noom *et al*., 2007). Similar to *C. crescentus*, both the *E. coli* and the *Bacillus subtilis* genomes are also folded such that the linear order of genes on the DNA is preserved (Teleman *et al*., 1998; Wang *et al*., 2006). On top of this particular chromosomal arrangement, the *E. coli* genome appears to be folded into a higher-order structure containing four so-called macrodomains and two less structured regions (Valens *et al*., 2004). Two of the macrodomains that were originally identified by *in situ* hybridization are about 1 Mb and are located at the origin of replication (the Ori domain, from 80 to 100 min) and at the termination site (the Ter domain, from 25 to 45 min) (Niki *et al*., 2000). Two additional domains were found flanking the Ter domain (termed left and right domain) and two less structured domains flank the Ori domain (Valens *et al*., 2004). There are contacts between different DNA regions within one domain (up to more than 500 kb away), but interactions between different domains are restricted. In addition, the less structured

regions interact with the adjacent macrodomains, but not with other regions of the chromosome (Boccard *et al*., 2005). There is no evidence for macrodomains in *C. crescentus* (Viollier *et al*., 2004;Espeli and Boccard, 2006). The folding of the bacterial genome in macrodomains and the arrangement of loops that preserve the linear order of genes along the DNA imposes severe constraints on the way the bacterial chromosome is folded in living cells.

Loop Structure of Archaeal Genomes

Higher order organization of archaeal genomes has to date not been explicitly addressed. Nevertheless, it seems likely that it shares features with both bacterial and eukaryotic organisms as a consequence of the prominent similarities in the intrinsic properties of the architectural proteins. Although speculative, *Crenarchaea* (which generally lack histones) may exhibit an organization in looped domains similar to that found in bacteria, where a key role is envisioned for DNA bridgers (such as Alba, which may be involved in looped domain formation) and benders (such as Cren7-like proteins). On the other hand the chromatin of *Euryarchaea* (which do encode histones), may share more similarities with eukaryotes, including chromatin fibers that fold in higher order structures. These higher order structures are likely less densely packed (since archaeal histones wrap less DNA than their eukaryotic counterparts). In addition there are probably regions devoid of archaeal nucleosomes, since the ratio of histones – and other architectural proteins—to DNA is not sufficient to cover the whole genome.

Loop Structure of Eukaryotic Genomes

The organization of chromatin in eukaryotic cells is often suggested to involve nucleosomal arrays that coil up to form the 30 nm fiber, which is subsequently stabilized by histone H1 and folded into a higher order structure that eventually generates the interphase chromosomes and finally the mitotic ones (Varga-Weisz and Becker, 2006). In reality, however, very little is known about the organization of chromatin beyond the nucleosomal array, although some of its organizing principles are being slowly unveiled. The emerging view is that chromosomes are confined to discrete territories ($\sim$2 μm in diameter) that show little intermingling with chromatin from neighboring territories (Cremer and Cremer, 2001). The chromatin fiber within territories is organized in loops that may be attached at their base to a structural network of scaffold proteins, including SMC proteins (Cremer and Cremer, 2001; Maeshima and Eltsov, 2008). In fact, DNA sequences that bind this nuclear scaffold as well as specific scaffold-binding proteins have been identified (Cai *et al*., 2006; Galande *et al*., 2007; Nickerson, 2001). For instance, SATB proteins (special AT-rich binding protein) are assumed to organize chromatin into discrete loops by anchoring regions of high AT content to a nuclear scaffold (Galande *et al*., 2007). Expression of several (mainly T cell-specific) genes was shown to depend on a specific arrangement of occupied SATB binding sites (Cai *et al*., 2006; Kumar *et al*., 2007). An other example is CTCF (CCCTC-binding factor), which mediates long-range chromatin–chromatin interactions (due to looping) among others between distant regions of the β-globin locus in erythrocytes (Splinter *et al*., 2006), although the mechanistic basis of CTCF action remains obscure. The precise nature of the proposed nuclear scaffold is unclear, but it may comprise the nuclear lamina. Indeed, specific regions of the human genome (which are on average 500 kbp in size) bind the nuclear lamina, suggesting that lamina-binding domains serve as a structural framework to organize the genome inside the nucleus (Guelen *et al*., 2008). The genomic regions associated with the nuclear lamina are in a repressive chromatin state and are therefore transcriptionally inactive. Relocating genes to the nuclear periphery may thus be a mechanism to silence genes (Reddy *et al*., 2008). Surprisingly, more than 40% of the human genome is found to interact with the lamina, although it is not likely that these regions are bound to the lamina at all times (Guelen *et al*., 2008). Scaffold-binding proteins (or proteins that link chromatin to the lamina) play important roles in organizing chromatin in loops of several tens of Mbp or larger (Fransz *et al*., 2002; Iarovaia *et al*., 2004; Galande *et al*., 2007). It is expected that proteins such as BAF (that can link chromatin to the lamina), SATB proteins, CTCF and SMC proteins play key roles in this process. These proteins appear to cooperate as well since cohesin (an SMC protein) binding to genomic sites depends on CTCF (Rubio *et al*., 2008). Chromatin loops from different positions along the same chromosome or even from different chromosomes have been proposed to come together in shared regions of ongoing transcription (often referred to as transcription factories) (de Laat and Grosveld, 2007; Fraser and Bickmore, 2007), suggesting that transcriptional activity is a major determinant in the higher-order folding of the genome in eukaryotes.

At a higher organizational level, individual chromosomes that are present in territories of $\sim$2 μm in diameter contain numerous irregularly-shaped dense chromatin domains (visible by electron microscopy) that are about 0.1–0.5 μm in size and are surrounded by inter-chromatin space, which contains little or no chromatin (Fakan and van Driel, 2007). The surface of dense chromatin domains has been suggested to constitute a functional barrier that allows single proteins to enter, but not larger protein complexes above a certain threshold (Cremer and Cremer, 2001). However, chromatin domains are readily accessible to macromolecules in the range of several hundred kDa, arguing against this notion (Verschure *et al*., 2003). At the interface between the dense chromatin domains and inter-chromatin space is the perichromatin region that constitutes a shell ($\sim$80 nm wide) of partly decondensed chromatin that loops outwards (Fakan and van Driel, 2007). Interestingly, major chromatin-associated processes, such as replication, transcription and DNA repair occur predominantly in the perichromatin region (Cmarko *et al*., 1999; 2003; Jaunin *et al*., 2000; Solimando *et al*., 2008). This suggests that chromatin is relocated from the interior to the surface of condensed domains to allow DNA transactions to occur.

FUNCTIONAL IMPLICATIONS OF GENOME ORGANIZATION IN ALL KINGDOMS OF LIFE

Crowding and Supercoiling: Regulators of Genome Function?

Organisms in the three kingdoms have evolved mechanisms to organize the genome and to fit it within the structure that contains it, which can be the nucleoid of a prokaryotic cell or the nucleus of a eukaryotic cell (Figure 1). A major force driving genome compaction is exerted by the high concentration of macromolecules in prokaryotes and eukaryotic nuclei. However, this crowding force drives compaction non-specifically, and therefore only serves in compacting genomes and cannot be used to regulate genome function (Figure 3). On the other hand due to its general effects on the DNA association constants of proteins (Zimmerman, 1993), occupancy of regulatory sites by transcription factors (repressors or activators) will be enhanced. A second essential mechanism that may serve to compact genomic DNA is to reduce its effective volume by supercoiling (Figure 2) (Stuger *et al*., 2002). In addition, supercoiling serves a regulatory purpose and increases the rate at which proteins locate binding sites on the genome (Gowers and Halford, 2003). Expression of many bacterial genes is dependent on superhelicity (Hatfield and Benham, 2002; Peter *et al*., 2004). This is due to the fact that supercoiling facilitates promoter melting during transcription initiation (Borowiec and Gralla, 1985). Although DNA wrapping around histones in eukaryotes induces significant supercoiling, this superhelicity is constrained by extensive DNA–histone interactions. The absence of free supercoiling in organisms from the eukaryotic kingdom suggests that this mechanism is not relevant in promoting global genome compaction. However, local supercoiling may serve an important regulatory function in gene expression. For example, supercoiling induced by RNA pol II during transcription through nucleosomes has been shown to recruit structure-sensitive regulatory proteins to sequence elements sensitive to supercoiling (Kouzine *et al*., 2008). It is feasible that supercoiling is involved in the regulation of divergent promoters (i.e. adjacent oppositely oriented promoters). In this scenario, expression from one promoter would induce changes in the superhelicity of the other promoter, which in turn would affect its activity by the binding of structure-specific factors. In *E. coli*, where 50% of supercoiling is unconstrained, these effects do occur (Chen *et al*., 1992; Rhee *et al*., 1999). Local modulation of transcriptional activity by means of supercoiling may be more widespread in eukaryotes than previously anticipated, since more than 10% of all human promoters are estimated to be divergent. Moreover, the gene products of many of these divergent promoters are often co-expressed (Trinklein *et al*., 2004). In addition, several studies have indicated that nucleosomes are partially or fully disassembled during transcription (Boeger *et al*., 2003; Bruno *et al*., 2003; Henikoff, 2008). Nucleosome disassembly results in a release of the supercoils that were constrained by that nucleosome and this free superhelicity may aid in strand separation during transcription initiation (Figure 2) (Travers and Muskhelishvili, 2007) or promote recruitment of structure-sensitive regulatory proteins (Kouzine *et al*., 2008).

Principles of Genome Organization by Architectural Proteins

In addition to the above-mentioned concepts for organizing and modulating the activity of genomic DNA, cells of all kingdoms of life encode DNA and chromatin-binding proteins. These proteins serve functions in genome compaction as well as in regulation of genome function. The individual contributions of crowding, supercoiling and architectural proteins to genome compaction are not clear. These three mechanisms are tightly interwoven and therefore act in concert rather than in parallel. This is illustrated by the fact that macromolecular crowding increases the association constants of (architectural) proteins (Zimmerman, 1993). Moreover, binding of architectural proteins is enhanced by supercoiling, either by changing the free energy required to bend or wrap the DNA or by changing the local effective DNA concentration by plectoneme formation, which favors DNA bridging (Bates and Maxwell, 2005). Due to the physical properties of DNA there is a limited number of ways in which DNA structure can be modulated. Reducing the effective volume and simultaneously imposing functional organization on the genome can be achieved mechanically by either bending and/or bundling the DNA. It seems that cells of organisms across the three kingdoms have—at least in part—independently evolved architectural factors capable of modulating DNA structure following these principles. Here, it is argued that architectural functions of genome packaging proteins are conserved throughout the kingdoms of life, with different types of proteins having emerged to carry out the same functions. According to these principles, architectural proteins can be generically classified in three functional groups based on the effect they exert on the DNA: (1) DNA benders, (2) DNA wrappers and (3) DNA bridgers (Table 1, Figure 4A). This classification is key to understanding the fundamental role of architectural proteins in the dynamic organization of the genome. As a consequence of their conserved functionality, architectural proteins from one domain of life are often able to substitute (at least partially) for proteins from another domain. Thus, it is known that yeast HMG proteins can substitute for HU in *E. coli* (Becker *et al*., 2008; Paull and Johnson, 1995). It has also been shown that the mouse Btcd (binding to curved DNA) protein complements at least partially the phenotype of *E. coli* cells deficient for the DNA bridger H-NS (Timchenko *et al*., 1996). In both cases there is no structural similarity between the two proteins. These studies suggest that the architectural functions of DNA packaging proteins are—in principle—similar across the three kingdoms of life.

Control of Genome Function by Architectural Proteins

The organization of genomes is not static. On the contrary, regulation of essential genome functions including gene expression, replication and DNA repair requires a high degree of

chromatin plasticity. This plasticity is achieved through mechanisms that lead to remodeling of chromatin a at least two hierarchical levels. Either the interactions between an architectural protein and its binding site on the DNA are modulated or the higher-order folding of the genome is modulated, using a large variety of approaches.

Modulation of Binding Site Occupancy

Modulation of binding site occupancy in its simplest form is mediated by competition or cooperative binding of two (or more) proteins at the same binding site. This may involve two proteins that have affinity for the same site (Figure 4C). A variation on this theme is competition between two proteins with different sizes of DNA binding sites. For instance, the binding site may be wrapped as part of a larger DNA region around, for instance, an Lrp-like protein or an archaeal (tetrameric) or eukaryotic (octameric) nucleosome (Figure 4D) (Pul *et al*., 2007). In eukaryotes, wrapping in nucleosomes appears to be the main mechanism to repress transcription. Alternatively, a binding site may be located within a larger region along which a protein has formed an extended filament. An example of such a protein is H-NS, which binds along extended AT-rich regions that are bridged to other regions along the DNA (Dame *et al*., 2001). In fact, binding of H-NS occurs in the promoter regions of many genes, thus hindering access of RNA polymerase and other proteins to the promoter and interfering with transcription initiation (Figure 4E) (Dorman, 2004; Fang and Rimsky, 2008). More sophisticated mechanisms include chemical modification of the binding sites (Figure 4F) or modification of the protein (Figure 4G), each resulting in a change of affinity of the protein to its binding site on the DNA. In bacteria, methylation of DNA modulates the interaction with DNA of a broad range of proteins, the most well known being the host restriction enzymes that are selective for the unmethylated invading DNA of viruses (Murray, 2002). DNA methylation also reduces the affinity for DNA of proteins such as Lrp (Nou *et al*., 1995; Camacho and Casadesús, 2002). Binding of H-NS interferes with methylation (White-Ziegler *et al*., 1998) and this may globally modulate the binding of other proteins within regions of preferential binding for H-NS (Lucchini *et al*., 2006; Navarre *et al*., 2006; Main-Hester *et al*., 2008). While the effect of methylation on the binding of these proteins has to date only been explicitly addressed in relation to gene regulation, it seems likely that the global organization of the genome is similarly modulated by changing the affinity of architectural proteins. DNA methylation (at both A and C) is also found in several archaeal lineages and appears to serve a similar protective function against invading viral DNA as in bacteria (Barbeyron *et al*., 1984; Grogan, 2003; Prangishvili *et al*., 1985). It is likely that DNA methylation in the archaea also affects the binding of architectural proteins to chromatin, but this issue awaits further studies. In most eukaryotes, methylation of DNA is associated with gene repression, likely by preventing the binding of transcription factors. Vertebrate and plant genomes especially are globally methylated, while other eukaryotes (such as *Drosophila* and *C. elegans*) lack DNA methylation (Hendrich and Tweedie, 2003). Specialized proteins containing methyl-binding domains (MBD) have emerged (such as MBD1/2 and MeCP2), which bind to methylated DNA and subsequently recruit repressor complexes (Jones *et al*., 1998), rendering the DNA inaccessible to the transcription machinery. MeCP2 has been suggested to form dimers or larger oligomers containing two or more independent MBD domains. Hence, MeCP2 can be considered a bridger that specifically brings methylated DNA regions together. In agreement with such an architectural function, expression of either MeCP2 or MBD2 in mouse cells resulted in clustering of methylated chromatin during differentiation (Brero *et al*., 2005).

In addition to modification of DNA by methylation, histone proteins are subject to post-translational modification. The evolutionary conserved SET domain proteins are widespread amongst eukaryotes and function as site-specific histone methyltransferases. Proteins containing SET domains are also found in bacteria and archaea. In eukaryotes, histone tails (especially the N-termini of H3 and H4) are extensively modified and this alters the interaction between many chromatin-binding proteins and nucleosomes. A typical example of how modification of a binding site on eukaryotic chromatin modulates the binding of a protein is methylation of histone 3 (H3) at lysine 9 (H3K9me). This chromatin mark recruits the heterochromatin 1 (HP1) protein that contains a motif (called a chromodomain), which specifically binds H3K9me$_3$ (Nielsen *et al*., 2002). Moreover, binding of HP1 can be further modulated by modification of the serine residue directly adjacent to its binding site on H3. Phosphorylation of this residue triggers dissociation of HP1 even if lysine 9 is methylated (Hirota *et al*., 2005). It is interesting to note that the functional unit of HP1 is a dimer and that it can thus function as a bridger linking two methylated tails of H3 (at K9) on different nucleosomes, thereby possibly stabilizing nucleosomes and higher-order chromatin folding (Henikoff, 2008). Another example of a frequent histone modification in eukaryotes is acetylation, occurring at the N-terminal tails of H3 and H4. Acetylated histones serve as binding site for a set of specific proteins (containing a bromodomain) often resulting in activation of genes. Histone acetylation is therefore generally associated with transcriptional activity (Clayton *et al*., 2006). Archaeal histones lack the N-terminal tails (flexible tails extending from the nucleosome core) that are subject to extensive modification in eukaryotes and studies of archaeal histones have indeed not revealed any post-translational modification (Forbes *et al*., 2004). Finally, a chromatin binding protein itself can also be modified, resulting in an altered interaction with its binding site. In the case of HP1, a residue located close to the chromodomain of the HP1 protein is phosphorylated, thereby triggering dissociation from histone H3 methylated on K9 (Ayoub *et al*., 2008). In bacteria, post-translational modification of architectural proteins is rare. The only known example is H-NS, which has been shown to exist in two or three isoforms (Spassky *et al*., 1984; Donato

and Kawula, 1999). Although the location of the modifications has been mapped to the N-terminal dimerization domain, the nature of these modifications is unclear (Donato and Kawula, 1999). Their position suggests an effect on dimerization of H-NS, but there is no evidence that these isoforms actually exhibit altered characteristics resulting in different *in vivo* activity. In archaea, there are currently only a few cases known of modifications of DNA-binding proteins. These include acetylation of Alba in *S. solfataricus* (Bell *et al*., 2002) and methylation of Sul7 and Cren7 in *Crenarchaea* (Guo *et al*., 2007). Whereas there is no evidence for methylation of archaeal histones, the chromatin protein MC1 was found to be methylated by an archaeal SET domain protein *in vitro*. It is currently unclear whether this serves a regulatory purpose *in vivo* (Manzur and Zhou, 2005). Several bacterial genomes encode SET proteins. In *Chlamydia*, a SET protein methylates the Hc1 and Hc2 proteins (linker H1 homologues) *in vitro*, showing that SET proteins potentially function as methyltransferases in bacteria (Murata *et al*., 2007). However, if such a mechanism is indeed operating *in vivo* (and exploited to modulate the properties of proteins) in bacteria, it is probably restricted to only a few bacterial species.

Regulation of Genome Functions by the Opposing or Concerted Action of Architectural Proteins: A Struggle amongst the Architects

Modulating the occupancy of binding sites can also involve competition (antagonism) or joint cooperative activity (synergism) between different architectural proteins (Figure 5A–D). While in the first case the effectiveness of competition is predominantly determined by the DNA binding affinity of the individual proteins, in the second case the specific architectural features of a set of architectural proteins determines how effectively they can work together in forming a multi-protein complex. A common theme appears to be the competition between a bridger and a DNA bender (Figure 5A). Bridgers are often functionally linked to repression of transcription, which can be counteracted by a DNA bender. In bacteria, at many of the genes repressed by H-NS the action of this protein is antagonized (and repression is alleviated) by the binding of architectural proteins that bend DNA (HU, IHF, Fis) (Dame and Goosen, 2002; Dame, 2005). In the case of IHF this occurs by binding at a well-defined recognition site within the region of H-NS binding. Interestingly, this relieves binding not only at this specific site, but all through the region of H-NS binding (van Ulsen *et al*., 1996). The underlying mechanism is as yet unknown, but may involve a decrease in bridging probability by bending DNA duplexes away from each other, thereby lowering the local effective DNA concentration. In the case of Fis it appears that its binding occurs at a site exhibiting large similarity to that recently defined as a high affinity (and proposed nucleation) site for H-NS (Bouffartigues *et al*., 2007; Cho *et al*., 2008; Lang *et al*., 2007). This suggests that at least at a subset of promoters these proteins directly compete for bind to this site and that Fis binding may thus prevent nucleation and subsequent cooperative binding of H-NS along the DNA. These types of mechanisms are generally linked to genes of which the expression is altered in response to environmental signals (available nutrients, salt, temperature, etc.), that affect the ratio of expression of H-NS and its antagonists (Atlung and Ingmer, 1997; Luijsterburg *et al*., 2006; Talukder *et al*., 1999). Conceptually similar mechanisms have been shown to exist in eukaryotes. For instance, HMG proteins directly compete with histone H1 (Figure 5B), which binds to linker DNA and is responsible for folding chromatin fibers into more compact structures (Catez *et al*., 2002, 2004; Brown *et al*., 2006). The underlying mechanisms may be similar to that employed by bacterial benders and involve bending the linker region in such a way that binding (and bridging) of H1 becomes unfavorable. This provides a mechanism that explains how HMG proteins can destabilize higher-order chromatin structures. ChIP experiments suggest that binding of HMG and H1 is mutually exclusive and that association of HMG (coupled with dissociation of H1) to nucleosomes assembled on promoters is associated with transcriptional activation (Ju *et al*., 2006). The competition of HMG with H1 is probably prominent in undifferentiated cells and during early stages of development when the ratio at which the two proteins are expressed is in favor of HMG (Muller *et al*., 2004). An alternative generic mechanism for antagonism between different types of architectural proteins has been proposed to operate on the global scale and to be important in remodeling of the DNA loops stabilized by H-NS in bacteria. According to this model, the binding of a DNA bender at a position away from the apex of the loop will impose a configuration where the DNA-bound bender is translocated to the apex and where the regions involved in bridging become shifted (Dame, 2005; Luijsterburg *et al*., 2006). Analogously, if polymerization of proteins along the DNA leads to its local stiffening (van Noort *et al*., 2004), such a region will preferentially localize away from the apex of the loop (Dame, 2005; Luijsterburg *et al*., 2006).

Besides competition or opposing structural effects, architectural proteins can also act synergistically, giving rise to multiprotein assemblies on the DNA. Within these complexes, binding of different factors is cooperative. DNA bridging will be stimulated between the DNA arms extending from a protein-induced bend due to an increased effective DNA concentration. Vice versa, DNA bending proteins can be more easily accommodated within a loop that results from DNA bridging (Figure 5C). This type of complex, containing a bender (IHF) and a bridger (H-NS), sometimes complemented with a third binding partner (for instance Fis) has been demonstrated in bacteria (Browning *et al*., 2000; Gerstel *et al*., 2003), where they act to repress transcription. The co-localization of many architectural proteins found by ChIP-on-chip experiments (Grainger *et al*., 2006), suggests that such complexes are also formed on a global scale, where they have differential effects on genome organization that go beyond the properties of the individual players. Analogously, DNA wrapping proteins can promote bridging by effectively bending the DNA

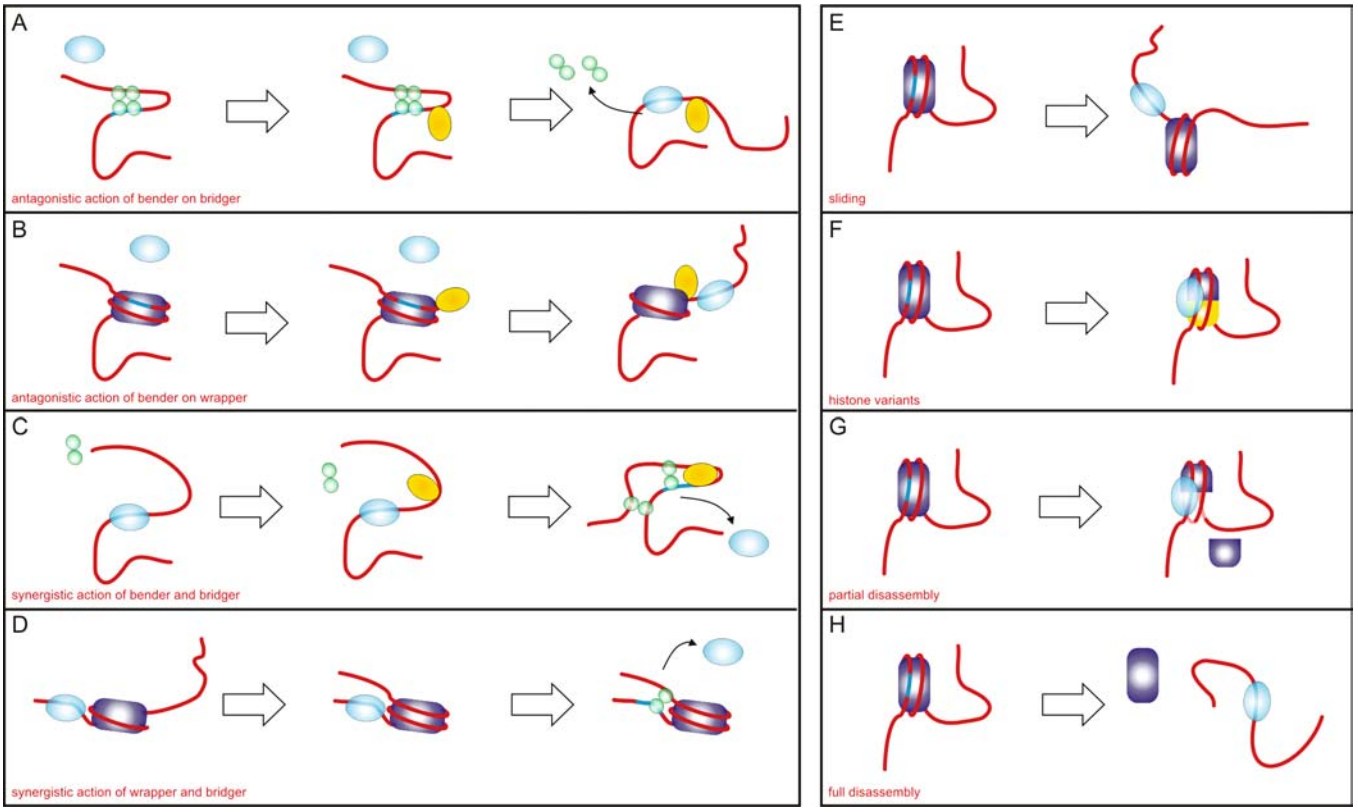

FIG. 5. Modulation of binding site accessibility by the concerted or opposing action of architectural proteins and energy-dependent displacement of DNA wrappers. A) Opposing action of a bender and bridger. The binding of bridgers is often very cooperative, as binding depends on a high effective DNA concentration. A bender can destabilize a complex formed by bridging proteins by bending two DNA duplexes away from each other. B) Opposing action between a bender and wrapper: the bender bends the DNA away from the surface of the wrapper. For instance, HMG proteins facilitate unwrapping of DNA from nucleosomes by ATP-dependent remodelling complexes (Bonaldi *et al*., 2002). C) Concerted action of a bender and bridger. This is analogous to (A) but with a different outcome. If a bender bends DNA duplexes towards each other this can create a site suited for binding of a bridger. D) Concerted action of wrapper and bridger. Wrapping DNA results in a local high concentration of DNA, for instance, where DNA enters and exits the nucleosome, which can facilitate bridging. A clear example is the binding (and bridging) of DNA that enters and exist the nucleosome by linker H1. E) A nucleosome can be "pushed" away from its binding site, which then becomes exposed. This type of "sliding" is mediated by remodelling complexes of the SWI/SNF (that randomize nucleosomes by sliding) and ISWI families (that promote equal spacing between nucleosomes by sliding) (Cairns 2007). F) Incorporation of histone variants that alter the stability of nucleosomes (depicted in yellow). Several variants of H2A (H2A.Z, macroH2A and H2A.X) as well as of H3 (H3.3 and cenH3) exist that destabilize the nucleosomes into which they are incorporated to replace the "standard" H2A and H3 proteins (Henikoff 2008). G) Partial disassembly of nucleosomes by release of H2A-H2B dimers. This mechanism results in partial unwrapping of the DNA and is often employed during transcription. H) Complete disassembly of nucleosomes, which can be mediated by ATP-dependent remodelling complexes of the SWI/SNF family (Cairns 2007), resulting in full unwrapping of the DNA. This may release supercoils constrained by that nucleosome, as explained in fig 2 and the main text.

and bringing sections of the wrapped DNA close together (Figure 5D). This occurs, for instance, in eukaryotes where H1 bridges the linker DNA to a DNA segment approximately one full turn away inside the nucleosome. In this case, H1 binding is facilitated due to the locally high effective DNA concentration, while the wrapped DNA becomes more stably bound within the nucleosome.

Displacing Wrappers by Brute-Force Methods: Plowing through Nucleosomes with Bulldozers

As described in the previous section, competition between architectural proteins is an effective means to regulate the functioning of the genome dynamically. Whereas bridgers and benders seem to be able to antagonize each other's action (Figure 5A), wrappers appear to be, at least in part, resistant to this type of

regulation (Figure 5B). This is due to the extensive protein–DNA interactions that usually exist in wrapped complexes. Benders and bridgers simply cannot effectively compete with wrappers for binding sites on the genome, due to their tight binding. Thus, it appears that similar architectural mechanisms operate on the level of DNA, but that accessibility of DNA is regulated by wrapping and unwrapping of DNA from nucleosomes. Accessibility to genomic DNA wrapped in nucleosomes is achieved in several ways: (1) nucleosome sliding (thereby exposing DNA regions that were wrapped) (Figure 5E) (Langst *et al*., 1999); (2) incorporation of chromatin activity-dependent histone variants (such as H2A.Z or H3.3) that destabilize the nucleosome (Figure 5F) (Bruno *et al*., 2003); (3) partial nucleosome disassembly (by dissociation of H2A-H2B dimers) resulting in partial unwrapping (Figure 5G) (Bruno *et al*., 2003); and/or (4) complete disassembly of nucleosomes ensuring full exposure of the wrapped DNA region (Figure 5H) (Boeger *et al*., 2003; 2004). It is worth noting that a partially disassembled eukaryotic nucleosome (which consists of an H3–H4 tetramer), is similar to the archaeal tetrameric nucleosome. This may suggest that the default state of the tetrameric nucleosomes in archaea is more permissive or accessible than the restrictive octameric nucleosomes found in eukaryotes. Mobilization, restructuring and ejection of nucleosomes is mediated by ATPase-containing chromatin remodeling complexes (Cairns, 2007). These large (several MDa) complexes convert chemical energy from ATP hydrolysis into mechanical motion by forcing DNA around the nucleosome and thereby disrupting histone-DNA contacts (Saha *et al*., 2006). A single nucleosome is at all times retained at active promoters *in vivo*, suggesting that a remodeler bound to one of the nucleosomes in the promoter region "peels" the DNA of the surface of neighboring nucleosomes (Cairns, 2007; Boeger *et al*., 2008). ATP-dependent remodelers are abundant in eukaryotes and also found in some archaeal lineages (Flaus *et al*., 2006). Finally, members of this family of remodelers are also found in bacteria, where, for instance, RapA plays a role in recycling stalled RNA polymerase (Shaw *et al*., 2008; Sukhodolets *et al*., 2001). There is to date, however, no evidence that these bacterial homologues have a chromatin remodeling function as well. Once in eukaryotic organisms the DNA is partially removed from the nucleosome surface, it is feasible that other architectural proteins stabilize the partially unwrapped state by binding to the DNA and, for instance, bend it further away from the nucleosome surface (Figure 5B) (Travers, 2003). In support of such a mechanism, HMGB was found to facilitate chromatin remodeling by ATP-dependent complexes (Bonaldi *et al*., 2002). DNA sequences that position nucleosomes far more stably than natural sequences have been engineered, suggesting that nucleosome positioning sequences have evolved to be metastable (Lowary and Widom, 1998; Segal *et al*., 2006; Henikoff, 2008), allowing modulation of histone-DNA contacts. Moreover, nucleosome-free regions (regions lacking positioning sequences) are found specifically upstream of promoters (Lee *et al*., 2004; Sekinger *et al*., 2005), and these may serve as nucleation sites for the binding of transcription factors that subsequently initiate chromatin remodeling.

CONCLUDING REMARKS

One of the major challenges of any cell is to reduce the volume of its genome effectively by several orders of magnitude without compromising its functionality and plasticity. Several principles appear to underlie the dynamic shaping of genomes. A key role is envisioned for the numerous architectural proteins that fold and compact genomes. Proteins that wrap, bend or bridge DNA are widespread, although they often do not exhibit sequence or structural conservation across kingdoms. Rather it appears that these architects of chromatin are functionally conserved. The existing literature regarding these architects of chromatin from various organisms seems to emphasize the differences between modes of chromatin organization rather than the similarities. It is often not acknowledged that chromatin in bacteria and archaea is organized and compacted in similar ways as it is in eukaryotes. This can possibly be traced back to the lower abundance of DNA wrapping proteins (yielding nucleosome-like structures in these organisms) rather than that it is based on a lack of architectural proteins. The overview given here aims to bring across the message that genome organization and compaction is essential to organisms in all three kingdoms, but that not all organisms have evolved similar solutions in terms of what type of architectural proteins are used. Nevertheless, it appears that the number of options to solve the problem of DNA compaction is limited and that similar mechanisms are used in all forms of life. Thus, the underlying mechanisms to dynamically compact genomes are remarkably similar throughout the three kingdoms. Obviously, these different solutions are also related to differences in "lifestyle" between single-celled bacteria and archaea, where a permissive organization of the genome aids in adaptation to changing environmental conditions, and higher eukaryotes, where such issues are less prominent. The approaches taken to study chromatin structure in the bacterial, archaeal and eukaryotic fields differ significantly and this leads at least in part to emphasis on different aspects in different organisms. Currently, in the prokaryotic field most researchers employ a bottom-up approach, where the role of individual proteins in chromatin organization is dissected. There are only few studies at the cellular level that address these issues in prokaryotes. In eukaryotes, on the other hand, the majority of studies examine chromatin organization at the cellular level, while the architectural role of individual proteins is often not understood in detail. In order to gain progress in the field of chromatin research it is therefore essential to reach a large degree of cross-insemination between these fields. The prokaryotic field would benefit from adopting approaches such as chromosome conformation capture (3C) (Splinter *et al*., 2004; Tolhuis *et al*., 2002) and *in vivo* fluorescence imaging of living cells, which help in elucidating three-dimensional genome organization and dynamic aspects of architectural proteins. On the other hand, the powerful reductionist single-molecule approaches currently available (Dame,

2008; Moffitt *et al*., 2008; van Mameren *et al*., 2008), could be more widely adopted to study eukaryotic chromatin and the processes that shape and reshape it.

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