Function of Nucleoid-Associated Proteins in Chromosome Structuring and Transcriptional Regulation

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Abstract
Nucleoid-associated proteins typically are abundant, low-molecular-mass polypeptides that bind DNA and alter its shape and its ability to participate in transactions such as transcription. Some can bind RNA and influence the gene expression profile of the cell at a posttranscriptional level. They also have the potential to model and remodel the structure of the nucleoid, contributing to chromosome packaging within the cell. Some nucleoid-associated proteins have been implicated in the facilitation of chromosome evolution through their ability to silence transcription, allowing new genes to be integrated into the nucleoid both physically and in a regulatory sense. The dynamic composition of the population of nucleoid-associated proteins in model bacteria such as Escherichia coli and Salmonella enterica links nucleoid structure and the global regulation of gene expression, enhancing microbial competitive fitness and survival in complex environments.

The Nucleoid and Its Associated Proteins

One of the defining features of prokaryotes is the absence of a nuclear membrane and the presence instead of a nucleoid consisting of the genetic material and its attached macromolecules where the processes of transcription and translation are coupled. Early electron microscopic studies of the nucleoid in freeze-fractured Escherichia coli cells revealed little evidence of organization: the nucleoid appeared to be simply that part of the cell interior that was ribosome-free [Kellenberger et al., 1958]. Electron micrographs of chromosomes released from E. coli cells that had been gently lysed were suggestive of an underlying structure that involved subdivision of the circular chromosome into loops, but the details were obscure [Kavenoff and Bowen, 1976; Kavenoff and Ryder, 1976]. The large size of the chromosome and the small volume of the cell suggested that packaging was necessary and the need to solve the packaging problem in a way that met simultaneously the needs of DNA replication, chromosome segregation and gene transcription implied that a nucleoid organizing principle was likely to be at work. Over a period of several decades, a multidisciplinary approach involving genetics, biophysics and the use of imaging methods of ever-greater resolution has led us to a
NAPs and Chromosome Structuring and Transcriptional Regulation

model of the bacterial nucleoid in which the millimetre-scale chromosome is seen to be organized on a micrometre-scale macrodomain level and a nanometre-scale microdomain level [Benza et al., 2012; Dorman, 2013; Espéli and Bocard, 2006; Junier et al., 2014; Macvanin and Adhya, 2012; Waldminghaus, 2014; Wang et al., 2011, 2013].

Nucleoid-associated proteins (NAPs) are abundant, low-molecular-mass polypeptides that bind DNA using either direct or indirect readout mechanisms [Azam et al., 1999; Browning et al., 2010; Dillon and Dorman, 2010]. Most have been shown to alter DNA shape at least locally and many can affect transcription and other DNA-based transactions such as replication, transposition and recombination [Browning et al., 2010; Chodavarapu et al., 2008a; Dillon and Dorman, 2010; Freundlich et al., 1992; Haykinson and Johnson, 1993; Liu et al., 2011; Makris et al., 1990; Swingle et al., 2004]. The fact that many NAPs have been discovered during investigations of site-specific recombination mechanisms or in studies of gene regulation is indicative of the wide-ranging contributions that NAPs make in the lives of bacteria and of the bacteriophage that parasitize them. This point is usefully illustrated by the case of the gene that encodes H-NS, one of the most intensively studied NAPs. The hns gene was discovered and re-discovered several times and given a variety of names (e.g. bglY, pilG, dxdR, virR, osmZ) that were specific to individual genes or sets of genes whose expression it affected [Defez and De Felice, 1981; Dorman et al., 1990; Göransson et al., 1990; Freundlich et al., 1992; Haykinson and Johnson, 1993; Liu et al., 2011; Makris et al., 1990; Swingle et al., 2004]. The functional form of HU is dimeric and the αβ-heterodimer is the dominant form. The composition of the dimer changes through the growth cycle and reflects changes in the relative abundances of the monomers [Claret and Rouvière-Yaniv, 1997]; it also reflects differential responses of the two hup genes to environmental signals [Claret and Rouvière-Yaniv, 1996; Giangrossi et al., 2002]. Results from atomic force microscopy studies of cells lysed in situ show that the higher-order structure of the E. coli chromosome in the nucleoid is altered when the genes encoding HU are inactivated [Ohniwa et al., 2013]. Single molecule studies have shown recently that when HU proteins act cooperatively in a side-by-side binding mode [van Noort et al., 2004], they stabilize the DNA helix in the nucleoid; while individual HU dimers create bends in DNA, cooperative binding of HU leads to higher-order complexes through dimer-dimer interaction [Dame et al., 2013]. In addition to global effects on chromosome structure, HU can also have important influence at a local level through its ability to facilitate the looping of short lengths of DNA. This loop-enhancing activity is useful in genetic switches that rely on site-specific recombination or on loop closure by DNA-binding transcription factors [Becker et al., 2007; Czapla et al., 2013; Merickel and Johnson, 2004; Semsey et al., 2006].

The Kar and Adhya laboratories have isolated a mutant derivative of HUs that introduces positive supercoils into DNA [Guo and Adhya, 2007; Kar et al., 2006; Koli et al., 2011]. The mutant protein contains just two amino acid substitutions (E38K and V42L), but the resulting changes to global DNA topology in the E. coli cells that express it causes a number of infection-related cryptic genes to become expressed. This may be the basis of a lifestyle switch where commensal E. coli becomes pathogenic [Koli et al., 2011].

Wild-type HU has a significant impact on gene expression at both the transcriptional and posttranscriptional levels [Mangan et al., 2011; Oberto et al., 2009; Prieto et al., 2012], the latter reflecting its RNA binding activity [Balandina et al., 2001; Macvanin et al., 2012]; HU also possesses efficient single-stranded DNA binding activity [Kamashev et al., 2008]. Thus, HU is a highly versatile protein, acting in an architectural mode to

Nucleoid Shape-Determining Protein HU

One of the first NAPs to be described in detail was HU, whose name is derived from histone-like protein from E. coli strain U93 (HU). In E. coli and related bacteria, HU is encoded by hupA and hupB, with these two genes encoding its α- and β-subunits, respectively. The functional form of HU is dimeric and the αβ-heterodimer is the dominant form. The composition of the dimer changes through the growth cycle and reflects changes in the relative abundances of the monomers [Claret and Rouvière-Yaniv, 1997]; it also reflects differential responses of the two hup genes to environmental signals [Claret and Rouvière-Yaniv, 1996; Giangrossi et al., 2002]. Results from atomic force microscopy studies of cells lysed in situ show that the higher-order structure of the E. coli chromosome in the nucleoid is altered when the genes encoding HU are inactivated [Ohniwa et al., 2013]. Single molecule studies have shown recently that when HU proteins act cooperatively in a side-by-side binding mode [van Noort et al., 2004], they stabilize the DNA helix in the nucleoid; while individual HU dimers create bends in DNA, cooperative binding of HU leads to higher-order complexes through dimer-dimer interaction [Dame et al., 2013]. In addition to global effects on chromosome structure, HU can also have important influence at a local level through its ability to facilitate the looping of short lengths of DNA. This loop-enhancing activity is useful in genetic switches that rely on site-specific recombination or on loop closure by DNA-binding transcription factors [Becker et al., 2007; Czapla et al., 2013; Merickel and Johnson, 2004; Semsey et al., 2006].

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impose structure on the nucleoid while simultaneously influencing transcription and translation. Among its targets is rpoS, the gene that encodes the stationary phase and stress sigma factor of RNA polymerase, RpoS or σ38. HU stimulates the translation of rpoS mRNA and thus indirectly influences the expression of the large RpoS regulon [Balandina et al., 2001]. HU cooperates with DNA gyrase at specific sequence motifs in the E. coli chromosome called REP elements [Yang and Ames, 1990]. These elements are found at the ends of some open reading frames and may represent a means of positioning gyrase so that this type II topoisomerase can extinguish positive supercoils that are created by the trafficking of RNA polymerase along the open reading frames during transcription. HU seems to recognize and bind distortions in B-DNA such as the Holliday junctions that arise during recombination [Pontiggia et al., 1993]. The extraordinary abilities of HU to interact with DNA and RNA, and to act both as a structural and a regulatory element in the nucleoid should be considered in the context of its conservation across the prokaryotes. Mycoplasma genitalium possesses one of the smallest known self-replicating genome [Zhang and Baseman, 2011]. This organism has no transcription factors and has just one NAP: HU. Perhaps this is illustrative of a genome of unusual simplicity where DNA supercoiling (M. genitalium has a similar complement of topoisomerases to E. coli) and HU cooperate to manage simultaneously the nucleoid architectural and gene regulatory needs of the organism without contributions from a variety of sigma factors or any conventional transcription factors [Dorman, 2011].

The HU protein does not have a specific nucleotide sequence to which it prefers to bind. HU interacts with DNA through a mechanism that involves insertion of a β-ribbon into the minor groove of DNA, a mechanism that is reminiscent of the one used by the related protein IHF [Swinger and Rice, 2004] (fig. 1). Minor groove width is important for HU binding with the narrower groove found in A+T-rich DNA offering the better substrate for binding [Swinger and Rice, 2007]. The proteins differ in that IHF distorts the DNA to a greater extent than HU. Both proteins bend the sugar-phosphate backbone of DNA, but only IHF perturbs the bases at the DNA target, doing so within a sequence that matches the IHF consensus for DNA binding sites (fig. 1). In addition, once it has bound to DNA HU can recruit additional copies of the HU dimer through a cooperative binding mechanism, whereas IHF dimers act alone [Benevides et al., 2008].

### DNA Bending Specialist IHF

Early insights into IHF molecular biology came from investigations of its interactions with three high-affinity binding sites in bacteriophage lambda [Rice et al., 1996; Yang and Nash, 1989]. The heterodimeric IHF protein is composed of the products of the ihfA and the ihfB genes, and its cellular concentration seems to fluctuate during the growth cycle, achieving a maximum at the transition from exponential growth to stationary phase in E. coli cells growing in batch culture [Bushman et al., 1985]. It is also important in managing the transition from exponential growth to stationary phase in Pseudomonas putida [Silva-Rocha et al., 2013] and in Salmonella enterica [Mangan et al., 2006]. IHF is also involved in the cell cycle where it interacts with the DnaA protein to determine its position within the origin of chromosomal replication; HU also contributes to this process [Polaczek et al., 1998] but operates by a distinct mechanism [Ryan et al., 2002]. Although IHF is contrasted routinely with HU on the grounds of the DNA sequence specificity shown by the former in binding site selection, it is becoming apparent that IHF can also interact nonspecifically with DNA and has the potential to be even more involved in organizing the structure of the nucleoid than was thought hitherto [Ali et al., 2001; Lin et al., 2012].

The early work with lambda revealed that IHF contributed to phage site-specific recombination by functioning as an architectural element and that its primary role is to introduce bends of up to 180° at specific sites in the phage DNA so that a functional folded intasome is formed [Oppenheim et al., 2005]. The DNA bending function can be supplied by other, unrelated proteins once the preferred binding sites for those proteins had been substituted for the ones normally used by IHF [Goodman and Nash, 1989]. The phasing of the IHF-induced bends in the phage DNA is also crucial for efficient recombination [Snyder et al., 1989]. These insights continue to inform much of our understanding of what IHF does and how it does it at its many targets in the bacterial genome.

The contributions of IHF range far beyond lambda integration and excision. It is involved in other site-specific recombination systems [Corcoran and Dorman, 2009; Dorman and Higgins, 1987; Eisenstein et al., 1987], in transposition [Haniford, 2006; Makris et al., 1990; Saha et al., 2013], plasmid replication [Biek and Cohen, 1989; Fekete et al., 2006; Filutowicz and Appelt, 1988], and conjugation-mediated plasmid transfer [Karl et al., 2001; Wil-
liams and Schildbach, 2007]. IHF also makes many important contributions to transcription control (see below).

IHF affects transcription principally through its ability to bend DNA, just as DNA bending is usually at the heart of its contributions to other DNA transactions such as site-specific recombination or transposition [Engelhorn and Geiselmann, 1998; Goosen and van de Putte, 1995; Parekh and Hatfield, 1996]. DNA bending provides a means to introduce physical contact between distant segments of DNA or proteins that are bound to those segments of DNA. This mechanism is particularly important at enhancer-activated σ54-dependent promoters, where DNA bending by IHF can facilitate physical contact between a transcription activator bound at an upstream enhancer and RNA polymerase bound to the target promoter [Bertoni et al., 1998; Carmona and Magasanik, 1996;
H-NS, Genome Guardian and Transcription Silencer

The H-NS protein, encoded by the hns gene in the Ter macrodomains of E. coli and S. enterica, is expressed at all stages of growth and contributes both to the structure of the nucleoid [Dorman, 2013; Hardy and Cozzarelli, 2005; Hromockyj et al., 1992] and to the repression of the hns gene by H-NS is exerted tightly when the movement of the chromosome replication fork is arrested either genetically or by drug treatment, suggesting that hns transcription is sensitive to the progression of the bacterial cell cycle [Free and Dorman, 1995]. Expression of H-NS is further modulated negatively at a posttranscriptional level by the Hfq-dependent DsrA small regulatory RNA (sRNA), an sRNA molecule that is involved in the stimulation of translation of the mRNAs specifying expression of the stress and stationary phase sigma factor RpoS [Majdalani et al., 2005]. H-NS also binds directly to, and modifies the half-lives of, the DsrA and the RpoS mRNA molecules [Brescia et al., 2004]. These links to RpoS expression are likely to be of physiological importance in the global modification of the transcription profile of the cell as it undergoes growth phase transitions and responds to environmental stresses. H-NS is also active in influencing gene expression posttranscriptionally by targeting translation; it is an efficient RNA-binding protein and has been shown to modulate positively translation initiation efficiencies in mRNA molecules possessing poor translation initiation signals [Park et al., 2010].

H-NS has been implicated by chromosome conformation capture experiments, by super resolution imaging and by genetic studies as one of the architectural elements that determines nucleoid structure [Hardy and Cozzarelli, 2005; Wang et al., 2011]. The precise details of the structural contribution made by H-NS are still unclear and may be conditional on the growth and/or experimental conditions used immediately prior to the measurements [Caglieri et al., 2013; Wang et al., 2014]. H-NS has been classified as a ‘domainin’ protein, one that closes the 10- to 15-kb microdomains that contribute to the organization of the folded bacterial chromosome in the nucleoid [Hardy and Cozzarelli, 2005; Waldminghaus, 2014] (fig. 2). Thus, H-NS is likely to play a key role simultaneously in the shaping of the nucleoid and in determining the transcription profile of the cell in any given set of growth conditions [Dorman, 2013].

Single molecule studies have shown that H-NS can form bridges within and between DNA molecules [Dame et al., 2006], and this observation has been supported by atomic force microscopy work [Dame et al., 2000; Dame and Goosen, 2002; Maurer et al., 2009]. Bridging in vitro is conditional and is sensitive to magnesium cation concentrations. At 10 mM MgCl₂, bridging of DNA duplexes by H-NS is observed, whereas at lower concentrations of MgCl₂, H-NS coats the DNA and stiffens it without bridging [Liu et al., 2010]. Single molecule analyses of the 52%-identical H-NS paralogue StpA indicated that that protein too shows Mg²⁺-sensitive DNA bridging behaviour [Lim et al., 2012]. This is an interesting observation in light of the fact that StpA is an excellent RNA chaperone and has the ability to bridge RNA molecules [Rajkowitsch and Schroeder, 2007], suggesting that it might perform at the RNA level a role analogous to that performed by H-NS with DNA. Bridging lends itself to a facile explanation of microdomain formation wherein H-NS closes the chromosomal loops by binding DNA together in a bridged structure; it also provides a mechanism for transcription silencing wherein H-NS imprisons RNA polymerase in a looped structure closed by DNA-H-NS-DNA bridges, with the H-NS protein polymerizing in the space between the aligned DNA duplexes, bridging them together (fig. 2).

Both H-NS polymerization along a single DNA duplex and the bridging by H-NS of DNA duplexes lead to easy-to- appreciate models of transcription silencing in which RNA polymerase is either excluded from a promoter or held prisoner at a promoter, respectively (fig. 2) [Dame, 2014]. How are these silenced promoters to be activated? A survey of the literature reveals that the mechanisms of H-NS antagonism are legion, typically involving a remodelling of the repressive nucleoprotein complex such that H-NS tenure there becomes unsustainable [Stoebel et al., 2008]. Linking the remodelling to an environmental signal makes the anti-silencing mechanism physiologi-
cally responsive. This is achieved in some cases through an environmental-stress-mediated alteration to local DNA structure or, more typically, the activation of a DNA-binding protein whose intervention disrupts the H-NS nucleoprotein transcription-silencing complex [Kane and Dorman, 2011; Stoebel et al., 2008; Stonehouse et al., 2011; Walthers et al., 2011].

**Fig. 2.** H-NS, transcription silencing and chromosome microdomain formation. **a** A standard representation of RNA polymerase bound to a transcription promoter is shown consisting of the principal components of the holoenzyme: the α-subunit, in two copies with their carboxyl-terminal domains (CTD) and amino terminal domains (NTD) shown connected by flexible linkers. The β-, β′- and σ-subunits are also illustrated. The locations of the transcription start site (TSS, +1), the −10 and −35 elements are also shown together with the consensus DNA sequences for the −10 and −35 motifs of promoters that are bound by the RpoD sigma factor of RNA polymerase. **b** The same promoter sequence is shown decorated by the H-NS protein in its DNA stiffening mode, excluding RNA polymerase and silencing transcription. Here H-NS polymerizes along the DNA duplex, and the two DNA-binding motifs of each H-NS dimer bind to the same DNA molecule in cis. The H-NS monomers are arranged in an antiparallel orientation within each dimer. **c** H-NS is shown bound to the same promoter element in its bridging mode. Here, the DNA-binding domains of each H-NS dimer (shown in antiparallel configuration) bind to spatially widely separated segments of the same DNA molecule, creating a DNA-protein-DNA bridge that excludes RNA polymerase from the promoter. **d** The bridging function of H-NS can also form loops in DNA, including the 10- to 15-kb microdomain loops that contribute to the higher-order structure of the bacterial nucleoid. The drawings are not to scale.

**H-NS Family Proteins, Transcription and Genome Evolution**

Horizontal gene transfer (HGT) is one of the forces driving bacterial genome evolution, but acquiring novel genetic elements is a mixed blessing for the receiving cell. The newly acquired genes may confer new capabilities on
the host, but their (inappropriate) expression may also impair the competitive fitness of the bacterium, costing it its place in the ecosphere and putting at risk its survival. There is also the problem of the physical integration of the new genes into the genome, an aspect of HGT that has been little studied. Can new genes be placed at random into the chromosome, are there preferred locations for new arrivals or is it better to maintain the new genes on extrachromosomal element such as plasmids? There is a marked association between genes that have been acquired via HGT and genes that express transfer RNA (tRNA), with many pathogenicity islands being found adjacent to tRNA genes or operons [Dobrindt et al., 2002; Guo et al., 2014]. Recent discoveries concerning barriers to the free diffusion of proteins in the cytoplasm [Montero Llopis et al., 2010; Parry et al., 2014] and the finding that folding of the nucleoid brings together in space many genes that interact suggest that inserting new genes into the genome in a completely random fashion may not produce optimal gene–gene communication and regulatory integration [Berlatzky et al., 2008; Dorman, 2013; Janga et al., 2009; Jeong et al., 2012; Képès, 2004; Mathelier and Carbonne, 2010; Muskhelishvili, 2014; Wright et al., 2007; Xiao et al., 2011].

The principle mechanisms of HGT are conjugation (plasmid self-transmission or trans-acting plasmid mobilization), transformation (uptake of naked DNA, including plasmids, by cells) and transduction (uptake of bacterial viruses or bacteriophage by bacterial cells) [Dorman, 2009]. Some mobile genetic elements encode NAPs of their own [Dorman, 2014; Paytubi et al., 2014; Takeda et al., 2011], and the arrival in naive cells of large genetic elements such as A+T-rich high-molecular-mass plasmids can distort the H-NS-DNA balance, leading to a loss of competitive fitness [Dillon et al., 2010; Doyle et al., 2007]. The H-NS protein targets those horizontally acquired genetic elements whose DNA has a higher-than-average A+T content and intrinsic curvature [Bouffartigues et al., 2007; Lang et al., 2007]. This issue has been explored in some detail in the facultative intracellular pathogen S. enterica serovar Typhimurium, where a number of A+T-rich pathogenicity islands encode virulence factors that are essential for host cell invasion and host defence evasion [Lucchini et al., 2006; Navarre et al., 2006]. In all cases, the H-NS protein silences virulence gene transcription, leaving their expression to be activated by mechanisms that interfere with H-NS silencing activity [Stoebel et al., 2008]. A similar pattern of silencing and anti-silencing has been described in pathogens such as Shigella flexneri [Beloin and Dorman, 2003; Tran et al., 2011; Turner and Dorman, 2007], Vibrio cholerae [Stonehouse et al., 2011; Yu and DiRita, 2002], Yersinia spp. [Baños et al., 2008; El-lison and Miller, 2006], disease-causing strains of E. coli [Martínez-Santos et al., 2012; Trachman and Yasmin, 2004; Winardhi et al., 2014] and the plant pathogen Dickeya [Reverchon and Nasser, 2013]. In each case, the anti-silencing mechanism is triggered by an environmental signal, or a set of signals, that characterise the niche in the host where infection occurs [Rhen and Dorman, 2005; Stoebel et al., 2008]. Other NAPs, such as Hu, IHF and Fis play more or less well-characterized roles in the modulation of H-NS-mediated silencing in these pathogens, working in concert with conventional transcription factors to switch on virulence gene expression [Cameron et al., 2012; Falconi et al., 2001; Karamanoglov et al., 2011; Ouafa et al., 2012; Porter and Dorman, 1997; Schechter et al., 2003; Walthers et al., 2011; Winardhi et al., 2014]. Some pathogenic strains of E. coli encode H-NS paralogues such as H-NSB and Hfp that can confer new and subtle phenotypes on the bacterium. The genes for these proteins are found within horizontally acquired gene islands on the chromosome [Müller et al., 2010; Williamson and Free, 2005]. The locus of enterocyte effacement (LEE) is a pathogenicity island in enteropathogenic strains of E. coli that employs a protein called Ler to antagonize H-NS-mediated silencing of LEE genes [Abe et al., 2008; Bingle et al., 2014; García et al., 2012]. Ler has a DNA-binding domain that is similar to that of H-NS, but the proteins are otherwise non-identical. Ler competes with H-NS for access to a subset of H-NS target sites that includes those within the LEE island [Cordeiro et al., 2011; Winardhi et al., 2014].

Such horizontally acquired islands frequently contain vestiges of mobile genetic elements, suggesting a phage or plasmid origin [Williamson and Free, 2005]. Some self-transmissible plasmids encode a full-length orthologue of H-NS [Dorman, 2014; Doyle et al., 2007; Paytubi et al., 2014; Sherburne et al., 2000; Takeda et al., 2011] and these full-length H-NS-like proteins can form associations with DNA that are distinct from those formed by H-NS itself [Dillon et al., 2010; Fernandez-de-Alba et al., 2013]. These differences are thought to arise from properties of the linker domains of these proteins whose flexibility influences their capacity to form stable complexes with curved and non-curved DNA sequences [Fernandez-de-Alba et al., 2013].
Modulation of H-NS Activity by Hha-Like and H-NST-Like Proteins

H-NS activity is modulated by interaction with different NAPs that have varying degrees of amino acid sequence similarity to it. These are usually shorter proteins with sequence similarity to the parts of H-NS that are involved in dimerization and higher-order oligomerization. The prototypic member of this group is Hha, which is accompanied in model bacteria such as E. coli and Salmonella by its paralogue YdgT [Ali et al., 2013; Paytubi et al., 2014; Ueda et al., 2013]. Orthologues such as the YmoA protein from Yersinia have also been investigated as modulators of H-NS activity [Baños et al., 2008; McFeeters et al., 2007]. The genes for the chromosomally encoded Hha and YdgT proteins have counterparts on certain self-transmissible plasmids, with the IncHI1 plasmid R27 being the most intensively studied [Dorman, 2014; Paytubi et al., 2013; Takeda et al., 2011]. In many cases, these same plasmids encode an H-NS-like protein, leading to complex interactions among the chromosomally and plasmid-encoded proteins and H-NS targets in the genome [Dillon et al., 2010; Doyle et al., 2007; Paytubi et al., 2013; Takeda et al., 2011].

The H-NST group of proteins resembles superficially the Hha group in having some amino acid sequence similarity to the dimerization and oligomerization domain of H-NS but differs from the Hha-like proteins in having independent DNA binding activity [Levine et al., 2014; Williamson and Free, 2005]. Genes coding for proteins of the H-NST family have been found in horizontally acquired genomic islands in pathogenic strains of E. coli [Levine et al., 2014; Müller et al., 2010; Williamson and Free, 2005]. Whereas Hha probably affects H-NS activity through protein-protein interaction alone, with one Hha dimer forming a complex with one H-NS dimer [Ali et al., 2013], H-NST may be able both to form a complex with H-NS and to bind to DNA, perhaps displaying a wider range of activities in modulating H-NS-mediated gene silencing [Dorman, 2014].

The Fis Protein, the Great Integrator

Fis is the Factor for Inversion Stimulation [Johnson et al., 1986; Koch and Kahmann, 1986], a small DNA-binding protein consisting of four α-helices that exists as a homodimer in solution [Koch et al., 1991; Kostrewa et al., 1991, 1992]. Like most NAPs, the Fis protein is not essential for survival, yet it is involved in very many of the fundamental aspects of the life of the cell. These include the initiation of chromosomal DNA replication, transcription initiation, the expression of the translational machinery of the cell, transposition and site-specific recombination [Chintakayala et al., 2013; Gille et al., 1991; Hillebrand et al., 2005; Lei et al., 2007; Teras et al., 2009; Zhi et al., 2003].

The Fis protein exerts a global influence on the transcription profile of the cell and can have positive or negative effects on promoter activity [Grainger et al., 2008; Kahramanoglou et al., 2011; Kelly et al., 2004; Nilsson et al., 1990; Schnetz, 2008] (fig. 3). It is expressed at maximal concentrations as bacteria exit the lag phase of batch culture growth and enter the exponential growth phase [Ball et al., 1992; Keane and Dorman, 2003]. Fis concentrations then decline rapidly until the protein is almost undetectable by the stationary phase of growth. This expression pattern is sensitive to the degree of aeration of the culture and fis gene expression can be sustained into stationary phase under micro-aerobic growth conditions [Cameron et al., 2013; Ó Cróinín and Dorman, 2007]. Transcription of the fis gene is negatively autoregulated [Ball et al., 1992; Keane and Dorman, 2003; Ninnemann et al., 1992; Osuna et al., 1995] and is controlled by the stringent response [Mallik et al., 2004, 2006; Ninnemann et al., 1992], nucleotide concentrations [Walker et al., 2004] and negative supercoiling of the DNA [Schneider et al., 2000]. Stringent regulation links expression of fis to that of the genes that encode ribosomal components and other elements of the translational apparatus of the cell; Fis itself acts to stimulate the activities of the promoters of these same genes [Hillebrand et al., 2005; Lazarus and Travers, 1993; Opel et al., 2004; Zhi et al., 2003].

An important link exists between the Fis protein and the superhelical state of bacterial DNA [Cameron et al., 2011; Rochman et al., 2004] (fig. 3). Many of the promoters that Fis targets are sensitive to variations in DNA superhelical density, including the promoter of the fis gene [Schneider et al., 2000]. DNA is negatively supercoiled by DNA gyrase through an ATP-dependent double-stranded DNA cleavage and passage mechanism [Bates et al., 2011; Champoux, 2001]. The dependency of this reaction on ATP and its sensitivity to inhibition by ADP link gyrase-mediated supercoiling to the metabolic flux of the cell [Hsieh et al., 1991a, b; van Workum et al., 1996] making DNA supercoiling levels physiologically responsive [Cameron and Dorman, 2012; Dorman, 1991]. In rapidly growing bacteria, a higher [ATP]/[ADP] ratio results in DNA having a higher superhelical density (i.e. being more negatively supercoiled) than DNA in stationary...
phase cells [Bordes et al., 2003; Cameron et al., 2013; Dorman et al., 1988]. These fluctuations in DNA supercoiling have an important modulatory effect on transcription throughout the genome [Peter et al., 2004; Quinn et al., 2014; Sobetzko et al., 2012]. Fis exerts influence through its action as a transcription repressor of the gyrA and gyrB genes and through its ability to modulate the transcription of topA, the gene that encodes DNA topoisomerase I, an enzyme that relaxes negatively supercoiled DNA [Keane and Dorman, 2003; Schneider et al., 1999; Wein-
stein–Fischer and Altuvia, 2007] (fig. 3). Fis also acts as a topological buffer throughout the genome by attenuating the ability of gyrase and topoisomerase I to either over-supercoil or to over-relax the Fis-decorated DNA, respectively [Schneider et al., 1999]. The Fis protein can also play this buffering role at a local level at promoters where it has binding sites [Auner et al., 2003; Rochman et al., 2004].

The Fis protein has a preference for binding to A+T-rich DNA, and its interaction with DNA is affected by the width of the minor groove, something that is narrower in A+T-rich sequences [Hancock et al., 2013; Stella et al., 2010]. Fis bends the DNA at its binding site [Hübner et al., 1989; Verbeek et al., 1991], and this allows Fis to perform an architectural role in the genome with both local and global effects. Fis has also been identified in a genetic screen as a domainin, a protein that closes the looped minor groove [Hancock et al., 2013; Stella et al., 2010]. Fis bends the DNA at its binding site [Hübner et al., 1989; Verbeek et al., 1991], and this allows Fis to perform an architectural role in the genome with both local and global effects. Fis has also been identified in a genetic screen as a domainin, a protein that closes the looped minor groove [Hancock et al., 2013; Stella et al., 2010].

In the absence of environmental stress during exponential growth, the H-NS protein and Fis collaborate to repress RpoD-dependent transcription of dps. Here, each NAP performs a separate function: Fis traps the RpoD-containing form of RNA polymerase at the dps promoter while H-NS displaces RpoD-containing RNA polymerase but not RpoS-containing RNA polymerase from the promoter [Grainger et al., 2008]. In this way, Fis and RpoS link Dps expression to the growth phase of the culture, Dps and the End of Growth

In the NAP literature, it is common to see Dps (DNA binding protein from starved cells) and Fis contrasted in terms of the periods in the growth cycle when each appears: Fis expression is associated with the very early stages of exponential growth, whereas Dps is most abundant in stationary phase cultures [Dorman, 2013]. The Dps protein is a ferritin-like iron-binding protein that accumulates in stationary phase and is thought to protect the genomic DNA from chemical damage [Grant et al., 1998; Jeong et al., 2008; Wolf et al., 1999]. Dps expression can be triggered at other stages of growth by oxidative stress, an environmental insult that can damage DNA [Altuvia et al., 1994]. Manganese levels also control dps transcription in *E. coli*, and this is facilitated by the MntR transcription factor, which binds at the dps promoter, one of only a very few targets that are bound by this protein [Yamamoto et al., 2011]. Dps forms a complex with DNA that has crystalline properties, and the structure of this crystalline array may account for its protective properties [Frenkel-Krispin et al., 2004; Grant et al., 1998; Wolf et al., 1999]. Fis may indirectly disrupt Dps-DNA complexes through its regulatory effects on the transcription of the genes that encode DNA gyrase and DNA topoisomerase I (fig. 3), altering the topology of the chromosomal DNA in ways that compromise the stability of the Dps-DNA complexes [Ohnwa et al., 2006]. This would provide an attractive mechanism for resetting the cell when exponential growth restarts. Surprisingly for a protein that binds to and protects the entire genome, there are few data clearly linking Dps directly to the regulation of transcription. The *dps* gene is the target for an interesting transcription control circuit that involves other NAPs and conventional transcription factors. In exponentially growing bacteria, *dps* transcription is stimulated in response to oxidative stress by the OxyR transcription factor through a mechanism that targets RNA polymerase containing the RpoD sigma factor; in stationary phase, *dps* expression is dependent on the stress-and-stationary-phase sigma factor RpoS and the NAP IHF [Altuvia et al., 1994]. Dps sequesters and oxidizes Fe^{2+}, preventing the generation of free radicals that could damage DNA. In addition, the appearance of Dps in exponential growth reduces the number of initiations of chromosome replication in the bacterial population through a mechanism in which Dps interacts with the DnaA protein to block DNA duplex opening at the origin of replication, oriC [Chodavarapu et al., 2008b].

In the absence of environmental stress during exponential growth, the H-NS protein and Fis collaborate to repress RpoD-dependent transcription of *dps*. Here, each NAP performs a separate function: Fis traps the RpoD-containing form of RNA polymerase at the *dps* promoter while H-NS displaces RpoD-containing RNA polymerase but not RpoS-containing RNA polymerase from the promoter [Grainger et al., 2008]. In this way, Fis and RpoS link Dps expression to the growth phase of the culture,
with Fis acting as a proxy for early exponential growth phase and downregulation of *dps* combined with the presence of RpoS signalling cessation of growth and the onset of stationary phase.

**Perspective**

The important roles that NAPs play in the lives of bacterial cells have become much better understood in recent years as more and more advanced methods have been employed to study them. Interdisciplinary approaches relying on insights from biophysics, computational biology, mechanobiology, sophisticated imaging methods and whole-genome molecular biology are bringing us closer to a fully integrated picture of the nucleoid within the context of the living bacterial cell. This picture will enhance our ability to manipulate microorganisms to our benefit. It will also provide blueprints from successful natural living cells that can be applied in the quest to build synthetic ones for specific beneficial purposes.

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NAPs and Chromosome Structuring and Transcriptional Regulation

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