Function of nucleoid-associated proteins in chromosome structuring and transcriptional regulation

Charles J Dorman

Department of Microbiology
Moyne Institute of Preventive Medicine
Trinity College Dublin
Dublin 2
Ireland

Tel +353 1 896 2013

Email cjdorman@tcd.ie

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 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., 2013; Dorman, 2013; Espéli and Boccard, 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; Higgins et al., 1988; Spears et al., 1986). It is now understood that H-NS (encoded by *hns*) binds to hundreds of targets in the genome and affects the expression of hundreds of genes at the level of transcription, usually negatively (Dillon et al., 2010; Lucchini et a., 2006; Navarre et al., 2006; Oshima et al., 2006). Similarly, the integration host factor (IHF) is an important NAP that was originally identified as a protein encoded by *E. coli* that facilitates both the integration and the excision of bacteriophage lambda by site-specific recombination at the lambda attachment site on the bacterial chromosome (Oppenheim et al., 2005). Subsequently IHF was found to influence the expression of large numbers of bacterial genes and other DNA transactions in addition to lambda integration and excision (Dorman and Higgins, 1987; Eisenstein et al., 1987; Mangan et al., 2006; Silva-Rocha et al., 2013). This article

will consider those NAPs that have been studied in greatest detail both from the standpoint of chromosome structuring and as participants in gene regulation.

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 $\alpha\beta$ 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 (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

105 (Becker et al., 2007; Czapla et al., 2013; Merickel & Johnson, 2004; Semsey et al.,
106 2006).

107 The Kar and Adhya laboratories have isolated a mutant derivative of HUα
108 that introduces positive supercoils into DNA (Guo and Adhya, 2007; Kar et al.,
109 2006; Koli et al., 2011). The mutant protein contains just two amino acid
110 substitutions (E38K and V42L) but the resulting changes to global DNA topology
111 in the *E. coli* cells that express it causes a number of infection-related cryptic
112 genes to become expressed. This may be the basis of a life-style switch where
113 commensal *E. coli* becomes pathogenic (Koli et al., 2011).

114 Wildtype HU has a significant impact on gene expression at both the
115 transcriptional and posttranscriptional levels (Mangan et al., 2011; Oberto et al.,
116 2009; Prieto et al., 2012), the latter reflecting its RNA binding activity (Balandina
117 et al., 2001; Macvanin et al., 2012); HU also possesses efficient single-stranded
118 DNA binding activity (Kamashev et al., 2008). Thus HU is a highly versatile
119 protein, acting in an architectural mode to impose structure on the nucleoid
120 while simultaneously influencing transcription and translation. Among its
121 targets is *rpoS,* the gene that encodes the stationary phase and stress sigma
122 factor of RNA polymerase, RpoS or sigma-38. HU stimulates the translation of
123 *rpoS* mRNA and thus indirectly influences the expression of the large RpoS
124 regulon (Balandina et al., 2001). HU cooperates with DNA gyrase at specific
125 sequence motifs in the *E. coli* chromosome called REP elements (Yang and Ames,
126 1990). These elements are found at the ends of some open reading frames and
127 may represent a means of positioning gyrase so that this type II topoisomerase
128 can extinguish positive supercoils that are created by the trafficking of RNA
129 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 beta ribbon into the minor groove of DNA, a mechanism that is reminiscent of the one used by the related protein integration host factor (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 chromosome replication; HU also contributes to this process (Polaczek et al., 1998) but operates by a distinct mechanism (Ryan et al., 2004). 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 non-specifically 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

180 by IHF (Goodman and Nash, 1989). The phasing of the IHF-induced bends in the
181 phage DNA is also crucial for efficient recombination (Snyder et al., 1989). These
182 insights continue to inform much of our understanding of what IHF does and
183 how it does it at its many targets in the bacterial genome.
184 The contributions of IHF range far beyond lambda integration and
185 excision. It is involved in other site-specific recombination systems (Corcoran
186 and Dorman, 2009; Dorman and Higgins, 1987; Eisenstein et al., 1987), in
187 transposition (Haniford, 2006; Makris et al., 1990; Saha et al., 2013), plasmid
188 replication (Biek and Cohen, 1989; Fekete et al., 2006; Filutowicz and Appelt,
189 1988), and conjugation-mediated plasmid transfer (Karl et al., 2001; Williams
190 and Schildbach, 2007). IHF also makes many important contributions to
191 transcription control (see below).
192 IHF affects transcription principally through its ability to bend DNA, just
193 as DNA bending is usually at the heart of its contributions to other DNA
194 transactions such as site-specific recombination or transposition (Engelhorn and
195 Geiselmann, 1998; Goosen and van de Putte, 1995; Parekh and Hatfield, 1996).
196 DNA bending provides a means to introduce physical contact between distant
197 segments of DNA or proteins that are bound to those segments of DNA. This
198 mechanism is particularly important at enhancer-activated sigma-54-dependent
199 promoters, where DNA bending by IHF can facilitate physical contact between a
200 transcription activator bound at an upstream enhancer and RNA polymerase
201 bound to the target promoter (Bertoni et al., 1998; Carmona and Magasanik,
202 1996; Shingler, 2011) (Fig. 1A). The relationship is not necessarily positive in all
203 cases and there are examples where IHF activity can be both positive and
204 negative (Wassem et al., 2000).

205

206 **H-NS, genome guardian and transcription silencer**

207 The H-NS protein, encoded by the *hns* gene in the Ter macrodomains of *E. coli*
208 and *S. enterica,* is expressed at all stages of growth and contributes both to the
209 structure of the nucleoid (Dorman, 2013; Hardy and Cozzarelli, 2005) and to the
210 repression of hundreds of genes (Dillon et al., 2010; Kahramanoglou et al., 2011;
211 Lucchini et a., 2006; Navarre et al., 2006; Oshima et al., 2006). The *hns* gene is
212 negatively autoregulated and is under the positive control of the Fis NAP, with
213 Fis antagonizing H-NS-mediated repression (Falconi et al., 1993; 1996).
214 Transcription of *hns* is also sensitive to the iron regulator protein Fur (Troxell et
215 al., 2011) and to the cold shock protein CspA (Brandi et al., 1994; La Teana et al.,
216 1991). In the plant pathogen *Dickeya dadantii,* the PecS protein is a regulator of
217 *hns* expression (Reverchon and Nasser, 2013). Autorepression of *hns* by H-NS is
218 exerted tightly when the movement of the chromosome replication fork is
219 arrested either genetically or by drug treatment, suggesting that *hns*
220 transcription is sensitive to the progression of the bacterial cell cycle (Free and
221 Dorman, 1995). Expression of H-NS is further modulated negatively at a
222 posttranscriptional level by the Hfq-dependent DsrA small regulatory RNA
223 (sRNA), an sRNA molecule that is involved in the stimulation of translation of the
224 mRNA specifying expression of the stress and stationary phase sigma factor
225 RpoS (Majdalani et al., 2005). H-NS also binds directly to, and modifies the half-
226 lives of, the DsrA and the RpoS mRNA molecules (Brescia et al., 2004). These
227 links to RpoS expression are likely to be of physiological importance in the global
228 modification of the transcription profile of the cell as it undergoes growth phase
229 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 (Cagliero 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 $MgCl_2$, bridging of DNA duplexes by H-NS is observed whereas at lower concentrations of $MgCl_2$ 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

255 too shows Mg^{2+}-sensitive DNA bridging behaviour (Lim et al., 2012). This is an
256 interesting observation in light of the fact that StpA is an excellent RNA
257 chaperone and has the ability to bridge RNA molecules (Rajkowitsch and
258 Schroeder, 2007) suggesting that it might perform at the RNA level a role
259 analogous to that performed by H-NS with DNA. Bridging lends itself to a facile
260 explanation of microdomain formation wherein H-NS closes the chromosomal
261 loops by binding DNA together in a bridged structure; it also provides a
262 mechanism for transcription silencing wherein H-NS imprisons RNA polymerase
263 in a looped structure closed by DNA-H-NS-DNA bridges, with the H-NS protein
264 polymerizing in the space between the aligned DNA duplexes, bridging them
265 together (Fig. 2).

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

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., 2004; Junier et al., 2012; Kepes, 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

305 viruses or bacteriophage by bacterial cells) (Dorman, 2009). Some mobile
306 genetic elements encode NAPs of their own (Dorman, 2014; Paytubi et al., 2014;
307 Takeda et al., 2011) and the arrival in naïve cells of large genetic elements such
308 as A+T-rich high-molecular-mass plasmids can distort the H-NS-DNA balance,
309 leading to a loss of competitive fitness (Dillon et al., 2010; Doyle et al., 2007). The
310 H-NS protein targets those horizontally acquired genetic elements whose DNA
311 has a higher-than-average A+T content and intrinsic curvature (Bouffartigues et
312 al., 2007; Lang et al., 2007). This issue has been explored in some detail in the
313 facultative intracellular pathogen *Salmonella enterica* serovar Typhimurium
314 where a number of A+T-rich pathogenicity islands encode virulence factors that
315 are essential for host cell invasion and host defence evasion (Lucchini et al.,
316 2006; Navarre et al., 2006). In all cases, the H-NS protein silences virulence gene
317 transcription, leaving their expression to be activated by mechanisms that
318 interfere with H-NS silencing activity (Stoebel et al., 2008). A similar pattern of
319 silencing and anti-silencing has been described in pathogens such as *Shigella*
320 *flexneri* (Beloin and Dorman, 2003; Tran et al., 2011; Turner and Dorman, 2007),
321 *Vibrio cholerae* (Stonehouse et al., 2011; Yu and DiRita, 2002), *Yersinia* spp.
322 (Baños et al., 2008; Ellison and Miller, 2006), disease-causing strains of *E. coli*
323 (Martínez-Santos et al., 2012; Trachman and Yasmin, 2004; Winardhi et al.,
324 2014) and the plant pathogen *Dickeya* (Reverchon and Nasser, 2013). In each
325 case the anti-silencing mechanism is triggered by an environmental signal, or a
326 set of signals, that characterise the niche in the host where infection occurs
327 (Rhen and Dorman, 2005; Stoebel et al., 2008). Other NAPs, such as HU, IHF and
328 Fis play more-or-less well-characterized roles in the modulation of H-NS-
329 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; Kahramanoglou 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; Garcia 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., 2004; 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 alpha 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;

405 Keane and Dorman, 2003; Ninnemann et al., 1992; Osuna et al., 1995) and is
406 controlled by the stringent response (Mallik et al., 2004; 2006; Ninnemann et al.,
407 1992), nucleotide concentrations (Walker et al., 2004) and negative supercoiling
408 of the DNA (Schneider et al., 2000). Stringent regulation links expression of *fis* to
409 that of the genes that encode ribosomal components and other elements of the
410 translational apparatus of the cell; Fis itself acts to stimulate the activities of the
411 promoters of these same genes (Hillebrand et al., 2005; Lazarus and Travers,
412 1993; Opel et al., 2004; Zhi et al., 2003).

413 An important link exists between the Fis protein and the superhelical
414 state of bacterial DNA (Cameron et al., 2011; Rochman et al., 2004) (Fig. 3). Many
415 of the promoters that Fis targets are sensitive to variations in DNA superhelical
416 density, including the promoter of the *fis* gene (Schneider et al., 2000). DNA is
417 negatively supercoiled by DNA gyrase through an ATP-dependent double-
418 stranded DNA cleavage and passage mechanism (Bates et al., 2011; Champoux,
419 2001). The dependency of this reaction on ATP, and its sensitivity to inhibition
420 by ADP links gyrase-mediated supercoiling to the metabolic flux of the cell
421 (Hsieh et al., 1991a; 1991b; van Workum et al., 1996) making DNA supercoiling
422 levels physiologically responsive (Cameron and Dorman, 2012; Dorman, 1991).
423 In rapidly growing bacteria, a higher [ATP]/[ADP] ratio results in DNA having a
424 higher superhelical density (i.e. being more negatively supercoiled) than DNA in
425 stationary phase cells (Bordes et al., 2003; Cameron et al., 2013; Dorman et al.,
426 1988). These fluctuations in DNA supercoiling have an important modulatory
427 effect on transcription throughout the genome (Peter et al., 2014; Quinn et al.,
428 2014; Sobetzko et al., 2012). Fis exerts influence through its action as a
429 transcription repressor of the *gyrA* and *gyrB* genes and through its ability to

430 modulate the transcription of *topA,* the gene that encodes DNA topoisomerase I,
431 an enzyme that relaxes negatively supercoiled DNA (Keane and Dorman, 2003;
432 Schneider et al., 1999; Weinstein-Fischer and Altuvia, 2007) (Fig. 3). Fis also acts
433 as a topological buffer throughout the genome by attenuating the ability of
434 gyrase and topoisomerase I to either over-supercoil or to over-relax the Fis-
435 decorated DNA, respectively (Schneider et al., 1999). The Fis protein can also
436 play this buffering role at a local level at promoters where it has binding sites
437 (Auner et al., 2003; Rochman et al., 2004).

438 The Fis protein has a preference for binding to A+T-rich DNA and its
439 interaction with DNA is affected by the width of the minor groove, something
440 that is narrower in A+T-rich sequences (Hancock et al., 2013; Stella et al., 2010).
441 Fis bends the DNA at its binding site (Hübner et al., 1989; Verbeek et al., 1991)
442 and this allows Fis to perform an architectural role in the genome with both local
443 and global effects. Fis has also been identified in a genetic screen as a domainin, a
444 protein that closes the looped microdomains of *E. coli* (Hardy and Cozzarelli,
445 2005). The preference of Fis for A+T-rich DNA allows it to target the many A+T-
446 rich genes that are found among the virulence operons and regulons of
447 pathogens. In many cases Fis has been shown to antagonize the silencing of these
448 genes by H-NS, often working in association with conventional regulatory
449 proteins that transmit a specific environmental signal to control virulence gene
450 expression under growth conditions that are relevant to infection (Cameron and
451 Dorman, 2012; Duprey et al., 2014; Falconi et al., 2001; Goldberg et al., 2001;
452 Kelly et al., 2004; Labandeira-Rey et al., 2013; Ó Cróinín et al., 2006; Prosseda et
453 al., 2004). In this way Fis activity represents an important integrating principle
454 in the genome, acting both as a structural element that helps to organize the

nucleoid and as a global regulator that ties together the control of both housekeeping genes and genes involved in specialist, infection-related functions. In addition, it connects these functions to the cell cycle and to genome maintenance. Although knockout mutations in the *fis* gene are tolerated, a mutant deprived of the Fis protein fails to compete with its otherwise isogenic ancestor (Schneider et al., 1999).

Dps and the end of growth

In the NAP literature it is common to see Dps 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 (DNA binding protein from starved cells) 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 (Frenkiel-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

480 of the chromosomal DNA in ways that compromise the stability of the Dps-DNA
481 complexes (Ohniwa et al., 2006). This would provide an attractive mechanism
482 for resetting the cell when exponential growth restarts. Surprisingly for a protein
483 that binds to and protects the entire genome, there are few data clearly linking
484 Dps directly to the regulation of transcription. The *dps* gene is the target for an
485 interesting transcription control circuit that involves other NAPs and
486 conventional transcription factors. In exponentially growing bacteria, *dps*
487 transcription is stimulated in response to oxidative stress by the OxyR
488 transcription factor through a mechanism that targets RNA polymerase
489 containing the RpoD sigma factor; in stationary phase, *dps* expression is
490 dependent on the stress-and-stationary-phase sigma factor RpoS and the NAP
491 IHF (Altuvia et al., 1994). Dps sequesters and oxidizes Fe^{2+}, preventing the
492 generation of free radicals that could damage DNA. In addition, the appearance
493 of Dps in exponential growth reduces the number of initiations of chromosome
494 replication in the bacterial population through a mechanism in which Dps
495 interacts with the DnaA protein to block DNA duplex opening at the origin of
496 replication, *oriC* (Chodavarapu et al., 2008b).
497 In the absence of environmental stress during exponential growth, the H-
498 NS protein and Fis collaborate to repress RpoD-dependent transcription of *dps*.
499 Here, each NAP performs a separate function: Fis traps the RpoD-containing
500 form of RNA polymerase at the *dps* promoter while H-NS displaces RpoD-
501 containing RNA polymerase but not RpoS-containing RNA polymerase from the
502 promoter (Grainger et al., 2008). In this way, Fis and RpoS link Dps expression to
503 the growth phase of the culture, with Fis acting as a proxy for early exponential

504 growth phase and down-regulation of *dps* combined with the presence of RpoS
505 signalling cessation of growth and the onset of stationary phase.
506
507 **Perspective**
508 The important roles that NAPs play in the lives of bacterial cells have become
509 much better understood in recent years as more and more advanced methods
510 have been employed to study them. Interdisciplinary approaches relying on
511 insights from biophysics, computational biology, mechanobiology, sophisticated
512 imaging methods and whole-genome molecular biology are bringing us closer to
513 a fully integrated picture of the nucleoid within the context of the living bacterial
514 cell. This picture will enhance our ability to manipulate microorganisms to our
515 benefit. It will also provide blueprints from successful natural living cells that
516 can be applied in the quest to build synthetic ones for specific beneficial
517 purposes.
518
519 **Acknowledgement**
520 Work on nucleoid-associated proteins in the author's laboratory has been
521 supported by research grants from Science Foundation Ireland.

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Figures

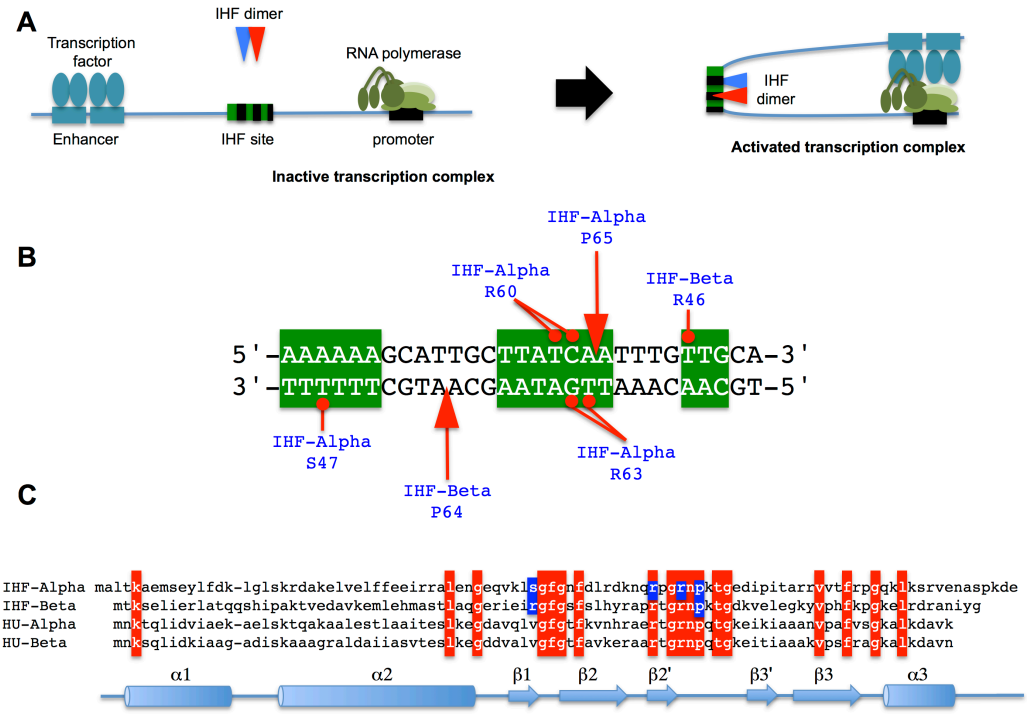
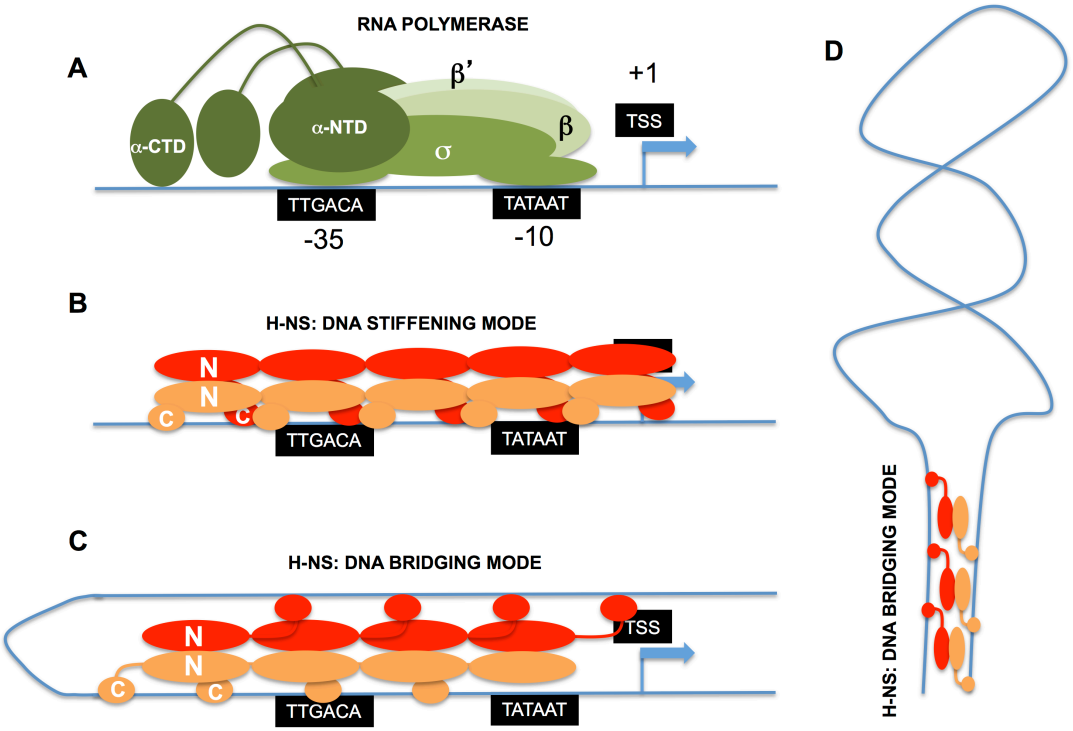


Fig. 1. IHF and its paralogue HU. (A) The interaction of IHF with its target site and the consequences for the pathway of the DNA are shown. Here RNA polymerase (containing sigma-54) has formed an inactive complex with a promoter and the bending of the DNA by IHF causes a transcription factor, bound as two dimers to two copies of the upstream-located enhancer sequence, to make physical contact with RNA polymerase, activating transcription. Not to scale. (B) The details of the IHF site sequence are shown, with the highlighted residues being the conserved members of the IHF binding site consensus. The amino acids in the alpha and the beta subunits of IHF that interact with the DNA sequence are shown. In the cases of proline residues P65 (alpha subunit) and P64 (beta subunit) the protein makes an insertion into the minor groove of the DNA duplex, bending it by up to 180˚. (C) An alignment of the alpha and beta

subunits of the paralogous IHF and HU proteins from *E. coli* strain W3110 is shown together with a summary of the main structural features of each monomer. Amino acids that are completely conserved in all four proteins are highlighted. The information in (B) and (C) is based on data from Swinger and Rice (2004; 2007). The NCBI reference numbers for the four protein sequences are: IHF alpha, YP_489974.1; IHF beta, YP_489184.1; HU alpha, YP_491460.1; HU beta, YP_488732.1.



1150

1151 **Fig. 2.** H-NS, transcription silencing and chromosome microdomain formation.

1152 (A) A standard representation of RNA polymerase bound to a transcription

1153 promoter is shown, consisting of the principal components of the holoenzyme:

1154 the alpha subunit, in two copies with their carboxyl-terminal domains (CTD) and

1155 amino terminal domains (NTD) shown connected by flexible linkers. The beta,

1156 beta prime and sigma subunits are also illustrated. The locations of the

1157 transcription start site (TSS, +1), the -10 and -35 elements are also shown

1158 together with the consensus DNA sequences for the -10 and -35 motifs of

1159 promoters that are bound by the RpoD sigma factor of RNA polymerase. (B) The

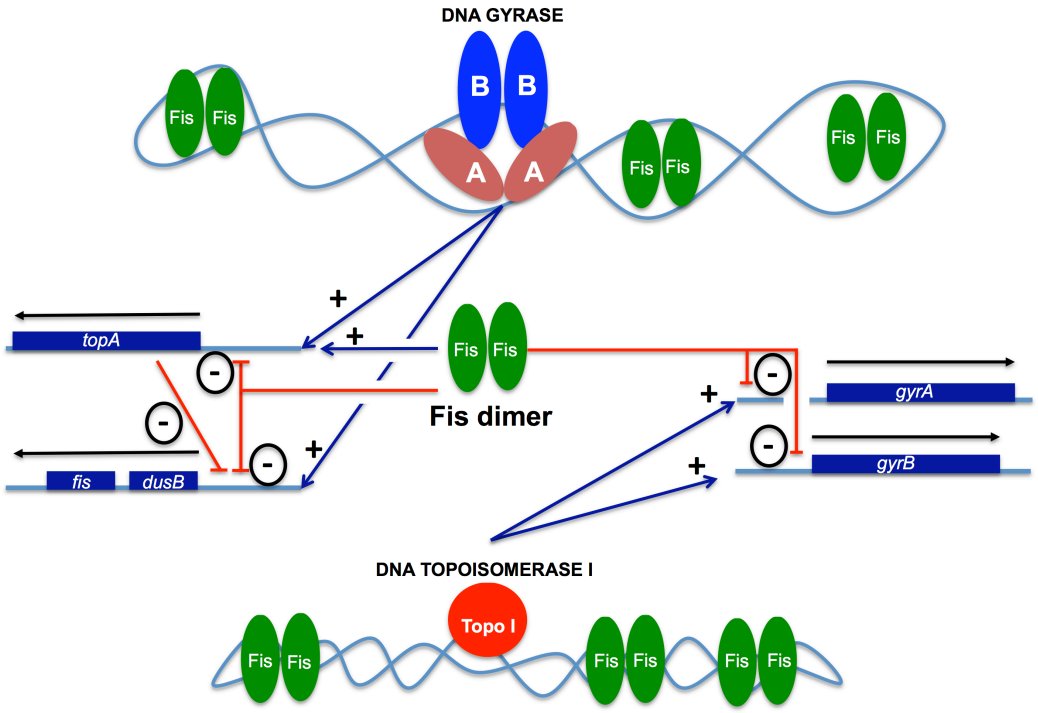
1160 same promoter sequence is shown decorated by the H-NS protein in its DNA

1161 stiffening mode, excluding RNA polymerase and silencing transcription. Here H-

1162 NS polymerizes along the DNA duplex and the two DNA binding motifs of each H-

1163 NS dimer bind to the same DNA molecule in *cis*. The H-NS monomers are

1164 arranged in an antiparallel orientation within each dimer. (C) H-NS is shown
1165 bound to the same promoter element in its bridging mode. Here the DNA binding
1166 domains of each H-NS dimer (shown in antiparallel configuration) bind to
1167 spatially widely-separated segments of the same DNA molecule, creating a DNA-
1168 protein-DNA bridge that excludes RNA polymerase from the promoter. (D) The
1169 bridging function of H-NS can also form loops in DNA, including the 10-to-15-kb
1170 microdomain loops that contribute to the higher-order structure of the bacterial
1171 nucleoid. The drawings are not to scale.
1172



1173

1174 **Fig. 3.** The Fis protein and the management of DNA supercoiling in the bacterial
1175 genome. Fis is a repressor of transcription at the *gyrA* and *gyrB* genes that
1176 encode the alpha and beta subunits of DNA gyrase. Gyrase uses energy from ATP
1177 hydrolysis to supercoil DNA negatively and this stimulates the promoters of the
1178 *dusB-fis* operon and *topA,* the gene that encodes DNA topoisomerase I.
1179 Topoisomerase I in turn relaxes negatively-supercoiled DNA and this stimulates
1180 the transcription of *gyrA* and *gyrB* while down-regulating transcription of *topA*
1181 and the *dusB-fis* operon. These interactions create a homeostatic balance in the
1182 cell, keeping global DNA superhelicity within limits that are beneficial for the
1183 cell. Fis acts as a dual-functional transcriptional regulator at *topA* where it uses
1184 alternative binding sites to activate or to repress *topA* transcription depending
1185 on the stage of growth and the nature of the environmental stresses being
1186 experienced by the cell (Weinstein-Fischer and Altuvia, 2007). Fis also

1187 modulates the global level of DNA supercoiling through its many interactions
1188 with the chromosome, limiting the degree to which gyrase and topoisomerase I
1189 can alter the linking number of the DNA. The dependence of gyrase on the ratio
1190 of the concentrations of ATP to ADP and the sensitivity of the *dusB-fis* promoter
1191 to metabolic flux in the cell through its stringent response control connects DNA
1192 supercoiling levels to cellular physiology. Rapidly growing cells have high levels
1193 of Fis, a high [ATP]/[ADP] ratio and DNA that is negatively supercoiled,
1194 conditions that favour a large subset of genes; cells entering stationary phase
1195 have few molecules of Fis, low [ATP]/[ADP] levels and DNA that is relaxed,
1196 favouring the expression of an alternative set of genes, but with some overlap
1197 with the exponential phase group. This provides the cell with the basis of a far-
1198 reaching command and control system for the governance of its transcription
1199 programme. The drawings are not to scale.