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H-NS and RNA polymerase: a love–hate relationship?

Robert Landick[2,3], Joseph T Wade[4,5] and David C Grainger[1]

Histone-like nucleoid structuring (H-NS) protein is a component of bacterial chromatin and influences gene expression both locally and on a global scale. Although H-NS is broadly considered a silencer of transcription, the mechanisms by which H-NS inhibits gene expression remain poorly understood. Here we discuss recent advances in the context of a 'love–hate' relationship between H-NS and RNA polymerase, in which these factors recognise similar DNA sequences but interfere with each other's activity. Understanding the complex relationship between H-NS and RNA polymerase may unite the multiple models that have been proposed to describe gene silencing by H-NS.

Addresses
[1] Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[2] Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA
[3] Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA
[4] Wadsworth Center, New York State Department of Health, Albany, NY 12208, USA
[5] Department of Biomedical Sciences, University at Albany, State University of New York, Albany, 12201, USA

Corresponding authors: Landick, Robert (landick@bact.wisc.edu), Wade, Joseph T (joseph.wade@health.ny.gov) and Grainger, David C (d.grainger@bham.ac.uk)

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Introduction

All life forms must organise their chromosomes within the confines of the cell or its compartments. This requires that DNA is folded, so it can be stored in a confined space, whilst simultaneously remaining accessible, so that the genetic code can be utilised. In eukaryotes, histone proteins interact with DNA to form nucleosomes [1]. The nucleosomes are further folded into chromatin fibres [2]. Our understanding of nucleosome formation at the molecular level is well developed. Furthermore, it is known that i) nucleosomes can impede access of cellular machinery to genes and ii) that this process is carefully regulated by the cell [2]. In prokaryotes, where DNA is folded into a structure known as the nucleoid, mechanisms of chromosome folding are also best understood at the molecular level; a group of 'nucleoid-associated' DNA-binding proteins impose constraints on DNA topology [3]. The effects on other DNA transactions are complex and poorly defined.

The Histone-like nucleoid structuring (H-NS) factor is a 15.5 kDa protein found in the bacterium *Escherichia coli* and its close relatives [4,5]. Initially referred to as protein H1, H-NS was first isolated on the basis of its propensity to bind DNA [6,7]. Subsequent studies showed that this activity was biased towards AT-rich sequences [8]. The intracellular abundance of H-NS, alongside its DNA binding properties, immediately suggested a role in DNA organisation and the regulation of gene expression [5]. Thus began decades of painstaking research into these distinct functions. What followed revealed that H-NS plays diverse roles in bacterial cells, including not only the control of gene expression and DNA folding, but also the facilitation of bacterial genome evolution [4,5,9,10••]. The generally accepted view is that H-NS binds to several hundred high-affinity nucleation sites dispersed across the chromosome before oligomerising across AT-rich DNA segments to exert its various effects. In many cases, these effects are intertwined and manifest themselves at the same genomic loci. For example, the *E. coli* K-12 *ygeH* gene is located within a remnant of a horizontally acquired pathogenicity island. Such regions are frequently silenced by H-NS, reducing their toxicity and facilitating genome evolution [10••]. However, in the case of *ygeH*, H-NS binding not only results in transcriptional repression but also co-localisation of this genomic locus with other H-NS-bound regions of the chromosome [11].

Over the past three decades it has become apparent that H-NS is part of a family of proteins with similar properties [5]. In *E. coli*, 'H-NS-like' proteins such as StpA, Hha, YdgT and Ler have now been characterised. These proteins generally interact with H-NS and modulate its DNA binding or oligomerisation properties. For instance, recent structural work has shown that Hha binds to the H-NS oligomerisation domain and alters the ability of H-NS to regulate a subset of genes, possibly by influencing the DNA binding activity of H-NS via modified oligomerisation [12,13]. Orthologs of H-NS have been identified in diverse bacteria, including *Bacillus subtilis* (Rok), *Pseudomonas aeruginosa* (MvaT), *Burkholderia vietnamiensis* (Bv3F) and *Mycobacterium tuberculosis* (Lsr2) [14–17]. In Gram-positive bacteria and mycobacteria, functional analogues

of H-NS exhibit similar DNA binding properties despite only partial structural similarity and the absence of sequence similarity [16••]. These divergent factors have only been identified recently; their discovery suggests that H-NS-like proteins may be a near universal feature of bacterial chromatin.

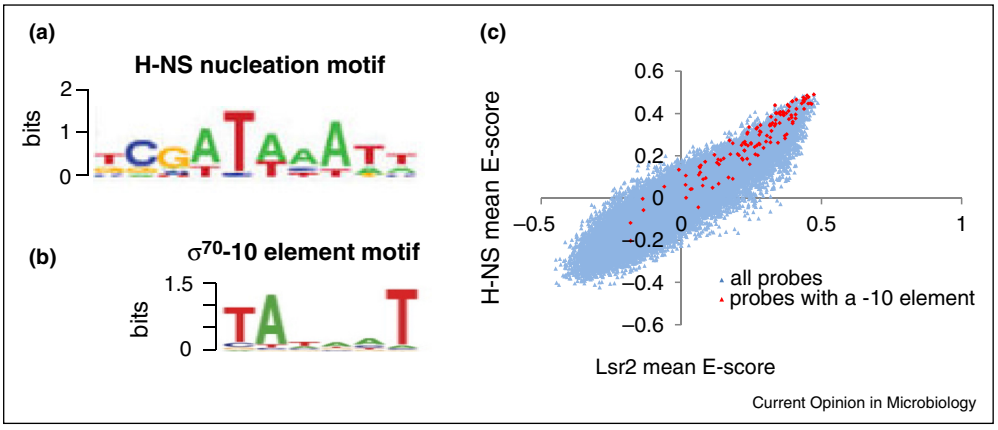
Although the influence of H-NS on DNA folding, gene expression, and horizontal gene transfer is broadly accepted, the molecular mechanism by which H-NS exerts these effects remains controversial. Multiple models purport to explain H-NS function, and gaping holes in our knowledge are still evident. For example, it is unclear if H-NS represses transcription by occluding RNA polymerase targets or by trapping RNA polymerase in unproductive complexes [18–20]. Furthermore, the toxic effects of AT-rich DNA, and the mechanism by which H-NS negates this toxicity, remain only partially defined. In this review, we focus on the 'love–hate' relationship that exists between H-NS and RNA polymerase and the need for a better understanding of their uneasy partnership. In particular, we discuss reasons why both H-NS and RNA polymerase have a propensity to bind (i.e. a 'love' for) AT-rich DNA and why H-NS-bound AT-rich DNA 'hates' to be transcribed.

DNA recognition by H-NS and RNA polymerase: a shared 'love' for AT-rich DNA

Recent structural work has unveiled the precise organisation of the H-NS, Lsr2 and Bv3F DNA binding determinants [16••]. Strikingly, although these proteins have a different overall configuration, a surface exposed loop in all three factors adopts an almost identical conformation. This loop contains a conserved Q/RGR amino acid motif that is essential for DNA binding [16••]. The first (Q/R) and last (R) side chains of the motif dock with the DNA minor grove. This docking is facilitated by the narrowing of the minor grove associated with AT-rich DNA. However, minor grooves that are too narrow (e.g. those in A-tract DNA) are sub-optimal targets [16••]. Studies of the Ler protein support this view [21]. In complementary genomic experiments, Gordon and co-workers examined the DNA sequence specificity of H-NS and Lsr2 by measuring binding to a double-stranded DNA oligonucleotide microarray [16••]. The microarrays consisted of 32 896 possible 8-mer sequences, each represented multiple times on the array. For each 8-mer sequence an 'E-score' was determined. The E-score describes the ranking of probes containing a particular 8-mer, relative to all other probes, upon protein binding. Put simplistically, the E-score represents the binding preference of H-NS or Lsr2 for a given 8-base sequence. This analysis revealed very similar DNA-binding specificities for H-NS and Lsr2. Moreover, the results agreed with previous work proposing that the best nucleation sites for H-NS contain a central T–A step and lack extended A-tract or T-tract sequences [22–24]. Lang and co-workers had previously derived a DNA sequence logo for H-NS nucleation (Figure 1a) on the basis of *in vitro* DNA footprinting analysis and chromosome-wide analysis of H-NS binding [22,23]. Whilst this logo may not represent the only sequence that H-NS can recognise with a high affinity, it further supports the requirement for a T–A step at H-NS nucleation sites.

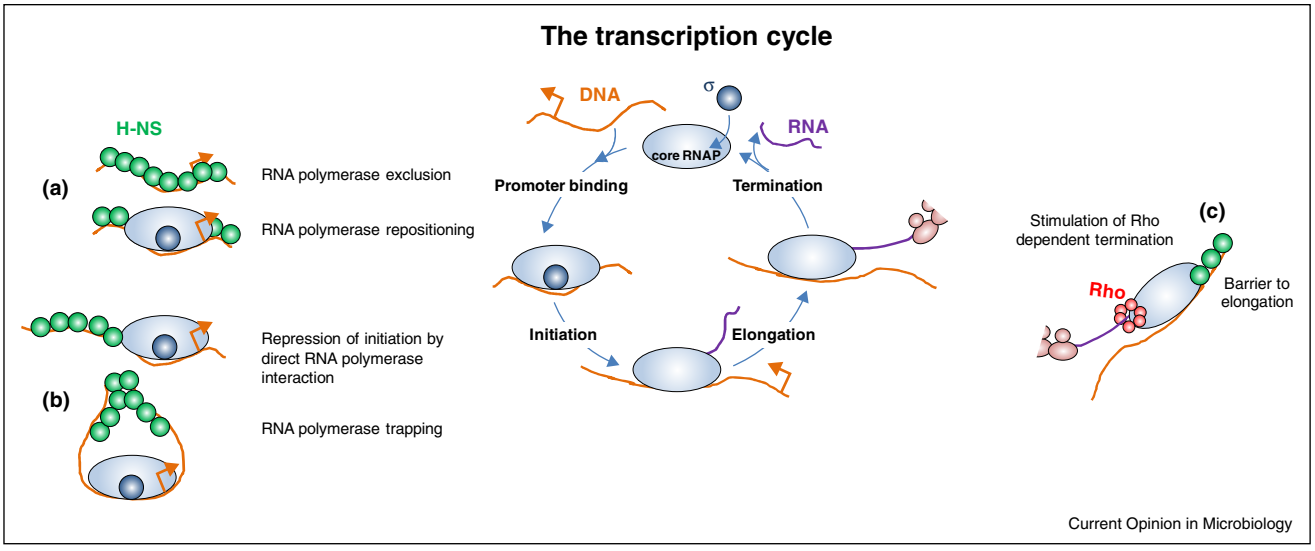
The DNA binding properties of H-NS are particularly intriguing when considered alongside those of RNA polymerase. Briefly, the role of RNA polymerase in the cell can be described by the transcription cycle (Figure 2).

Figure 1



DNA binding properties of H-NS and RNA polymerase. **(a)** The H-NS DNA nucleation motif. The panel shows a DNA sequence logo generated from high-affinity H-NS nucleation sites (adapted from Lang *et al.* [22]). **(b)** The DNA sequence logo represents sequence conservation in promoter −10 elements recognised by the housekeeping σ^{70} factor (25). **(c)** Promoter −10 elements are enriched in high affinity H-NS targets. The scatter plot shows H-NS and Lsr2 binding signals generated by the binding of each protein to the double stranded DNA microarray of Gordon *et al*. Each data point represents a different DNA octamer sequence. Data points highlighted in red are those that contain a promoter −10 element (as defined in Ref. [30]).

Figure 2



H-NS can influence all aspects of the transcription cycle. The figure shows a cartoon of the transcription cycle and the potential involvement of H-NS at different stages. At the step of promoter binding **(a)** H-NS can exclude RNA polymerase by occluding promoter DNA elements or bind DNA in conjunction with RNA polymerase to influence its position on the DNA. Transcription initiation **(b)** may be blocked by H-NS mediated stalling of RNA polymerase at promoters and elongation of RNA polymerase, through tracts of H-NS-bound DNA, may impede transcription to enhance termination by Rho **(c)**.

In this model, RNA polymerase must recognise and interact with two core promoter DNA elements [25]. Thus, the RNA polymerase σ^{70} subunit contacts the −10 (5′-TATAAT-3′) and −35 (5′-TTGACA-3′) hexamers [25]. Recognition of −10 hexamers drives DNA unwinding during transcription initiation as elegantly demonstrated by recent structural work [26,27]. During promoter opening, the second and final positions of the −10 hexamer are flipped out of the DNA base stack and accommodated by pockets in the sigma factor. Although details of this transition remain to be defined, these DNA opening events are clearly facilitated by the highly conserved T–A step found in promoter −10 hexamers. This T–A step is easily observed in a DNA sequence logo, generated from >500 experimentally verified σ^{70}-dependent promoters (Figure 1b) [28]. Interestingly, direct comparison of an H-NS nucleation motif (Figure 1a) and RNA polymerase promoter −10 element (Figure 1b) reveal the potential for overlapping DNA sequence preference. Indeed, it was recently shown that many H-NS-bound regions of the *E. coli* chromosome are enriched for sequences that resemble promoter −10 elements [29••,30]. Similarly, identification of putative promoters using genomic SELEX with the *E. coli* RNA polymerase holoenzyme revealed an abundance of promoters in H-NS-bound regions of the genome [31]. The overlapping DNA sequence preference of H-NS and RNA polymerase is also evident in the DNA oligonucleotide array data of Gordon *et al.* [16••]. These data are shown in Figure1c where i) each data point represents a different 8-mer and ii) 8-mers containing -10 elements (defined as 5′-TAnAAT-3′, 5′-TATnAT-3′ or 5′-TATAnT-3′) are highlighted red. There is a statistically significant overrepresentation of −10 elements in the top 10% of Lsr2 and H-NS binding targets (P = <0.0001 for both H-NS and Lsr2, Chi-square test). For example, whilst only 122 of the 32 896 8-mer sequences examined by Gordon *et al.* contained −10 elements, 90 of the −10 elements fell in the top 10% of all 8-mers ranked according to the E-score for H-NS binding. Given the overlapping DNA-binding specificities of RNA polymerase and H-NS, it is unsurprising that they frequently target intergenic non-coding DNA and genes enriched for intragenic promoters. As discussed in more detail below, this shared 'love' of AT-rich DNA gives rise to conflict between RNA polymerase and H-NS.

Conflict between H-NS and RNA polymerase: interactions at different stages of the transcription cycle

H-NS interactions with transcription initiation complexes

A variety of mechanisms have been described for H-NS modulation of transcription initiation. The simplest mechanism involves occlusion of promoter sequences by H-NS filaments (Figure 2a). Genome-scale analysis of RNA polymerase binding, and transcription start-site mapping, are consistent with this being a common mechanism by which H-NS represses transcription initiation. Surprisingly, the majority of repressed promoters are located either inside genes or far from a nearby gene start, suggesting that H-NS suppresses spurious transcription initiation in AT-rich regions [29••,30]. Several alternative models for

transcription regulation by H-NS are focused on interaction with the transcription initiation complex after promoter binding by RNA polymerase. The principal 'post-binding' model posits that H-NS represses transcription by binding upstream and downstream of an initiating RNA polymerase, thus ensnaring the polymerase in a repression loop (Figure 2b) [20]. Recent work with *E. coli* O157:H7 suggests the action of H-NS may involve even more complex interactions and intriguing regulatory possibilities. For example, at the LEE5 promoter, H-NS appears to interact directly with the RNA polymerase alpha subunit to prevent transcription initiation [32••]. Furthermore, at the *ehxCABD* operon regulatory region, H-NS appears to prevent RNA polymerase binding to spurious promoter-like sequences located adjacent to the genuine *ehxCABD* promoter. In this way, H-NS may 'focus' binding of RNA polymerase to the correct sequence [33]. Interestingly, the gene regulatory effects of H-NS are not restricted to direct control of RNA polymerase. Two recent studies showed that many binding sites for the transcription activators FNR and CRP are occluded by H-NS [34••,35].

The various mechanisms by which H-NS can control transcription initiation are illustrated in Figure 2b. It is worth noting that the regulatory mechanisms ascribed to H-NS are not mutually exclusive. Structural studies of the H-NS N-terminal oligomerisation domain identified different modes of H-NS dimerisation [36,37]. Whilst both studies agreed on the presence of three helical segments (α1, α2 and α3), the coiled-coil formed between the two α3 helices in an H-NS dimer was proposed to be anti-parallel in one structure and parallel in the other. Recent molecular simulations suggest that both structures may be relevant, that each could dictate different regulatory outcomes, and that switching between the two conformations could be controlled by temperature and divalent cations [19,38]. Given that transcription regulation by H-NS is known to be sensitive to osmolarity, it is possible that transitions in H-NS oligmerisation may determine which mode of regulation predominates in a particular condition.

H-NS interactions with transcription elongation complexes

Although much attention has focused on suppression of transcription initiation by H-NS, many active transcription elongation complexes are likely to encounter H-NS (Figure 2c). Such encounters arise both in coding regions where significant H-NS binding occurs [39,40] and in non-coding regions, bound by H-NS, that are transcribed as the result of antisense transcription of neighbouring genes [41,42]. It is unclear, however, whether bound H-NS impacts transcript elongation or if active elongation can perturb H-NS filaments. Several recent studies suggest that both these phenomena may occur. By inhibiting the Rho termination factor, Peters *et al.* [41] found that most Rho termination *in E. coli* affects noncoding, antisense transcription, and that a substantial fraction of these transcription termination events coincide with sites of H-NS binding. Conversely, deletion of *hns* results in increased RNA polymerase binding at 334 chromosomal loci, of which 222 coincide with Rho-dependent terminators. Thus, it appears that H-NS aids Rho-dependent transcription termination, especially in untranslated segments of DNA such as antisense transcription units or horizontally transferred genes. It seems likely that H-NS impedes the progress of elongating RNA polymerase and hence makes it more susceptible to termination by Rho (Figure 2c); such a view is supported by *in vitro* experiments showing H-NS stimulation of RNA polymerase pausing and Rho-dependent termination [43].

If H-NS can promote removal of RNA polymerase from DNA upon close encounter, is the reciprocal also true? Exciting recent results from Chandraprakash and Seshasayee [44] suggest this may well be the case. These authors report that when RNA polymerase invades H-NS-bound regions of the chromosome, H-NS binding is reduced. Using a similar strategy of chemical inhibition of Rho, but instead monitoring H-NS binding levels using ChIP-seq, Chandraprakash and Seshasayee [44] observed that the increased read-through of RNA polymerase into large tracts of H-NS-bound DNA occurred concomitantly with destabilisation of H-NS–DNA interactions. Whilst this result shows that RNA polymerase and H-NS perceive each other's presence during transcription elongation, the molecular details of the interplay remain undefined (Figure 2c). Thus, careful studies such as single-molecule approaches that can distinguish effects of H-NS on transcript elongation rates and different classes of transcriptional pausing as well as effects of RNAP on H-NS release are now desirable.

H-NS interactions with RNA

Remarkably, the potential roles of H-NS in transcription elongation also extend to a direct interaction with the RNA. There have been numerous reports of H-NS, and orthologs such as StpA, binding to RNA [45,46]. In a particularly notable piece of work, Park and colleagues recently showed that H-NS can stimulate the expression of some genes by binding to AU-rich 5′ UTRs of some mRNAs with suboptimal ribosome binding sites [47••]. When H-NS targets these mRNAs, it results in repositioning of the ribosome and more effective mRNA translation. We speculate that these properties of H-NS, which have been long overlooked, may well be as important as the interactions between H-NS and RNA polymerase at promoters. Moreover, these H-NS–RNA interactions are likely to occur on nascent RNA, since transcription and translation are coupled in bacteria. Thus, H-NS–RNA interactions likely represent an additional connection between H-NS and RNA polymerase.

Summary

We propose that H-NS and RNA polymerase are engaged in an inharmonious matrimony with squabbling over shared interests being commonplace. Whilst the two factors share certain properties (most notably, a propensity to recognise AT-rich sequences) and participate in all aspects of the transcription cycle, their union frequently has a disruptive outcome: H-NS interferes with promoter binding by RNA polymerase, can trap RNA polymerase in DNA loops, may disrupt transcription elongation complexes, and can enhance termination. H-NS has previously been described as promoting 'xenogeneic silencing', that is, suppression of transcription of horizontally acquired genes [48–50]. The model has since been expanded to include suppression of spurious promoters within these genes [29••,30]. We speculate that the DNA sequence preferences of H-NS and related proteins have evolved to match those of RNA polymerase, as a mechanism for silencing transcription from horizontally acquired DNA. However, once horizontally acquired DNA has been stably inserted in the genome, the function of H-NS can evolve to accommodate more complex regulatory outcomes, such as the focusing of RNA polymerase to the correct transcription start site as observed for the *ehxCABD* operon in *E. coli* O157:H7 [33].

The relationship between H-NS and RNA polymerase can be modulated by other proteins. For example, 'invasive' DNA can encode proteins that counteract the repressive effects of H-NS. Such H-NS inhibitors are known to be encoded in several bacteriophage genomes [51,52]. Furthermore, host-encoded proteins frequently interfere in the relationship between RNA polymerase and H-NS. Proteins such as Ler and Hha can disrupt or stabilise repressive H-NS–DNA complexes on a global scale [12,13,53]. Understanding these partner proteins will likely prove key in determining the precise details by which H-NS functions. This may take longer for organisms where functional homologs of H-NS have only recently been found [54]. On a local scale, many transcription activators have evolved to counteract H-NS-mediated repression. These proteins can function by preventing H-NS oligomerisation, bridging, or simply disrupting H-NS–DNA interactions [55]. At many promoters, transcription activators function solely by displacing H-NS [55]. Thus, widespread repression of transcription by H-NS represents an opportunity to activate transcription of genes in a highly specific manner. This is a common strategy for regulating virulence genes, and is likely to evolve rapidly since many promoter architectures can be accommodated and any DNA-binding protein can activate transcription in this manner [56,57]. Given the wide variety of mechanisms by which H-NS can repress transcription, it is likely that many mechanisms of anti-repression have yet to be discovered.

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