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

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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.

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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.

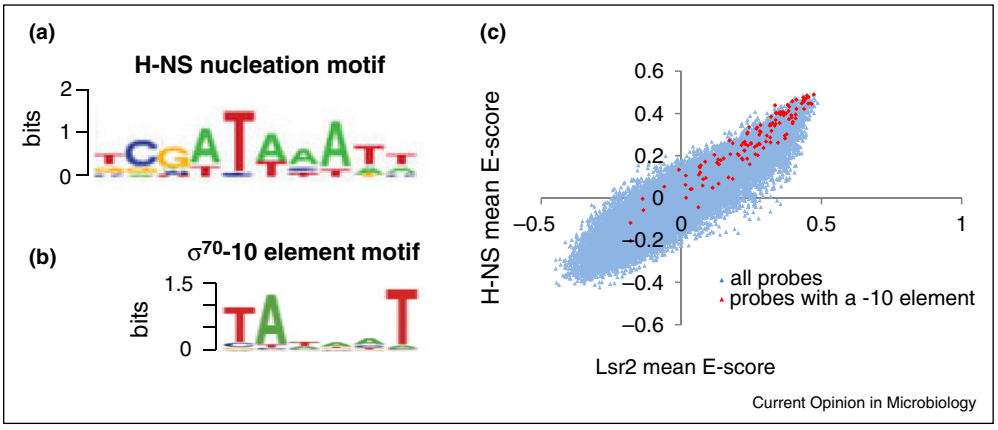
Although the influence of H-NS on DNA folding, gene expression, and horizontal gene transfer is broadly accepted, the molecular mechanism by which H-NS exerts these effects remains controversial. Multiple models purport to explain H-NS function, and gaping holes in our knowledge are still evident. For 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]).