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Bacterial H-NS Protein: Structure and Function

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Summary
The histone-like nucleoid structuring (H-NS) protein constitutes the major portion of the *Escherichia coli* (*E. coli*) chromosome. H-NS has been involved in controlling transcription, DNA condensation, and genome evolution. Given that H-NS is constructed from small peptide-protein with basic self-association, hetero-oligomerization, and DNA binding characteristics, its broad impact is noteworthy. With an emphasis on these structural components, the information will be evaluated in this article. Mainly, the interaction surfaces that allow H-NS to exert its various effects will be analyzed.

Introduction
Escherichia coli (*E. coli*) and closely related bacteria both share a 15.5 kDa peptide known as the histone-like nucleoid structuring (H-NS) protein (Singh *et al.*, 2016). H-NS, which was first known as protein H1, was discovered to be a plentiful component of cell extracts that could be separated due to its DNA-binding ability (Laine *et al.*, 1984). The results of further research have demonstrated that H-NS binds AT-rich DNA by first recognizing a high-affinity nucleation site, which can be identified by the presence of a T:A step, and then oligomerizing over contiguous AT-rich DNA (Kahramanoglou *et al.*, 2011). H-NS can also affect translation initiation and target RNA (Park *et al.*, 2010). The rules for binding this nucleic acid have not yet been established. The prevalence of H-NS within cells shows that it affects several DNA-related processes. It has been demonstrated that H-NS has an impact on transcription, DNA folding, and genome evolution (Winardhi *et al.*, 2014). It might be challenging to distinguish these various tasks since they are so closely intertwined. Therefore, it is unclear whether H-NS was developed to simultaneously govern several aspects of cell life or if H-NS has a single fundamental role, with other functions serving as incidentals. This article concentrate on how the structure of H-NS and its many functions are related.

The structural aspects of H-NS
The 137 amino acid long *E. coli* H-NS protein has two distinct domains. A flexible linker connects the domains. There are four-helices (numbered 1, 2, 3, and 4) in the 83 amino acid long N-terminal domain (NTD). These allow for "head-to-head" and "tail-to-tail" self-association. Helices 1 to 3 are necessary for the head-to-head encounter. The creation of a coiled-coil, including a sub-section of three from each promoter, stabilizes the contact (Bloch *et al.*, 2003). Both parallel and antiparallel versions of the coiled-coil have been seen. The C-terminal region of helixes 3 and 4 mediates the tail-to-tail contact (Arold *et al.*, 2010). A helix-turn-helix motif is formed when they fold. The motif can interlock twice in an antiparallel arrangement (Esposito *et al.*, 2002). Importantly, head-to-head and tail-to-tail interactions can take place at the same time. H-NS can oligomerize and create filaments as a

result. A linker and the C-terminal domain (CTD), which binds DNA, come after the helix-turn-helix. Two-sheets (numbered 1 and 2), a-helix (5), and a 310 helix are all included in this domain. In the highly conserved sequence "TWTXGRXP," a loop between position 2 and the side-chain R114 that contains α-helix is the critical factor for DNA binding (Gordon, *et al*., 2011). These amino acids combine to create an AT-hook motif, which introduces R114 to the DNA's minor groove.

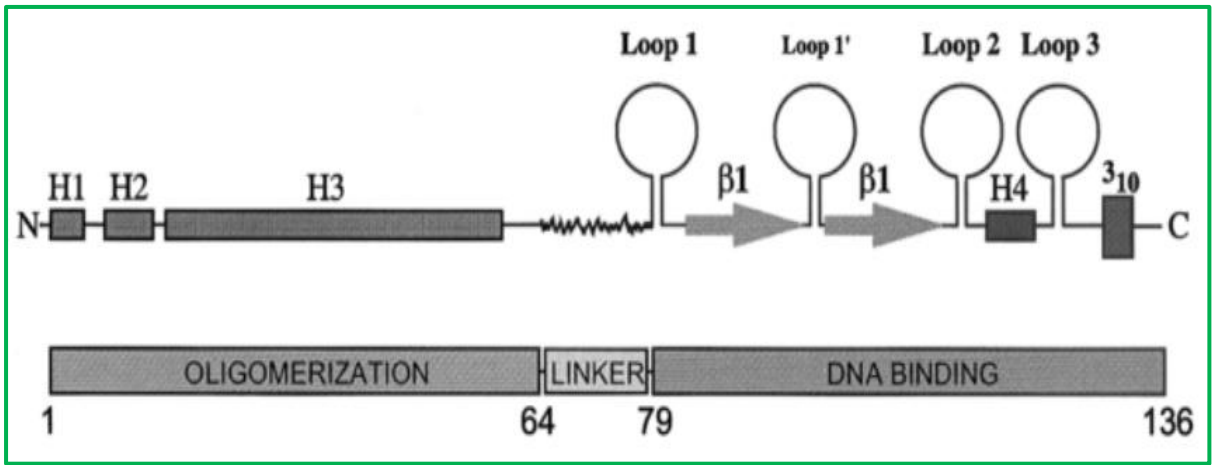

Figure 1. DNA binding and oligomerization caused by H-NS (Schröder & Wagner, 2002)

The known structural components of the protein are illustrated schematically in *Figure 1*. Three α-helical segments (H1, H2, and H3), covering residues 2 to 8, 12 to 20, and 22 to 53, respectively, are found in the N-terminal oligomerization domain, which spans residues 1 to 64. The DNA binding domain extends from residue 79 to the protein's C-terminus after the flexible linker region, which is found between residues 64 and 79. Together with loop 2 (T109-A116), which carries the majority of the consensus core DNA binding motif, loop 1 of the DNA binding domain creates a DNA binding surface. Two short-strands, 1 (Y96-D100) and 2 (T105-W108), are situated between loops 1 and 2. Loop 1' separates the two strands. Loop 2 and loop 3 (residues 125–128) are connected by a -helix spanning from A116 to E124 (H4), which is followed by a 310-helix structure (L129-F132). Note that the residue counts may vary by 1 depending on the nomenclature used in the relevant research (Schröder & Wagner, 2002).

These amino acids combine to create the AT-hook motif, which brings R114 to the DNA's minor groove. AT-rich sequences that show slight groove narrowing are more favorable for this interaction. Numerous Gram-negative bacteria produce H-NS-like proteins that can be recognized by the AT-hook motif. Some of these proteins, such as VicH from *Vibrio cholera*, are similar to the *E. coli* H-NS protein, whereas others have distinct functions (e.g., Ler, an antagonist of H-NS found in enterohaemorrhagic *E. coli*). Several H-NS-like proteins are encoded by various Gram-negative bacteria (Madrid *et al.*, 2007). Cross-talk between these components frequently plays a crucial role, as is covered in greater depth below. In Gram-positive bacteria and mycobacteria, it is less clearly characterized; analogs of H-NS show comparable DNA-binding characteristics, but the structural similarity is restricted to the AT-hook motif (Gordon *et al*., 2010).

Topological aspects of H-NS

Nucleoprotein filaments are produced when H-NS oligomerizes with DNA (Amit *et al*., 2003). These filaments can be organized in a bridged or linear fashion. Therefore, H-NS can unroll DNA into an extended shape or condense DNA. Although these structures have been observed using atomic force microscopy and can be found using several different biophysical methods, it is still unclear exactly how they are organized (Liu *et al.*, 2010). The structural

characterization of the H-NS NTD served as the basis for the current models (Arold et al., 2010). Here, H-NS filamentation is made possible in the absence of DNA through back-to-back head-to-head and tail-to-tail contacts. Therefore, alternating H-NS protomers' AT-hook motifs are ideally suited to facilitate DNA bridging. Successive AT-hooks likely bind neighboring double helix portions in linear filaments. This can include rearranging the coil that serves as a mediator between head-to-head interaction (Arold *et al*., 2010). This concept has been proposed because the availability of divalent cations and perhaps temperature affect the response of switching between bridging and linear filaments (Liu *et al*., 2010). The character of the head-to-head competition may also be influenced by these variables (Esposito *et al*., 2002).

Regulation of transcriptional parameters by H-NS

H-NS probably affects a variety of procedures that need access to the DNA template. Transcription initiation has received the most attention out of all the potential outcomes. In a nutshell, the promoter is a DNA region that an enzyme called RNA polymerase binds to start transcription (Lee *et al*., 2012). DNA unwinding and the beginning of an RNA chain occur next. Determinants that make contact with sequence elements in the promoter DNA mediate the first binding of RNA polymerase (Lee *et al*., 2012). Thus, the promoter 35 (50-TTGACA-30) and 10 elements (50-TATAAT-30) are in touch with the dissociable RNA polymerase σ^{70} subunit. The subunit CTD interacts with AT-rich regions further upstream at some promoters (Lee *et al*., 2012). H-NS can affect these interactions by imposing constraints on DNA architecture. The first instance of this was shown at the H-NS-repressed pros and bgl loci. Notably, interactions between the distal H-NS nucleation sequences on either side of the promoter are frequently necessary for suppression (Dole *et al*., 2004). It is unclear, nevertheless, if this is a typical kind of suppression or if H-NS works through a variety of different methods. The many types of transcriptional repression by H-NS that have so far been identified are described in the sections that follow.

RNA polymerase working with other factors can occasionally overcome H-NS repression (Grainger *et al*., 2008). For instance, the housekeeping phase (σ^{70} related) and starvation phase (σ^{38} associated) forms of RNA polymerase can both employ the same *E. coli* DPS promoter. To stop the σ^{70} RNA polymerase derivative from attaching, H-NS attaches to sites that overlap the DPS promoter (Grainger *et al.*, 2008). On the other hand, co-binding is shown when RNA polymerase is coupled to σ^{38} and is not susceptible to the presence of H-NS (Grainger *et al.*, 2008). Significantly, this ternary complex is capable of initiating transcription. At the *E. coli* hdeAB promoter, a distinct mechanism was found (Shin et al., 2005). In this situation, DNA bending by RNA polymeraseσ^{70} promotes the creation of a repression loop. Repression doesn't happen and bending is less apparent for RNA polymerase that is attached to σ^{38} (Shin *et al*., 2005).

The regulation of elongation by H-NS

The H-NS-bound DNA must also be encountered by the RNA polymerase during transcription elongation (Landick *et al*., 2015). It is unclear whether such nucleoprotein filaments restrict transcription elongation or whether they are damaged by the passage of the transcriptional machinery. One study analyzed how RNA polymerase moved over a region of the bgl operon. It was demonstrated that the structure of the H-NS:DNA complex affected how H-NS affected transcription elongation (Kotlajich *et al*., 2015). Thus, transcription elongation was unimpeded by linear H-NS:DNA filaments. On the other hand, bridging complexes caused RNA polymerase to pause. Based on *in vivo* data, it appears that the presence of H-NS-bound DNA predisposes transcription elongation to premature termination (Chandraprakash & Seshasayee,2014). This termination is probably brought about by a factor called, which binds to the developing RNA and prevents transcription elongation. As the rate

of transcription elongation decreases, Rho is expected to function more effectively (Epshtein et al., 2010).

The anti-silencing activity of H-NS

The genomes of Gram-negative bacteria have been shown to include a range of H-NS-like proteins, as was previously mentioned. For H-NS-bound genes to express as much as possible, certain of these are necessary. H-NST of enteropathogenic *E. coli* and Ler of enterohaemorrhagic *E. coli* are two well-known instances. These proteins seem to control the complexes that DNA and H-NS form (Williamson & Free, 2005). For instance, Ler may remove H-NS from the DNA by preferentially binding to the same AT-rich DNA regions. Variations in the NTD of Ler that inhibit cooperative DNA-binding and direct interactions with H-NS may be to blame for this (Mellies *et al*., 2008). When H-NS interacts with H-NST, heterodimers are created that stop nucleoprotein filament formation (Williamson & Free, 2005). This happens as a result of H-ability NST's forming head-to-head interactions with H-NS but not engaging in tail-to-tail contacts (Williamson & Free, 2005).

Global investigations of transcription regulation by H-NS have been possible during the past ten years thanks to genome-scale techniques (Oshima *et al*., 2006). These results demonstrate that H-NS mostly inhibits transcription at horizontally acquired loci with a high AT-content, which may assist the chromosomal integration of such genes. It was supposed that mRNA synthesis was the cause of the discovery that horizontally acquired genes are transcribed more often in cells lacking H-NS. It is now evident that the issue is significantly more complicated (Wade & Grainger, 2014). Particularly, several sequences that mimic promoter regions may be found in AT-rich genes. Recent research has confirmed this, demonstrating that the bulk of H-NS-repressed promoters are found within open reading frames or far from the start of genes (Wade & Grainger, 2014). This might help to explain why H-NS can neutralize the harmful effects of AT-rich DNA. As a result, these experiments demonstrated that H-crucial NS's role in ensuring transcriptional specificity. For instance, H-NS inhibits transcription from erroneous promoters inside genes at the ehxCABD operon from *E. coli* O157:H7, enabling proper identification of a canonical promoter in the upstream gene regulatory region (Wade & Grainger, 2014).

Conclusion

A little peptide known as the H-NS protein can self-associate and bind DNA. This simple structure allows for astonishingly varied impacts on bacterial chromosomes. Understanding the exact organization of the in vivo nucleoprotein filament containing H-NS is a continuing issue. This will probably show how to circumvent silencing and accomplish precise transcription of H-NS bound genes without simultaneously producing erroneous transcripts from intragenic promoters. For instance, it's conceivable that H-NS:DNA filaments might take on a shape that facilitates transcription elongation but prevents initial RNA polymerase binding to gene promoters. The knowledge of the field's experts in genetics, genomics, structural biophysics, and modeling must be brought together to meet these issues, which is important.

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