MiniReview

Molecular aspects of the *E. coli* nucleoid protein, H-NS: a central controller of gene regulatory networks

Roy M. Williams 1, Sylvie Rimsky *

Unité de Physicochimie des Macromolécules Biologiques (URA 1149 du Centre National de la Recherche Scientifique), Institut Pasteur, 75724 Paris Cedex 15, France

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Abstract

The nucleoid-associated protein H-NS has a central role in the structuring and control of the enteric bacterial chromosome. This protein has been demonstrated to contribute to the regulation of expression for approximately thirty genes. In this article, the molecular aspects of H-NS structure and function are briefly reviewed. H-NS contains at least two independent structural domains: a C-terminal domain, involved in the DNA-protein interactions, and a N-terminal domain, likely involved in protein–protein interactions. Recent reports have revealed that H-NS is a key factor in a multi-component gene regulatory system. Factors have now been discovered which can backup or antagonise H-NS action at certain promoters. These recent findings are summarised and discussed in relationship to the role of H-NS in DNA packaging and nucleoid structure.

Keywords: H-NS; StpA; Domain structure; Chromosome structure; Nucleoid protein; Regulatory network

1. Introduction

The H-NS protein is a major component of the enteric bacterial chromosome with roles in many disparate cellular processes (summarised in reviews [1,2]). Our understanding of H-NS has recently advanced in two main ways. First, structure to function relationships in H-NS are now starting to provide insights into the molecular mechanism of H-NS action. Secondly, the discovery of regulatory factors linked to H-NS, for example the H-NS analogue, StpA [3], and the untranslated small RNA species, DsrA, [4] suggest the existence of an extensive genetic control system in which H-NS plays a central role. The aim of this review is to illustrate how the molecular study of H-NS offers a way of probing the structure-function relationships in bacterial chromatin and the global control mechanisms of bacterial gene expression.

2. The bacterial nucleoid and its associated proteins

Both eukaryotic and prokaryotic organisms are faced with the problem of condensing a large quantity of genetic material into a nucleus, or cell, whilst retaining biological activity. In many prokaryotes this task is accomplished through the formation of
a structure called the nucleoid, in which the level of DNA packaging or the local concentration of DNA (20–50 mg/ml) is similar to that observed for an interphase eukaryotic nucleus [5]. For eukaryotic chromatin, extensive studies of chromosomes have led to the determination of a well defined and stoichiometric basic repeat unit, the nucleosome, around which the first level of DNA compaction is organised [6]. However, the physico-chemical parameters leading to the higher order structures of eukaryotic chromatin are still largely unknown. In the prokaryotic nucleoid, no basic repeat unit such as the nucleosome has ever been identified and, because of its absence (or its peculiar lability), the precise roles of proteins participating in the process of prokaryotic DNA condensation have been much harder to characterise. Relatively few proteins seem to be associated with the nucleoid, namely IHF, FIS, HU and H-NS [7]. The two most abundant of these, HU and H-NS [8], do not exhibit specific sequence DNA-binding [7], a primary requirement for a protein involved in nucleoid organisation. However, they are both able to recognise particular DNA structures such as intrinsically curved sequences (H-NS, HU) [9–12] or cruciform DNA (HU) [13]. In the last decade, H-NS has been found to be involved in many disparate cellular processes, ranging from cold-shock [14] to being a general repressor and/or silencer of transcription [15–17]. This protein, which is very abundant (20 000 copies/cell) [18], thus possesses the particularly interesting dual functions of DNA packaging and global regulation. A fuller understanding of the molecular mechanisms involved in H-NS reactions is required. In this review, we discuss the current knowledge concerning the molecular details of H-NS structure, how these influence H-NS function, and how the coupling between H-NS and other cellular factors builds up a complex regulatory network in the cell.

3. H-NS: a molecular overview

3.1. General aspects

The initial characterisation of E. coli H-NS (or H1) [19,20] revealed that it was an abundant, heat-stable protein, with a molecular mass of 15.6 kDa [21]. Further work has shown that the native protein carries almost no net charge (pl 7.5) at neutral pH, despite the presence of many patches of basic and acidic amino acids in its sequence. Interestingly, three H-NS isoforms have been identified which differ by approximately 0.1 pH unit [18], suggesting that H-NS may undergo some kind of post-translational modification. The characteristics, and possible significance, of these isoforms has been reviewed recently [1].

3.2. Tertiary structure

There are a number of results suggesting that the H-NS protein is organised into two distinct domains (Fig. 1). First, its C-terminal domain alone, containing amino acids 91–137, can bind DNA, however, this is with a much reduced affinity compared to full-length H-NS [22]. The solution structure of this domain has been deduced from NMR studies, which reveal a stretch of antiparallel beta-sheet followed by short helical regions, characteristics which have not been observed in any DNA-binding domain reported so far [22]. Hence, the exact whereabouts of the H-NS DNA-binding motif is not deducible from these studies. Second, chemical cross-linking studies with a purified N-terminal 64 amino acid fragment of H-NS (H-NSΔ64) reveal that it contains an H-NS dimer interface, which enables protein–protein interactions [23]. For the N-terminal domain per se there is currently no structural information, although structure predictions suggest that it probably has a high alpha-helical content and contains heptad repeats capable of forming an alpha-helical coiled-coil structure [1]. This would be consistent with the observation that this domain has a role in protein–protein interactions [23,24].

3.3. Quaternary structure

The oligomeric state of native H-NS is unclear. Both dimers and oligomers of H-NS have been detected in solution by chemical cross-linking techniques. H-NS dimers are stable even under high salt conditions, indicating that the monomer–monomer interaction has a marked hydrophobic character [25]. Small angle neutron scattering results (Rimsky unpublished data) together with large zone gel per-
Fig. 1. H-NS is represented as a bar running from amino acid 1 to 136 with the positions of the N- and C-terminal domains shown shaded and cross-hatched respectively. Indicated above the bar are the locations and nature of amino acid substitutions, and the H-NSΔ64 truncation, found to cause severe defects in H-NS repressor function or dominant negative activity. A square next to a particular alteration signifies that it was isolated as a dominant negative mutant. A diamond signifies a mutation which leads to H-NS loss of function. Six of the substitutions mapping functionally important residues fall in the N-terminal domain, while fifteen fall in the C-terminal domain. Independently isolated mutations include those at positions 110, 113 and 116 and at the near neighbour positions 53, 54 and 55 [23,24].

mention chromatography (Angeletti, paper in preparation), revealed that the tetramer is the dominant form in solution (at concentration in the range of 10–100 μM monomer protein), although larger aggregates are also present. Formation of tetramers seems more sensitive to high ionic strength compared to dimer suggesting the involvement of electrostatic interactions upon their formation [21]. However, at lower H-NS concentrations, chemical cross-linking and small zone gel chromatography data support the dissociation of these forms into dimers [21,24–27].

Further uncertainty surrounds the oligomeric state of H-NS in the presence of DNA. Indeed, both DNase I footprinting studies and electron microscopy demonstrate that H-NS has the ability to cover DNA molecules from one end to the other [28,29]. Such observations, together with the characterised highly cooperative mode of DNA binding [29–31] used by H-NS imply that H-NS can form an oligomeric structure on the DNA. For H-NS to polymerise in this way, a protein–protein interaction not implicated in, but perhaps induced by, the initial dimerisation would be required. Further biophysical data are necessary to characterise first the various DNA-H-NS complexes, as well as the oligomericisation states which prevail in the presence as well as in the absence of DNA. The possibility that H-NS oligomerisation and DNA-binding functions can be coupled has been highlighted recently [24,27]. This was proposed after C-terminal mutants, already demonstrated to be defective in curved DNA recognition were also shown to have a fault in oligomerisation function. It remains to be determined if this substitution was directly affecting a site for protein–protein interaction.

4. H-NS protein-nucleic acid interactions

4.1. The effect of H-NS on DNA structure

Initial studies on the nucleic acid-binding activity of H-NS revealed that it has the ability to bind both double and single stranded DNA, and RNA [32] with decreasing affinity respectively. Additional studies have demonstrated that H-NS can also affect the overall structure of DNA by: (i) strongly compacting DNA [18], (ii) altering DNA superhelicity (H-NS has been found to constrain negative DNA supercoils in vivo as well as in vitro in a manner dependent on the concentration of potassium glutamate ([28] and references therein)), and (iii) induction of DNA bending [27]. These phenomena apparently occur re-
4.2. The effect of H-NS on DNA function

Genetic data, from studies on *E. coli* and other enteric bacteria, form the first line of evidence demonstrating that H-NS is playing the role of a global control element. It is striking that the *hns* gene has been characterised as a controller in the expression of about 30 genes [33–35]. Recently several products of these genes have been identified using two-dimensional electrophoresis [36]. Many of the genes regulated by H-NS are involved in bacterial adaptation to environmental stress, including bacterial virulence [2,36].

DNase I footprinting studies performed at a number of relevant promoters have revealed that specific binding sites are first populated, followed at higher concentrations by a complete protection against DNase I attack. Results obtained from electron microscopy are consistent with the footprinting data, since they also show end-to-end coating of DNA molecules by H-NS [28]. Specific DNA-binding by H-NS is usually associated with the presence of either: (i) AT rich DNA sequences or (ii) regions of intrinsically curved DNA. Many groups have now reported that the affinity of H-NS for curved DNA is significantly greater than that for linear DNA [9–11]. These observations initially suggested that the ability of H-NS to discriminate between curved and linear DNA sequences could be essential for its in vivo activity. This possibility has been investigated by the construction of a series of semi-synthetic promoter constructs containing curved DNA sequences inserted upstream of a constitutive test promoter. The in vivo activity of the downstream promoters was found to be modulated in a H-NS dependent manner, depending on the properties of the upstream DNA curvature [37]. The activities of the test promoters and the relative affinity of H-NS for the different curved sequences revealed a good correlation between curved DNA-binding and promoter repression by H-NS (Rimsky et al., in preparation).

Certain *hns* responsive promoters contain regions of intrinsic DNA curvature located either upstream, or downstream, of the transcription start point [11,30,31,38]. The preference of H-NS for some of these curved promoter elements has now been directly demonstrated in vitro by competitive gel shift assays [31]. Altogether, the available data support the idea that the presence of curved DNA sequences near promoters can confer the specificity required by H-NS to modulate gene expression in vivo. Recently, it was shown that, in addition to recognising curved DNA, wild-type H-NS can induce curvature in non-bent DNA [27].

5. The molecular genetic analysis of H-NS

5.1. The identification of functionally important residues

Recent mutational analyses have contributed to a more detailed understanding of the structure and function of H-NS [23,24]. Two different approaches have been employed to map amino acid residues important for H-NS function, namely: (i) selection of dominant negative H-NS derivatives [23] and (ii) systematic characterisation of mis-sense alleles of *hns* [24]. The genetic screens used in these studies exploited the well-characterised *proU* promoter, known to be repressed by the binding of H-NS (for a review [39]), fused to a downstream copy of the lacZ reporter gene. Mutations in *hns* resulting in the de-repression of *proU-lacZ* expression were selected and the sequences of the altered *hns* genes determined. The location of the mutations indicate that amino acid substitutions mapping along the length of the *hns* gene can cause either dominant negative activity or loss of H-NS function (see Fig. 1 for examples). Specific regions and residues in the protein have been consistently identified in these independent studies as being functionally important (Fig. 1).

5.2. A molecular interpretation of the genetic data

Most of these mutations occur in the C-terminal domain of the protein, as defined by NMR studies, although amino acid substitutions in both the N- or the C-terminal domains may cause H-NS dominant negative activity [23] and large defects in H-NS repressor function [24] (Fig. 1). The results of in vitro studies on the N-terminally substituted H-NS mu-
ants demonstrate that they retain the preference of
the native protein for curved DNA and H-NS target
promoters ([24] and R. Williams et al., unpublished
result). These results provide further support for the
idea that whatever the function of the N-terminal
domain of H-NS it has no direct role in DNA-bind-
ing. The C-terminally substituted H-NS mutants
have also been analysed in vitro, and, in contrast
to the N-terminal mutants, they are all severely def-
ective in DNA-binding and in the preferential rec-
ognition of H-NS target sites ([24], and RW unpub-
lished data). These observations reinforce the
proposition that the C-terminal region of H-NS
largely directs H-NS DNA-binding activity. The lo-
cation of many of these C-terminal substitutions on
the three-dimensional structure reveals that they are
clustered around Trp109, a residue suggested to be in
close proximity to the DNA by fluorescence analysis
[40].

On the other hand, it clearly appears that the ef-
fect of the mutations partly depends upon which
promoter is involved, i.e. whether the proU promoter
or another promoter such as bgl or the 5.46Agal
synthetic promoter is used for reporting H-NS func-
tion [23,24]. H-NS mutants modified in their N-
terminal domain induce a clear de-repression on the
collection of H-NS governed promoters studied,
whilst the effect of C-terminal mutations appears to
be more specifically on proU promoter activity. These
results indicate that the architecture of a given
promoter governs its H-NS specific regulation.

6. H-NS involvement in global cellular regulation

6.1. Mechanisms of transcription regulation by H-NS

The available genetic and biochemical data are
largely consistent with the hypothesis that H-NS is
a general negative regulator of transcription [17,41].
Indeed H-NS behaves as a pleiotropic repressor in
the original sense of the word [42], being capable of
repressing transcription from all promoters tested in
vitro, and when absent in vivo, results in the en-
hanced expression of thirty genes. Two models
have been proposed to explain the molecular mech-
anism underlying this activity. These models have
been described respectively as, 'transcriptional silenc-
ing' [16] and 'repression via DNA topology' [28,43].
Both hypotheses include the observation that H-NS
first 'nucleates' at a preferred site and proceeds to
form a nucleoprotein complex in the vicinity of a
target promoter. This process is illustrated schemati-
cally in Fig. 2. Step 1 shows how H-NS could exploit
its enhanced affinity for curved DNA to initially nu-
cleate on the DNA, and step 2 shows how coopera-
tive DNA-binding by H-NS would lead to the occu-
pation of the region of the DNA around a promoter,
and hence to the inhibition of promoter activity (see
Fig. 2). Included in Fig. 1 are the characteristics of
H-NS mutants which are apparently defective in one
or other of these functions; these mutants provide
support for the idea that H-NS action can be re-
duced to two components. The two models differ
in respect to how the H-NS-DNA complex might
affect the activity of RNA polymerase. In the 'tran-
scriptional silencing' model, H-NS is proposed to act
directly as a transcriptional repressor via its ability
to polymerise linearly along the DNA and thus oc-
clude RNA polymerase access. Here the term 'silenc-
ing' is used in a very broad sense, and does not imply
that H-NS has the ability to indefinitely switch off
transcription over large regions of the chromosome.

The 'repression via DNA topology' model comes
from the observation that H-NS can directly affect
DNA topology and that many promoters are known
to be sensitive to DNA superhelical density [28,44].
In this instance, the H-NS nucleoprotein complex
has been suggested to act as a scaffold for a topo-
logical form of DNA that is incapable of favourable
interactions with RNA polymerase. Consequently,
the effect of H-NS need not be direct, and DNA
topological effects could influence promoter activity
at a distance. These two models are not mutually
exclusive and it is possible that elements of both
could be involved in the action of H-NS at its var-
ious targets. It has been shown previously that a
ternary complex can be formed by H-NS, RNA pol-
lymerase and the non-specific promoter lac L8UV5.
It was suggested that H-NS can hinder the rotational
flexibility of the DNA double helix, preventing the
isomerisation from the closed complex to the open
complex competent for transcription [18].

An additional, and poorly understood, aspect of
transcriptional regulation by H-NS is its ability to
discriminate between two different forms of RNA
polymerase; Eσ and Eσ70, at a subset of E. coli promoters [45,46]. Such promoters, normally preferentially recognised by Eσs, can be transcribed by Eσ70 in the absence of H-NS [45]. This raises questions concerning how H-NS can govern promoter selectivity by the different forms of RNA polymerase. It has been suggested that the presence of RNA polymerase α-subunit-binding sites (the AT rich UP-elements) at these promoters may play a role [46]: interestingly, these sites are also preferred H-NS target sites [31].

6.2. The H-NS analogue, StpA

6.2.1. StpA as an RNA chaperone

The stpA gene was originally isolated as a suppres-
isor of a T4 phage phenotype associated with RNA splicing [3]. In vitro studies with purified StpA and RNA substrates revealed that StpA has a novel RNA chaperone activity (as defined by Herschlag, 1995) [47]. This appears to arise from the ability of StpA to bind RNA non-specifically; this interaction somehow encourages their untangling, and thus favours the specific interaction of the bases involved in the self-splicing reaction.

The cloning and sequencing of stpA revealed that it encoded an H-NS analogue, with 58% identity to H-NS [3]. Genetic studies have shown that stpA can be isolated as a suppressor of hns phenotypes, thus indicating that the structural similarity between these two proteins is functionally significant [35,48]. A biochemical comparison of StpA and H-NS revealed that the two proteins share many properties, including the abilities to inhibit transcription from promoters containing curved DNA and to constrain DNA supercoils [35]. Significantly, StpA RNA chaperone activity was also discovered for H-NS. However, a clear functional difference between the two proteins has been observed in vitro, since the RNA splicing activity of H-NS is about one order of magnitude lower than that of StpA, and the kinetics of the H-NS reaction two orders of magnitude slower.

6.2.2. The H-NS/stpA network

The comparison of StpA and H-NS dominant negative mutants revealed two key points: (i) the homology between H-NS and StpA apparently extends down to the domain structure and amino acid function, and (ii) dominant negative StpA proteins can compromise the activity of H-NS [23] (the converse is also true in that dominant negative H-NS mutants inhibit repression by StpA). In vitro cross-linking experiments provided firm support for the idea that the dominant negative activity of the H-NS and StpA mutants occurs at the level of protein-protein interactions, since the H-NSΔ64 polypeptide was found to interact with StpA [23].

The extensive and close similarity between H-NS and StpA thus reflects their intricate functional relationship. In standard laboratory strains of E. coli, StpA is normally very poorly expressed [35,49]. However during growth in exponential phase in rich medium, there is a transient burst of the stpA expression, and in minimal media stpA expression is also significantly enhanced in a manner which is dependent on the leucine responsive regulatory protein (LRP) [49,50]. Other environmental changes such as temperature and osmotic shock lead to the activation of the stpA expression. In addition it is clear that the expression level of StpA is enhanced several fold in an hns strain. Reciprocally, hns expression can be shut off in a strain over-expressing StpA. Further studies looking at the global effects of hns and stpA mutations have revealed that this simple cross regulatory loop can be extended. The comparison of the expressed proteins (by 2D PAGE analysis) seen in hns and stpA strains reveals that: (i) StpA can act as a molecular backup for H-NS in the control of many genes, and (ii) for a subset of genes the simultaneous presence of both proteins is required for the wild-type pattern of gene expression [35]. This observation indicates that in certain situations, StpA has a regulatory function which is clearly distinct from that of H-NS. This, added to the fact that both hns and stpA exert parallel autogenous control and simultaneous cross-regulation, led to the proposal of a global scheme for the regulatory loops involving H-NS and StpA [35]. Furthermore the presence of the FIS protein and the cold-shock regulator, CspA, in the regulation of hns [51,52], and the fact that stpA expression is affected by H-NS, Lrp, temperature and growth phase [49,50] indicate that H-NS action (and hns expression) is integrated and probably controlled within that of many additional factors (see Fig. 3 for an overview).

6.2.3. Regulatory networks: multiple factors

The existence of such a regulatory network raises a number of fundamental points concerning H-NS activity in vivo. Many of these questions remain unsolved, but their study promises the most fruitful prospects for our understanding of the precise role of H-NS in cellular regulation. In most cases studied, the StpA protein is present at very low levels in the cell compared to H-NS. Considering the existence of H-NS/stpA hetero dimers (see above), it is likely that the majority of the cellular StpA is complexed with H-NS. In this situation, RNA chaperone activity could occur through the binding of H-NS/stpA oligomers to RNA molecules. In addition to StpA, two proteins: the T4 phage 5.5 gene product [53] and the protein HF-I [54] have been suggested to interact
directly with H-NS. Gene 5.5 product antagonises H-NS action, increasing the frequency with which chromosomal recombination events occur, and hence aiding T4 phage propagation. In contrast, the expression of HF-I can complement a hns phenotype. The mechanism underlying this complementation is unknown, however it is striking that both H-NS and HF-I have been implicated in the post-translational regulation of rpoS [55,56]. This gene encodes σs, an alternative RNA polymerase σ factor; it has been shown that hns strains increase the efficiency of translation of the rpoS mRNA. In vitro H-NS binds tightly to the RNA-binding HF-I protein which is known to play a major role in rpoS translation [45]. Its role in influencing rpoS mRNA translation could thus occur via the promotion of H-NS-HF-I interactions, although the possibility that the influence of H-NS in this process could occur through the control of hfg expression (coding for the HF-I protein) can not yet be ruled out [4]. Finally, DsrA, a small untranslated RNA, acts as an antisilencer of the H-NS silenced in rcsA gene (rcsA codes a regulator of capsular polysaccharides [57]). Although in none of these cases has a direct H-NS-RNA interaction been demonstrated, they provide further evidence for H-NS involvement at many levels of regulation in E. coli.

7. Conclusion

The H-NS protein, whose role has long been limited to maintaining nucleoid structure through the condensation of DNA, appears now as one of the most important regulatory factors in enteric bacteria. Together with the discovery of its multiple regulatory functions, H-NS appears to be able to interact with an increasing number of partner molecules. The existence of these co-factors could in turn influence the binding of H-NS to DNA, providing the nucleoid with those dynamic aspects necessary to ensure its full genetic activity.
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