

The nucleoid-associated protein StpA binds curved DNA, has a greater DNA-binding affinity than H-NS and is present in significant levels in *hns* mutants

J.M. Sonnenfield^{b*}, C.M. Burns^b, C.F. Higgins^c, J.C.D. Hinton^{a**}

^aMolecular Microbiology, Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK

^bNuffield Department of Clinical Biochemistry, Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, UK
^cMRC Clinical Sciences Centre, Imperial College School of Medicine, The Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

(Received 21 December 2000; accepted 15 January 2001)

Abstract — The StpA protein is closely related to H-NS, the well-characterised global regulator of gene expression which is a major component of eubacterial chromatin. Despite sharing a very high degree of sequence identity and having biochemical properties in common with H-NS, the physiological function of StpA remains unknown. We show that StpA exhibits similar DNA-binding activities to H-NS. Although both display a strong preference for binding to curved DNA, StpA binds DNA with a four-fold higher affinity than H-NS, with K_{ds} of 0.7 μ M and 2.8 μ M, respectively. It has previously been reported that expression of *stpA* is derepressed in an *hns* mutant. We have quantified the amount of StpA protein produced under this condition and find it to be only one-tenth the level of H-NS protein in wild-type cells. Our findings explain why the presence of StpA does not compensate for the lack of H-NS in an *hns* mutant, and why the characteristic pleiotropic *hns* mutant phenotype is observed. © 2001 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS

E. coli / H-NS / StpA / nucleoid-associated protein

1. Introduction

The *stpA* gene was first identified as a multicopy suppressor of the *td* phenotype of a splicing-defective phage T4 *td* intron mutant [1]. StpA stimulates splicing of the T4 *td* intron in vitro, acting as an RNA chaperone, to prevent RNA mis-folding and thereby promoting formation of the catalytically-active self-splicing intron [2]. The 133 amino acid StpA protein of *E. coli* is 52% identical to H-NS, the abundant protein which plays an important role in chromatin packing and gene regulation [3–5]. H-NS influences the expression of many genes dispersed throughout the genome (for reviews see [6, 7]).

StpA was also identified as a multicopy suppressor that can complement the effect of an *hns* mutation on arginine decarboxylase gene expression [8]. Subsequently, *stpA* was shown to cause multicopy suppression of the H-NS-dependent genes *pap*, *hns*, *proU* and *bgl*, and of the cold-sensitive phenotype of *hns* mutants. However, chromosomal *stpA* mutations do not affect *pap* or *proU* expression, showing that *stpA* has no direct effect on the expression of these H-NS-dependent genes in a wild-type

background [9, 10]. Under laboratory conditions *stpA* mutants have no obvious phenotype, although *hns stpA* double mutants do produce low levels of ppGpp [11]. The expression of *stpA* in *hns*⁺ *E. coli* is SOS-inducible and is affected by temperature, Lrp, medium osmolarity, DNA supercoiling [10, 12–14].

It is clear that expression of the *stpA* gene is derepressed in *hns* mutants of *E. coli* [10, 13, 14]. Johansson and Uhlin [11] studied the fate of StpA in *hns* mutants, and showed that wild-type StpA was rapidly degraded by the Lon protease in the absence of H-NS. This is because StpA is not normally present in wild-type cells as a homomer, but is able to form heteromeric complexes with H-NS which are refractory to cleavage by Lon [3, 11, 15]. Given the rapid turnover of StpA in H-NS-deficient strains, we wanted to know whether significant levels of StpA protein remained in an *hns* mutant. We have now determined the relative level of StpA produced in the absence of H-NS, and our results show that the concentration of StpA in an *hns* mutant is much less than the concentration of H-NS in a wild-type cell.

H-NS is a crucial protein involved in nucleoid structure, but the viability of *hns* mutants suggests that it is not essential for cellular survival. Because StpA is present in *hns* mutants, we wanted to determine whether StpA could perform similar biochemical functions to H-NS. StpA has previously been shown to bind DNA [16, 17] and we have now accurately compared the DNA-binding properties of

* Present address: Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA

**Correspondence and reprints.

E-mail address: jay.hinton@bbsrc.ac.uk (J.C.D. Hinton).

H-NS and StpA. We show here that StpA is a more efficient DNA-binding protein than H-NS, and that StpA also shows a preference for curved DNA. These data shed light on the important relationship between StpA and H-NS.

2. Materials and methods

2.1. Bacterial strains, genetic methods and growth media

The *E. coli* K12 strains used for this study were MC4100 (F⁻ *araD139* Δ (*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*) [18] and MC4100 *hns::Kan* [19]. The *E. coli hns::Kan* mutation has an insertion at codon 33 [19], resulting in a 32 amino acid truncate that contains the first two of the three alpha-helical regions of the trimerisation domain of H-NS (Renzoni et al., submitted).

The plasmids pAF453 and pAF456 carry an AT-planar curve and the *proU* DRE respectively [20]. Rich media was LB [21], supplemented with 100 μ g/mL ampicillin or 50 μ g/mL kanamycin as appropriate. Cells were grown aerobically with vigorous shaking at 37 °C.

2.2. Purification of *E. coli* StpA protein

The *E. coli stpA* gene was amplified from MC4100 chromosomal DNA by the polymerase chain reaction (PCR). The primers STPA1 (CAAACCTGGAGACTCATATGTCGTAATGTTACAA) and STPAR (ATCGCGGATCCTTAATCAGGAAATCGTC) were based on the published sequence of the *E. coli stpA* gene [1] and introduced *Nde*I and *Bam*HI sites 5' and 3' of the *stpA* gene, to facilitate cloning into the pT7-7 protein expression vector [22]. Following confirmation of the *stpA* sequence, the resulting plasmid, pT7STPA, was transformed into BL21(λ DE3) (pLysS) (Novagen). StpA expression was induced by the addition of 1 mM IPTG to a culture ($A_{600} = 0.8$) that had been grown at 37 °C from a freshly transformed colony, and followed by a further 3 h of incubation. Induced cells were pelleted by centrifugation at 10 000 *g* and rapidly frozen at -80 °C. Cells were resuspended in 1.5 M KCl, 6 mM Tris-Cl, pH 7.5, lysed by freeze-thaw in the presence of protease inhibitors (0.2 mM AEBSF, 0.2 mM PMSF, 1 mM EDTA) and the insoluble material removed by centrifugation at 370 000 *g* for 1 h. Following treatment with DNAase I, the soluble material was dialysed against 20 mM Tris-Cl, 20 mM potassium phosphate, pH 7.6, (Buffer A) containing 200 mM NaCl, and loaded onto an 80-mL DNA cellulose column (Sigma D8515). StpA did not bind to this column in the presence of less than 200 mM NaCl (data not shown). The column was washed with Buffer A containing 0.5 M NaCl, and StpA was eluted with Buffer A containing 1 M NaCl.

Fractions containing approximately 85% pure StpA protein, as confirmed by tricine SDS-polyacrylamide electrophoresis, were pooled and dialysed against Buffer A containing 200 mM NaCl. The protein was then loaded onto a heparin-Sepharose column (Pharmacia 17-0467-01), and washed with Buffer A containing 200 mM NaCl, and eluted with a linear gradient of Buffer A containing 200 mM to 1 M NaCl, run over six column volumes at a low flow rate. Under these conditions StpA protein eluted between 0.6 to 0.7 M NaCl, and was separated from contaminating nucleases. The purified StpA was >98% pure and nuclease-free, with a final yield of 11 mg per litre of culture. StpA was dialysed against 10 mM potassium phosphate, 50% (v/v) glycerol (pH 7.6), and stored at -20 °C at a concentration of < 0.2 mg/mL. H-NS protein was prepared from *Salmonella enterica* sv. *Typhimurium* as described previously [23].

2.3. Gel electrophoresis and Western blotting

Tricine-SDS polyacrylamide gel electrophoresis was performed on 15% gels [24]. Cell cultures were centrifuged and normalised to a standard concentration by resuspension in an appropriate volume of water (calculated by the applying the formula $A_{600} \times 100 =$ resuspension volume in μ L). An equal volume of Laemmli loading buffer [25] was added, and samples were heated at 70 °C for 5 min before loading. After transfer of proteins to Hybond C Super membrane (Amersham) [26], two antibodies were used as probes for Western blotting. The anti-H-NS monoclonal antibody H113 was raised against the first 64 residues of H-NS from *S. enterica* sv. *Typhimurium* [27], the anti H-NS polyclonal antibody H200 was raised in a rabbit against the whole H-NS protein from *S. enterica* sv. *Typhimurium* (purified as [23]). Western blots were developed with the horseradish peroxidase-based ECL system (Amersham). Prestained broad-range SDS-PAGE standards and protein concentration assay reagents were obtained from BioRad. Relative quantification of Northern and Western blots was performed on a BioImage densitometer, using Whole band Analyser II software. Background values were subtracted throughout.

2.4. DNA methods

Gene cloning techniques were as [28], and plasmid DNA was prepared using Qiagen columns. Bacterial chromosomal DNA was prepared as [29].

2.5. DNA-binding assays

Gel mobility shift experiments were done using 1 μ g of pAF453 digested with *Hae*III as described [20]. The 189-bp fragment contains an AT-planar curve [30]. Samples were electrophoresed in a 3% Metaphor agarose

gel and visualised by ethidium bromide staining. Filter retention assays using radioactively labelled DNAs were as described [20]. The DNA fragments were prepared from the plasmids pAF453 (403 bp AT-planar curve) and pAF456 (309 bp *proU* DRE). Poly [d(I-C)] DNA (Boehringer Mannheim) was used as a non-specific competitor, as indicated.

3. Results

3.1. *StpA* protein expression in *E. coli*

We wanted to determine how the level of StpA protein in an *hns* mutant compared with the level of H-NS in an *hns*⁺ cell. We used monoclonal and polyclonal antibodies raised against H-NS to investigate StpA protein expression (2. *Materials and methods*). Purified StpA and H-NS proteins were used to show that the anti-H-NS monoclonal antibody H113 recognised H-NS but not StpA (figure 1A), whereas the anti-H-NS polyclonal antibody H200 recognised both StpA and H-NS (figure 1B). Densitometric analysis of figure 1B showed that the H200 polyclonal antibody has a 10-fold lower affinity for StpA compared with H-NS (compare lanes containing 50 ng of H-NS and StpA), presumably reflecting the absence of certain H-NS-specific epitopes from StpA. This polyclonal antibody permitted identification of StpA in an *hns* mutant in which full-length H-NS protein was not produced (see below).

StpA expression was determined in an *hns* mutant with samples taken throughout the growth phase. A Western blot was probed with anti-H-NS monoclonal antibody H113, which is unable to recognise StpA (figure 2A). This confirms the absence of H-NS in the *hns* mutant. When probed with the anti-H-NS polyclonal antibody H200, which can recognise StpA, the StpA protein was shown to be present at different levels during the growth phase. Figure 2BC shows StpA at a lower level at early log phase and at a 50% higher level at mid and late log phase. Under all conditions tested, the amount of protein correlated with the relative amount of mRNA, suggesting the absence of significant post-transcriptional control (data not shown). Figure 2C shows a densitometric analysis of figure 2B, compensating for the dilution factor used for the lysate of the host strain. Since the anti-H-NS antibody H200 recognises StpA with a ten-fold lower affinity than for H-NS (figure 1), the relative concentration of StpA in an *hns*⁻ strain is about ten-fold less than the concentration of H-NS in a wild-type *E. coli* cell at mid-log phase.

3.2. DNA-binding characteristics of *StpA* and H-NS

To aid our understanding of the respective physiological roles of H-NS and StpA we compared their DNA-binding properties. Previously published work suggested that StpA was a DNA-binding protein, and bound DNA at

similar levels to H-NS [14, 16, 17]. We have now performed an accurate quantification of DNA binding by StpA and H-NS. To compare the DNA binding properties of the two proteins, *E. coli* StpA was purified to homogeneity following over-expression, as described in 2. *Materials and methods*. Two complementary DNA-binding assays were used, gel mobility shift and filter binding.

It was first necessary to confirm that the purified H-NS and StpA proteins could be compared meaningfully. Thus, the relative DNA binding capacities of the purified StpA and H-NS proteins were determined by examining the displacement of radioactive *proU* DRE DNA by non-specific competitor (2. *Materials and methods*). Similar amounts of generic poly-[d(I-C)] DNA were necessary to displace the radioactive DNA, indicating that both protein preparations have a similar binding capacity (data not shown). This demonstrates that the two protein preparations have equivalent numbers of active protein molecules, and hence validates subsequent experiments to compare the relative DNA-binding affinities of StpA and H-NS.

Increasing concentrations of StpA or H-NS were incubated with *Hae*III-digested pAF453 (plasmid containing an AT-planar curve; 2. *Materials and methods*). Under these conditions, six-fold more H-NS than StpA (5.9 μ M versus 1.0 μ M) was required to bind to the 189 bp AT-planar curve and other DNA fragments (figure 3). In general (with the exception of curved DNA fragments), as the StpA concentration was increased it bound to the other fragments as a function of DNA fragment size. At high concentrations of H-NS or StpA all DNA fragments are shifted (data not shown). H-NS has previously been reported to show a preference for binding to this AT-planar curve in gel mobility shift assays [20]. Figure 3 shows that both StpA and H-NS require approximately four-fold lower concentrations of protein to shift the 189 bp AT-planar curve, compared with other DNA fragments of similar size. Thus, like H-NS, StpA has a slight preference for binding this particular curved DNA fragment. These properties are similar to that reported previously for H-NS [20]. Thus, apart from the observation that StpA appears to have a slightly higher affinity for DNA, the DNA binding properties of H-NS and StpA appear similar in gel mobility shift assays.

Because the use of gel mobility shift assays for the determination of DNA binding constants has limitations, filter retention assays were used to determine the DNA binding affinities of StpA and H-NS for an AT-planar curve fragment. The downstream regulatory element (DRE) DNA fragment of the *proU* operon was also studied, as it is a well characterised region of DNA that is required for the binding of H-NS in vivo [20, 23].

As expected, StpA and H-NS retained the AT-planar curve DNA on the nitrocellulose filters as a function of protein concentration (figure 4). DNA binding affinities were determined by curve fitting analyses. H-NS bound

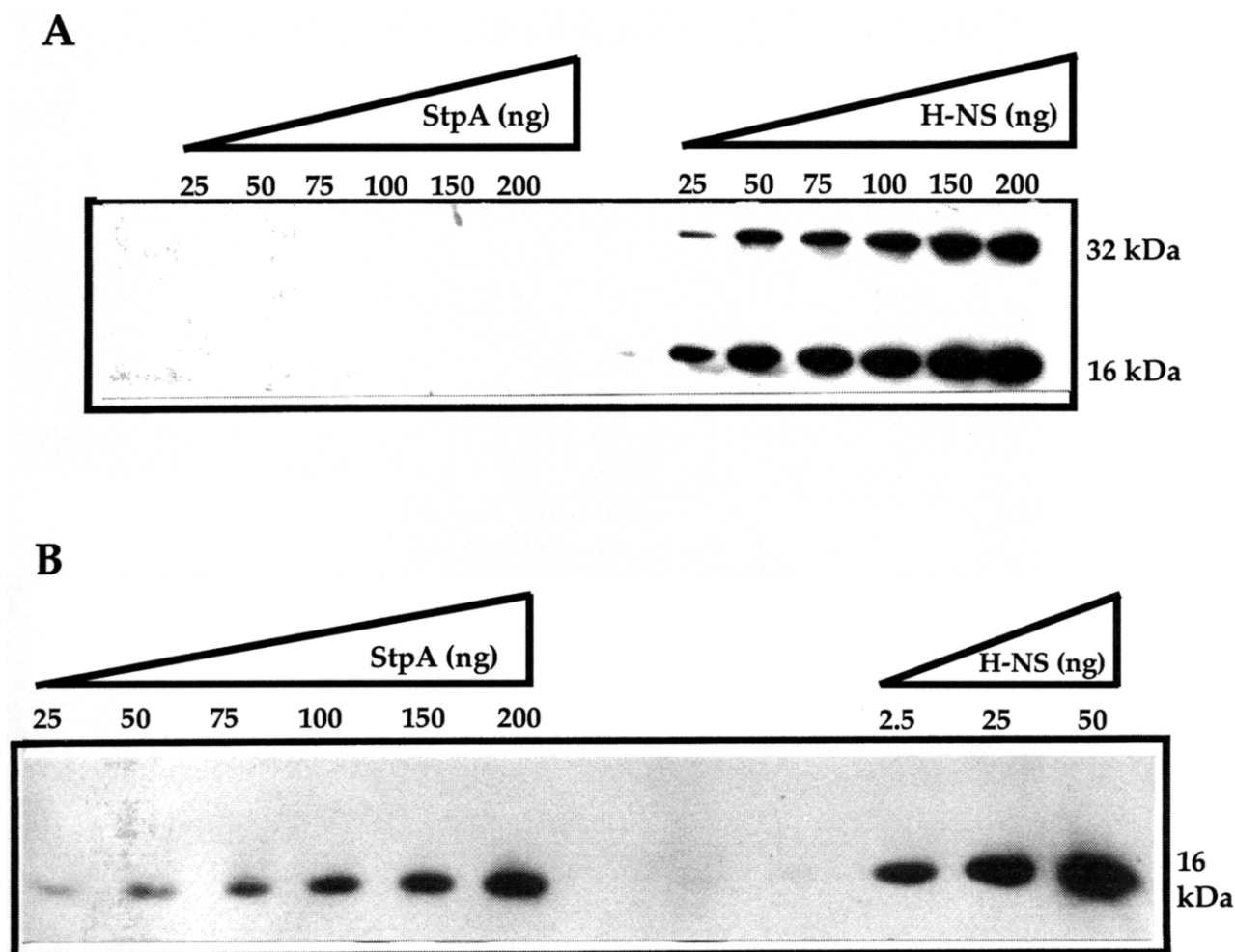


Figure 1. H-NS and StpA are differentiated by anti-H-NS monoclonal and polyclonal antibodies. **A.** Western blots of purified StpA and H-NS protein probed with the anti-H-NS monoclonal antibody H113. The 32 kDa dimer of H-NS is apparent in this case. **B.** Western blots of StpA and H-NS probed with the anti-H-NS polyclonal antibody H200. Note that less H-NS was loaded than StpA, as indicated.

the DNA fragments with a K_d of 2.8 μM while StpA showed a higher affinity with a K_d of 0.7 μM . These binding affinities are consistent with the gel mobility shift assays, and with K_d values previously determined for H-NS (figure 3) [20]. The StpA protein did not discriminate between the AT-planar curve and the DRE DNA fragments, as has been previously reported for filter retention assays involving H-NS [20]. In summary, StpA has a four- to six-fold higher affinity for DNA than H-NS, but its specificity is similar.

4. Discussion

Comparison of DNA-binding by StpA and H-NS shows that StpA has a broad specificity for DNA which matches

that of H-NS. This suggests that StpA could affect gene expression directly, in the same way as H-NS, by binding to DNA. It is clear that StpA has a four- to six-fold greater affinity for DNA than H-NS, which is consistent with the observation that StpA is more active than H-NS at constraining negative supercoils in DNA [14]. Furthermore, StpA has been shown to act as a more efficient RNA chaperone than H-NS [31]. Thus, H-NS and StpA have broadly similar biochemical properties, but StpA has a slightly higher affinity for nucleic acids. Such a difference in affinity could account for differential effects of StpA and H-NS on promoter function and gene expression.

Another group has recently surveyed the binding of several nucleoid-associated proteins from *E. coli* and their

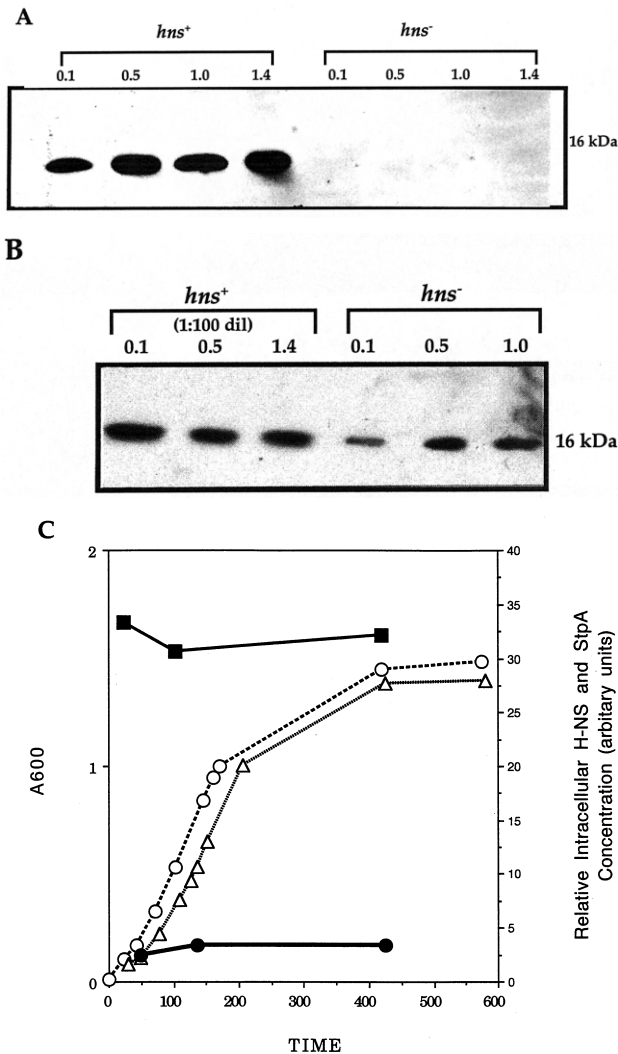


Figure 2. Effect of *hns* on expression of StpA in *E. coli*. **A.** Total cell protein was extracted from MC4100 and MC4100 *hns*::Kan at four stages of the growth phase in LB medium (A_{600} values, as indicated). Samples were Western blotted with anti H-NS monoclonal antibody H113. **B.** MC4100 and MC4100 *hns*::Kan samples from three stages in the growth curve were Western blotted with anti-H-NS polyclonal antibody. Protein was prepared at various A_{600} values, as indicated. To allow visualisation of StpA-specific bands in the MC4100 *hns*::Kan sample, a 100-fold dilution of the MC4100 sample was loaded. **C.** Densitometric analysis of figure 2B. The growth curves of MC4100 (O) and MC4100 *hns*::Kan (Δ) are shown. The relative proportion of H-NS plus StpA in MC4100 (\blacksquare) to StpA in MC4100 *hns*::Kan (\bullet) was calculated by densitometric analysis allowing for sample dilution, and using the relative affinity of the anti-H-NS polyclonal antibody for H-NS and StpA (figure 1).

results for StpA and H-NS appear to be somewhat different from ours [16]. They found that both proteins

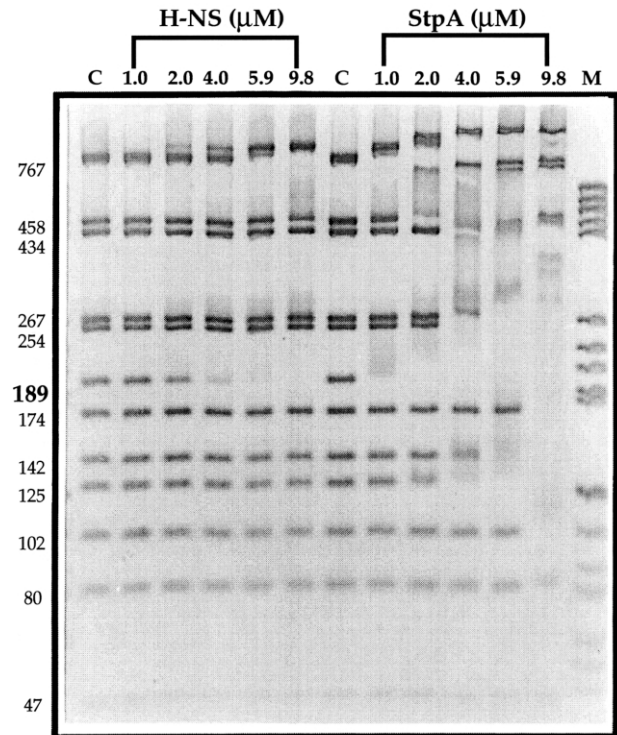


Figure 3. Gel mobility shift assays. The concentration dependence of the interaction of StpA and H-NS with different DNA fragments was examined by gel mobility shift. The DNA fragments were produced by digestion of plasmid pAF453 with *Hae*III (2.Materials and methods). The fragment containing the 189-bp AT-planar curve is indicated in bold. Sizes of bands (bp) are indicated. The differences in binding to particular fragments were consistently observed in four independent experiments.

bound DNA with indistinguishable affinity and that only H-NS showed any specificity of binding. As their measurements were done using different buffer conditions, and different DNA ligands, direct quantitative comparison is not possible. If we assume that the preparations of H-NS and StpA used for these studies are similarly active, as we have shown above, then why do our experiments reveal an affinity difference that is not observed by Azam and Ishihama [16]? We believe that the size of the DNA ligands may be responsible. Azam and Ishihama [16] found that for short (ca. 50 bp) ligands, H-NS and StpA bound DNA with an equal affinity, but that only H-NS showed specificity for curved DNA, while we find that with a longer ligand (189 bp), StpA binds with significantly higher affinity than H-NS, and both proteins show preference for curved DNA. Taken together, these data suggest that StpA recognises a longer curved DNA segment than H-NS. This difference may relate to the ability of H-NS and StpA to differentially regulate gene expression from certain virulence gene regulons (Raupach et al.,

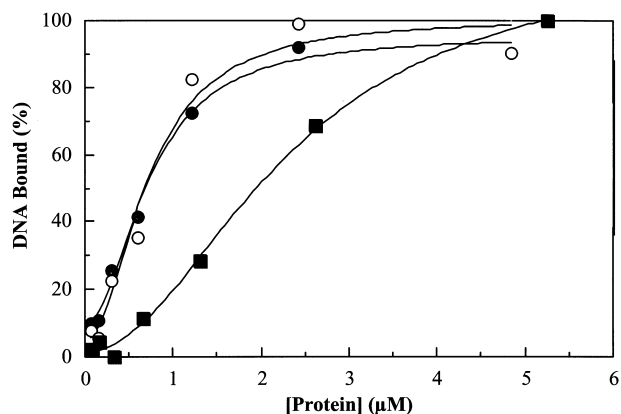


Figure 4. Filter retention assays. The concentration dependence of the interaction of StpA (●, ○) and H-NS (■) with different radiolabelled DNA fragments was determined by filter retention. The DNA interactions were examined using the AT-planar curve (●, ■) or the *proU* DRE fragment (○). The data are expressed, for comparative purposes, as the percentage of the maximal fraction bound. The values are the mean of five independent experiments and the error was less than 5%.

in preparation). The function of StpA and H-NS is thought to be linked to their ability to form hetero-oligomers [3, 9, 15]. If H-NS-StpA hetero-oligomers have novel properties, environmental regulation of StpA expression could modulate the function of H-NS, and hence also affect H-NS-dependent gene regulation. We are currently investigating this possibility.

As StpA expression is induced in an H-NS mutant, and as over-expression of StpA can suppress many of the effects of H-NS on gene expression [8, 10], we determined the level of StpA present in cells in the absence of H-NS. Our results show that despite significant Lon-dependent degradation of StpA in the absence of H-NS [11], there is still only 10% as much StpA protein in an *hns* mutant as there is H-NS in a wild-type strain. In spite of the fact that StpA has a four- to six-fold greater affinity for DNA than H-NS, it is clear that StpA cannot directly substitute for H-NS in *hns* mutants. We already knew that the ability of StpA to suppress certain Hns⁻ phenotypes when expressed in multicopy showed that StpA and H-NS shared a degree of functional similarity. It is important to remember that more than 60 genes show altered expression in *hns* strains of *E. coli* [32, 33], despite the presence of the low level of StpA. In agreement with preliminary findings for H-NS [34], our results indicate that the cellular concentration of H-NS or StpA is critical in determining the effect on gene expression.

Acknowledgments

We are grateful to Georgina Berridge and Clare Smyth for preparing H-NS protein, to Takeshi Mizuno for bacterial strains,

to Bart Jordi, Beatrice Py, Julie Sidebotham, Loredana Soceanantu and David Ussery for helpful advice, and to Thérèse Bown for assistance in preparing the manuscript. C.F.H is a Howard Hughes International Research Scholar. This work was supported by an award from the Brasenose Scholar Foundation (to J.S.) and a Programme grant from the Wellcome Trust (Ref. 045490).

References

- [1] Zhang A., Belfort M., Nucleotide sequence of a newly-identified *Escherichia coli* gene, *stpA*, encoding an H-NS-like protein, *Nucleic Acids Res.* 20 (1992) 6735.
- [2] Zhang A., Derbyshire V., Galloway Salvo J.L., Belfort M., *Escherichia coli* protein StpA stimulates self-splicing by promoting RNA assembly in vitro, *RNA* 1 (1995) 783–793.
- [3] Dorman C., Hinton J.C.D., Free A., Homo- and hetero-oligomerization among H-NS-like proteins in bacteria, *Trends Microbiol.* 7 (1999) 124–128.
- [4] Higgins C.F., Hinton J.C.D., Hulton C.S.J., Owen-Hughes T., Pavitt G.D., Seirafi A., Protein H1: a role for chromatin structure in the regulation of bacterial gene expression and virulence? *Mol. Microbiol.* 4 (1990) 2007–2012.
- [5] Hulton C.S.J., et al., Histone-like protein H1 (H-NS), DNA supercoiling and gene expression in bacteria, *Cell* 63 (1990) 631–642.
- [6] Ussery D.W., et al., The chromatin-associated protein H-NS, *Biochimie* 76 (1994) 968–980.
- [7] Williams R.M., Rimsky S., Molecular aspects of the *Escherichia coli* nucleoid-associated protein H-NS: a central controller of gene regulatory networks, *FEMS Microbiol. Lett.* 156 (1997) 175–185.
- [8] Shi X., Bennett G.N., Plasmids bearing *hfq* and the *hns*-like gene *stpA* complement *hns* mutants in modulating arginine decarboxylase gene expression in *Escherichia coli*, *J. Bacteriol.* 176 (1994) 6769–6775.
- [9] Free A., Williams R.M., Dorman C.J., The StpA protein as a molecular adapter to mediate repression of the *bgl* operon by truncated H-NS in *Escherichia coli*, *J. Bacteriol.* 180 (1998) 994–997.
- [10] Sonden B., Uhlin B.E., Coordinated and differential expression of histone-like proteins in *Escherichia coli*: regulation and function of the H-NS analog StpA, *EMBO J.* 15 (1996) 4970–4980.
- [11] Johansson J., Uhlin B.E., Differential protease-mediated turnover of H-NS and StpA revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*, *Proc. Natl. Acad. Sci. USA* 96 (1999) 10776–10781.
- [12] Benson N.R., Wong R.M., McClelland M., Analysis of the SOS response in *Salmonella enterica* serovar *Typhimurium* using RNA fingerprinting by arbitrarily primed PCR, *J. Bacteriol.* 182 (2000) 3490–3497.
- [13] Free A., Dorman C.J., The *Escherichia coli stpA* gene is transiently expressed during growth in rich medium and is induced in minimal medium and by stress conditions, *J. Bacteriol.* 179 (1997) 909–918.
- [14] Zhang A., Rimsky S., Reaban M.E., Buc H., Belfort M., *Escherichia coli* protein analogs StpA and H-NS: regulatory loops, similar and disparate effects on nucleic acid dynamics, *EMBO J.* 15 (1996) 1340–1349.
- [15] Williams R.M., Rimsky S., Buc H., Probing the structure, function, and interactions of the *Escherichia coli* H-NS and StpA proteins by using dominant negative derivatives, *J. Bacteriol.* 178 (1996) 4335–4343.
- [16] Azam T.A., Ishihama A., Twelve species of the nucleoid-associated protein from *Escherichia coli*. Sequence recognition specificity and DNA binding affinity, *J. Biol. Chem.* 274 (1999) 33105–33113.

- [17] Bertin P., et al., The structural and functional conservation of H-NS-like proteins is evolutionarily conserved in Gram-negative bacteria, *Mol. Microbiol.* 31 (1999) 319–329.
- [18] Casadaban M., Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage Lambda and Mu, *J. Mol. Biol.* 104 (1996) 541–555.
- [19] Yamada H., Toshida T., Tanaka K.I., Sasakawa C., Mizuno T., Molecular analysis of the *Escherichia coli hns* gene encoding a DNA-binding protein, which preferentially recognises curved sequences, *Mol. Gen. Genet.* 230 (1991) 332–336.
- [20] Jordi B.J.A.M., Fielder A.E., Burns C.M., Hinton J.C.D., Dover N., Ussery D.W., Higgins C.F., DNA binding is not sufficient for H-NS-mediated repression of *proU* expression, *J. Biol. Chem.* 272 (1997) 12083–12090.
- [21] Roth J.R., Genetic techniques in studies of bacterial metabolism, *Methods. Enzymol.* 17A (1970) 3–35.
- [22] Tabor S., Richardson C., A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes, *Proc. Natl. Acad. Sci. USA* 82 (1985) 1072–1078.
- [23] Owen-Hughes T.A., Pavitt G.D., Santos D.S., Sidebotham J.M., Hulton C.S.J., Hinton J.C.D., Higgins C.F., The chromatin-associated protein H-NS interacts with curved DNA to influence DNA topology and gene expression, *Cell* 71 (1992) 255–265.
- [24] Schagger H., Von Jagow G., Tricine sodium dodecyl-sulfate polyacrylamide-gel electrophoresis for the separation of proteins in the range from 1-kda to 100-kda, *Anal. Biochem.* 166 (1987) 368–379.
- [25] Laemmli U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* 227 (1970) 680–685.
- [26] Towbin H., Staehlin T., Gordon J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, *Proc. Natl. Acad. Sci. USA* 76 (1979) 4350–4354.
- [27] Hinton J.C.D., Santos D., Seirafi A., Hulton C.S.J., Pavitt G.D., Higgins C.F., Expression and mutational analysis of the nucleoid-associated protein H-NS of *Salmonella typhimurium*, *Mol. Microbiol.* 6 (1992) 2327–2337.
- [28] Sambrook J., Fritsch E.F., Maniatis T., *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press, 1989.
- [29] Blum E., Holzschu D., Kwan H.S., Riggs D., Artz S., Gene replacement and retrieval with recombinant M13mp bacteriophages, *J. Bacteriol.* 171 (1989) 538–546.
- [30] Ulanovsky L., Bodner M., Trifonov E.N., Choder M., Curved DNA: design, synthesis and circularization, *Proc. Natl. Acad. Sci. USA* 83 (1986) 862–866.
- [31] Cusick M., Belfort M., Domain structure and RNA annealing activity of the *Escherichia coli* regulatory protein StpA, *Mol. Microbiol.* 28 (1998) 847–857.
- [32] Atlung T., Ingmer H., H-NS: a modulator of environmentally regulated gene expression, *Mol. Microbiol.* 24 (1997) 7–17.
- [33] Laurent-Winter C., Ngo S., Danchin A., Bertin P., Role of *Escherichia coli* histone-like nucleoid-structuring protein in bacterial metabolism and stress response. Identification of targets by two-dimensional electrophoresis, *Eur. J. Biochem.* 244 (1997) 767–777.
- [34] Goyard S., Bertin P., Characterization of BpH3, an H-NS-like protein in *Bordetella pertussis*, *Mol. Microbiol.* 24 (1997) 815–823.



Paris, December 2000

To the contributors of *Biochimie*

The first two issues of *Biochimie* volume 83 (2001) will be devoted to 'Cell cycle and nucleoid organization in bacteria'. You will find following this letter the contents of both issues.

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Béatrice Fournier

(tel: +33 1 45 58 90 33

fax: +33 1 45 58 94 21

e-mail: b.fournier@elsevier.fr)

Cell cycle and nucleoid organization in bacteria

Part I. Cell cycle

Coordinated by J. Rouviere-Yaniv

Foreword

<i>J. Rouviere-Yaniv (Paris, France)</i>	000
Bacterial replication initiator DnaA. Rules for DnaA binding and roles of DnaA in origin unwinding and helicase loading <i>W. Messer, F. Blaesing, D. Jakimowicz, M. Krause, J. Majka, J. Nardmann, S. Schaper, H. Seitz, C. Speck, C. Weigel, G. Wegrzyn, M. Welzcek, J. Zakrewska-Czerwinska (Berlin, Germany; Wroclaw, Gdansk, Poland)</i>	000
Multiple pathways regulating DnaA function in <i>Escherichia coli</i> : Distinct roles for DnaA titration by <i>datA</i> locus and regulatory inactivation of DnaA <i>T. Katayama, K. Fujimitsu, T. Ogawa (Fukuoka, Japan)</i>	000
DnaA protein. Phospholipid interactions: In vitro and in vivo <i>E. Crooke (Georgetown, USA)</i>	000
Defective initiation in an <i>Escherichia coli dnaA (Cs, Sx)</i> mutant <i>E. Boye, A. Blinkova, J.R. Walker (Austin, USA)</i>	000
DnaA protein dependant denaturation of negative supercoiled <i>oriC</i> DNA minicircles <i>A. Landoulsi, M. Kohiyama (Paris, France)</i>	000
Partitioning of the <i>Escherichia coli</i> chromosome: Superhelicity and condensation <i>K. Nordström, S. Dasgupta (Uppsala, Sweden)</i>	000
The <i>Escherichia coli</i> SeqA protein binds specifically to two sites in fully and hemimethylated <i>oriC</i> and has the capacity to inhibit DNA replication and affect chromosome topology <i>K. Skarstad, N. Torheim, S. Wold, R. Lurz, W. Messer, S. Fossum, T. Bach (Oslo, Norway)</i>	000
SMC proteins in bacteria: Condensation motors for chromosome segregation ? <i>P.L. Graumann (Marburg, Germany)</i>	000
Models of movement of DNA regions in <i>Escherichia coli</i> evaluated by computer simulation <i>M. Roos, R. Lingeman, C.L. Woldringh, N. Nanninga (Amsterdam, the Netherlands)</i>	000
Coordinating DNA replication with cell division: Lessons form outgrowing spores <i>E.J. Harry (Sidney, Australia)</i>	000
Cell-cycle research with synchronous cultures. An evaluation <i>C.E. Helmster, M. Thornton, N.B. Grover (Jerusalem, Israel)</i>	000
Hypothesis: Membrane domains and hyperstructures control bacterial division <i>V. Norris, I. Fishov (Mont-Saint-Aignan, France; Be'er Sheva, Israel)</i>	000
Approaching the physiological functions of penicillin-binding proteins in <i>Escherichia coli</i> <i>K.D. Young (Grand Forks, USA)</i>	000
Enzymology of elongation and constriction of the murein sacculus of <i>Escherichia coli</i> <i>J.V. Höltje, C. Heidrich (Tubingen, Germany)</i>	000
Transcription of the <i>Escherichia coli dcw</i> cluster: Evidence for distal upstream transcripts being involved in the expression of the downstream <i>ftsZ</i> gene <i>A. de la Fuente, P. Palacios, M. Vicente (Madrid, Spain)</i>	000
Conserved sequence motif at the C-terminus of the bacterial cell-division protein FtsA <i>J. Löwe, F. van den Ent (Cambridge, UK)</i>	000
Perpendicular planes of FtsZ arcs in spheroidal <i>Escherichia coli</i> cells <i>E. Pas, M. Einav, C.L. Woldringh, A. Zaritsky (Beersheva, Israel)</i>	000
FtsZ rings in <i>mukB</i> mutants with or without the Min system <i>X.-C. Yu, Q. Sun, W. Margolin (Houston, USA)</i>	000

Cell cycle and nucleoid organization in bacteria

Part II. Nucleoid organization

Coordinated by J. Rouviere-Yaniv

Isolation of the <i>Escherichia coli</i> nucleoid <i>S. Cunha, T. Odijk, E. Sueleymanoglu, C. Woldringh (Amsterdam, the Netherlands)</i>	000
Transcription induces a supercoil domain barrier in bacteriophage Mu <i>K.E. Scheirer, N.P. Higgins (Birmingham, USA)</i>	000
Polarization of the <i>Escherichia coli</i> chromosome. A view from the terminus <i>H. Capiiaux, F. Cornet, J. Corre, M.-I. Guijo, K. Pérals, J. Emilio Rebollo, J.-M. Louarn (Toulouse, France; Badajoz, Spain)</i>	000
DNA degradation in the terminus region of resolvase mutants of <i>Escherichia coli</i> , and suppression of this degradation and the Dif phenotype by <i>recD</i> <i>J. Prikryl, E.C. Hendricks, P.L. Kuempel (Boulder, USA)</i>	000
The form of chromosomal DNA molecules in bacterial cells <i>A.J. Bendich (Seattle, USA)</i>	000
Chromosome separation and segregation in dinoflagellates and bacteria may depend on liquid crystalline states <i>Y. Bouligand, V. Norris (Angers, Mont-Saint-Aignan, France)</i>	000
HU-GFP and DAPI colocalize on the <i>Escherichia coli</i> nucleoid <i>M. Wery, J. Rouviere-Yaniv (Paris, France)</i>	000
Genome organisation and chromatine structure in <i>Escherichia coli</i> <i>D. Ussery, T.S. Larsen, K.T. Wilkes, C. Friis, P. Worning, A. Krogh, S. Brunak (Copenhagen, Denmark)</i>	000
DNA supercoiling and transcription in <i>Escherichia coli</i> . The FIS connection <i>A. Travers, R. Schneider, G. Muskhelishvili (Cambridge, UK; München, Germany)</i>	000
Structural basis for preferential binding of H-NS to curved DNA <i>R. Thei Dame, C. Wyman, N. Goosen (Leiden, Rotterdam, the Netherlands)</i>	000
Does the parallel evolution pattern between the replication-segregation proteins and HU have a biological signification? <i>J. Oberto J. Rouviere-Yaniv (Paris, France)</i>	000
H-NS and H-NS-like proteins in Gram-negative bacteria and their multiple role in the regulation of bacterial metabolism <i>P. Bertin, F. Hommais, E. Krin, O. Soutourina, C. Tendeng, S. Derzelle, A. Danchin (Paris, France)</i>	000
The nucleoid-associated protein StpA binds curved DNA and has a greater DNA-binding affinity than H-NS <i>J.M. Sonnenfield, C.M. Burns, C.F. Higgins, J.C. Hinton (Norwich, UK)</i>	000
Mutagenesis of the downstream region of <i>Escherichia coli</i> hns promoter <i>M. Giangrossi, C. Gualerzi, C.L. Pon (Camerino, Italy)</i>	000
The GemA protein of phage Mu and the GyrB gyrase subunit of <i>Escherichia coli</i> : The search for targets and interactions leading to the reversion of Mu-induced mutations <i>C. Abbes, D. Joseleau-Petit, J.-C. Liébart, R. D'Ari, G. Sezonov (Paris, France)</i>	000
A possible role of L24 of <i>Bacillus subtilis</i> in nucleoid organization and segregation <i>R.M. Exley, M. Zouine, J.J. Pernelle, C. Beloin, F. Le Hegarat, A.M. Deneubourg (Orsay, France)</i>	000
Molecular components of the archaeal nucleosome <i>K. Sandman, D. Soares, J.N. Reeve (Columbus, USA)</i>	000