

Hypothesis

HU: promoting or counteracting DNA compaction?

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Abstract The role of HU in *Escherichia coli* as both a protein involved in DNA compaction and as a protein with regulatory function seems to be firmly established. However, a critical look at the available data reveals that this is not true for each of the proposed roles of this protein. The role of HU as a regulatory or accessory protein in a number of systems has been thoroughly investigated and in many cases has been largely elucidated. However, almost 30 years after its discovery, convincing evidence for the proposed role of HU in DNA compaction is still lacking. Here we present an extensive literature survey of the available data which, in combination with novel microscopic insights, suggests that the role of HU could be the opposite as well. The protein is likely to play an architectural role, but instead of being responsible for DNA compaction it could be involved in antagonising compaction by other proteins such as H-NS.

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1. Introduction

In bacteria the chromosomal DNA is organised into a condensed structure called a nucleoid. A considerable number of proteins, which have been collectively named histone-like proteins, or nucleoid-associated proteins, are thought to be involved in DNA compaction. The best characterised and most abundant members of this group of proteins are HU, H-NS, IHF and Fis. The proposed function of these proteins has been derived from co-purification with isolated nucleoids. In addition, their role in DNA compaction was (initially) based on superficial similarities with eukaryotic histones, such as DNA-binding capacity, small size, basicity and abundance. Later, co-localisation with the bulk DNA of many of these proteins has in vivo been shown microscopically by using fluorescently labelled protein [1,2] or by using antibodies directed against these proteins in fixed cells [3].

The bacterial nucleoid is usually considered to be a regular oval structure. This has been deduced from light-microscopic visualisation of the nucleoid using either DNA or protein

markers and from 'early' electron microscopic (EM) investigations (for an extensive review see [4]). However, it has been suggested that light microscopy does not allow visualisation of the 'exact' structure of the nucleoid, and that in fact the structure is somewhat different. Reconstruction from EM images of serial sections of cryofixed and freeze-substituted *Escherichia coli* cells shows that the nucleoid has a coralline shape [5].

Two proteins associated with the nucleoid, HU and H-NS, are particularly abundant (in logarithmic phase 50 000 and 20 000 copies, respectively [6]), and are therefore likely to contribute significantly to bacterial nucleoid structure. HU has been identified in all eubacteria analysed so far. In most of these bacteria it exists as a 18-kDa homodimer, but in *E. coli* (and other enterobacteriaceae) HU is predominantly found as a heterodimer composed of two subunits, HU α and HU β , encoded by the *hupA* and *hupB* genes. Next to the heterodimeric form of HU, homodimers of each of the two subunits are also found in *E. coli*. The reason for this is unclear, but the differential regulation of the expression of both subunits suggests a mechanism for fine-tuning of the properties of the HU dimer. HU is a structural homologue of IHF, a protein that can induce bends in DNA of up to 160° [7] when bound to its recognition site. Binding of HU is sequence unspecific but, as is the case with IHF [7], binding to DNA probably involves its two arms being inserted into the minor groove.

H-NS is similar in size to HU (15.6 kDa) and also binds DNA without sequence specificity. Unlike HU, this protein can self-associate to form dimers, trimers or larger oligomers and this property is probably essential for its architectural function. Evidence for a role of H-NS in DNA compaction has been obtained by employing numerous methods. It has been shown in vitro that H-NS induces compaction [8–10] and that it affects DNA topology [11]. In addition, overproduction of H-NS was shown to induce a strong level of compaction of the nucleoid [12]. The situation is different for HU and, in fact, real evidence for a role of HU in DNA compaction is practically lacking. It has been shown in vitro that HU can constrain supercoils [13,14], but, for example, overproduction of HU does not lead to an increased level of compaction of the bacterial chromosomal DNA [15], as observed with H-NS [12]. Nevertheless, HU is the 'textbook' prokaryotic equivalent of eukaryotic histones. In order to understand how HU got to be considered a histone-like protein, the developments leading to and following its discovery should be put in a historical context.

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2. The creation of a dogma

At the time that HU was discovered [16] the understanding of eukaryotic chromatin was advancing rapidly. The organisation of eukaryotic chromatin in nucleosomes had been elucidated not only by employing biochemical methods, such as nuclease protection assays [17,18], but also by direct electron microscopic visualisation [19–21]. It was noticed that HU was in some aspects similar to histones: it is a small basic DNA-binding protein and is abundant. This led to the idea that HU could play a histone-like role.

Visualisation of bacterial chromosomal DNA upon cell lysis had been done [22,23], but due to the preparation procedure this did not reveal any residual higher order organisation of bacterial chromatin. However, rosette-like structures were observed in which about 50 plectonemically interwound DNA loops were emanating from a central core. The images were interpreted as reflecting an organisation of the chromosome in supercoiled loops [23,24]. Further evidence for an organisation of the bacterial chromosome in independent loops stems from the observation that complete relaxation of the chromosomal DNA requires the introduction of multiple nicks [25]. On the basis of those experiments it was proposed that a chromosome in a living cell is composed of 50- to 100-kb modular domains [25]. These experiments could not provide insight into whether the looped organisation is of static or dynamic nature. However, *in vivo* recombination experiments revealed that the recombination efficiency is only dependent on the distance between the two recombination sites and did not reveal the presence of fixed boundaries in the proposed size range of the domains (up to 100 kb) [26]. This suggests that the placement of the supercoil domain barriers is a stochastic process and that the organisation of bacterial chromatin in supercoiled loops is of a highly dynamic nature [26]. Many attempts to reveal a higher order organisation of the bacterial chromosome were made hereafter, but these were not successful or were later disregarded as artefacts [27]. The apparent lack of organisation has been attributed to a low stability of histone-like protein–DNA complexes and to a very dynamic nature of bacterial chromatin [13].

Initial attempts to characterise HU–DNA complexes by electron microscopy failed in the sense that they did not indicate clear structural effects of HU binding (apart from DNA thickening) [16]. Shortly after, nucleosome-like structures were visualised on bacterial chromosomal DNA upon cell lysis [28]. The observation of these nucleosome-like structures (also called compactosomes) [28] was, however, later suggested to have been the result of dehydration of the sample [27], a procedure which can even induce the formation of nucleosome-like structures on naked DNA [29]. Nevertheless, this was followed by new attempts to microscopically visualise HU–DNA complexes. If complexes were glutaraldehyde-fixed a ‘beads on a string’ structure could be observed [30], which was in fact very similar to images of eukaryotic chromatin [20,30]. Thus it was concluded that HU has the capacity to form nucleosome-like structures. However, to date no further evidence has been found that compactosomes truly exist in bacteria, or that HU *in vivo* is a functional and/or structural equivalent of eukaryotic histones.

3. Structural role of HU

In the case of eukaryotic chromatin an organisation into regular units (nucleosomes) is evident from nuclease digestion, which results in a regular pattern reflecting the length of the DNA wound around the histone-octamer. In the case of HU–DNA complexes, nuclease digestion products are obtained with a characteristic 8.5–9 bp repeat, and this has been interpreted as being the result of a reduction in helical pitch (increase in twist) as a result of HU binding [13]. Later binding studies indicate that the size of the repeat of the digestion products corresponds to the size of the HU-binding site [31] and our unpublished results), which suggests that the observed nuclease digestion patterns do not necessarily reflect a change in helical pitch, but rather are footprints of individual HU molecules.

It was also shown that HU binds preferentially to negatively supercoiled DNA [32], that it constrains supercoils and that one negative superhelical turn is constrained every 120 to 290 bp [13,14]. The effect of HU on DNA topology is further underlined by the observation that the absence of this protein is compensated by mutations in gyrase [33], the enzyme responsible for the induction of negative supercoils in DNA. Together the *in vitro* data were taken to provide evidence for DNA being wrapped around an HU protein core [34]. An alternative explanation is that each of a large number of bound HU molecules introduces a small increase in helical pitch (decrease in twist), which would be sufficient to explain the observed numbers of supercoils being restrained. The previously proposed reduction in helical pitch as a consequence of HU binding [13] would lead to the introduction of negative supercoils. This would be incompatible with the fact that HU binds preferentially to negatively supercoiled DNA and that the protein can constrain negative supercoils.

4. DNA bending by HU

A specific regulatory role for HU, which involves ‘stable’ DNA binding and bending at a specific position, has been reported for a number of systems. It is likely that in each of these systems a single HU dimer is responsible for the introduction of a bend or for an increase in local flexibility of the DNA. The use of an HU converted into a chemical nuclease [35–37] indeed indicates that only one HU dimer is required for Mu transposome assembly and for GalR loop formation [38]. The finding that HU can replace IHF in excision recombination of bacteriophage lambda and that the need for either of these proteins is abolished by insertion of curved/flexible DNA near the IHF sites [39] provides further support for the idea that a single HU dimer is sufficient to bend DNA. Finally, HU has also been shown to play a role in Hin invertosome assembly, where it facilitates interaction between two sites separated by an ~90-bp tract [40], and probably also in this system only the binding of a single HU dimer is required. It has been suggested that the preferential binding by HU in this region could be a consequence of it being curved [41,42].

In the systems described above the binding of a single HU dimer is sufficient for bending. In earlier studies, however, it was proposed that DNA bending by HU is achieved by protein–protein interactions between adjacent HU dimers [14,43]. This model was supported by circularisation experiments, in which HU stimulates ligation of substrates as short as 78 bp

[44,45], whereas in the absence of HU substrates shorter than 150 bp are not efficiently circularised [46]. Interestingly, circularisation efficiency of short substrates in the presence of HU relies heavily on the length of the DNA, and thus on the relative orientation of the DNA ends. Circularisation maxima are observed on substrates with a difference in length corresponding to the helical repeat, which could mean that the helical pitch of the DNA is not changed while bending [44]. The proposed side-by-side binding of several HU dimers [43] would be an explanation for the stimulatory effect of HU on the circularisation of short DNA substrates [45]. An alternative explanation is that this stimulation is mediated by bending induced by the binding of single HU dimers. Transient binding and bending by single HU dimers at random positions would increase the apparent flexibility (lower the persistence length) of a piece of DNA and would thus permit this piece to randomly sample more extreme conformations and increase the probability that the two DNA ends come close. A mechanism in which single dimers are responsible for DNA bending would also explain why a change in helical pitch cannot be detected in circularisation experiments [44].

Interestingly, it has been shown that HU can be functionally replaced by members of the eukaryotic family of HMG1/2-proteins. For instance, segregation defects of *hupA**hupB* mutants can be relieved by production of the mitochondrial yeast protein HM [47] and the nuclear yeast NHP6A/B (both HMG proteins) in *E. coli* [48,49]. Like HU, these proteins bind DNA without apparent sequence specificity and can induce bends into DNA. The role of HU in *hin* invertasome assembly can be taken over by such HMG-proteins [44,49] and also in excision recombination of bacteriophage lambda HMG proteins (like HU) can replace IHF [50]. Furthermore, HMG1 [44] and NHP6A/B [49] stimulate the circularisation of short DNA substrates. There is no evidence that bending by HMG involves the binding and cooperative action of a number of these proteins: binding of a single HMG protein is sufficient for bending [51]. The observed capability of these HMG proteins to functionally replace HU provides further support for DNA bending being induced by a single (transiently) bound HU dimer.

5. Compaction or decompaction?

The evidence for compaction by HU and in particular the formation of nucleosome-like structures (or DNA being wrapped around an extended HU core) is very limited. In fact, based on the available data, the proposed role of HU in DNA compaction should be considered controversial. In order to contribute to solving this controversy, we applied scanning force microscopy (SFM) to visualise HU–DNA complexes. Previously this technique was used for the visualisation of H-NS–DNA complexes and this showed that H-NS compacts DNA and that this primarily occurs by bridging adjacent DNA helices [9].

In initial experiments supercoiled DNA (95% s.c.) and saturating amounts of HU (1 dimer: 9 bp) were used. Nucleosome-like structures were not formed. Instead, the observed HU–DNA complexes were characterised by an in general more relaxed appearance of the DNA, which reflects constraining of supercoils by HU. Surprisingly, a considerable number of complexes with a ‘perfectly’ circular open shape was also observed (compare Fig. 1A and B). This effect of HU

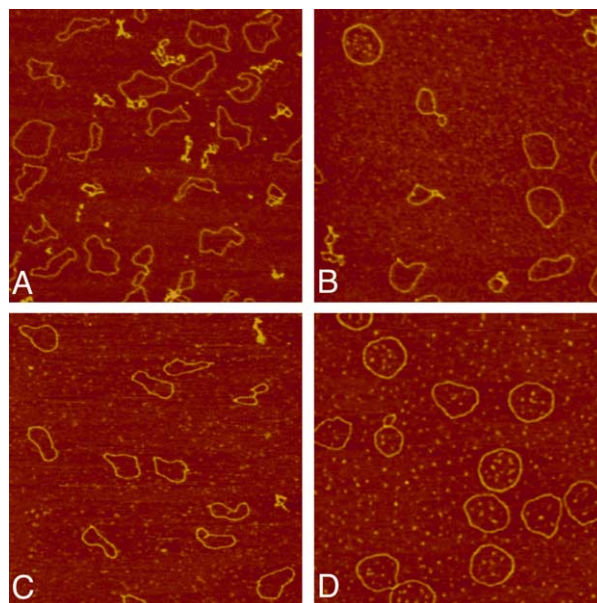


Fig. 1. Structural effect of HU binding to plasmid DNA. A: Supercoiled pUC19. B: Supercoiled pUC19+HU (1 HU: 9 bp). C: Relaxed pUC19. D: Relaxed pUC19+HU (1 HU: 9 bp). Images show a $2 \times 2\text{-}\mu\text{m}$ surface area. Method: Relaxed DNA was prepared by DNase I treatment as described [9]. Following this procedure no more than one nick is present on each molecule. Sample preparation for scanning force microscopy was essentially as described [9].

on DNA plasmid structure was even more apparent when relaxed DNA containing a single strand nick was used, which resulted in the formation of almost 100% perfectly circular open molecules (see Fig. 1C and D). This may be explained by the presence of the nick in these molecules, which can allow the release of any superhelical tension built up by HU binding. The contour length of the naked relaxed DNA molecules and the HU–DNA complexes has been measured. Contour length values were estimated by fitting a Gaussian curve to the data. Following this approach the contour length of naked relaxed DNA molecules is estimated at $903 \pm 31\text{ nm}$ ($n=48$), whereas the contour length of the HU–DNA complexes is $934 \pm 35\text{ nm}$ ($n=49$). The observed difference in DNA contour length can be explained by an increase of the helical pitch from 10.5 to 10.9 bp/helical turn. Such an increase in helical pitch would correspond to the constraint of one negative supercoil every $\sim 300\text{ bp}$, which is in agreement with the previously reported value of 290 bp [13]. If less HU (1 dimer: 72 bp or 1 dimer: 36 bp, instead of 1 dimer: 9 bp) is used, the molecules show progressively less perfect circularity (not shown). The effect of HU on the structure of the plasmid DNA is in strong contrast with the compactive effects observed with H-NS [9], which is shown for comparison in Fig. 2. The relative amounts of HU and H-NS used for these experiments are in the same order as the ratio of these proteins in vivo [6].

The observed effects of HU binding are, at first sight, puzzling, but they are fully reproducible and indicate that nucleosome-like structures are *not* formed and that *no* compaction (in fact the DNA becomes longer) of the DNA occurs. In particular on supercoiled molecules, to which HU binds preferentially [32], one would have expected efficient assembly of such complexes. The fact that the supercoiled DNA in these complexes attains a more relaxed appearance indicates that

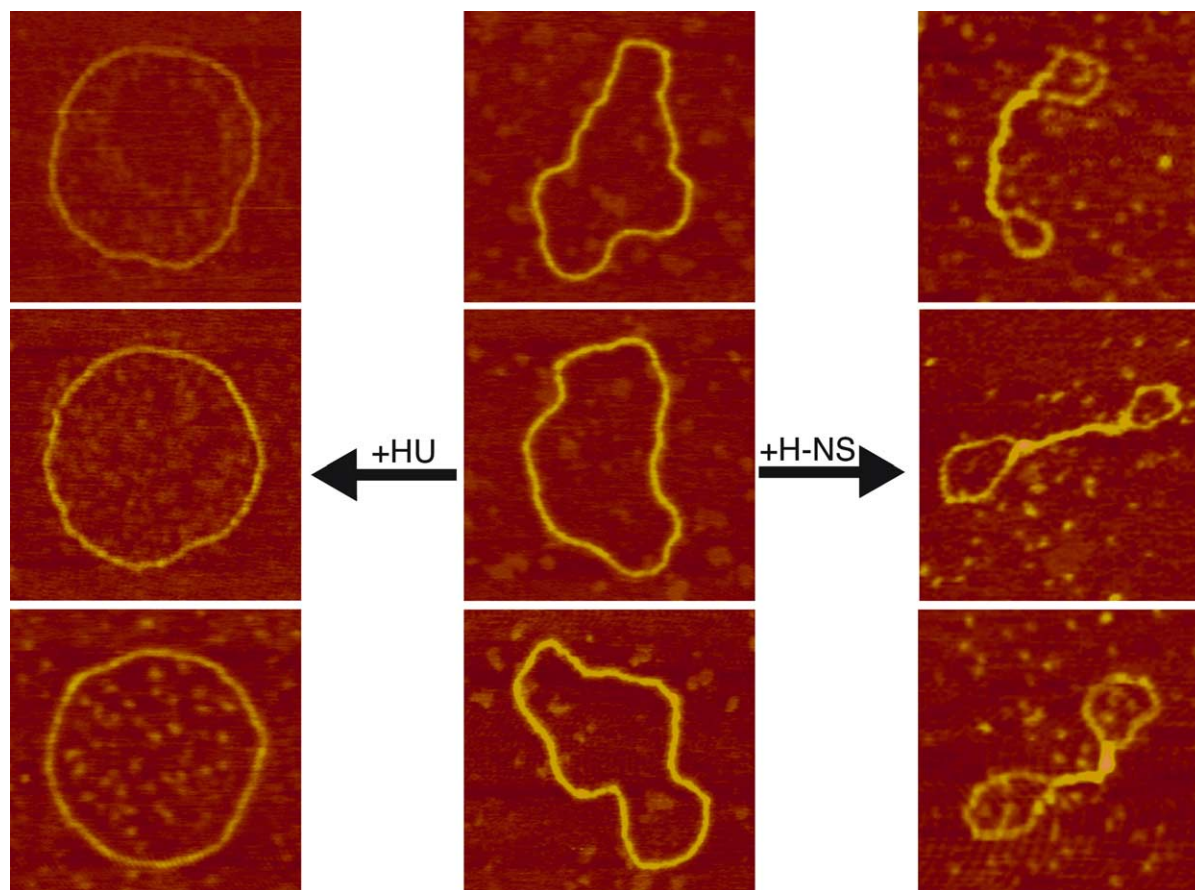


Fig. 2. Illustration of the opposite effects of H-NS and HU on DNA structure. H-NS compacts DNA molecules, whereas HU 'opens up' DNA molecules. Left panel: relaxed pUC19+HU (1 dimer: 9 bp). Centre panel: relaxed pUC19. Right panel: relaxed pUC19+H-NS (1 dimer: 12 bp). Greytone represents height ranging from 0.0 to 1.5 nm (from dark to bright).

supercoiling is nevertheless constrained [13,14], which can only be explained by increasing the pitch (decreased twist) of the DNA as a result of HU binding, as observed on relaxed circular DNA (10.5 $\rightarrow$ 10.9 bp).

In principle, the formation of the observed circles could be explained by active bending by HU dimers arranged side-by-side [43]. This would mean that formation of a perfect circle can only take place with DNA of a specific length. Experiments with plasmids of much bigger size than pUC19, however, show exactly the same effect of HU binding (not shown). A more likely explanation for circle formation therefore is that HU causes rigidification (an increase in persistence length) of the DNA.

The properties of the HU protein as observed with SFM may at first sight seem peculiar because bending and rigidification of DNA are seemingly conflicting effects. Probably the effect exerted by HU depends on the concentration of the protein. Bending is likely to occur at subsaturating protein:DNA ratios. HU is then not stably associated with the DNA and does not induce a static bend. Rather, it increases the effective flexibility of the DNA by transient binding at random positions, thus allowing it to sample more extreme conformations (see section 4). Rigidification of the DNA probably occurs at relatively high protein:DNA ratios, when HU dimers bind to adjacent positions on the DNA. Because the DNA contour length is not reduced (but rather increased) upon HU binding, we propose that the protein is bound

around the DNA helix [34], as has been described for the DBP–DNA complex [52].

6. Conclusion

Based on the SFM results described above, the effects of H-NS and HU could be considered opposing: H-NS induces the formation of a more compact structure, whereas HU 'opens up' the DNA molecules (see Fig. 2). In addition to a role in DNA compaction, H-NS is known to specifically repress transcription of a whole set of genes by its ability to modulate DNA structure [53,54]. If H-NS is overproduced, global silencing of transcription occurs [15], which is lethal. It is also known that many of the H-NS sensitive promoter regions are organised such that other specific DNA-binding proteins can antagonise the H-NS-mediated repression. This has been demonstrated for the promoters of *virF* [55–57], *hns* [58], the early promoter of phage Mu [59] and the ribosomal RNA P1 promoters [60,61], in which relief of repression occurs by the binding of Fis or IHF. We hypothesise that HU plays a similar role, but primarily at a more general level: HU non-specifically antagonises H-NS action such that regions compacted by H-NS will be of limited size and stability, which could be important for transcriptional activity. Indeed, preliminary SFM experiments with both HU and H-NS suggest that H-NS-mediated compaction can be efficiently counteracted by HU (not shown). A role of this kind would be sup-

ported by the finding that HU is not associated with the bulk DNA [62] but mostly localised to the area where transcription takes place [63]. In addition, locally HU and H-NS could compete for the same preferential binding sites as formed by curved DNA [41,64,65]. An example which could clearly point to such a role for HU is the *proU* operon, the expression of which is repressed by H-NS [66,67]. In *hupAhupB* mutants *proU* expression is more strongly repressed, which could be due to a higher effective repressive activity of H-NS, whereas overproduction of HU apparently alleviates the repression by H-NS [68]. Similar observations have been reported for transcription regulation of the *micF* antisense RNA [69]. Transcription of *micF* is repressed by H-NS. In *hns* mutants this repression is relieved [70,71], whereas in *hupAhupB* mutants transcription again seems to be more effectively repressed than in wt strains [72]. Another recent study demonstrates that in vivo the transcriptional activity of T7 RNA polymerase is enhanced by the presence of HU, and that this stimulation most likely occurs at the level of initiation [73]. This could be related to the finding that in vivo transcription by T7 RNA polymerase is particularly sensitive to repression by H-NS [74]. Our hypothesis is further supported by the fact that overproduction of HU does not lead to strong nucleoid compaction, is well tolerated and compatible with transcription [15]. In this context it is relevant that the relative amounts of HU and H-NS are growth-phase dependent: in the logarithmic phase the HU/H-NS ratio is ~ 2.5 , whereas it drops to 1 in the stationary phase, which is mostly the result of a large decrease in the amounts of HU. This change in relative amounts of H-NS and HU may in part explain that in stationary phase the nucleoid is more condensed [6].

In conclusion, a thorough survey of the existing literature supplemented with novel microscopic data reveals that HU probably has a dual effect on DNA structure, which depends on the local concentration of the protein. Binding of single HU dimers at random positions can induce local DNA bending and increase the average flexibility of the DNA. Adjacent binding of multiple HU dimers is not likely to be involved in compaction of the bacterial chromosomal DNA as has long been thought. Instead, we propose that it plays a role as a modulator of nucleoid compactness by antagonising the compactive effects of H-NS in *E. coli*. Thus variations in HU expression levels are likely to indirectly affect the expression of a large number of genes. However, among prokaryotic genomes HU seems to be more conserved than H-NS [75]. Possibly in organisms lacking H-NS the compaction of the bacterial chromosomal DNA is mediated by other proteins, which may also be counteracted by HU. A conclusive answer with regard to the structural properties of HU awaits a more physical approach using single molecule experiments, as have been recently reported for IHF [76].

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