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Current Opinion in Microbiology

The role of nucleoid-associated proteins in mediating responses to environmental changes

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Bacteria face diverse environmental challenges, such as changes in temperature, pH, and osmolarity, and exposure to antibiotics, which necessitate adaptive responses for survival. The chromosome-structuring nucleoid-associated proteins (NAPs) are key to these responses owing to their role in global gene regulation. In this review, we summarize the functional interplay between environmental challenges and NAPs, and the adaptive responses mediated by NAPs. Specifically, physicochemical environmental factors modify the transcription level of NAP genes and affect protein activity, which facilitates bacterial adaptation via a short-term strategy. Additionally, NAPs regulate horizontally transferred genes, such as those involved in antibiotic resistance and virulence, by affecting their expression and integration into the host genome. Via this long-term strategy, NAPs contribute to both stress resilience and the evolution of bacterial traits, ensuring survival under environmental stress while facilitating genetic diversity through horizontal gene transfer.

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Current Opinion in Microbiology 2025, **87**:102628

This review comes from a themed issue on **Special section**

Edited by **Anthony Vecchiarelli**

Available online xxxx

https://doi.org/10.1016/j.mib.2025.102628

Introduction

Bacteria inhabit diverse environments and are frequently exposed to varying and often challenging conditions. For instance, bacteria experience changes in pH when transiting through the human gastrointestinal tract: the pH in the stomach is between 1.5 and 3.0, in the small intestine is between 7.0 and 8.5, and in the colon between 6.7 and 8.5 [1,2]. A bacterium entering the gastrointestinal tract also experiences a sudden increase in osmotic pressure, and a bacterium coming from the ambient (10°C–25°C), infecting a mammalian host, experiences a change in temperature (36°C–40°C) [3]. Such changes impose stress on the cell, but bacteria exhibit remarkable adaptability to such challenges and have evolved sophisticated strategies to sense, respond to, and survive under adverse circumstances [4]. A comprehensive understanding of bacterial environmental response and adaptation mechanisms provides tools for optimization of culturing in biotechnology, development of novel antibiotics, and bioremediation in environmental science.

Nucleoid-associated proteins (NAPs) are architectural proteins that structurally organize and compact DNA into the small cellular volume of bacteria [5]. NAPs also function as regulators in many cellular processes, such as gene transcription, DNA replication, DNA repair, and biofilm formation [5,6]. Unlike in eukaryotic cells, where DNA is wrapped around nucleosomes and chromatin segments interact via long-range loops, bacterial chromatin organization by NAPs is more diversified: NAPs bridge, bend, loop, or stiffen DNA [5–8]. This is attributed to the diversity of NAPs and their structural properties. Environmental cues play a pivotal role in modulating the expression level, structural conformation, and post-translational modifications (PTMs) of NAPs. This impacts the structural properties of NAPs and affects how NAPs organize the chromosome. Reorganization of chromatin structure affects (global) gene expression and enables bacteria to adapt to changing environmental conditions.

This mini-review summarizes the environmental regulation by a selection of NAPs. The focus is on studies related to *E. coli* NAPs, with an occasional excursion to studies on similar proteins from other species. We highlight the gaps in the current understanding of the interplay between environmental cues and NAPs, and discuss potential directions for the future.

Environment regulates the expression of nucleoid-associated protein genes

The expression level of most NAPs changes depending on the growth phase. In *E. coli*, for instance, H-NS (histone-like nucleoid structuring protein), StpA

(suppressor of td- phenotype A), HU (histone-like protein from *E. coli* strain U93), Fis (factor for inversion stimulation), and Hfq (host factor for Qb RNA replication) are highly expressed during log phase but not during stationary phase. IHF (integration host factor) and Dps (DNA protection during starvation protein) are abundant in the stationary phase but not in the log phase [9,10]. Northern blots and RNA-seq experiments that measure transcript levels rather than protein abundance show that genes encoding NAPs are differentially expressed when cells are subjected to different environmental stimuli. For example, transcription of *dps* decreases under heat stress [11]. Transcription of *stpA*, *hupB*, *fis*, and *dps* is higher at a mildly acidic condition (pH 5.8) [12,13]. Transcription of *stpA* is repressed in conditions of carbon starvation but strongly induced upon osmotic shock [14].

Environmental factors affecting nucleoid-associated protein activity

In this section, we focus on a subset of classical NAPs, for which information on the interplay with environmental factors is available, and summarize the effects of environmental cues on the protein levels of NAPs. Specifically, we discuss the effects of physicochemical conditions on the surface charge properties of NAPs, their conformation and oligomerization, and PTMs. We describe how these changes influence the function of NAPs *in vitro* and *in vivo*. There is limited evidence for a direct response to environmental factors by the H-NS homolog protein StpA, which has been implied in (co-)regulation (repression) of many H-NS regulated genes [15], and the factor for inversion stimulation protein, Fis, which is associated with fast growth due to its direct implication in the regulation or rRNA and tRNA operons [10]. Hence, we do not discuss these proteins in the following section.

H-NS

H-NS is a small NAP conserved in gram-negative bacteria. Even though the length of the protein and its amino acid sequence differ among species, it is structurally conserved [16]. The protein comprises an N-terminal dimerization domain, a central dimer–dimer interaction domain, and a C-terminal positively charged DNA-binding domain [17]. The dimerization and dimer–dimer interaction domains are connected by a long α-helix (helix α3). H-NS binds preferentially to AT-rich DNA. Its *in vivo* functionality requires multimerization along a DNA duplex to form an H-NS-DNA filament in which up to half the DNA-binding domains are involved; the other DNA-binding domains are available for recruitment of a second DNA duplex, resulting in a bridged DNA-H-NS-DNA complex (Figure 1a) [5].

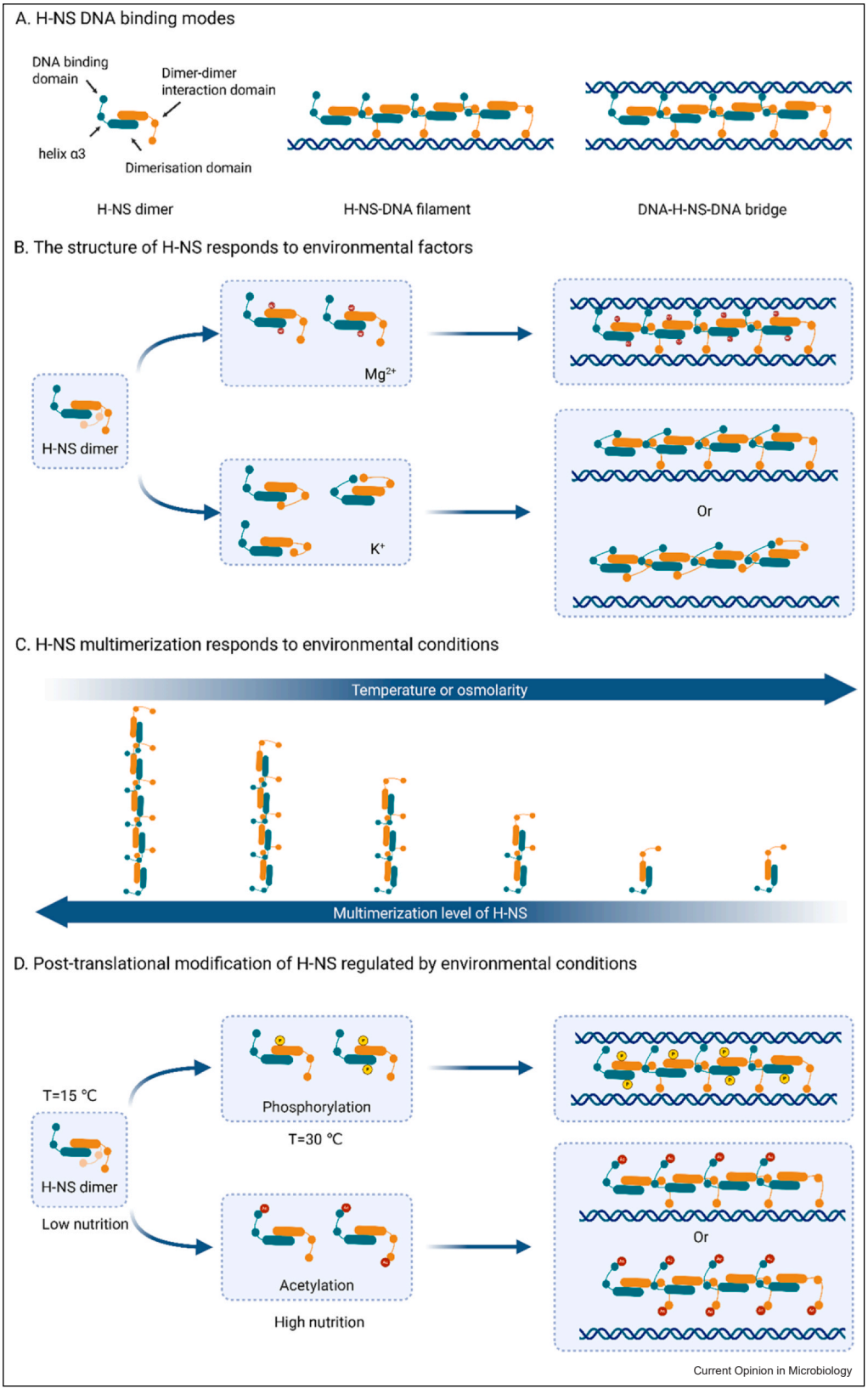
Recent models attribute specific functions to the DNA-binding modes of H-NS described above: the H-NS–DNA filament represses transcription initiation but not elongation, while DNA-H-NS-DNA bridges repress both steps [18]. In this light, it is relevant that H-NS structure, multimerization, and H-NS–DNA interaction can all be modulated by environmental cues. One of the better-known examples is that of Mg^{2+} affecting H-NS structure. In the absence of Mg^{2+}, the buckling of helix α3 facilitates the interaction between the DNA-binding domain and the dimerization domain, yielding a 'closed', bridging incompetent conformation [19,20]. In the context of multimeric H-NS-DNA complexes, this weakens DNA bridging due to a reduction in available DNA-binding domains [15]. In the presence of Mg^{2+}, the interaction between H-NS DNA-binding and dimerization domains is shielded, and the conformation of helix α3 is stabilized, facilitating DNA bridging (Figure 1b) [19,20]. K^+ also modulates H-NS structure. K^+ ions favor the interaction of the DNA-binding domains of the H-NS dimer with each other, with the dimerization domain, and with helix α3. These changes in conformation weaken DNA bridging [19,20].

Other studies reveal additional effects of osmolarity on intramolecular interactions [21,22]. The current consensus is that H-NS multimerizes in a concentration-dependent manner with a head-to-head/tail-to-tail conformation (Figure 1a). The dimerization domains have been proposed to exist in a parallel [23,24] or an antiparallel [17,25–27] conformation. Switching between these two distinct dimerization states was proposed as another mechanism of modulation of H-NS based on Molecular Dynamics simulations showing that the parallel conformation might exist only under specific conditions, such as at high salt (500 mM NaCl) [28]. While attractive, the model of parallel/antiparallel conformational switching, controlled by changes in physicochemical conditions, is not supported by experimental evidence *in vitro* or *in vivo*: small-angle X-ray scattering (SAXS) studies demonstrated that the conformation of the dimerization site of H-NS from *Salmonella typhimurium* [21] and in other H-NS orthologs [29] is not affected by temperature, osmolarity, or pH.

The dimer–dimer interaction domain of H-NS is also sensitive to physicochemical environmental changes. An increase in NaCl concentration from 150 mM to 500 mM, or in temperature from 5°C to 42°C, perturbs the quaternary structure (Figure 1c) [21,22]. Perturbation of H-NS multimerization in response to pH occurs at high salinity (500 mM NaCl) only, upon lowering of the pH from 8 to 6 [21].

Besides structural changes in response to physicochemical conditions, PTM has emerged as a mechanism that modulates H-NS function [30–33]. In *Shewanella*, H-NS is phosphorylated at Ser42 in its dimerization domain. The modification is temperature-dependent. It is

Figure 1


A. H-NS DNA binding modes
DNA binding domain
Dimer-dimer interaction domain
helix α3
Dimerisation domain
H-NS dimer
H-NS-DNA filament
DNA-H-NS-DNA bridge
B. The structure of H-NS responds to environmental factors
H-NS dimer
Mg2+
K+
Or
C. H-NS multimerization responds to environmental conditions
Temperature or osmolarity
Multimerization level of H-NS
D. Post-translational modification of H-NS regulated by environmental conditions
T=15 °C
H-NS dimer
Low nutrition
Phosphorylation
T=30 °C
Acetylation
High nutrition
Or
Current Opinion in Microbiology

Models for the response of H-NS to changes in environmental conditions. **(a)** H-NS-DNA-binding modes. H-NS forms dimers via its N-terminal dimerization domain. It multimerizes via its dimer–dimer interaction domain. H-NS multimers bind along a DNA duplex to form an H-NS-DNA filament. Under favorable conditions, this filament recruits a second DNA duplex, forming a bridged DNA-H-NS-DNA complex. **(b)** The structure of H-NS responds to environmental factors. In the H-NS multimer, the DNA-binding domain can interact with the dimerization domain. The presence of Mg^{2+} blocks this interaction, enhancing DNA bridging. High concentrations of K^+ oppose the formation of bridging-competent conformations, consequently perturbing bridging. **(c)** H-NS multimerization responds to environmental conditions. The dimer–dimer interaction site of the H-NS multimer is sensitive to changes in temperature and osmolarity. With the increase of temperature and osmolarity, this interaction is perturbed, lowering the multimerization level of H-NS. **(d)** Post-translational modification of H-NS is regulated by environmental conditions. The phosphorylation of the dimerization domain at Ser 42 is temperature-regulated. At 30°C, a bridged DNA-H-NS-DNA complex is formed, attributed to an effect of Ser phosphorylation level on helix α3 stability, facilitating an open conformation, which represses the transcription of H-NS target genes. The acetylation level of H-NS is positively correlated with the acetyl-CoA concentration, a measure of nutrition status, of the cytoplasm. Acetylation of H-NS at Lys120 neutralizes its positive charge at the DNA-binding domain, which disrupts the electrostatic interaction between the DNA-binding domain and the DNA duplex. Figure created in BioRender. Ge, P. (2025) https://BioRender.com/r7v4jeh.

detected at 30°C but not at 15°C (Figure 1d) [32]. Phosphorylation of Ser42 yields a negatively charged residue, which potentially allows for stronger interactions with Mg^{2+} that stabilize helix α3. This permits both DNA-binding domains of H-NS to interact with DNA, enhancing DNA bridging. In line with this, EMSA (electrophoretic mobility shift assay) and RNA-seq studies indicate that phosphorylation of Ser42 increases DNA binding by H-NS and increases transcription silencing by H-NS *in vivo* [32]. Interestingly, only a subset of H-NS variants is subject to phosphorylation at Ser42. The H-NS proteins of *E. coli*, *Salmonella*, *Vibrio*, and some *Shewanella* variants carry a conserved Glu residue at position 42, which mimics the phosphorylation state of Ser [32]. H-NS can also be acetylated, and the acetylation level of H-NS is an indicator of nutritional status; the increase in lysine acetylation is correlated with acetyl-CoA levels [34]. H-NS is acetylated at Lys120 in its DNA-binding domain in *Edwardsiella piscicida*. Acetylation neutralizes the positively charged lysine, perturbing the interaction with DNA. Consequently, acetylated H-NS exhibits reduced DNA binding across the whole genome, and H-NS-mediated transcription repression is relieved (Figure 1d) [33].

HU/integration host factor proteins

HU is another NAP widely found in prokaryotes, playing a crucial role in DNA packaging, transcription regulation, and DNA repair. HU exists as a dimer in bacteria [35]. *E. coli* genes *hupA* and *hupB* encode the HUα and HUβ subunits, respectively, which assemble into HU (αβ) heterodimers or HU(αα) homodimers. Exponential phase cells contain a mixture of HU(αα) and HU(αβ). In stationary cells, the predominant form is HU(αβ) [13,36].

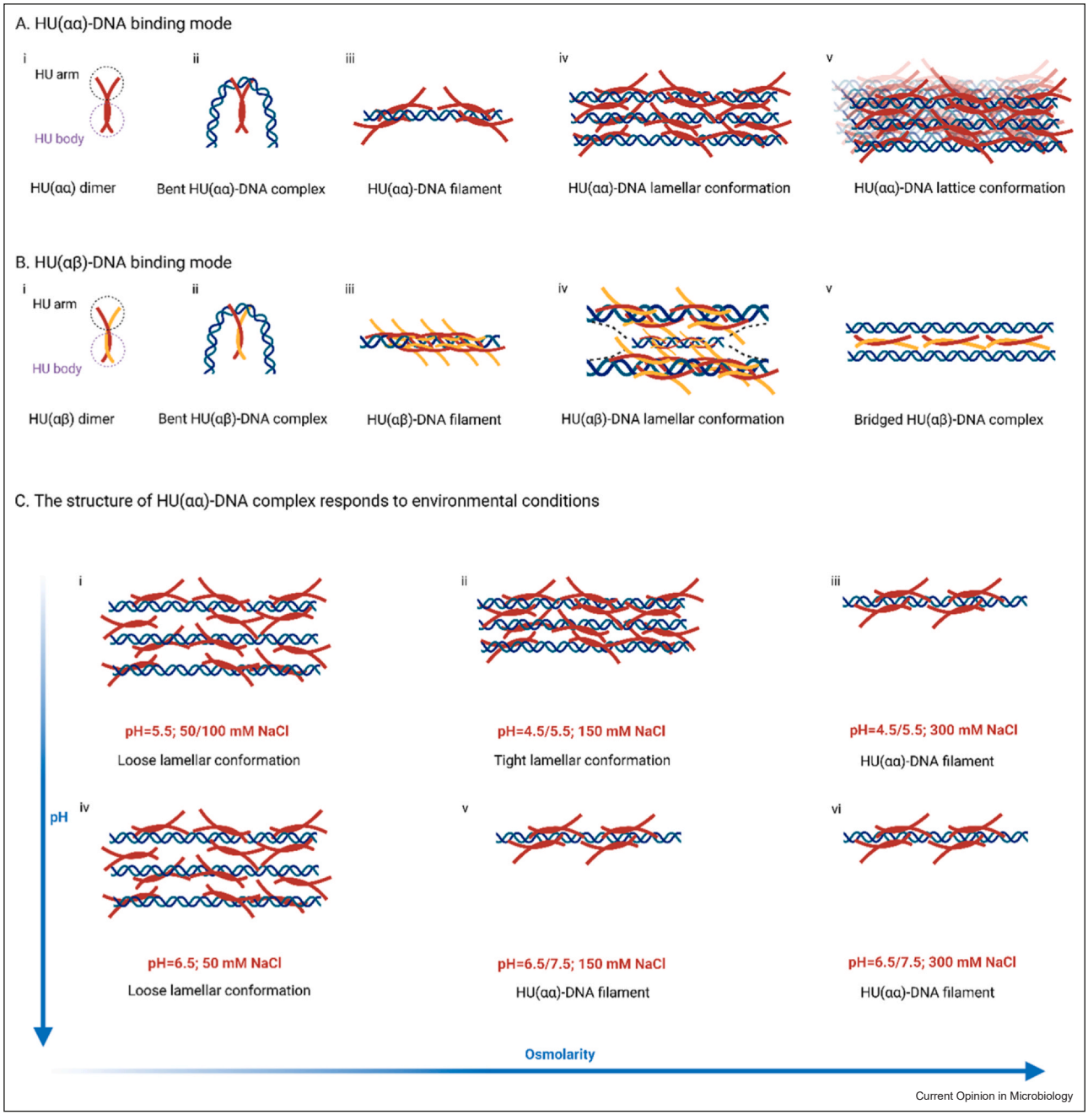
HU binds to DNA without sequence specificity. The protein's binding affinity to DNA depends on DNA structure. HU has a higher affinity for distorted DNA (such as DNA with nicks and three- or four-way junctions), and negatively supercoiled DNA than for canonical, intact, B-DNA, in line with the type of structural changes induced into DNA by HU binding [5,37–39]. Based on the HU-DNA co-crystal structure, HU bends DNA by ~105–140° through contacts between the β-hairpin arms of the HU dimer and the minor groove of DNA (Figure 2a, ii and b, ii) [39,40]. At high protein densities, HU assembles into a super-helical protein filament around DNA, yielding complexes in which the DNA is straightened rather than bent [40]. Unlike the DNA bending mediated by the HU dimer β-hairpin arms described above, a rigid filamentous conformation has also been observed, resulting from the direct binding of the HU dimer 'body' to DNA (Figure 2a, iii and b, iii) [13].

Single molecule DNA manipulation experiments have shown that low concentrations of HU compact DNA, whereas larger HU concentrations result in a stiffened HU-DNA filament [40,41]. This bimodal binding behavior is sensitive to salt concentration. At 150 mM NaCl and higher, the affinity of HU for DNA is reduced, and only DNA compaction is observed [42].

X-ray crystallography studies using nonspecific DNA show that HU dimers can multimerize via hydrogen bonds in body-to-body contacts, which promotes the straightening of the DNA axis, forming an HU-DNA filament. Through the multimerization mediated by the HU(αα)-HU(αα) interface (K38, V42, E34), HU(αα)-DNA filaments align into lamellar and lattice conformations (Figure 2a, iv and v) [13,38]. The lattice conformation can be observed at low HU(αα):DNA ratios (Figure 2a, v) [38]. Unlike HU(αα), there are two interfaces in the HU(αβ)–HU(αβ) interaction, an α-to-α and an α-to-β interface. The difference of residues at positions 12, 15, and 90 between the α and β chains (α: E12, E15, and K90; β: A12, D15, and N90) disrupts the α-to-β interaction but not the α-to-α interaction. Accordingly, HU (αβ) heterodimers assemble into lamellar structures but not lattice structures. A noteworthy observation is that the lamellar conformation mediated by HU(αβ) is not parallel but exhibits a pronounced curvature (Figure 2b, iv). In addition, two kinds of HU(αβ)-DNA structures have been identified depending on the HU(αβ)/DNA ratio. HU(αβ) fully covers DNA at high HU(αβ)/DNA ratios and bridges two DNAs at low ratios [38] (Figure 2b, v).

HU(αα)-body and HU(αα)-arms-based multimerization are dependent on pH and salt concentration. At pH 4.5 and 5.5, HU(αα) readily forms ordered lamellar structures with DNA (Figure 2c, i and ii). At pH 6.5, the

Figure 2



Models for the response of HU to environmental factors. **(a)** HU(αα) exhibits different modes of DNA binding: DNA bending, filament conformation, lamellar conformation, and lattice formation. **(b)** HU(αβ) exhibits different modes of DNA binding: DNA bending, filament formation, lamellar conformation, and bridge formation. **(c)** The structure of the HU(αα)-DNA complex under different conditions. From top to bottom represents an increase in pH. From left to right represents an increase in osmolarity. At acidic pH, the structure of HU(αα)-DNA shows a transition from a loose lamellar conformation to a tight lamellar conformation to an HU(αα)-DNA filament with an increase in salt concentration. At 150 mM NaCl, the conformation of HU(αα)-DNA changes from a tight lamellar conformation to a filament upon pH increase. Figure created in BioRender. Ge, P. (2025) https://BioRender.com/jocx8rj.

formation of lamellar structures is largely abolished (Figure 2c, v), except at low salt concentrations (50 mM NaCl) (Figure 2c, iv). At pH 7.5, the formation of lamellar structures is further reduced (Figure 2c, v and vi). This pH-dependent behavior is thought to be due to changes in the protonation state of surface residues like

Glu34, altering the hydrogen bond network that stabilizes HU(αα)-HU(αα) multimerization. This conformation transition is also salt-dependent. At pH 4.5 and 5.5, an increase in ionic strength from a physiologically relevant salt concentration (150 mM NaCl) to a high salt concentration (300 mM NaCl) yields a transition from the lamellar structure of HU(αα)-DNA to a filament structure (Figure 2c, i-iii). At pH 5.5, the parallel DNA strands within the lamellar structure were observed to be more distantly spaced at 100 mM NaCl than at 150 mM NaCl (Figure 2c, i and ii) [13].

IHF is a homolog of HU and also assembles as hetero- and homodimers of α and β subunits [6]. The subunits of IHF are encoded by *ihfA* and *ihfB* genes in *S. enterica* and *E. coli* [43,44]. IHF specifically binds to the consensus sequence (TAAnnnnTTGATW, where W is A or T) [45]. X-ray crystallography, Molecular Dynamics simulations, and AFM (atomic force microscopy) studies have revealed multiple IHF-DNA-binding modes, including 180° bending of DNA and DNA bridging [46,47]. Single molecule DNA manipulation experiments reveal bimodal sequence unspecific DNA-binding behavior of IHF similar to that of HU: at low protein concentrations IHF compacts DNA, and at higher concentrations a stiff IHF–DNA filament is formed. The binding behavior of IHF is similarly sensitive to salt concentration as HU: at high KCl concentration, only DNA compaction is observed [48]. In the presence of 2 mM Mg^{2+}, IHF-mediated DNA bending brings remote DNA sites together, inducing DNA condensation, but how Mg^{2+} concentration affects IHF–DNA interaction is unclear [49]. The IHF–DNA interaction is also affected by pH. At pH 6.5, IHF has a higher DNA-binding affinity than at pH 8.5. IHF–DNA interaction is insensitive to temperature changes in the range of 30°C to 37°C [49].

Dps

Dps is a dual-function protein. It behaves as a NAP that compacts DNA to maintain nucleoid structure and as a ferritin to catalyze the oxidation of Fe^{2+} to produce Fe^{3+}. Iron oxidation by Dps uses up H_2O_2, which prevents the synthesis of reactive oxidative species. Collectively, these properties of Dps prevent DNA damage from stress conditions [9,50]. The structure, function, regulation, and protein–protein interactions of Dps have been discussed extensively in recent reviews [9,51,52]. Here, we focus on the interplay between environmental factors and the DNA condensation properties of Dps *in vitro* [49,53].

Single molecule DNA stretching experiments show a destabilization of Dps–DNA interactions with increasing ionic strength, regardless of whether the increase is due to changing the concentration of monovalent cations, Na^+ or K^+, from 50 mM to 150 mM, or divalent cations, Mg^{2+} from 0 mM to 2 mM. At 10 mM $MgCl_2$, Dps even loses its DNA compaction ability [49,53]. The Dps–DNA interaction is also weakened by an increase in pH from 6.5 to 8.5 [49,53]. The addition of a molecular crowding agent such as PEG8000 stabilizes the Dps–DNA complex [53]. How the Dps–DNA interaction responds to changes in the environment *in vivo* is still unclear.

Role of nucleoid-associated proteins in bacterial adaptation, survival, and evolution

Environmental adaptation strategies for prokaryotes can be grouped into two categories: short-term and long-term. The quintessential short-term strategy is NAP-dependent transcriptional regulation. For instance, H-NS, StpA, and HU repress transcription of multiple environmental stress response operons/genes [13,30,54]. In *E.coli*, H-NS represses transcription of the acid response genes (*hdeAB*, *gadAB*, *gadXW*, *gadE*, *adiA*, *adiC*, *cadC*, *yhiM*, and so on). H-NS is subject to lysine 2-hydroxyisobutyrylation (Khib) at Lys121 in the DNA-binding domain in response to growth at low pH. Because of this modification, the transcription of acid response genes is upregulated, which enhances bacterial adaptation to extreme acid conditions [30]. H-NS also acts as an osmotic stress sensor-effector that regulates the transcription of the osmo-responsive *proVWX* (*proU*) operon by remodeling the local chromatin structure [54]. The gene product of this operon, the ProVWX transporter, facilitates osmotic stress adaptation of *E. coli* [54]. NAPs can also regulate gene transcription by modulating DNA supercoiling levels [55–57]. In *Dickeya dadantii*, the transcription of the virulence gene *pelE* is supercoiling level dependent in that DNA relaxation reduces *pelE* transcription [56]. Acidic and oxidative stress both reduce the average supercoiling density of the DNA. The transcription of the *pelE* gene goes down accordingly [56]. With the deletion of H-NS, DNA supercoiling levels are no longer affected by environmental cues. Consequently, the regulation of DNA supercoiling on *pelE* expression is lost in a Δ*hns* strain [56]. However, it is not clear how StpA acts, alone and in conjunction with H-NS, and how gene specificity is achieved [15,54]. It is also unclear mechanistically how HU affects transcription, but it has been proposed to act as H-NS counter silencer [58,59].

Unlike most NAPs, which function in transcription regulation, Dps does not function as a regulator of gene expression to facilitate bacterial adaptation under stressful conditions [9,60]. Nevertheless, it is essential for bacterial survival under these conditions. *In vivo*, the growth of Dps mutant strains is sensitive to stressful conditions (e.g. changes in pH, oxidative stress, temperature shock, UV or gamma irradiation, and metal shock) [50,61]. However, how Dps detects environmental stimuli and how that affects the Dps–DNA

interaction remain unclear. These observations underline that NAPs may employ yet unknown mechanisms to sustain bacterial survival under harsh conditions.

Long-term strategies for environmental adaptation of bacteria involve bacterial evolution, specifically, horizontal gene transfer (HGT). HGT is the movement of genetic material between organisms in a manner other than inheritance from the parent [62]. This strategy can imbue bacteria with new characteristics like antibiotic resistance [63,64]. It is essential to highlight that NAPs are important contributors to the processes of regulatory integration that accompany HGT. Transposons are mobile genetic elements that can change position within a genome. The insertion of a transposon can disrupt or alter gene expression. Cooper et al. revealed that H-NS-mediated DNA bridging promotes capture of a transposon in *Acinetobacter baumannii* [65]. Also, conjugation is an important mechanism of spreading genetic material [66,67]. H-NS represses the conjugation of the F plasmid by binding to the promoters of conjugation-related genes (*traY*, *traJ*, and *traM*) [68,69]. A plasmid-encoded H-NS homolog, Sfx, has been reported to repress the R6K plasmid conjugation in an unclear way. Notably, the DNA-binding domain of Sfx is essential for the repression of conjugation, and the interaction of Sfx with the small H-NS modulator Hha enhances this function [70].

Conclusions and perspectives

Increasing evidence indicates that NAPs play a crucial role in bacterial adaptation to stress conditions and evolutionary processes, including but not limited to the regulation of gene expression, DNA protection, and chromatin organization. However, many questions remain regarding the role of NAPs in mediating responses to environmental changes. For instance, how does a NAP that responds to more than one environmental stress selectively regulate only that subset of genes required for stress response? How do NAPs coordinate stress responses in complex environments with multiple stress factors? How do environmental factors mediate the PTM of NAPs? What is the mechanism of NAP-mediated dynamic remodeling of nucleoid structure during environmental stress? Does this process involve an interplay between NAPs, or NAPs with other factors?

Important directions of future research, in our view, include studies in physiologically relevant environmental contexts, that is, for instance, to study pathogenic bacteria in pathogen–host infection systems using correlated *dual* proteomic, transcriptomic, and genomic analyses. This will elucidate the interplay between host and pathogen and facilitate a mechanistic understanding in terms of signals and responses involved. Also, application of live-cell fluorescence microscopy in microfluidic systems could yield valuable information on temporal effects of changes in physicochemical environment on genome organization and gene activity. Finally, we foresee that cryogenic electron tomography, whether correlated or not with fluorescence microscopy, will generate insight into changes in genome organization that alter gene activity and vice versa, and how genomic features change during evolution. Altogether, these approaches will form a foundation for a holistic and dynamic understanding of the interplay between genome organization, gene activity, and cellular environment.

CRediT authorship contribution statement

Pingzhuang Ge: Writing – original draft, Writing – review & editing, Visualization. **Fatema-Zahra M Rashid:** Writing – review & editing. **Remus T Dame:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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