Three-dimensional chromosome re-modelling: the integral mechanism of transcription regulation in bacteria

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Three-dimensional chromosome re-modelling: The integral mechanism of transcription regulation in bacteria

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Abstract

Nucleoid-associated proteins (NAPs) are architectural proteins of the bacterial chromosome and transcription factors that dynamically organise the chromosome and regulate gene expression in response to physicochemical environmental signals. While the architectural and regulatory functions of NAPs have been verified independently, the coupling between these functions in vivo has not been conclusively proven. Here we describe a model NAP – histone-like nucleoid structuring protein (H-NS) – as a coupled sensor-effector that directly regulates gene expression by chromatin re-modelling in response to physicochemical environmental signals. We outline how H-NS-binding partners and post-translational modifications modulate the role of H-NS as a transcription factor by influencing its DNA structuring properties. We consolidate our ideas in models of how H-NS may regulate the expression of the *proVWX* and *hlyCABD* operons by chromatin re-modelling. The interplay between chromosome structure and gene expression may be a common – but, at present, under-appreciated – concept of transcription regulation in bacteria.

KEYWORDS
chromatin assembly and disassembly [G05.308.095], chromosomes, bacterial [G05.360.162.190], gene expression regulation, bacterial [G05.308.300]

1 | INTRODUCTION

The bacterial chromosome is compacted and organised in the nucleoid of bacterial cells in a manner that allows every gene to be accessible on demand. The organisation is mediated by DNA-binding architectural proteins, commonly referred to as nucleoid-associated proteins, or NAPs, that exert their role by folding, bridging and twisting the chromosome, locally, at the scale of individual operons or regulatory elements, and over longer ranges resulting in compaction of macrodomains and the formation of inter-arm interactions. The chromosome is also compacted by DNA supercoiling that is maintained by DNA topoisomerases and gyrases. Actively transcribed genes and some NAPs behave as supercoil diffusion barriers, restricting supercoiling density to smaller chromosome segments (reviewed in Dame et al. (2020)). The steric effects of chromosome compaction limit the accessibility of open reading frames and regulatory elements, making chromatin structure a direct regulator of transcription. Being the architects of bacterial chromosomes, NAPs inherently play a dual role in the cell, functioning as chromosome structuring proteins and as transcription factors.

The biochemical properties of proteins are affected by fluctuations in temperature, pH and osmolarity. This can result in, among others, the loss or gain of functionality, a change in multimeric state and a change in protein stability. Often, in NAPs, susceptibility to

physicochemical changes affects the structure of the protein that, consequently, tunes DNA-binding affinity and the formation of higher order NAP structures. This makes chromosome compaction, organisation and accessibility dependent on the immediate environment of bacterial cells. NAPs, therefore, provide coupled sensor-effector systems that detect changes in the environment as changes to their DNA structuring properties and manifest these as changes in chromosome architecture, gene accessibility and gene expression profiles. This coupling suggests that the complexity of transcription regulation may have evolved around structural remodelling of the chromosome. The interplay between chromosome structure and gene expression is well established in eukaryotes and forms the foundation of the 4D nucleome project (Dekker et al., 2017). In prokaryotic systems, models of the structural regulation of transcription are limited.

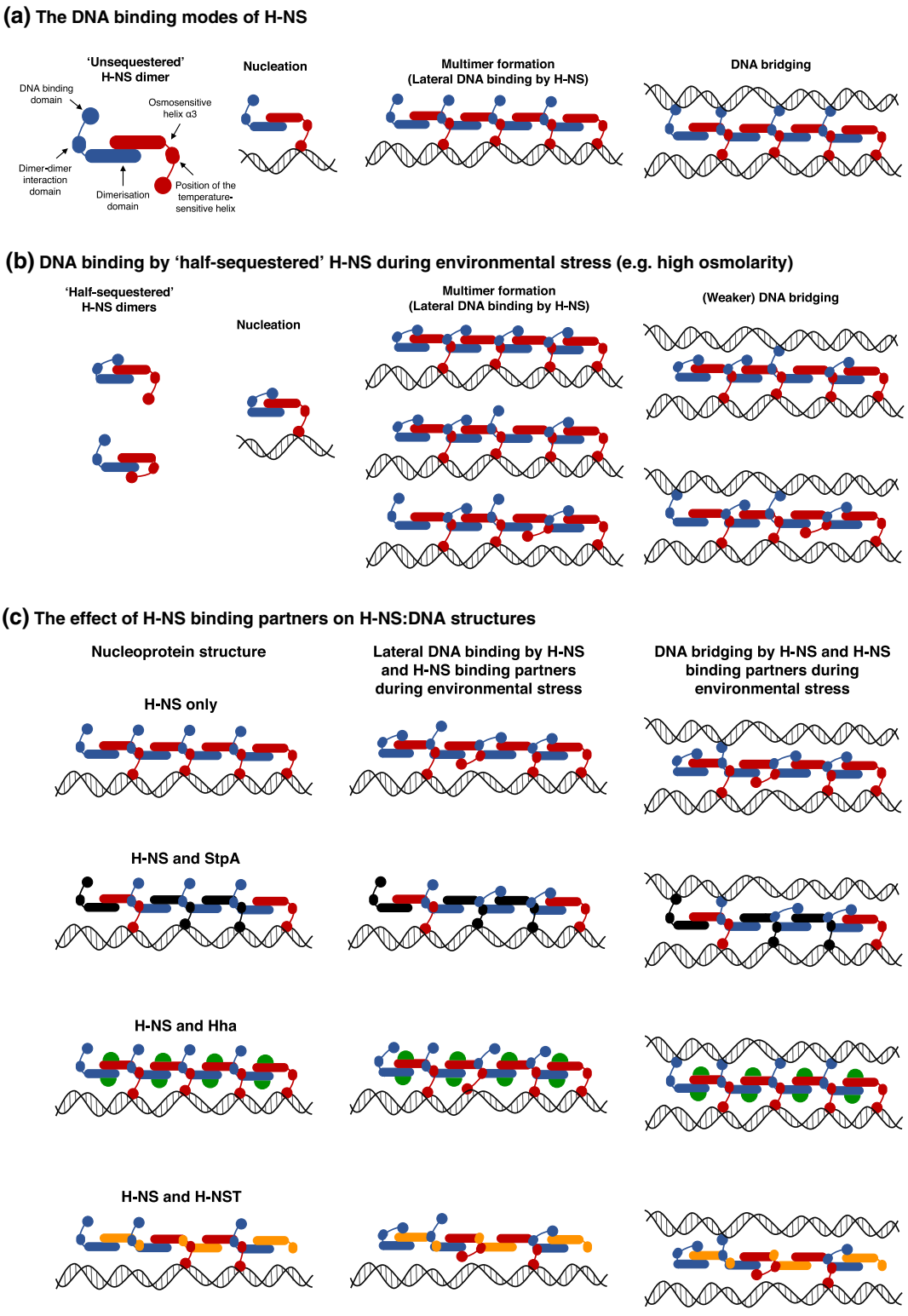
2 | THE COUPLED SENSOR-EFFECTOR SYSTEM OF H-NS

2.1 | H-NS

The coupled sensor-effector system of Histone-like nucleoid structuring protein (H-NS) is the best studied among bacterial NAPs. H-NS is a 137 amino acids long NAP that exists as a dimer in solution (Falconi et al., 1988) formed via the interaction between a pair of N-terminal dimerization domains (amino acids 1-41) (Bloch et al., 2003; Esposito et al., 2002; Ueguchi et al., 1996). The dimer is the smallest functional unit of H-NS and preferentially binds AT-rich DNA through its C-terminal DNA-binding domains (amino acids 96-137) (Shindo et al., 1995). This forms a 'nucleation point' from which H-NS dimers multimerise laterally over the DNA via dimer-dimer interaction domains (amino acids 52-84) forming an H-NS:DNA filament (Figure 1a). The H-NS DNA-binding domains in a filament contact the DNA in the minor groove every 8-17 base pairs at an average of one contact per 10 base pairs (Shen et al., 2022). The contact site is confined to a locus that can be physically accessed by the DNA-binding domain and is distinguished by its narrower minor groove width, lower electrostatic potential and TA step (Shen et al., 2022). H-NS multimerization is co-operative and allows the filament to extend towards lower affinity regions (Arold et al., 2010; Leonard et al., 2009). The α-helical structure at residues 71-74 in the dimer-dimer interaction domain of H-NS regulates the formation of H-NS:DNA filaments, as unfolding of the helix can disrupt multimerisation (Arold et al., 2010; Hameed et al., 2019). The presence of a pair of DNA-binding domains per dimer allows the H-NS multimer to recruit a second DNA molecule to form a DNA:H-NS:DNA bridge (Figure 1a) (Dame et al., 2000; Dame et al., 2006; Qin et al., 2020; van der Valk et al., 2017). The shift between the lateral DNA binding and DNA bridging modes of H-NS is mediated by an unstable helix at amino acids 42-50 of the protein (helix α3) that extends between the dimerization and dimer-dimer interaction domains (van der Valk et al., 2017). The stochastic unfolding of helix α3 (amino acids 42-50) causes H-NS to fold on to itself such that the N-terminal DNA-binding domain interacts with the C-terminal dimerization domain. In a dimer, this structural change forms a half-sequestered state where only one DNA-binding domain remains available per dimer, favouring the formation of an H-NS:DNA filament (Figure 1b) (van der Valk et al., 2017).

H-NS is a repressor. H-NS:DNA nucleoprotein structures occlude the binding of RNA polymerase (RNAP) to AT-rich regions of the chromosome (Myers et al., 2013) – a characteristic feature of horizontally-acquired genes and promoter regions. This property of non-specific repression and xenogeneic silencing prevents spurious transcription from promoter-like sequences that can place a heavy toll on cellular resources, and from horizontally acquired genes that may be toxic (Lamberte et al., 2017; Navarre et al., 2006; Singh et al., 2014). Interestingly, non-specific repression by H-NS at AT-rich regions is key in directing expression from promoters rather than promoter-like sequences. This is because in the competition between H-NS and RNAP for promoter binding, RNAP more efficiently competes H-NS away from AT-rich sites that match the −10 consensus sequence (Singh & Grainger, 2013). Elongating RNAP, on the other hand, can transcribe across H-NS:DNA by remodelling the structure as a consequence of the force exerted by the actively transcribing polymerase (Dame et al., 2006; Wang et al., 1998). In vivo, remodelling of the H-NS:DNA nucleoprotein relieves silencing of promoters contained within the structure (Rangarajan & Schnetz, 2018). For instance, the insertion of a cassette comprising the constitutive *lacUV5* promoter or arabinose-inducible pBAD promoter and the *tR1*-λN conditional terminator system upstream of the H-NS-repressed *proVWX* P2 promoter increases transcription initiating at P2 when expression from the upstream promoter increases and extends across the conditional terminator. Similar observations were made for other H-NS repressed promoters (Rangarajan & Schnetz, 2018). While the force exerted by transcribing RNAP is higher than the force required to break H-NS:DNA bridges with optical tweezers (Dame et al., 2006; Wang et al., 1998), in vitro transcription assays on linearised plasmid templates show that DNA:H-NS:DNA bridges, but not H-NS:DNA filaments, still function as effective transcription roadblocks (Kotlajich et al., 2015). H-NS-mediated DNA bridges promote RNAP pausing and backtracking, increase the dwell times of RNAP in its paused state, and favour Rho-dependent transcription termination by providing a wider kinetic window for Rho function (Kotlajich et al., 2015). H-NS bridges may exert their roadblocking effect by trapping positive supercoils generated by the polymerase in the downstream template. It is possible that H-NS:DNA filaments can also function as transcription roadblocks in the constrained system of the chromosome by trapping-positive supercoils, and that the use of a torsionally unconstrained, linearised template in the in vitro assays may have obscured the roadblocking effect.

The stability of H-NS, and hence the architecture that the H-NS:DNA nucleoprotein assumes, can be tuned. Helix α3 (amino acids 42-50) is osmosensitive in that high K^+ concentrations are



destabilising and favour H-NS:DNA filament formation, while Mg^{2+} is stabilising and promotes DNA:H-NS:DNA bridges (van der Valk et al., 2017). The α-helical structure at residues 71-74 in the dimer-dimer interaction domain of H-NS melts in response to increasing temperature. This disrupts H-NS:DNA nucleoprotein and folds the C-terminal DNA-binding domain of H-NS onto the N-terminal dimerization domain to form a sequestered structure (Arold et al., 2010; Hameed et al., 2019). Therefore, helix α3 (amino acids 42-50) and the α-helical structure formed by amino acids 71-74 are key elements of the coupled sensor-effector system of H-NS that regulate gene expression in response to environmental stimuli. This interplay between local chromatin architecture and gene expression is reminiscent of the mechanisms employed by conventional prokaryotic transcription factors such as the

FIGURE 1 The coupled sensor-effector system of H-NS. (a) The DNA-binding modes of H-NS. H-NS is a nucleoid-associated protein that exists as a dimer in solution. The dimer is formed by the interaction between pair of N-terminal dimerisation domains. H-NS binds the DNA via its C-terminal to form a nucleation point from which H-NS dimers multimerise laterally over the DNA via dimer-dimer interaction domains. The availability of two DNA-binding domains per dimer allows the H-NS multimer to recruit a second DNA molecule to form a bridge. (b) DNA binding by 'half-sequestered' H-NS during environmental stress. The osmosensitive helix α3 (amino acids 42-50) can unwind causing the DNA-binding domain of H-NS to fold onto the body of the protein and interact with the dimerisation domain. In such a conformation, only one DNA-binding domain remains available per dimer. Under conditions that favour the half-sequestered conformation of H-NS, H-NS can still bind DNA, multimerise laterally on a DNA substrate, and potentially, form DNA:H-NS:DNA bridges. However, the DNA-binding domains exist in an equilibrium between the sequestered and unsequestered states, hence, the availability of DNA binding domains is reduced. As a consequence, H-NS:DNA nucleoprotein structures are weaker. (c) The effect of H-NS binding partners on H-NS:DNA structures. StpA: StpA is a paralogue of H-NS and can form heterodimers with the protein. Owing to the presence of stable α-helices in StpA, its DNA-binding domain is not sequestered by heat and salt stress, hence, StpA stabilises H-NS:DNA structures against changes in temperature and osmolarity. In vitro studies show that StpA favours the formation of DNA:H-NS:DNA bridges. Hha: Hha is an H-NS modulator and is part of the YmoA family of proteins. Hha lacks a DNA-binding domain. It exerts its role by binding the the dimerisation domain of H-NS and sterically interfering with the sequestration of the H-NS DNA binding domain. Hha stabilises DNA:H-NS:DNA bridges at low temperature. H-NST: H-NST are truncates of H-NS that lack the DNA-binding domain. H-NST can form heterodimers with the full-length protein. This negatively affects the DNA structuring properties of the H-NS by reducing the number of DNA binding domains in an H-NS:DNA complex.

MerR-family regulators that control gene expression by altering promoter structure in response to changes in the concentrations of specific ligands, and CI-type lambda regulators that control gene expression through DNA binding and bridging in response to environmental cues (Brown et al., 2003; Révet et al., 1999). However, H-NS-mediated regulation is notably more complex, since, in contrast to conventional transcription factors, H-NS does not bind defined sites in defined oligomeric states.

2.2 | Binding partners

H-NS-binding partners such as its paralogue StpA (Zhang & Belfort, 1992), Hha of the YmoA family of proteins (Madrid et al., 2007), and H-NS truncates (Williamson & Free, 2005) modulate the regulatory role of H-NS. These proteins exert their effects by influencing the architectural properties of H-NS (Figure 1c).

StpA forms heteromers with H-NS and is distributed within H-NS:DNA nucleoprotein. Indeed, ChIP studies show that H-NS and StpA bind the same loci (Uyar et al., 2009). In vitro studies reveal that StpA favours the formation of DNA:H-NS:DNA bridges and stabilises the structures against changes in temperature and osmolarity (Boudreau et al., 2018). The robustness of DNA:H-NS/StpA:DNA bridges is expected to arise due to stable α-helices in StpA that do not unfold in response to heat and salt stress, and hence, resist the disruption of the multimer (Figure 1c) (Boudreau et al., 2018). This manifests as an increase in pausing and backtracking of elongating RNAPs, and pre-mature transcription termination under conditions that disrupt DNA:H-NS:DNA bridges (Boudreau et al., 2018). The stability of H-NS/StpA nucleoprotein may also reinforce the occlusion of RNAP from promoters enclosed within the structure, for instance, the H-NS and StpA-regulated *appY* gene is repressed 75-fold by H-NS and ~1000-fold by H-NS/StpA (Rangarajan & Schnetz, 2018). Nevertheless, actively transcribing RNAP can remodel H-NS/StpA:DNA and relieve repression of silenced promotes in the nucleoprotein (Rangarajan & Schnetz, 2018). Despite the effect of StpA on H-NS-regulated transcription, *stpA* deletion mutants of *E. coli* do not have a clear phenotype. The increase in StpA levels in Δ*hns* strains, and the mild repression of the H-NS regulon in these mutants suggest that in *E. coli*, StpA functions as a molecular back-up of H-NS (Browning et al., 2010; Dorman, 2014; Sondén & Uhlin, 1996; Zhang et al., 1996). It remains to be studied, however, if the stress response of Δ*stpA* mutants of *E. coli* to changes in temperature and osmolarity is affected. In *Salmonella*, the expression of several genes is affected upon *stpA* deletion (Lucchini et al., 2009).

The role of H-NS as a transcription factor is also modulated by Hha (high hemolysin activity) (Baños et al., 2009; Vivero et al., 2008). Hha lacks a DNA-binding domain and acts by forming a complex with H-NS. Unlike StpA, Hha only binds H-NS at a subset of loci. The signal that directs Hha localisation is unclear. Hha binds the N-terminal dimerization domain of H-NS and obstructs the interaction between the H-NS DNA binding and dimerization domains that form in the 'sequestered' H-NS structure (Figure 1c) (Ali et al., 2013; Hameed et al., 2019; van der Valk et al., 2017). Indeed, in vitro studies show that Hha improves the DNA-binding affinity of H-NS (Ali et al., 2013), and stabilises DNA:H-NS:DNA bridges (Figure 1c) (Boudreau et al., 2018). Hha also promotes the formation of multi-bridged complexes and reinforces the H-NS roadblock for RNAP (Boudreau et al., 2018). DNA:H-NS/Hha:DNA bridges are disassembled at higher temperatures consistent with the relief of repression of the H-NS/Hha-bound α-hemolysin operon in *E. coli*, and pathogenicity islands in *Salmonella* upon host infection (Ali et al., 2013; Boudreau et al., 2018; Mouriño et al., 1994).

H-NS is also modulated by truncates of the protein that lack the C-terminal DNA-binding domain. H-NS truncates (H-NST) naturally occur in pathogenic *E. coli* strains and play a role in the activation of the H-NS-repressed LEE (locus of enterocyte effacement) pathogenicity island (Williamson & Free, 2005). H-NSTs exert their role by forming heterodimers with H-NS. The truncates have a dominant negative effect on the DNA structuring properties of H-NS since the absence of a DNA-binding

FIGURE 2 H-NS may regulate the expression of *proU* by chromatin remodelling. (a) The regulatory region of the *proU* operon consists of the upstream regulatory element (URE; orange), 183 bp in length, positioned at −229 to −46 with respect to the σ^{70}-dependent *proU* P2 promoter (black right-angled arrow), and a 217 bp long downstream regulatory element (DRE; orange) at −40 to +177 relative to the P2 promoter. The DRE contains two 10 bp long high-affinity H-NS binding sites (green) at +20 to +30 and +127 to +137 relative to P2. The 1203 bp long *proV* open reading frame is positioned 60 bp downstream of the P2 promoter. (b) A model of the regulation of *proU* by the DRE independent of the URE. In the absence of the URE, t– DRE may regulate expression from P2 by interfering with multiple steps in the transcription process. At low osmolarity, the H-NS:DNA nucleoprotein at the DRE, represented here as a filament, may occlude the binding of RNA polymerase (RNAP; blue) to P2, prevent promoter escape, and reduce the processivity of elongating RNAP by trapping positive supercoils in the downstream template (not represented). The increase in intracellular K^+ at high osmolarity destabilises helix α3 (amino acids 42-50) and shifts the equilibrium of H-NS DNA binding domains from their unsequestered to their sequestered state. This results in fewer available DNA-binding domains, and hence, fewer H-NS-DNA contacts thus weakening the H-NS:DRE structure. This may increase the accessibility of P2 for RNAP, and reduce inhibitory effect of H-NS:DRE on promoter escape and transcription elongation. (c) A model of the regulation of *proU* by the URE independent of the DRE. In the absence of the DRE, an H-NS multimer extending from the URE may occlude the binding of RNAP to the P2 promoter. The weakening of H-NS:URE at high osmolarity due to the destabilisation of helix α3 (amino acids 42-50) and the shift of the equilibrium towards the sequestered state of the H-NS DNA-binding domain may increase the accessibility of P2 for RNAP. (d) A model of the regulation of *proU* by co-operativity between the URE and the DRE. Owing to the proximity of the pair of H-NS-binding regulatory regions of *proU*, H-NS may form a bridge between the URE and DRE. At low osmolarity, the stability of helix α3 (amino acids 42-50) favours the unsequestered state of H-NS. This increases the availability of H-NS DNA-binding domains in URE:H-NS:DRE for interaction with DNA and stabilises the bridge. The structure occludes the binding of RNAP to P2, inhibits promoter escape and functions as a strong roadblock to transcription elongation. The destabilisation of helix α3 (amino acids 42-50) at high osmolarity shifts the equilibrium of the DNA-binding domains in the H-NS multimer towards the sequestered state. This reduces the number of H-NS-DNA contacts in URE:H-NS:DRE and increases the accessibility of P2 for RNAP. The weaker URE:H-NS:DRE bridge behaves as a weaker inhibitor for promoter escape and transcription elongation. The AT-rich URE may facilitate 'peeling away' of the H-NS multimer from actively transcribed template on the DRE by providing accessible H-NS-binding sites.

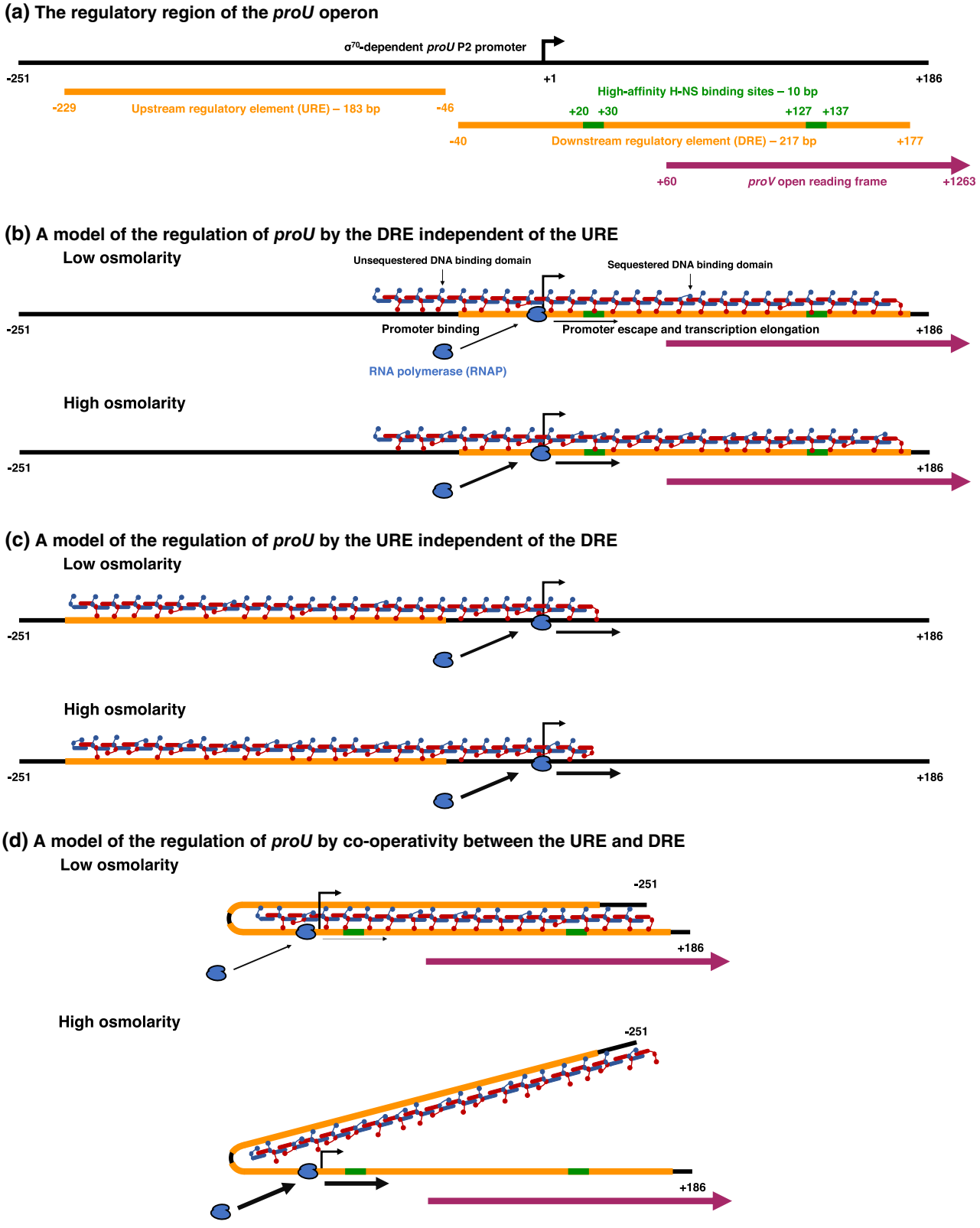
domain in an H-NS/H-NST dimer reduces DNA-binding affinity, weakens H-NS:DNA filaments and impairs the formation DNA:H-NS:DNA bridges (Figure 1c) (van der Valk et al., 2017; Williamson & Free, 2005). The effects may be exacerbated by the sequestration of the remaining DNA-binding domain by the stochastic unfolding of helix α3 (amino acids 42-50) (Figure 1c). Indeed, when overexpressed from a tunable promoter, H-NST imparts a fitness defect to *E. coli* by triggering global derepression of transcription (Williamson & Free, 2005). At physiological levels in enteropathogenic *E. coli*, H-NST disrupts the repressive H-NS:DNA structure at the LEE operon, facilitating the binding of the Ler – the LEE-specific activator (Williamson & Free, 2005). A clue to understand how the effects of H-NST are restricted to specific loci of the chromosome is found in studies of the regulatory elements of the *proU* operon. H-NS binds the upstream and downstream regulatory elements (URE and DRE) of *proU*, but exhibits stronger binding to the DRE. In a 1:1 ratio of H-NS:H-NST, H-NST disrupts the H-NS:DNA nucleoprotein at the URE, but not at the DRE (Williamson & Free, 2005). This suggests that H-NST affects low affinity H-NS-binding sites. At such regions, H-NST may easily disrupt weakly-bound H-NS homodimers and multimers. At high affinity H-NS-binding sites, weaker H-NS/H-NST structures may be displaced by H-NS homodimers and their multimerization on the DNA substrate.

2.3 | Post-translational modifications

Post-translational modification (PTM) of NAPs regulates transcription in bacteria (Carabetta et al., 2019; Dong et al., 2022; Sakatos et al., 2018). For several NAPs including H-NS, IHF, HU and Fis, PTMs may mediate this effect by modifying the architectural properties of the protein (Dilweg & Dame, 2018). In H-NS, for instance, the neutralisation of negatively charged lysines in the DNA-binding domain by acetylation and succinylation, and the acetylation of negatively-charged amino acids that contact DNA in the linker region of H-NS, may reduce the DNA-binding affinity of the protein. The introduction of negative charges by phosphorylation and de-amination of -OH and $-NH_2$ moieties in residues involved in the sequestration of the H-NS DNA-binding domain during the unfolding of helix α3 (amino acids 42-50) may stabilise the un-sequestered structure of H-NS by coordinating Mg^{2+} (Dilweg & Dame, 2018). While most of the models proposed in 2018 (Dilweg & Dame, 2018) have not been experimentally verified yet, a study on the hydroxyisobutyration of Lysine-121 in the DNA-binding domain of H-NS supports the predictions (Dong et al., 2022). Neutralisation of the negative charge of Lysine-121 of H-NS by hydroxyisobutyrylation (H-NS K121hib) reduces the DNA-binding affinity of the protein and globally alleviates H-NS-mediated repression (Dong et al., 2022). Increased H-NS K121hib improves the survival of *E. coli* at low pH by increasing in the basal level of expression of acid stress response genes (Dong et al., 2022). Despite its apparent fitness advantage, *E. coli* may have evolved to maintain a lower level of H-NS K121hib to reduce spurious transcription that is associated with an alleviation of H-NS repression.

3 | H-NS-LIKE PROTEINS

NAPs that fulfil similar functional roles as H-NS – H-NS-like proteins – have evolved in several bacteria. Lsr2 of *Mycobacteria* sp., MvaT



of *Pseudomonas* sp., Bv3f of *Burkholderia* sp., and Rok of *Bacillus* sp. share little sequence and structural similarity, but all function as xenogeneic silencers by preferentially binding to AT-rich DNA (Ding et al., 2015; Duan et al., 2018; Gordon et al., 2010; Smits & Grossman, 2010). Of these, MvaT and Lsr2 have been shown to regulate gene expression in response to environmental cues (Bartek et al., 2014; Diggle et al., 2002). In vitro studies highlight that, similar to H-NS, the modulation of the DNA binding and architectural properties of MvaT by physicochemical signals may contribute to its role as a transcription factor (Qin et al., 2020). In contrast, Rok of *Bacillus*

sp. is an atypical H-NS-like protein in that its DNA structuring properties are not affected by physiologically-relevant physicochemical cues in vitro (Erkelens et al., 2022). However, in the presence of small Rok (sRok) as a binding partner, the Rok:DNA nucleoprotein is sensitised to changes in osmolarity (Erkelens et al., 2022) – reminiscent of the interplay between H-NS and StpA (Boudreau et al., 2018). sRok is a naturally occurring Rok variant that lacks part of the linker between the DNA binding and dimerization domains (Erkelens et al., 2022).

4 | MODEL OPERONS

H-NS is a global regulator of gene expression. RNA-Seq studies show that the deletion of H-NS impacts the relative transcription levels of 5% of genes in the *Escherichia coli* chromosome (Hommais et al., 2001), while cappable RNA-Seq has shown that in addition to the above, H-NS deletion results in spurious transcripts arising from promoter-like sequences in AT-rich genomic segments (Forrest et al., 2022). An understanding of how transcriptional changes are directly coupled to in vivo chromosome architecture remains elusive.

4.1 | The *proVWX* (*proU*) operon

ProVWX (*proU*) is an H-NS-regulated operon that is activated in response to high osmolarity (Csonka, 1982; Gowrishankar, 1985; Rajkumari et al., 1996). H-NS exerts its effect on *proU* through the negative regulatory element (NRE) (Dattananda et al., 1991). A pair of cis regulatory elements in the NRE – the upstream and downstream regulatory elements (URE and DRE) – positioned around the σ^{70}-dependent P2 promoter of *proU* bind H-NS and are key in coordinating H-NS-mediated repression (Lucht et al., 1994; Nagarajavel et al., 2007). The DRE, in particular, consists of a pair of high-affinity H-NS-binding sites (5′-TCGATATATT-3′) that closely match the Lang motif for H-NS binding (5′-TCGATAAATT-3′) (Figure 2a) (Bouffartigues et al., 2007; Lang et al., 2007). β-galactosidase expression assays performed by Nagarajavel et al. show that expression from P2 in response to increasing osmolarity in the presence of both the URE and the DRE is sigmoidal. *ProU* is silenced by the URE and DRE at low osmolarity, and repression is abrogated at higher osmolarities (Nagarajavel et al., 2007). In the presence of only the DRE, expression from P2 increases with osmolarity, but, H-NS exerts its repressive effect at all osmolarities. Interestingly, at low osmolarity, the DRE functions as a weaker repressive element than the URE and DRE combined, but at higher osmolarities, the repression via the DRE only is stronger than with both regulatory elements (Nagarajavel et al., 2007). In the presence of only the URE, H-NS weakly represses P2 at low osmolarity, with a small increase in salt concentration alleviating repression (Nagarajavel et al., 2007). The repressive effects on *proU* P2 mediated by H-NS via the URE and the DRE are not additive, suggesting that they function cooperatively. Cooperativity may be observed if the URE and DRE separately repress different steps of transcription – initiation and elongation, respectively. However, cooperativity between a pair of regulatory elements that are bound by an architectural protein – H-NS – may also be achieved by a physical interaction.

Mechanistically, the DRE may repress P2 by interfering with both, transcription initiation and elongation. In the absence of the URE, the H-NS:DNA filament formed at the DRE may occlude the binding of RNAP to the promoter and reduce the processivity of RNAP by behaving as a roadblock (Figure 2b). Increasing extracellular osmolarity triggers the influx of K^+ (Sleator & Hill, 2002), and up-regulates expression from P2 due to the inherent osmosensitivity of the promoter, and weakening of the H-NS:DRE filament by the destabilisation of helix α3 (amino acids 42-50) (Figure 2b). In vitro studies only show that the destabilisation of helix α3 (amino acids 42-50) shifts the equilibrium of H-NS:DNA nucleoprotein from DNA:H-NS:DNA bridges to H-NS:DNA filaments (van der Valk et al., 2017). However, it is conceivable that DNA-binding domains in an H-NS:DNA filament that are oriented towards the DNA molecule exist in an equilibrium between DNA-bound, and sequestered states. The destabilisation of helix α3 (amino acids 42-50) may shift the equilibrium towards the sequestered state, weakening H-NS:DNA filaments to a structure that can be more easily displaced by RNAP. Repression by the URE independently of the DRE can be achieved by the occlusion of RNAP binding to P2 by an H-NS multimer that extends from the URE (Figure 2c). Due its positioning upstream of the P2 transcription start site, the structure may have a minimal impact on transcription elongation. The weakening of the H-NS:URE multimer at higher osmolarities due to the destabilisation of helix α3 (amino acids 42-50) abrogates H-NS:URE repression (Figure 2c).

Regulation of P2 by co-operativity between the URE and DRE may involve a physical interaction between the two elements. Repression at low osmolarity may be maintained by an H-NS multimer that bridges the URE and the DRE to form a structure that occludes RNAP from the promoter, and, in the case of RNAP bound to the promoter, traps the positive supercoils generated by a transcribing enzyme on the downstream template stimulating RNAP pausing (Figure 2d). At higher osmolarities, associated with an increase in intracellular K^+, the destabilisation of helix α3 (amino acids 42-50) may shift the equilibrium of the H-NS dimer towards its half-sequestered state, dismantling the DNA:H-NS:DNA bridge, and alleviating H-NS repression (Figure 2d). At high osmolarities, repression of P2 by the DRE alone is stronger than in the presence of both the URE and the DRE. Structurally, this may occur if the URE facilitates 'peeling away' of the H-NS multimer from the DRE as H-NS shifts from its bridging mode towards a filament structure (Figure 2d).

4.2 | The *hlyCABD* (*hly*) operon

The *hlyCABD* operon is encoded by uropathogenic *E. coli* strains that express the hemolysin toxin (Goebel & Schrempf, 1971). *HlyCABD* is osmolarity- and temperature-sensitive and is co-regulated by H-NS and Hha (Mouriño et al., 1994). H-NS binds the regulatory region of

hlyCABD, which is positioned at the 5′ end of the operon extending into *hlyC*, and represses *hly*. Hha reinforces H-NS mediated repression. In vivo transcription assays show that Hha can repress *hly* even in a Δ*hns* background (Nieto et al., 2000). Owing to the absence of a DNA-binding domain in Hha, and because Hha mediates its effect via H-NS (Ali et al., 2013), in Δ*hns* strains, Hha may function via the H-NS paralogue – and molecular back-up – StpA.

In vivo, *hlyCABD* is repressed by high osmolarity (Nieto et al., 2000). The repression is weakly alleviated by the deletion of *hha* and strongly alleviated in Δ*hns* strains. Expression is highest in a Δ*hns* Δ*hha* background (Nieto et al., 2000). At high osmolarity, helix α3 (amino acids 42-50) of H-NS is destabilised, causing the H-NS DNA-binding domain to fold onto the body of the protein, effectively, weakening the repressive H-NS:DNA structures (van der Valk et al., 2017). Hha sterically interferes with the folding of H-NS, and stabilises H-NS:DNA (Ali et al., 2013; Hameed et al., 2019; van der Valk et al., 2017). While this explains why the repression of the *hly* operon is alleviated at high osmolarity in Δ*hha* strains, it does not explain how H-NS, in the absence of Hha, strongly represses *hly* at osmolarity conditions that disrupt H-NS:DNA nucleoprotein (Nieto et al., 2000). The presence of StpA in H-NS:DNA structures in vivo (Uyar et al., 2009) may contribute to the stability of the nucleoprotein complex (Boudreau et al., 2018). *Hly* is also repressed by high osmolarity in a Δ*hns* Δ*hha* strain (Nieto et al., 2000), indicating that either an additional *hly* repressor that exerts its effect at high osmolarity exists, or the *hly* promoter is inherently osmosensitive and is repressed by high osmolarity – reminiscent of the *proVWX* P2 promoter that is, conversely, activated by high osmolarity in a purified in vitro transcription system lacking additional transcription factors (Rajkumari et al., 1996). Due to the complexity of an in vivo system, the precise roles of H-NS and Hha and the potential roles of StpA and an osmosensitive promoter are difficult to discern. Disentangling the effects of these regulators on *hly* will require in vitro transcription studies.

HlyCABD is repressed at low temperature (Nieto et al., 2000). H-NS mediates the repression regardless of the genetic context of Hha (Nieto et al., 2000). At lower temperatures, the multimeric, non-sequestered structure of H-NS is more prevalent due to the stabilisation of the α-helix at position 71-74 of H-NS (Hameed et al., 2019). This favours the formation of DNA:H-NS:DNA bridges (Hameed et al., 2019) that function as transcription roadblocks (Kotlajich et al., 2015) and may also increase the DNA-binding affinity of an H-NS multimer in an H-NS:DNA filament by increasing the availability of DNA-binding domains in the filament. Hha augments repression by H-NS and exerts a stronger effect at 25°C than at 37°C (Nieto et al., 2000). Atomic force microscopy of H-NS/Hha:DNA structures shows that Hha may function by stabilising H-NS-mediated bridges and forming multibridged complexes (Boudreau et al., 2018). It is unclear if DNA:H-NS:DNA bridging is involved in the repression of *hly*. However, the involvement of Hha, and perhaps StpA, in the regulation of *hlyCABD* argue in favour of such a model. In that case, if bridging involves specific elements within the H-NS-bound regulatory region of *hly* akin to *proVWX*, non-specific looping, or a multibridged 'knot' remains to be examined.

5 | THE ELUSIVE CHROMOSOME

The models presented above to describe the interplay between local three-dimensional chromosome structure and transcription are proposed based on in vivo transcription assays (Nagarajavel et al., 2007; Nieto et al., 2000), in vitro biochemical and biophysical techniques (Ali et al., 2013; Arold et al., 2010; Boudreau et al., 2018; Hameed et al., 2019; Kotlajich et al., 2015; van der Valk et al., 2017), and in silico molecular dynamics simulations (van der Valk et al., 2017). Direct demonstration of such environment-dependent, structure-regulated transcription in the context of the bacterial chromosome is still elusive. A recent study of the role of H-NS-mediated bridging in regulating the expression of the *bgl* operon also does not yet provide explicit proof for transcription regulation by local chromatin re-organisation (Lam et al., 2022).

Chromosome conformation capture (3C)-based assays can provide direct evidence. 3C-based assays reveal in vivo three-dimensional chromosome architecture with sequence information. The variety of these assays enable studies of chromosome organisation from the scale of individual genes and operons to whole genomes. Time-resolved 3C experiments allow a study of chromosome re-organisation in response to stress (reviewed in Han et al. (2018)). Investigating the re-modelling of bacterial operons requires an assay that provides a resolution of individual regulatory regions that may be shorter than 100 bp. At the present time, this is difficult and costly to achieve with high-throughput assays such as 3C-Seq and Hi-C. However, the resolution can be achieved with 3C-qPCR. 3C-qPCR is a low-throughput assay that probes the relative interaction frequency between a pair of loci at the resolution of individual restriction fragments, and therefore, offers a reliable system to probe local chromosome structure (Hagege et al., 2007; Rashid et al., 2022). 3C-qPCR can conclusively prove if the local structure of the chromosome is remodelled in response to changes in the environment, and, in association with RT-qPCR can verify if these structural changes involve a change in transcription.

6 | CONCLUSION AND PERSPECTIVES

Bacteria have evolved to respond to physicochemical environmental cues selectively on a transcriptional level. NAPs are key in this response. The dependence of the biochemical and architectural properties of NAPs on the milieu tether the physical environment to a biological system. The consequential re-modelling of the chromosome in response to the environment and the steric changes in gene accessibility manifest as altered transcription profiles. This highlights that NAPs directly link a homeostatic system to the fickle environment to which it needs to respond to survive. The diversity of NAPs that respond to different physicochemical cues, and the evolution of mechanisms that modulate the role of NAPs by fine-tuning their architectural properties (binding partners and post-translational modifications), suggests that the principle of chromatin remodelling to regulate gene expression in bacteria may be ubiquitous. Towards a complete

understanding of transcription regulation in bacteria, gene expression cannot be uncoupled from chromatin structure.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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