The power duo: how the interplay between nucleoid-associated proteins and small non-coding RNAs orchestrates the cellular regulatory symphony

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Abstract

In bacteria, the regulation of gene expression involves complex networks that integrate both transcriptional and post-transcriptional mechanisms. At the transcriptional level, nucleoid-associated proteins (NAPs) such as H-NS, HU, Lrp, IHF, Fis and Hfq are key players as they not only compact bacterial DNA, but also regulate transcription. Small non-coding RNAs (sRNAs), on the other hand, affect bacterial gene expression post-transcriptionally by base pairing with the target mRNA. Interestingly, certain NAPs also influence the function of sRNAs and, conversely, sRNAs themselves can modulate the activity of NAPs, creating a complex bidirectional regulatory network. Here, we summarise the current knowledge of the major NAPs, focusing on the specific role of Hfq. Examples of the regulation of NAPs by sRNAs, the regulation of sRNAs by NAPs and the role of sRNAs in nucleoid structuring are also discussed. This review focuses on the cross-talk between NAPs and sRNAs in an attempt to understand how the interplay between cellular proteins and regulatory RNAs contributes to the fine-tuning regulation of bacterial gene expression.

1) Introduction

Regulation of gene expression occurs in all domains of life at different molecular levels. Although the transcriptional level with transcription factors has been extensively studied, other largely underestimated regulatory mechanisms also play an important role. One such mechanism is the relationship between DNA organisation and gene expression (Le Berre*et al.* , 2022). Chromosomal DNA forms a structure in the bacterial cell called the nucleoid. DNA compaction is achieved by a combination of mechanisms, including DNA supercoiling, DNA bridging, DNA bending, DNA wrapping, and self-assembly of nucleoid proteins. The proteins involved in this compaction are known as nucleoid-associated proteins (NAPs), which also regulate the transcription of a significant portion of the bacterial chromosome (Amemiya, Schroeder and Freddolino, 2021; Schwab and Dame, 2024). A dozen NAPs have been identified in *Escherichia coli* and most Gram-negative bacteria, and their architectural and regulatory activities have been relatively well characterised: H-NS, HU, IHF, Fis, and Lrp (Azam and Ishihama, 1999). Although they are highly abundant proteins, their cellular concentration can vary depending on the physiological state of the cell, indicating that the production of NAPs is regulated (Ali Azam *et al.* , 1999; Talukder and Ishihama, 2015).

In recent decades, there has also been a growing interest in post-transcriptional regulation by RNAs, highlighting the crucial role of such regulation in global regulatory networks (Papenfort and Melamed, 2023). In bacteria, post-transcriptional regulation by RNAs is carried out by small non-coding RNAs (sRNAs), which range in size from 50 to 500 nucleotides (nt) and typically do not encode peptides (Storz, Vogel and Wassarman, 2011). While some regulatory RNAs can be longer and encode peptides or proteins, the mechanism of action involves repression or activation of target genes through complementary base pairing with target

mRNAs. This interaction affects mRNA stability or translation by modifying the secondary structure of the RNA and affecting the accessibility of RNAses to cleavage sites or ribosomes to the ribosome binding site (RBS) (Storz, Vogel and Wassarman, 2011; Papenfort and Melamed, 2023) Antisense RNAs are a category of regulatory RNAs that are transcribed from the opposite DNA strand of their target gene, they have relatively long and precise base pairings with their RNA target, and each antisense RNA targets only one mRNA (Georg and Hess, 2011). The second category of regulatory RNA is called *trans*- acting RNA, which usually has multiple target mRNAs. In this case, the RNA-RNA binding is short (around 10 nt) and has imperfect base complementarity (Holmqvist *et al.* , 2018; Melamed *et al.* , 2020). These*trans*- acting sRNAs work in concert with RNA chaperones, such as Hfq and ProQ, which increase sRNA stability and facilitate base pairing with *trans* -encoded transcripts (Quendera *et al.* , 2020). It is worth noting that Hfq is also referred to as a NAP (Amemiya, Schroeder and Freddolino, 2021). In addition, one of the earliest examples of non-coding RNA regulation was the regulation of *hns* mRNA by the sRNA DsrA (Lease, Cusick and Belfort, 1998). Since then, several other examples have been studied, underscoring the interplay between NAPs and post-transcriptional RNA regulation.

The purpose of this review is to examine the cross-talk between NAPs and non-coding RNAs. Our focus is primarily on *E. coli* , as most research has been conducted in this model strain. This review will first provide a summary of the current knowledge base regarding the major NAPs, focusing on the specific role played by Hfq. We will then present examples of the regulation of NAPs by RNAs and the regulation of sRNAs by NAPs, illustrating the complexity of regulatory networks. The role of sRNAs in nucleoid structuring will also be discussed.

2) Description of the major nucleoid-associated proteins

This section describes the main functional and structural data on NAPs. For a comprehensive review on this topic, refer to Hustmyer and Landick, 2024.

2.1) H-NS

H-NS (Histone-like Nucleoid Structuring protein), originally named as histone-like protein H1, is a 15.5 kDa basic protein composed of two regions: a DNA-binding domain at the C-terminal side of the protein linked to the oligomerisation interfaces located at the N-terminal side of the protein (**Table 1**) (Grainger, 2016). H-NS is widely distributed in Gram-negative bacteria, and several H-NS-like proteins are often present in a single species, with possible cross-talks between these proteins (Leonard *et al.* , 2009). H-NS exhibits a binding preference for AT-rich curved DNA regions. This process involves the initial nucleation of H-NS at high affinity sites, followed by the subsequent spreading along the neighbouring AT-rich DNA (Lang *et al.* , 2007; Sette *et al.* , 2009). On binding DNA, H-NS can oligomerise, leading to the formation of nucleoprotein filaments that can alter DNA topology, i.e. the 3-D structure and spatial arrangement of DNA (Dame, Wyman and Goosen, 2000). Both linear (or "stiffened") and cross-bridged filaments have been observed *in vitro* (Boudreau*et al.* , 2018). In addition, H-NS affects the interactions between RNA polymerase (RNAP) and DNA, thereby repressing gene expression through a variety of mechanisms. H-NS can interact directly with RNAP, exclude RNAP from specific DNA localisation, or form repressive loops that trap RNAP and prevent it from entering the elongation phase of transcription (Grainger, 2016; Boudreau *et al.* , 2018). All of these regulatory mechanisms have a major impact on gene expression (Hommais *et al.* , 2001; Zghidi-Abouzid *et al.* , 2016). H-NS can also inhibit elongation: the bridged filaments strongly increase pausing by RNA polymerase at a subset of pausing sites with high potential for backtracking (Kotlajich *et al.* , 2015). Horizontally acquired genes, pathogenic operons, and antisense transcripts, all of which tend to be AT-rich, are typical targets of H-NS (Navarre *et al.* , 2006, 2007; Doyle *et al.* , 2007; Singh *et al.* , 2014). In addition, H-NS can bind nascent transcripts near translation initiation sites, which has been proposed to facilitate correct ribosome positioning and enhance translation (Park*et al.* , 2010). H-NS was initially designated as a modulator of environmentally regulated gene expression (Atlung and Ingmer, 1997) due to its involvement in acclimation to stress-inducing conditions. Environmental conditions, such as osmolarity and temperature, can modulate DNA curvature and alter H-NS binding to DNA (Shahul Hameed*et al.* , 2019; Zhao *et al.* , 2021).

The activity of H-NS can also be modulated by proteins such as StpA, YdgT, the Hha family of co-repressors, such as Hha, YmoA and Cnu. The formation of heteromeric protein–protein complexes with H-NS results in the modulation of H-NS activity (Stoebel, Free and Dorman, 2008; Uedaet *al.* , 2013; Hustmyer *et al.* , 2022; Lukose *et al.* , 2024). Finally, other DNA-binding proteins, such as LeuO and SlyA can act through anti-silencing mechanisms and prevent filament formation with DNA (Stoebel, Free and Dorman, 2008).

2.2) HU

HU (Heat Unstable) is a highly conserved small (9 kDa), basic histone-like protein that is abundantly expressed (**Table 1**) (Rouvière-Yaniv and Gros, 1975; Ali Azam *et al.* , 1999). Its main function is to control DNA topology by introducing bends into double-stranded DNA, making it a critical component of the bacterial nucleoid, *e.g.* a deficiency of HU protein results in a reduction in nucleoid condensation (Bensaid *et al.* , 1996). Investigation of the role of HU has shown that, like H-NS, it is involved in the global modulation of gene transcription (Oberto *et al.* , 2009). HU is composed of two homologous subunits, α and β, which are encoded by the*hupA* and *hupB* genes, respectively. It exists in three dimeric forms: HUα2, HUβ2, and HUαβ. The levels of the α and β subunits vary during the growth cycle, resulting in a different composition of HU at different stages. HUα2 is predominant in the exponential phase, whereas HUαβ is predominant in the stationary phase. HUβ2 is almost undetectable in any growth phase (Claret and Rouviere-Yaniv, 1997). HU exhibits two distinct modes of DNA binding, both of which are sequence non-specific. The first is a non-specific binding mode characterised by ionic bonds between positively charged amino acid residues and the DNA phosphate backbone, while the second is a structure-specific binding mode involving preferential binding of HU to contorted DNA, including DNA with kinks, nicks, gaps, or cruciform structures, as well as bent DNA within loops (Verma *et al.* , 2023). Bettridge et al., (2021) showed that the non-specific binding interactions with the DNA decondensed the DNA, while the specific structure-binding interactions with the DNA compacted the nucleoid, demonstrating a dual role for HU in maintaining a proper nucleoid volume. Accordingly, HU is known to play a significant role in DNA repair and recombination (Kamashev and Rouviere-Yaniv, 2000), but also in replication (Bahloul, Boubrik and Rouviere-Yaniv, 2001). In 2002, Balandina and colleagues investigated the general RNA binding properties of HU and showed that it has a non-specific binding affinity for double-stranded RNA, DNA, and DNA-RNA complexes. In addition, DsrA was identified as a specific RNA target for HU (Balandina, Kamashev and Rouviere-Yaniv, 2002). Subsequent RNA immunoprecipitation assays, followed by microarray analysis, revealed additional RNA molecules that interact with HU, including tRNA, rRNA, several mRNAs including *rpoS* , sRNAs and various *REPs,* which are repeated extragenic palindromic elements encoding RNAs molecules (Macvanin *et al.* , 2012).

2.3) Lrp

LRP, the Leucine-responsive Regulatory Protein (18 kDa), is a widely conserved global transcription factor that can activate or repress gene expression (**Table 1**) (Ziegler and Freddolino, 2021). Combined Chromatin-Immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) under different nutrient availability conditions revealed that Lrp regulates one third of *E. coli via* direct, cooperative and indirect routes, but little is known about the precise mechanism by which Lrp regulates its target genes (Kroner, Wolfe and Freddolino, 2019). Lrp regulates genes involved in metabolism, such as those involved in amino acid biosynthesis, nutrient transport, motility, virulence, stress response and antibiotic resistance (Ziegler and Freddolino, 2021). In *E.coli,* this protein is commonly referred to as a feast/famine regulatory protein due to its response to nutrient levels. Lrp is a highly expressed protein with 2,500 molecules per cell in the exponential phase in rich medium, and three-to-fourfold higher in minimal medium (Ali Azam *et al.* , 1999). Lrp is composed of a helix-turn-helix DNA-binding domain and a regulator of amino acid metabolism (RAM) domain (De Los Rios and Perona, 2007), which binds effector molecules and modulates oligomerisation. Under starvation conditions, Lrp forms hexadecamers through oligomerisation. Conversely, in conditions of feast, it dissociates into octamers *via* leucine-induced mechanisms (Chen *et al.* , 2001). While oligomerisation beyond dimers is required for Lrp's regulatory activity, exogenous leucine modulates Lrp activity at its target promoters exclusively by inhibiting Lrp binding to DNA (Ziegler and Freddolino, 2023). Lrp is also a DNA-organising protein, but the binding motifs remain difficult to elucidate. Those

proposed since its discovery share a central AT-rich stretch flanked by 5'-CAG-3' and/or 5'-CTG-3' (Ziegler and Freddolino, 2021). Furthermore, there is evidence that *E. coli* Lrp favours DNA wrapping (Pollak and Reich, 2015) and also loops DNA over length scales of multiple kilobases (Ziegler and Freddolino, 2023), demonstrating its ability to organise bacterial DNA. Finally, it is worth noting that while Lrp from *Vibrio vulnificus, Neisseria meningitidis, Mycobacterium tuberculosis, Clostridium difficile,* and*Streptomyces spiramyceticus* have been identified as global regulators. However, the set of regulated genes is partially distinct between species (Ren *et al.* , 2007; Reddy *et al.* , 2008; Song *et al.* , 2016; Ho *et al.* , 2017; Chen *et al.* , 2019; Lu *et al.* , 2019).

2.4) IHF

IHF, the Integration Host Factor was first discovered as an essential factor for site-specific recombination of phage λ (Miller and Friedman, 1980). Subsequent studies have shown that IHF can act as a regulator, either transcriptionally repressing or activating targets depending on the context (Arfin *et al.* , 2000). IHF is involved in the control of many functions including DNA replication, recombination and cell regulation (Friedman, 1988), as well as various metabolic processes, and adaptive processes (Freundlich *et al.* , 1992; Arfin*et al.* , 2000; Reverchon *et al.* , 2021). IHF forms a heterodimer with two subunits encoded by *ihfA* and *ihfB* and is mainly found in *Proteobacteria* (**Table 1**) (Nash*et al.* , 1987). IHF is one of the major bacterial NAPs whose intracellular concentration changes during bacterial growth, increasing during the transition to the stationary phase (Azam and Ishihama, 1999). IHF binds DNA with significant specificity, nevertheless its binding specificity appears to depend on the intrinsic structure of DNA rather than the base composition of the DNA (Swinger and Rice, 2004; Liu, Ma and Xu, 2018). Binding of IHF can induce sharp bends in DNA, up to 160° (Dhavan *et al.* , 2002). This could facilitate long-range interactions and affect the DNA topology. It should be noted that IHF and HU proteins are both members of the DNABII family of DNA-binding proteins and are strikingly similar to each other in sequence and in their unique structural fold (Prieto *et al.* , 2012). In addition, they share some similar targets (Bonnefoy and Rouvière-Yaniv, 1992), but interact differently with the DNA and have different roles (Prieto *et al.* , 2012).

2.5) Fis

Fis for Factor of Inversion Stimulation, a 98-amino-acid homodimeric protein (11.4 kDa), was named and first identified for its role in the G-loop inversion of the bacteriophage Mu (Koch and Kahmann, 1986). Like the other NAPs, Fis is involved in the organisation and the maintenance of the nucleoid structure (**Table 1**) (Schneider, 2001). It is composed of four helices and a β-hairpin arm in the N-terminal domain that facilitates DNA inversion. It bends DNA up to 90°, stabilising DNA looping and aiding in DNA compaction (Skoko*et al.* , 2006). Fis binds as a homodimer to regulate the transcription of topoisomerases (*topA* and *gyrB),* thereby affecting the level of supercoiling of the cell, i.e. is the over- or under-winding of the DNA double helix relative to the relaxed state (Ohniwa *et al.* , 2006). It binds to a highly degenerate 15-bp consensus sequence (GnnYAnnnnTRnnC, where Y is T or C and R is A or G) (Stella, Cascio and Johnson, 2010). Fis is one of the most abundant NAPs during the exponential phase (over 60,000 proteins per cell) and drops drastically upon entry into the stationary phase, to less than 100 copies per cell (Ali Azam *et al.* , 1999). This growth phase regulation explains the major role of Fis in the activation of stable RNA genes, required for rapid bacterial growth (Hirsch and Elliott, 2005; Lautier and Nasser, 2007). Fis abundance also varies depending on the metabolic state of the cell: for example, high levels of the alarmone guanosine tetraphosphate ((p)ppGpp) negatively regulate*fis* expression (Ninnemann, Koch and Kahmann, 1992). A partially overlapping function between Fis and H-NS should be noted, as Fis also represses xenogeneic regions of the genome (Karambelkar, Swapna and Nagaraja, 2012; Amemiya, Schroeder and Freddolino, 2021).

3) Hfq: a double function protein?

Hfq is an 11 kDa homohexameric protein present in approximately half of sequenced bacterial genomes and in some archaeal species (**Table 1**) (Sun, 2002; Mura *et al.* , 2013). It was first described as a host factor required for the replication of the bacteriophage Qβ RNA (Franze de Fernandez, Hayward and August, 1972). An *E. coli* cell contains approximately 10,000 Hfq hexamers, making it one of the most abundant proteins.

The concentration of Hfq in the stationary phase is controversial in the literature. Although western blot quantification by Azam and Ishihama, (1999) showed a decrease in Hfq protein during the stationary phase of growth, other studies agree on a twofold increase in the protein level during the stationary phase of growth (Tsui, Feng and Winkler, 1997; Diestra *et al.* , 2009). 10%-20% of these hexamers are found in the nucleoid, 50% are membrane-bound fractions and 30% are localised in the cytoplasm (Azam, Hiraga and Ishihama, 2000; Diestra*et al.* , 2009). A Hfq monomer is composed of two domains named the N-terminal region (NTR) and the C-terminal region (CTR) (**Figure 1**). The NTR is highly conserved across bacteria and in *E. coli,* it is a 65 amino acid long peptide that is structurally related to the eukaryotic Sm family of proteins (Mura *et al.* , 2013). The Sm proteins have been shown to participate in nucleic acid-related processes, including splicing, telomere replication, RNA decapping and decay (Wilusz and Wilusz, 2013). The structure of the NTR reveals that it folds into five β-sheets and one N-terminal ∝-helix. Six monomers assemble into a typical toroidal hexameric ring (**Figure 1**) (Sauter, 2003). This toroid can be divided into three parts: the proximal face, where the ∝-helix is located, the distal face where most of the β-sheets are exposed to the solvent and the lateral rim. The CTR is located at the periphery of the toroid, is predicted to be unstructured and has been reported to be present only in a subset of Gram-negative bacteria (Vogel and Luisi, 2011). Disruption of the*hfq* gene in most bacterial species, including *E. coli* , results in diverse phenotypes, highlighting the pleiotropic functions of this protein. These include reduced growth rate, virulence, motility and biofilm formation, as well as modifications in oxidative and osmotic sensitivity (Tsui, Leung and Winkler, 1994; Sittka *et al.* , 2007; Leonard *et al.* , 2021). However, the requirement for Hfq appears to be facultative in a few bacteria, most of which are Gram-positive, such as *Bacillus subtilis* or *Staphylococcus aureus (Jousselin, Metzinger and Felden, 2009)* .

3.1) Hfq, a RNA chaperone

The similarity between RNA-binding Sm proteins and Hfq has led to many studies focusing on the interactions of Hfq with RNA. *E. coli* Hfq has been reported to bind to the sRNAs *oxyS* (Zhang, 1998),*rprA* (Wassarman *et al.* , 2001) and *dsrA* (Sledjeski, Whitman and Zhang, 2001) and to modulate the translation of *rpoS* (Battesti, Majdalani and Gottesman, 2011). Considering that RpoS is a sigma factor expressed under various stress conditions, it was assumed that most of the reported phenotypes of the *hfq* mutants were due to Hfq mediating the stress response through the interaction between*rpoS* mRNA and sRNAs. Accordingly, the *hfq* mutant shows a strongly reduced expression of representative RpoS-regulated genes (Muffler *et al.* , 1997). Since these early studies, Hfq has been reported to facilitate base-pairing between a large number of*trans* -encoded sRNAs and their mRNA targets (Holmqvist *et al.* , 2016). In general, the U-rich RNA sequences, usually corresponding to the 3'-end of sRNAs, bind to the proximal face of the toroid, whereas A-rich sequences, mostly found in mRNAs, bind to the distal face of Hfq (**Figure 1**). In addition, the lateral rim binds U-rich sequences found in sRNAs (Sauer, Schmidt and Weichenrieder, 2012). Thus, Hfq's three faces allow it to bind two different RNA strands simultaneously, making it an effective RNA chaperone that brings the regulatory RNA and its mRNA target closer together. Several outcomes are possible following the sRNA:mRNA hybridisation : (i) prevention or promotion of translation by concealing or facilitating access to the ribosome binding site, respectively (Maki *et al.* , 2008; Fröhlich and Vogel, 2009); (ii) prevention or facilitation of RNase degradation of the target mRNA (Moll*et al.* , 2003; Mohanty, Maples and Kushner, 2004). Taken together, the role of Hfq in post-transcriptional regulation is crucial. For more on the different regulatory mechanisms exhibited by Hfq, see Vogel and Luisi, 2011.

3.2) Hfq, a nucleoid-associated protein

Hfq has also been described as one of the NAP that shape the bacterial chromosome (Azam and Ishihama, 1999). Hfq is capable of binding double-stranded and single-stranded DNA but with less affinity than RNA (Updegrove *et al.* , 2010; Geinguenaud *et al.* , 2011; Kubiak*et al.* , 2022). The majority of studies examining Hfq as a NAP have been conducted in *E. coli* . Fluorescence microscopy imaging of single DNA molecules and atomic force microscopy experiments have demonstrated the formation of a nucleoprotein complex between Hfq and double-stranded DNA (Jiang *et al.* , 2015). The nucleoprotein complex between Hfq and DNA remains flexible with a moderate increase in bending persistence length, compared to the rigid filaments

observed after H-NS binding (Boudreau *et al.* , 2018). However, as observed for H-NS, the nucleoprotein complex with DNA compacts the DNA into a condensed form by bridging DNA segments (Jiang *et al.* , 2015). The DNA binding involves the CTR domain (Updegrove *et al.* , 2010), a sequence of 35 amino acids that has been predicted to be unstructured, but whose crystal structure remains unknown (Vogel and Luisi, 2011). Upon interaction with DNA, these domains self-assemble and form amyloid-like fibrillar structures *in vitro* (Arluison *et al.* , 2006; Fortas *et al.* , 2015). These structures have been shown to be responsible for the self-assembly with DNA, the DNA bridging and compaction (**Figure 1**) (Malabirade *et al.* , 2018a). Although the full-length Hfq binds to DNA *via* the interface of its toroidal hexameric ring, the NTR by itself is not required for Hfq to bind to DNA and only the presence of the CTR is necessary for DNA compaction (Malabirade *et al.* , 2017). A model has been proposed whereby Hfq can form a bridge by anchoring one or more of its other CTR arms to another section of the same or another DNA molecule and/or by CTR-mediated self-interactions among multiple proteins (Malabirade*et al.* , 2017). Recently, it was confirmed that amyloid structures are formed *in vivo* (Partouche *et al.* , 2019), indicating that the CTR is responsible for the nucleoid remodelling *in vivo* through DNA binding, bridging and compaction (**Figure 1**) (Cossa*et al.* , 2022). In contrast to H-NS, the bridging formed by the nucleoprotein complex doesn't affect DNA topology (Malabirade *et al.* , 2018a). It has therefore been suggested that the effect of Hfq on DNA supercoiling observed *in vivo* (Tsui, Leung and Winkler, 1994) may be indirect. Indeed, Hfq post-transcriptionally regulates the expression of proteins that affect DNA topology (**Figure 1**) (Sledjeski, Whitman and Zhang, 2001). Finally, the CTR of Hfq has been shown to bind to G-quadruplexes, a type of alternative DNA and RNA structure. G-quadruplex structures consist of three or more guanine quadruplex rings that are held together by Hoogsteen hydrogen bonds (Gellert, Lipsett and Davies, 1962), forming highly stable four-stranded structures. Hfq enhances the stability of G-quadruplex structures, which can lead to the termination of DNA replication and a significant increase in the mutation rate. Therefore, the stabilisation of G-quadruplexes by Hfq may drive the evolution or alternation of bacterial gene expression (Parekh *et al.* , 2019, 2020).

3.3) How does Hfq compare to other NAPs?

The amount of Hfq in the bacterial cell is comparable to that of other NAPs such as HU or H-NS (**Table 1**). However, only 20% of the Hfq content is localised in the nucleoid, corresponding to approximately 2000 hexamers. Therefore, a rough estimate suggests that Hfq hexamers represent around 5% of the total NAPs, compared to 20 and 40% for Fis and HU, respectively, during the exponential phase of growth, respectively (Talukder and Ishihama, 2015). Furthermore, Hfq appears to have a heterogeneous localisation in the nucleoid, in contrast to the more uniform distribution of other NAPs (Azam, Hiraga and Ishihama, 2000). This heterogeneous distribution may be due to the preferred AT-rich Hfq-binding motif, whose consensus is (A/T)T(A/G)TGCCG (Updegrove *et al.* , 2010), or to the polyphosphate molecule, which was suspected to have a role in the site-specific DNA binding properties of Hfq in bacteria (Beaufay *et al.* , 2021). Recently, Hfq and Fis have also been reported to be highly concentrated on EPODs (Extended protein occupancy domains) in the *E. coli* genome to ensure the silencing of prophage acquired DNA (Amemiya, Schroeder and Freddolino, 2021). The distribution of Hfq in EPODs would be consistent with the observation that the fibre-like pattern generated by Hfq can cover large regions of DNA.

4) NAPs are regulators of sRNA expression

Several examples of sRNAs regulated by NAPs have been described in the literature. Here, we only illustrate the redundant, opposing, and synergistic roles that different NAPs play in regulating sRNAs.

4.1) 6S RNA is regulated simultaneously by H-NS, Lrp and Fis

The *E. coli* 6S RNA (SsrS) was first described by Brownlee in 1971 (Brownlee, 1971). This 184 nt long sRNA has an extended double-stranded structure with a large single-stranded bulge, similar to the DNA structure in an open promoter complex (Barrick *et al.* , 2005; Trotochaud and Wassarman, 2005). This secondary structure is a common feature of 6S RNAs, and in *E. coli* it preferentially interacts with the σ70-RNA polymerase (Eσ 70). This complex leads to the titration of RNAP and thus inhibition of the transcription of

σ70-dependent promoters (Wassarman and Storz, 2000). Given that Eσ 70 is an exponential phase-specific polymerase, and that cellular levels of 6S RNA increase during the stationary phase (Wassarman and Storz, 2000), it has been suggested that 6S RNA facilitates the shift of global gene expression in *E. coli* from the exponential to the stationary phase of growth. In addition, *in vivo* experiments have shown that 6S RNA serves as a template for the synthesis of a short RNA product (pRNA) that forms the 6S RNA:pRNA during exponential growth, realising the 6S RNA from the RNAP (Bonar *et al.* , 2022).

The function of 6S RNA depends on its maturation, which involves the sequential removal of the extra 3' and 5' sequences by exonucleases and RNase E/G (Kim and Lee, 2004; Chae *et al.* , 2011). Its transcriptional initiation is under the control of two tandem promoters, P1 and P2, which are regulated by H-NS, Lrp and Fis (**Figure 2**) (Neusser *et al.* , 2008). The extended region of upstream DNA between the P1 and P2 promoters is occupied by H-NS, resulting in repression of *ssrS* transcription. Six to seven Lrp binding sites were identified clustered around the two promoters. *In vivo* and*vitro* assays confirmed the repression of *ssrS* transcription initiation by H-NS and Lrp. The Fis protein acts more like a dual regulator activating the P1 promoter but inhibiting the P2 promoter. Fis binding sites are mainly clustered upstream of P1 (Hirvonen *et al.* , 2001; Hillebrand *et al.* , 2005), and overlapping and downstream of the P2 promoter (Neusser *et al.* , 2008). In addition, three sites overlap with Lrp binding sites, suggesting that Fis and Lrp interfere with each other (Neusser *et al.* , 2008). Although the precise mechanism involved has not been elucidated, it has been shown that transcription from the P1 and P2 promoters is feedback-activated and feedback-inhibited depending on the cellular context. This feedback regulation disappears in *Δφις* strains, suggesting that Fis is involved in the regulatory circuitry that ensures an appropriate cellular concentration of 6S RNA (Lee*et al.* , 2013). These findings underline that the regulation of 6S RNA transcription is under the control of a sophisticated network of bacterial regulation, which plays a pivotal role in facilitating adaptation to fluctuating growth conditions.

4.2) Rsm/Csr regulation by H-NS homologs and IHF

Different NAPs can have similar functions in different species. An example of this is the Csr/Rsm sRNA family, which regulates the CsrA/RsmA proteins and is regulated by either the H-NS family of proteins or IHF, depending on the species (**Figure 2**). Indeed, the central component of the carbon storage regulator (Csr) and the homologous repressor of secondary metabolites (Rsm) systems is an RNA binding protein (CsrA or RsmA) that inhibits protein translation by binding to a stem-loop RNA motif in the 5'-region of its mRNA targets, such as genes involved in metabolism, motility, biofilm formation and quorum sensing and/or pathogenesis. sRNAs such as CsrB/RsmB, CsrC, RsmZ, RsmY and RsmX (the names vary depending on the bacterial species), contain multiple sequence/structural motifs that mimic the RNA motif and sequester the CsrA/RsmA protein. These sRNAs have a redundant titration function, but distinct regulatory pathways. In *P. aeruginosa* PAO1, the GacS/GacA system transduces regulatory signals to downstream genes by directly controlling the expression of only two genes, *rsmY* and *rsmZ* . Castang et al., (2008) showed that MvaT and MvaU, two members of the H-NS family, interact with *rsmZ* but not with *rsmY* . The repression of RsmZ transcription by MvaT was validated using mutants defective for the *mvaT* and*mvaU* genes, and gene fusions (Brencic *et al.* , 2009). However, in *P. fluorescens, in vitro* experiments showed that the AT-rich promoter-linker region of the *rsmZ* gene has two IHF binding sites, suggesting that IHF is likely to be involved in the regulation of *rsmZ* rather than the H-NS homologues (Humair, Wackwitz and Haas, 2010). In *Salmonella* , the sRNAs that sequester CsrA are CsrB and CsrC, which are similar to those in *P. fluorescens,* as is the regulatory mechanism whereby IHF activates the transcription of *csrB* but it has no effect on the transcriptional regulation of *csrC* (Martínez *et al.* , 2014). Furthermore, in *E. amylovora,* IHF positively regulates the *rsmB* sRNA to control motility (Lee and Zhao, 2016). CsrA homologs and Csr/Rsm sRNAs are present in most *Gammaproteobacteria* species, including*Vibrio* species and *Legionella pneumophila* , as well as the*Pseudomonas* species, *Salmonella enterica* ,*Xanthomonas* spp. and *Erwinia carotovora* , suggesting that the regulation illustrated here involving either H-NS homologs or IHF may be widespread in *Gammaproteobacteria* species, including pathogenic and beneficial biocontrol species.

5) sRNAs are regulators of NAPs

5.1) H-NS is regulated by the sRNA DsrA in *E. coli*

The regulation of *hns* by DsrA (downstream of *rcsA* gene which is positive regulator of the capsular polysaccharide synthesis) was first studied by the group of Susan Gottesman. Their work demonstrated that an overproduction of this small (87 nt) Hfq-dependent RNA activates the initiation of *rcsA* transcription, thereby counteracting H-NS silencing (Sledjeski and Gottesman, 1995). DsrA was later found to interact with the *hns* mRNA through specific RNA-RNA base pairing near the translation initiation region (TIR) (Lease, Cusick and Belfort, 1998; Lalaouna *et al.* , 2015). This interaction promotes mRNA destabilisation by actively recruiting the RNA degradosome complex, which cleaves *hns* mRNA at 131 nt (**Figure 3a**). Furthermore, it reduces protein synthesis as DsrA pairs in the "five-codon window" immediately downstream of the start codon, sterically interfering with the ribosome binding site (TIR) (Lease, Cusick and Belfort, 1998; Lease and Belfort, 2000; Lalaouna*et al.* , 2015; Lalaouna and Massé, 2016; Wu *et al.* , 2017). DsrA has the typical structure of a *trans*- acting RNA, consisting of 3 domains (Sledjeski and Gottesman, 1995; Wu *et al.* , 2017). The first domain is a single-stranded AU-rich region located between stem-loop I and II that binds to Hfq (Sledjeski, Whitman and Zhang, 2001). The second domain corresponds to the second stem-loop and is involved in the base pairing with *hns* (Lease, Cusick and Belfort, 1998), and the third domain is a stem-loop that acts as a Rho-independent transcriptional terminator (**Figure 3a**). The interaction mechanism between DsrA and *hns* mRNA has been elucidated by Lalaouna et al., (2015). In summary, Hfq melts out the second stem-loop of DsrA and alters the sRNA conformation, allowing base pairing with the five-codon-window downstream of the start codon in the*hns* mRNA (**Figure 3a**). In response to low temperature or acidic pH stress, the transcription and stability of DsrA is enhanced, thereby modulating the translation of *hns* mRNAs (Repoila and Gottesman, 2001; Repoila and Darfeuille, 2009; Bak *et al.* , 2014).

5.2) H-NS, bifunctional RNA and pervasive transcription

A recent study by Zhao et al., (2021) identified the *hns* mRNA as a bifunctional RNA that down-regulates the expression of its neighbouring gene, *galU* , thereby attenuating the motility of*S. enterica serovar* Typhimurium. Bifunctional (or dual-function) RNAs are defined as RNAs that can both be translated into proteins and play regulatory roles. This group includes mRNAs that overlap with the transcript of neighbouring genes transcribed from the complementary DNA strand (Toledo-Arana and Lasa, 2020). Since these mRNA sequences are complementary, they allow a regulatory mechanism using antisense RNAs. In *E. coli* and *S. enterica* , the *hns* genes are located on the opposite strand to the *galU* gene (**Figure 3b**). The *galU* gene encodes a uridine triphosphate-glucose-1-phosphate uridyltransferase, an enzyme that facilitates the production of bacterial surface structures such as lipopolysaccharide, extracellular polysaccharide and capsular polysaccharide (Dean and Goldberg, 2002; Guo *et al.* , 2010; Zhao*et al.* , 2021). Zhao et al., (2021) showed that the deletion of*galU* reduced the motility in *S.* Typhimurium, whereas overexpression of *hns* mRNA inhibited motility and flagellar gene expression in a *galU* -related manner. At the molecular level, the*galU* mRNA was found to contain an extensive 3'-untranslated region that overlaps with the *hns* mRNA. This long 3' untranslated region (UTR) is cleaved, resulting in the production of short RNA fragments. The proposed mechanism involves the formation of an RNA duplex between *hns* mRNA and the 3'UTR of *galU* , leading to RNA cleavage and ultimately to *galU* -processed transcripts that may be translationally inactive (**Figure 3b**). Since bifunctional RNAs occur in pairs and have two modes of regulation, it was also suspected that the reverse regulation might also occur. Overexpression of GalU-3'-UTR resulted in a decrease in *hns* mRNA and protein levels compared to the control strain, leading to the assumption that*galU* mRNA could also act as an antisense RNA and regulate*hns* mRNA.

5.3) Fis and RgsA in *Pseudomonas*

Following the hypothesis that several sRNAs regulate Fis, the first demonstration of sRNA regulation of Fis was conducted in 2016 in the*P. aeruginosa* PAO1 strain (Lu *et al.* , 2016). RgsA, originally named P16, is a 122 nt sRNA that is phylogenetically conserved in *Pseudomonas* species (Livny *et al.* , 2006). RgsA is regulated directly by RpoS and indirectly by the two-component system GacA/GacS (González *et al.* , 2008). Its expression increases from the exponential to the stationary phase, consistent with RpoS regulation, and decreases as cells are grown into deep stationary phase (González *et al.* , 2008). RgsA has also been

found to be involved in oxidative stress response and swarming motility (González*et al.* , 2008) (Park *et al.* , 2013). The predicted secondary structure of RgsA consists of four stem-loop structures. The third stem-loop is preceded by a highly conserved single-stranded region, which is a key determinant for base-pairing to *fis* mRNAs. In particular, two functional start codons were identified in the*fis* mRNA, the second of which is predominantly used in *P. aeruginosa* PAO1, resulting in the production of two types of Fis proteins. RgsA represses the expression of both proteins by different mechanisms. RgsA can repress the expression of the longer Fis by binding to the CDS region, leading to an acceleration of the RNase E-dependent mRNA decay. This interaction also inhibits the expression of shorter Fis by translational blockage and RNase cleavage. As with many sRNAs, Lu et al., (2016) demonstrated that RgsA regulates *fis* mRNA in an Hfq-dependent manner. More interestingly, they also showed that RgsA promoter activity is reduced in an *hfq* mutant, implying that Hfq also regulates RgsA at the transcriptional level. Furthermore, a feed-forward regulatory loop between Fis and RgsA was suggested after observing that the ectopic overexpression of RgsA was much higher in an*rgsA* mutant compared to the wild-type strain. Knowing that Fis represses *rpoS* transcription in *Salmonella (Hirsch and Elliott, 2005),* the authors proposed to investigate the regulatory effect of Fis on RpoS in PAO1. If so, this would suggest a regulatory loop involving RpoS as a third player.

5.4) Lrp is the target of several regulations by sRNA

The expression level of *E. coli lrp* is controlled by different proteins and environmental conditions. H-NS and the nitrite-sensitive repressor NsrR could repress the transcription of the*lrp* gene (Oshima *et al.* , 1995; Partridge *et al.* , 2009), whereas GadE and the alarmone ppGpp activate the expression of*lrp* during amino acid starvation (Hommais *et al.* , 2004; Traxler *et al.* , 2011). Furthermore, the *lrp* gene is autoregulated (Wang *et al.* , 1994), and it has been reported that arginine-loaded ArgR indirectly interferes with the negative autoregulation of *lrp* (Torres Montaguth *et al.* , 2019).

In *E. coli* and *S. enterica,* the location of the transcription start site is more than 250 nt upstream of the start codon, making this 5'UTR a desirable site for sRNA binding and regulation (Wang *et al.* , 1994; McFarland and Dorman, 2008). Wright et al., (2013) developed CopraRNA, an algorithm that uses comparative genomics to predict mRNA targets for bacterial small RNAs. Indeed, they identified *lrp* as a putative target of 7 sRNAs: GcvB, MicF, DsrA, FnrS, MicA, MicC, and RprA-L. Experimental validation was performed on GcvB, MicF and DsrA. In addition, a novel sRNA called ArcZ, was discovered to post-transcriptionally repress *lrp* in*Erwinia amylovora* (**Figure 4a-b**) (Schachterle and Sundin, 2019). These regulations will be reviewed below.

5.4.1) Lrp and GcvB

GcvB is a 200 nt long Hfq-associated sRNA that is highly conserved in Gram-negative bacteria (Zhang *et al.* , 2003; Sharma *et al.* , 2007). It regulates amino acid metabolism and transport and short peptide transport by repressing several ABC transporters, including the*oppABCDF* and *dppABCDF* operons (Pulvermacher, Stauffer and Stauffer, 2008, 2009; Sharma *et al.* , 2011). In addition, GcvB directly interacts with the 5'UTR region of *lrp* mRNA (**Figure 4c**) (Lee and Gottesman, 2016). The sRNA consists of four stem-loops (SL) defining three regions: R1, R2 and R3 (Sharma*et al.* , 2011). R1 is a 30-nt-long G/U-rich single-stranded sequence that separates SL1 and SL2 and regulates 92% of the GcvB target mRNA (Lalaouna *et al.* , 2019). The R2 is a single-stranded decamer between SL3 and SL4. Although R1 and R2 are highly conserved, they are not required for *lrp* binding. However, the R3 region contains a CUGUC sequence that is crucial for the *lrp* binding (Lee and Gottesman, 2016). Indeed, the R3 portion of GcvB protects two GACAG regions on the *lrp* mRNA leader, located between -179 and -175 nucleotides and between -39 and -33 nucleotides from the ATG, respectively (**Figure 4c**) (Lee and Gottesman, 2016). The presence of two GcvB binding sites on the *lrp* mRNA suggests that two GcvB molecules are required for the maximal repression of *lrp* in rich medium conditions and under oxidative stress (**Figure 4a**) (Lee and Gottesman, 2016). However, it is still unknown how GcvB/*lrp* pairing leads to regulation. Although for the binding site located between -39 and -33 nt, it has been suggested that GcvB interferes with a transcriptional enhancer located between -69 and -40 nt (**Figure 4c**), the regulation for the other site remains unexplained. Nevertheless, the structure of the *lrp* leader may be critical for efficient translation, as certain mutations can interfere with translation and/or

GcvB regulation (Lee and Gottesman, 2016).

5.4.2) Lrp regulation by MicF and DsrA

MicF is a 90 nt sRNA that was discovered as a negative regulator of*lrp* expression (**Figure 4a**). The repressive activity of MicF on *lrp* was experimentally validated by analysing strains lacking or overexpressing MicF. In addition, the base pairing between MicF and *lrp* that were predicted by the Mfold programme was experimentally validated by (Holmqvist *et al.* , 2012). The conserved 5'-end of MicF represses the *lrp* mRNAs by seed pairing and binding to a region overlapping the AUG start codon and the early CDS region of *lrp,* and the binding process requires Hfq (**Figure 4d**) (Corcoran *et al.* , 2012; Lee and Gottesman, 2016). Thus, Lrp translation is repressed by the formation of a complex that obstructs the proper 30S positioning on TIRs in *lrp* mRNAs (Holmqvist *et al.* , 2012). The repression of Lrp synthesis by MicF indirectly regulates downstream genes in the Lrp regulon (Holmqvist*et al.* , 2012).

DsrA has also been predicted and demonstrated to directly bind*lrp* mRNA (Lee and Gottesman, 2016). Regulation of *lrp* by DsrA was only demonstrated in strains overexpressing DsrA, while deletion of *dsrA* results in little or no change in the translational expression of *lrp* (Lee and Gottesman, 2016). This result suggested either a possible repression of *lrp* under low temperature and acid stress (Repoila and Gottesman, 2001; Repoila and Darfeuille, 2009; Bak *et al.* , 2014); or no regulatory role for DsrA exists in physiological condition. DsrA pairs early in the*lrp* ORF using the same region known to repress *hns* mRNA (**Figure 4e**) (Lease, Cusick and Belfort, 1998). The role of this interaction is still under debate and it is expected to be intricate, considering that the binding site of DsrA on *lrp* overlaps with MicF binding site (**Figure 4b**). In summary, if *lrp* mRNA is the target of several bindings of sRNA, the effectiveness of their regulation *in vivo* still needs to be studied in depth.

In this study, we delineated the manner by which GcvB and MicF regulate Lrp at the post-transcriptional level. Nevertheless, Lrp has also been shown to regulate GcvB and MicF at the transcriptional level (**Figure 4a**) (Ferrario *et al.* , 1995; Modi *et al.* , 2011), implying the existence of a double-negative feedback loop. Considering that GcvB and MicF are highly expressed in fast-growing conditions (nutrient-rich medium), whereas Lrp is present in nutrient-poor environments, it could be suggested that MicF and GcvB repress *lrp* in nutrient-rich media and Lrp represses GcvB and MicF in nutrient-poor medium (**Figure 4a**). Since most of the Lrp regulon contains proteins involved in amino acid biosynthesis, this regulatory circuit is physiologically reasonable. In accordance, deletion of *gcvB* affects *lrp* in LB medium but not in minimal medium when GcvB is poorly expressed (Lee and Gottesman, 2016), and the overexpression of MicF in a minimum medium showed a severe growth defect similar to the one observed in a *lrp* depletion strain (Holmqvist *et al.* , 2012). GcvB repression of *lrp* was also found to be effective under oxidative stress conditions (**Figure 4a**), whereas only a modest increase in mRNA *lrp* stability and Lrp proteins was observed after oxidative stress in strains deleted for *micF,* indicating a modest repression of*lrp* by MicF under these conditions (Lee and Gottesman, 2016). Therefore, a double-negative feedback loop involving mainly GcvB could also occur under oxidative stress. This regulatory circuit also seems probable since Lrp regulates genes involved in oxidative stress (Kroner, Wolfe and Freddolino, 2019). Therefore, *E. coli* may use this dual repression scheme to promote a switch for adequate Lrp-dependent adaptation to nutrient availability and to oxidative stress. Further work is needed to determine exactly how this loop translates changes in MicF and GcvB abundance into changes in Lrp abundance. Considering that the binding sites of MicF and GcvB do not overlap, an additive repressive effect of both sRNAs on *lrp* mRNA stability needs to be tested.

5.4.3) Lrp and ArcZ

In *Erwinia amylovora* , the causal agent of fire blight disease that devastates apple and pear trees, Lrp modulates several virulence-associated traits and it is post-transcriptionally regulated by the Hfq-dependent sRNA ArcZ (Schachterle and Sundin, 2019). ArcZ interacts with the 103-nt-long 5'UTR of *lrp* to destabilise*lrp* mRNA. ArcZ was shown to positively regulate the motility phenotype and the transcription of flagellar genes indirectly through Lrp, while directly repressing the translation of *flhDC* mRNA. This incoherent

feed-forward loop could generate a uniquely shaped output pulse in flagellar motility in response to variations in Lrp quantity. Thus, Lrp could temporarily shift motility under specific environmental conditions, mostly through its regulation of *flhDC* . Other sRNAs, such as ArcZ, may be involved in *lrp* regulation, as described in *E. amylovora* . We can therefore postulate that post-transcriptional regulation of *lrp* involving sRNAs may be crucial, even if the sRNA varies.

6) sRNAs involved in chromosome structure: the case of HU and *REP* RNA

Since the 1970s, researchers have acknowledged that RNA molecules participate in nucleoid condensation through RNA-DNA interactions (Hecht and Pettijohn, 1976). It has been hypothesised that nascent RNAs, or a distinctive class of unknown RNAs, stabilise the nucleoid. Given the importance of RNA molecules, along with HU and other nucleoid proteins, in maintaining chromosome structure, Macvanin *et al.* used a ribonomic approach to identify RNAs bound to HU (Macvanin *et al.* , 2012). In addition to tRNA and rRNA, HU binds to 11 mRNAs encoding membrane-associated proteins, four sRNAs (SsrS, SsrA, Ffs, and RnpB) and 10 novel non-coding RNAs (nc1 to nc10). Some of the sRNA targets were found to be transcribed from regions containing repetitive palindromic extragenic DNA elements, also known as *REPs* . A subsequent study by the same group confirmed the presence of 30 sRNAs transcribed from REP elements, which were found to be associated with the nucleoid (Qian*et al.* , 2015). *REP* elements were first reported in enterobacterial genomes 30 years ago (Gilson *et al.* , 1991) and consist of single palindromes separated by linkers. The *E. coli* genome has nearly 350 annotated *REP* elements, more than 50% of which are transcribed (Qian *et al.* , 2015). Therefore, transcripts involved in chromosome condensation are referred to as nucleoid-associated ncRNAs or naRNAs. Further research on the relationship between RNA and chromosome condensation has mainly focused on REP_{325} , which corresponds to nc5 (Qian*et al.* , 2015). REP_{325} consists of six highly homologous repeats composed of palindromic cruciform motifs, called Y and Z_2 which generate motifs separated by five spacers with the same DNA sequence (**Figure 5a**). REP_{325} is transcribed into six RNAs, naRNA1 to naRNA6. Each naRNA consists of two potential hairpins (corresponding to motifs Y and Z_2) connected by a short linker (**Figure 5a**). Interestingly, a strain withoutREP_{325} shows a greater degree of nucleoid decompaction that can only be restored by the presence of naRNA4. In addition, a chromosome conformation capture (3C) targeting *REP* segments revealed a physical proximity between different *REP* elements. This physical connection depends on the presence of both HU and REP_{325} RNA. However, other NAPs and RNAs may also be involved. Molecular analyses have shown that the effect of RNAs on DNA condensation requires the presence of two hairpins (Y andZ_2) rather than a specific sequence (Qian, Zhurkin and Adhya, 2017), and that HU connects to naRNA4. The model suggests that naRNA4 uses its two bulged hairpins to form DNA-RNA complexes through potential secondary structure. This configuration is only possible when DNA is supercoiled and palindromic sequences are converted into cruciform structures, which aids with chromosome condensation (**Figure 5b**). Cruciform DNA and RNA hairpins can bind to the HU protein, allowing the formation of DNA-naRNA4 complexes (Qian, Zhurkin and Adhya, 2017), and recent data suggest that HU must be able to bind both HU and the DNA in order to execute its function (Bettridge et al., 2019, unpublished data). However, it is worth noting that after facilitating the formation of the DNA-RNA complex, the HU protein dissociates from the complex, suggesting a chaperon-like role for HU (Qian, Zhurkin and Adhya, 2017).

7) Concluding remarks

The regulation of gene expression is a complex process involving different levels that may interact with each other. This review discusses the interplay between nucleoid-structuring proteins, which also regulate transcription, and RNAs, which are involved in post-transcriptional regulation. It is highlighted that sRNA can be regulated by several NAPs and, conversely, one NAP can be the target of several sRNAs (**Figure 2**). Therefore, these regulatory molecules, either sRNAs or NAPs, could form hubs in regulatory networks. For example, DsrA regulates H-NS at the post-transcriptional level and binds to *lrp* mRNA. In addition, the HU protein also binds DsrA, suggesting that this sRNA may link these three NAPs. This raises questions about the function of these cross-talks. One hypothesis is that a sRNA could indirectly modify the chromosome conformation by regulating NAPs. This is partially illustrated by the result for Hfq, which compacts DNA

without altering its topology, but the disruption of the *hfq* gene affects plasmid supercoiling *in vivo* (Cech*et al.* , 2016), suggesting an indirect effect of Hfq on DNA topology through its RNA chaperone function on sRNA (**Figure 2**). The hypothesis is that an unknown sRNA post-transcriptionally regulates NAPs, which in turn affects DNA supercoiling (Malabirade *et al.* , 2018b). Taking this a step further, such regulation could promote the activity of one NAP with its DNA binding specificity rather than another. Regulation of NAPs by sRNAs may also be specific to certain environmental growth conditions, promoting a switch to ensure an appropriate level of NAPs. It is also worth considering the physiological impact of sRNA regulation of highly abundant proteins such as NAPs. Although some NAPs are regulated by several sRNAs, it is possible that their regulation is additive.

Cross-talk between NAPs and sRNAs is also highlighted by the protein Hfq, which functions both as an RNA chaperone and as a NAP. Although the two functions of Hfq have been studied separately, they may be connected. Questions about how, when and where this connection occurs remain to be answered.

In addition to Hfq, other NAPs such as H-NS-like proteins, or HU have been shown to bind RNA. It is unclear whether this affinity has a physiological function. However, some sRNAs, known as nucleoid-associated ncRNAs, are directly involved in DNA compaction and the affinity of HU to these RNAs is directly involved in this phenomenon. Further research is needed to fully understand the role of sRNA in the chromosome structure. In conclusion, this review highlights the intricate interrelationship between NAPs, sRNAs, and chromosome structure in bacterial gene expression regulation and sheds new light on the potential to develop an interdisciplinary field that could overcome current barriers to understanding the global cellular regulatory network.

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`MOUTACHARRIF-Table1.docx` available at https://authorea.com/users/862838/articles/1244907-the-power-duo-how-the-interplay-between-nucleoid-associated-proteins-and-small-non-coding-rnas-orchestrates-the-cellular-regulatory-symphony

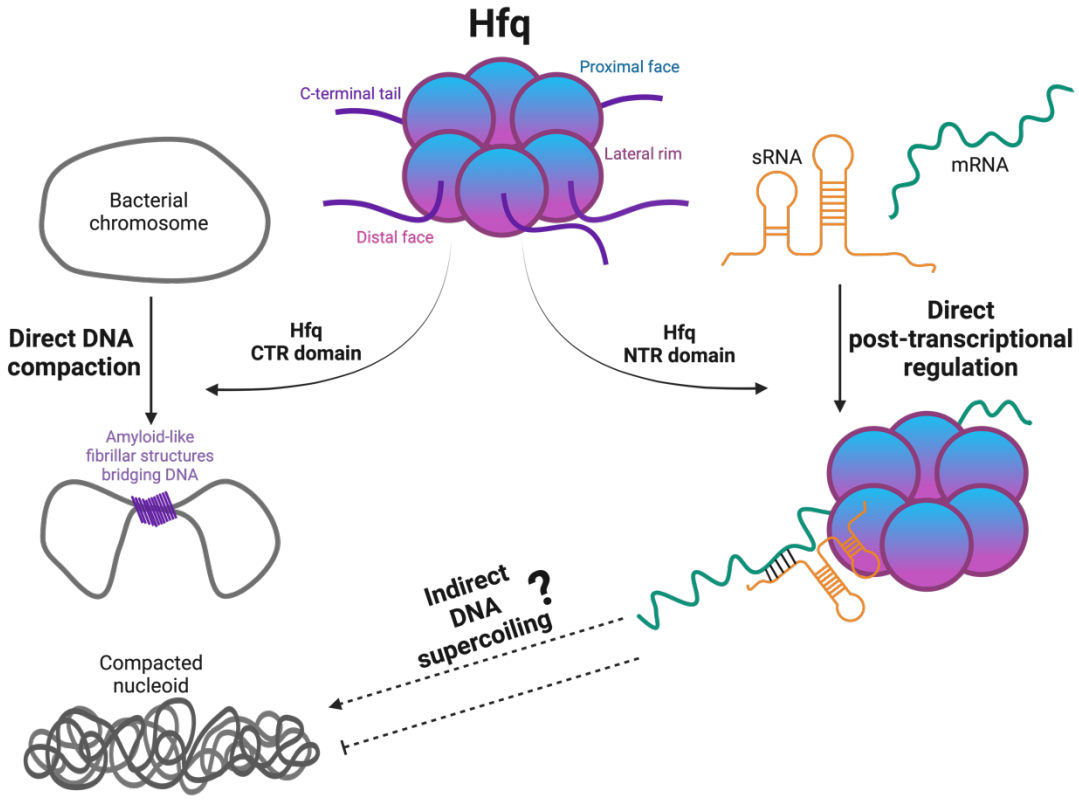


Figure 1. Hfq: a dual-function protein. Hfq is a homohexameric protein. Each monomer is composed of two domains named the N-terminal region (NTR) and the C-terminal region (CTR). The six NTRs forming the toroidal structure can be divided into three parts: the proximal face (in blue), the distal face (in pink) and the lateral rim. Hfq is an RNA chaperone that facilitates base pairing between sRNAs (in orange) and their mRNA target (in green): the U-rich 3'-end of sRNA binds to the proximal face, whereas A-rich sequences of mRNA bind to the distal face of Hfq (right panel). Hfq binds DNA *via* the CTR domain (C-terminal tail in purple), inducing the formation of long amyloid-like fibrillar structures that bridge the chromosome and lead to DNA compaction (left panel). Figure created using BioRender.com.

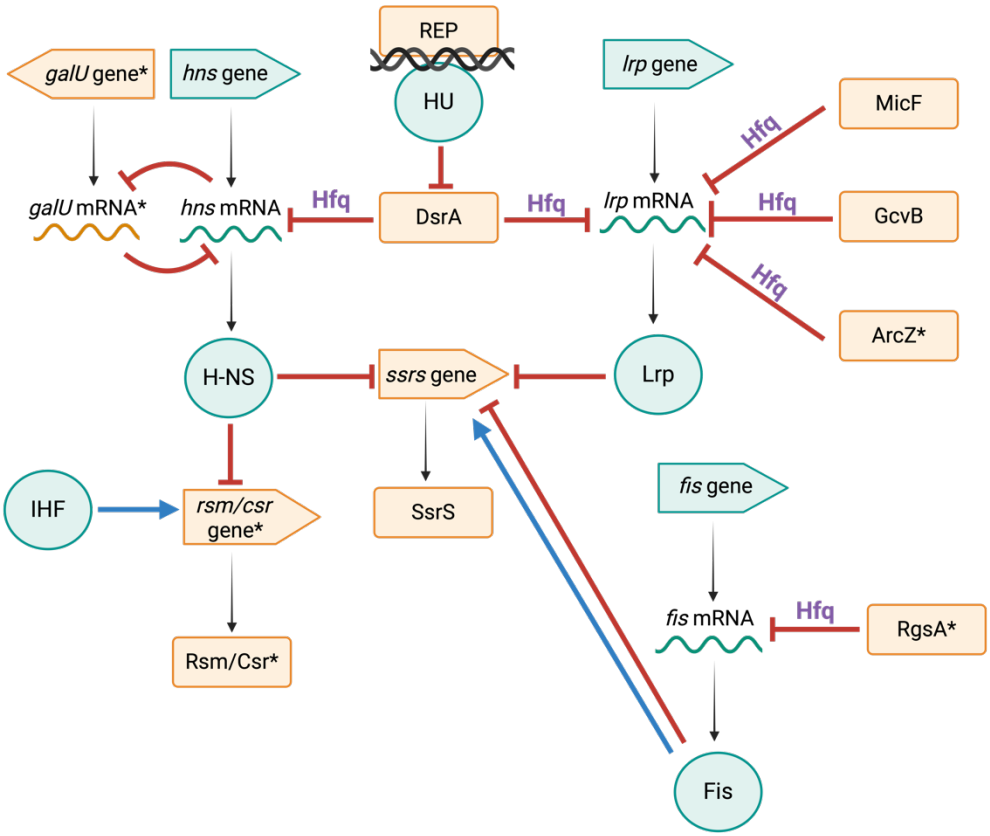


Figure 2. Regulatory network involving NAPs and sRNAs in Gram-negative bacteria. The regulation by NAPs occurs at the transcriptional level while the regulation by sRNAs occurs at the post-transcriptional level. NAPs are shown in green; regulatory RNAs in orange; Hfq acting as a RNA chaperon in purple; positive regulation is indicated by a blue arrow and negative regulation by a red bar. Asterisks indicate interactions that have not been demonstrated in *E. coli*. In particular, *rsm/csr* genes are inhibited by H-NS homologues in *P. aeruginosa* and activated by IHF in *P. fluorescens*, *S. enterica* and *E. amylovora.* RgsA repression of Fis is specific to *P. aeruginosa,* ArcZ repression of *lrp* is specific to *E. amylovora,* and mutual repression of the bifunctional RNAs *galU* mRNA and *hns* mRNA has only been demonstrated in *S. enterica*. Figure created using BioRender.com.

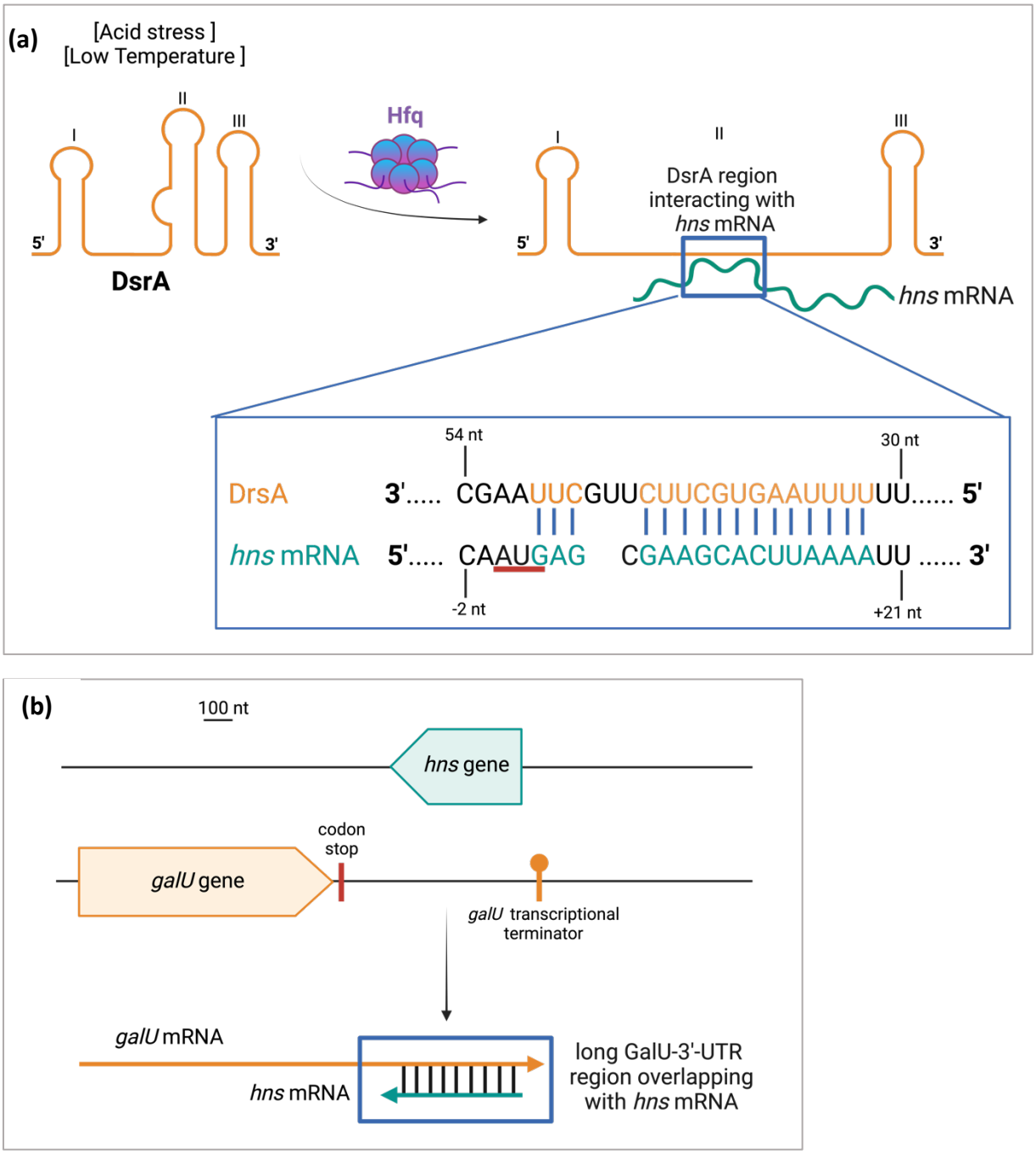
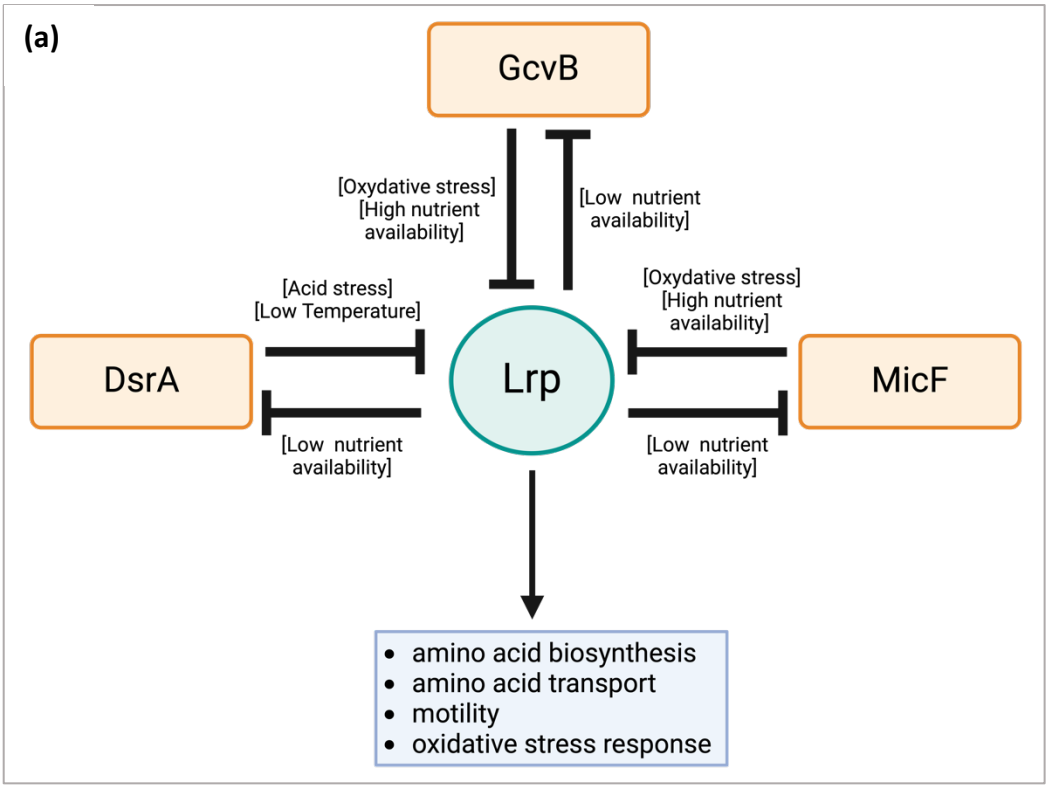
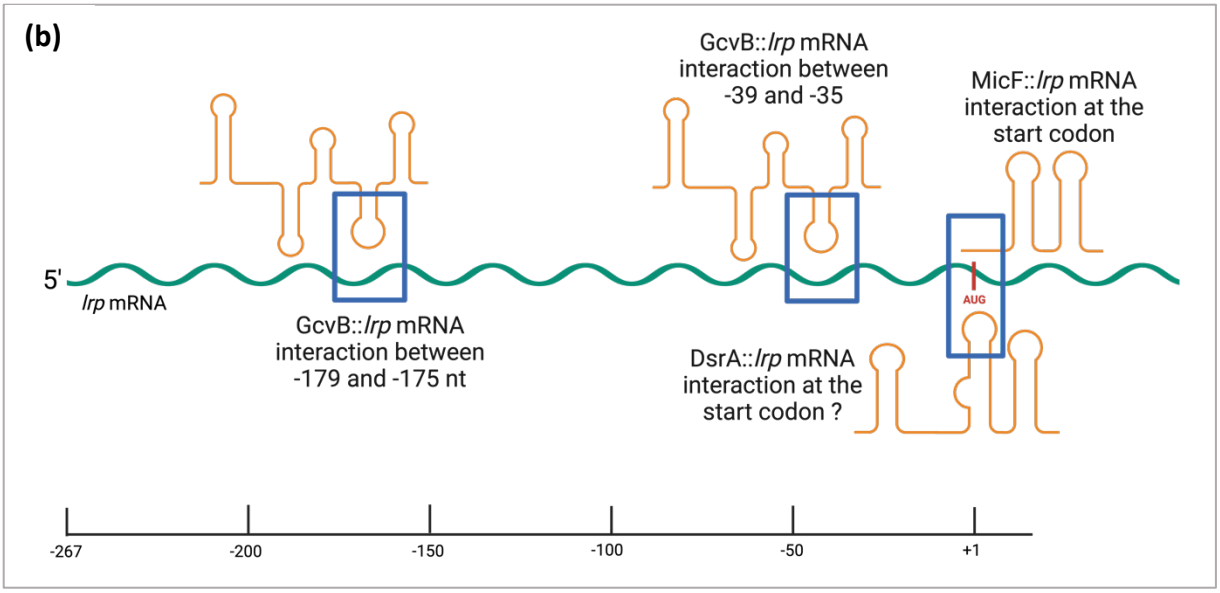
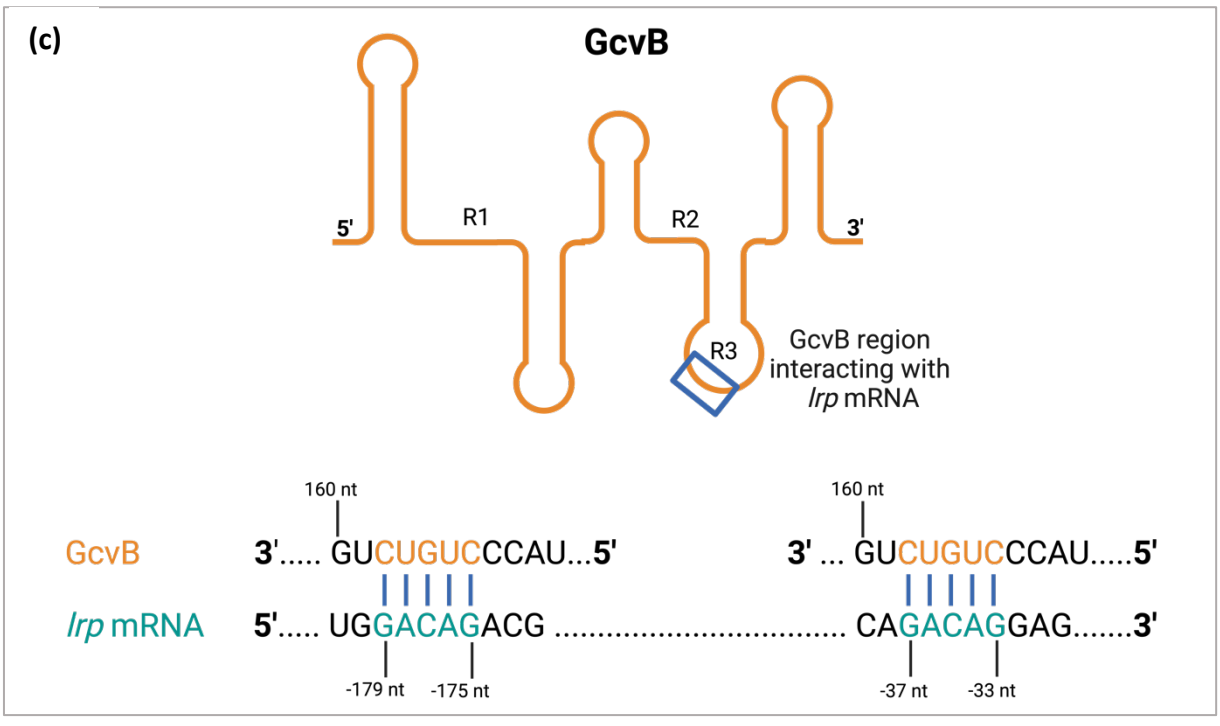


Figure 3. Post-transcriptional regulation of *hns* mRNA. (a) The DsrA sRNA, which is structured in three stem-loops (in orange), alters the stability of the *hns* mRNA and reduces the synthesis of the H-NS protein. Hfq alters the structure of the DsrA stem-loop II, allowing base pairing with *hns* mRNA at the translation initiation region (TIR). The interaction sequences between DsrA and *hns* mRNA are shown in the frame. The sequences are numbered according to the start codon for *hns* (in red). **(b)** The *hns* and *galU* genes are organized in a convergence on the chromosomal double-stranded DNA. The long 3'UTR region of *galU* overlaps with the *hns* mRNA. The transcriptional termination site of *galU* is estimated to be between 584 and 701 nucleotides downstream of the *galU* termination codon, represented by a horizontal red line. Figure created using BioRender.com.


(a)
GcvB
[Oxydative stress]
[High nutrient availability]
[Low nutrient availability]
[Acid stress]
[Low Temperature]
[Oxydative stress]
[High nutrient availability]
DsrA
Lrp
MicF
[Low nutrient availability]
[Low nutrient availability]
amino acid biosynthesis
amino acid transport
motility
oxidative stress response


(b)
GcvB::lrp mRNA interaction between -39 and -35
MicF::lrp mRNA interaction at the start codon
5'
lrp mRNA
AUG
GcvB::lrp mRNA interaction between -179 and -175 nt
DsrA::lrp mRNA interaction at the start codon ?
-267
-200
-150
-100
-50
+1


(c)
GcvB
5'
R1
R2
3'
R3
GcvB region interacting with lrp mRNA
160 nt
160 nt
GcvB
3'..... GUCUGUCCCAU...5'
3' ... GUCUGUCCCAU.....5'
lrp mRNA
5'..... UGGACAGACG ..... CAGACAGGAG.....3'
-179 nt
-175 nt
-37 nt
-33 nt

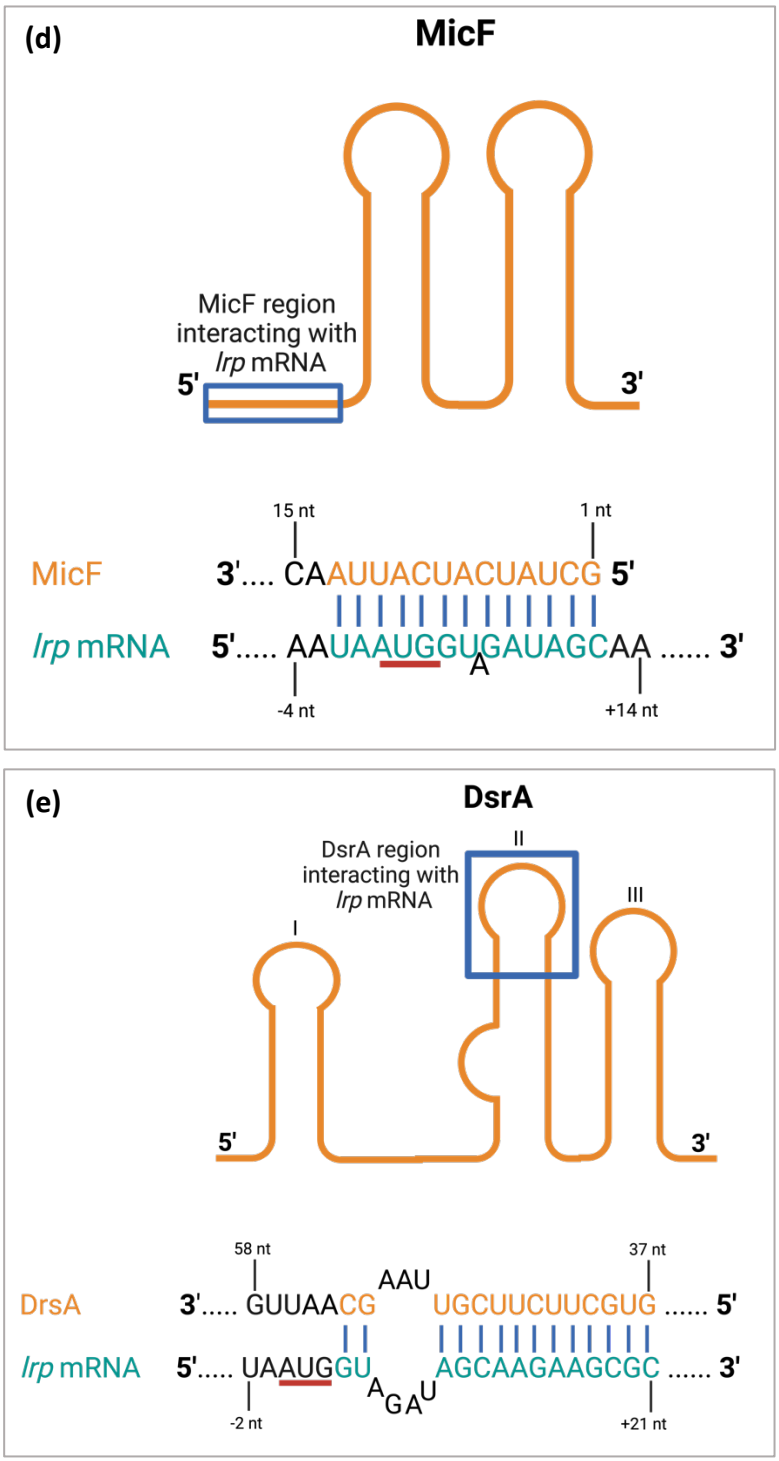


Figure 4. Regulation of Lrp by several sRNAs in *E. coli*. (a) The mutual regulation of Lrp and the sRNAs DrsA, GcvB and MicF. The physiological state that induces the regulation is shown between square brackets, negative regulation is shown by black bars and the Lrp regulon is summarized in the light blue box. **(b)** The three sRNAs (DsrA, GcvB and MicF) bind to the long 5'-untranslated leader region (5'UTR) of *lrp* and repress the *lrp* mRNA translation. **(c-d-e)** The binding sites of sRNAs and *lrp* mRNA, together with the positions of these binding sites within the structures of the sRNAs. Regions R1, R2 and R3 for GcvB and domains I, II and III for DsrA are indicated. sRNAs are shown in orange, mRNA in green. The numbering of *lrp* mRNA is relative to the AUG, which is underlined in red. Figure created using BioRender.com.

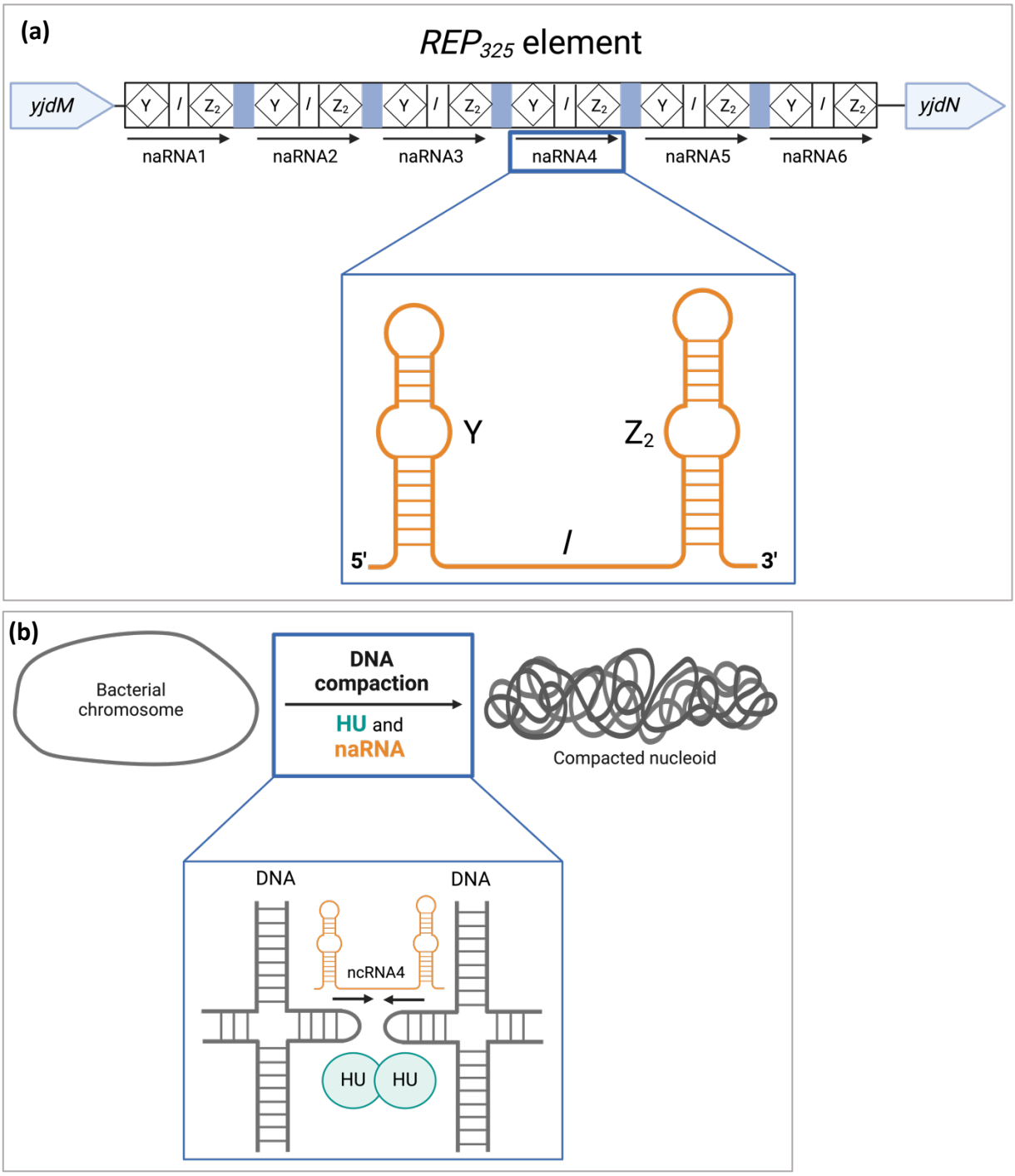


Figure 5. Schematic representation of HU- and naRNA-mediated DNA condensation. (a) The *REP*$_{325}$ element is located in the intergenic region between the *yjdM* and *yjdN*. The element consists of 6 homologous repeats separated by 5 unknown spacers with exactly the same DNA sequence (in blue). The transcripts of the 6 repeats are named naRNA1 to naRNA6. Each unit contains the palindrome Y and the palindrome Z_2, which are separated by a constant linker (*I*). The predicted secondary structure of naRNA4 contains a Y motif and a Z_2 motif, connected by a linker (*I*) (Qian et al., 2017). **(b)** Cruciform DNA structures may be bridged together by the ncRNA4 encoded by the *REP*$_{325}$ element. This interaction is facilitated by an HU dimer and leads to DNA condensation. Figure created using BioRender.com.