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REVIEW ARTICLE

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MucR protein: Three decades of studies have led to the identification of a new H-NS-like protein

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Abstract

MucR belongs to a large protein family whose members regulate the expression of virulence and symbiosis genes in α-proteobacteria species. This protein and its homologs were initially studied as classical transcriptional regulators mostly involved in repression of target genes by binding their promoters. Very recent studies have led to the classification of MucR as a new type of Histone-like Nucleoid Structuring (H-NS) protein. Thus this review is an effort to put together a complete and unifying story demonstrating how genetic and biochemical findings on MucR suggested that this protein is not a classical transcriptional regulator, but functions as a novel type of H-NS-like protein, which binds AT-rich regions of genomic DNA and regulates gene expression.

KEYWORDS
Brucella, H-NS, H-NS-like, MucR, nucleoid-associated proteins, rhizobia

1 | INTRODUCTION

The proteobacteria are the largest division of prokaryotes which comprises the class α-proteobacteria consisting of a diverse group of bacteria that occupy a wide array of habitats (Batut et al., 2004). Within this class, the agrobacteria, rhizobia, and brucellae represent important plant pathogens, plant symbionts, and mammalian pathogens, respectively.

The name "proteobacteria" derives from the Greek god Proteus, who could take many different shapes, thus reflecting the diversity of shape and physiology found within this group (Gupta, 2000; Murray et al., 1990; Stackebrandt et al., 1988; Zinder, 1998). Proteobacteria are also recognized as the closest ancestor of mitochondria. Indeed, it is currently accepted that mitochondria have arisen from an endosymbiosis established between α-proteobacteria and eukaryotic cells (Wang & Wu, 2015),

which root their origin in an Asgard archeon incorporating an α-proteobacteria (López-García & Moreira, 2020).

The division of proteobacteria is of great biological interest because it comprises many pathogens (Balows et al., 1992; Collier et al., 1998; Holt et al., 1994) whose virulence genes are mostly acquired by Horizontal Gene Transfer (HGT) (Dimitriu et al., 2014; Lee et al., 2022; McGinty & Rankin, 2012). In fact, a comparison of the genomes of bacterial pathogens and their close non-pathogenic relatives reveals that former differ from the latter mostly because of the presence in pathogens of horizontally-acquired virulence-associated genomic islets and islands (Groisman & Ochman, 1996, 1997; Will et al., 2015).

Uncontrolled expression of genes acquired by HGT can lead to fitness defects, and certain nucleoid-structuring proteins such as H-NS (Histone-like Nucleoid Structuring) and the H-NS-like proteins, known as Lsr2, MvaT, MvaU and Rok, have evolved to repress the expression of these genes until this repression can be overcome by counter-silencers which act as transcription activators by antagonizing repression (Castang & Dove, 2010; Dorman, 2007; Erkelens et al., 2022; Gordon et al., 2008; Li et al., 2009; Navarre et al., 2006). H-NS and the H-NS-like proteins compete with these counter-silencers thus ensuring that genes acquired by HGT do not have toxic effects (Groisman & Ochman, 1996, 1997; Will et al., 2015) and are only expressed in bacteria when their products provide a fitness benefit (Dorman & Dorman, 2018; Gordon et al., 2010; Newman et al., 2018; Williams McMackin et al., 2019). This relationship is particularly important for bacterial pathogens because it is well-established that the proper temporal expression of virulence genes plays a critical role in pathogenesis (Akerley et al., 1995).

In 1985, the transcriptional regulator Ros was discovered in *Agrobacterium tumefaciens* (Close et al., 1985) and subsequent genetic and biochemical studies demonstrated that homologs of this protein more widely known as MucR were present in many *Rhizobia* and *Brucella* species (Bittinger et al., 1997; Caswell et al., 2013; Janczarek, 2022; Janczarek & Skorupska, 2007; Keller et al., 1995; Wu et al., 2006; Zhan et al., 1989).

MucR and its homologs were studied as classical transcriptional regulators in the 1990s, mainly as repressors of fundamental symbiosis and virulence genes. Classical transcriptional regulators usually form homodimers and recognize specific DNA target sequences through DNA-binding domains, such as helix-turn-helix domain, able to interact with the major groove (Fernandez-Lopez et al., 2022; Sahota & Stormo, 2010). Very recent publications provide functional data showing that MucR acts as a new type of H-NS-like protein (Barton et al., 2023; Shi et al., 2022) rather than as a classical transcriptional regulator.

In this review, we report the earlier data about MucR and review them in light of the new findings that have revealed that MucR can structure the bacterial genome.

The story of MucR teaches us how every experimental detail is to be considered in order to understand the true nature of a protein factor and its working mechanism.

2 | THE EARLY STUDIES ABOUT MUCR REVEALED THAT THIS PROTEIN REGULATES VIRULENCE, SYMBIOSIS, AND CELL CYCLE GENES IN THE α-PROTEOBACTERIA

2.1 | The first discovered MucR homologs is Ros from *A. tumefaciens*

The first MucR homolog was originally identified in *A. tumefaciens* during a genetic screening designed to identify regulators of the *virC* and *virD* genes, which are required for the virulence of this bacterium in plants (Close et al., 1987). This gene was named *ros* (rough outer surface) based on the appearance of the colonies produced by the mutant (Close et al., 1985). Subsequent studies identified Ros as a 15.5 kDa transcriptional repressor with a sequence resembling the classical eukaryotic Cys_2His_2 zinc finger DNA-binding domain (Chou et al., 1998; Cooley et al., 1991). Zinc finger domain was first discovered in the transcription factor IIIA (TFIIIA) from *Xenopus laevis* (Klug & Schwabe, 1995) and later found in a very large number of eukaryotic sequence-specific transcription factors (Gamsjaeger et al., 2007; Laity et al., 2001). The protein Ros was shown to bind the intergenic region between the *virC* and *virD* genes (Chou et al., 1998; Cooley et al., 1991; D'Souza-Ault et al., 1993) through the part of the protein resembling the eukaryotic zinc finger domain (Isernia et al., 2020; Malgieri et al., 2007). This ability of Ros to interact with DNA through a domain resembling the eukaryotic zinc finger, which serves as a sequence-specific DNA-binding domain, led investigators to study the DNA sequence recognized by Ros with the aim of finding a DNA consensus. A 40 bp AT-rich sequence was identified as the region recognized by Ros in the promoters of the *virC* and *virD* genes, of its own gene *ros* and in that of *ipt*, an oncogene transferred by *A. tumefaciens* into its plant host during infection (Chou et al., 1998; Cooley et al., 1991; D'Souza-Ault et al., 1993). The presence in Ros of an eukaryotic zinc finger-like domain led to the proposition that Ros and its homologs may be the ancestors of the eukaryotic Cys_2His_2 zinc-finger proteins (Netti et al., 2013). Another telling and prescient finding during these studies were that the transcriptional activator VirG can overcome Ros repression of *virC* and *virD* expression when *A. tumefaciens* is exposed to the plant phenol acetosyringone (Close et al., 1987). This suggests that Ros and VirG can function as a silencer/counter-silencer pair and modulate the expression of these virulence genes in response to a biologically relevant stimulus from its plant host.

2.2 | MucR in the *Sinorhizobium* and *Rhizobium* species

A few years after the discovery of the *ros* gene in *Agrobacterium*, Zhan et al. described a *Sinorhizobium meliloti* mutant that displayed altered exopolysaccharide (EPS) production. They labeled the gene *mucR* based on the mucoid colonies produced by the mutant (Zhan et al., 1989). The gene sequence revealed that the encoded protein

shared 80% identity with the *A. tumefaciens* Ros protein (Keller et al., 1995).

MucR directly represses the transcription of its own gene (Keller et al., 1995) just like *A. tumefaciens* Ros (Cooley et al., 1991). A direct interaction between MucR and the promoter of its own gene was demonstrated and a sequence of 44 nucleotides was identified as the MucR target. It has been also noticed that the identified MucR target was in close proximity of the *mucR* coding region, but approximately 350 bp downstream of this gene's transcriptional start site. This observation led the authors to the conclusion that a simple interaction between MucR and the RNA polymerase could not explain the MucR repression of its own gene. Thus, Bertram-Drogatz and coworkers proposed that DNA bending could play an important role in the capacity of this protein to function as a transcriptional repressor and suggested that further investigation was necessary for understanding the mechanism of *mucR* gene repression (Bertram-Drogatz et al., 1998).

The role of MucR in regulating the production of exopolysaccharides biosynthesis was investigated in *S. meliloti*. This bacterium produces two different types of exopolysaccharides (EPS), succinoglycan (EPS I) (Reinhold et al., 1994) and galactoglucan (EPS II) (Her et al., 1990). Genes necessary for EPS I and EPS II synthesis are located in the symbiotic megaplasmid pSymB and form the *exo* and *exp* gene clusters (Glazebrook & Walker, 1989; Reuber & Walker, 1993). MucR was found to be an essential factor for the EPS biosynthesis. In fact, *S. meliloti mucR* mutants produce high levels of the EPS II HMW fraction and low levels of EPSI. Later, a direct MucR interaction with the promoter regions of two EPSI genes, *exoH* and *exoY*, was demonstrated, but the MucR binding sites in these promoters did not share a consensus sequence with those present in the *S. meliloti mucR* or *A. tumefaciens ros* promoters. The authors also noted that the positive effect of MucR on *exoH* and *exoY* expression at the transcriptional level was minimal and did not explain the positive impact that MucR has on EPSI production. Additionally, the authors demonstrated that MucR could directly regulate expression of a gene involved in LPS biosynthesis (*envA*) in *E. coli* and proposed that this regulation might explain the growth defect observed when recombinant MucR is produced in this bacterium (Bertram-Drogatz et al., 1998).

Bahlawane et al. (2008a, 2008b) demonstrated that MucR represses the EPSII biosynthetic genes (*exp*) by directly binding to their promoters. They also observed that a high concentration of MucR was required to see the complex with DNA in Electrophoretic Mobility Shift Assays (EMSAs) and speculated that MucR could bind DNA as a multimer. This hypothesis was further supported by the observation of a strong retardation of DNA electrophoretic mobility upon MucR binding in EMSAs. The direct binding of MucR to EPS II biosynthesis gene promoters was proposed to be responsible for the repression of the transcription.

MucR was also reported to bind directly to the promoter of the *rem* gene, which is required for *S. meliloti* motility. This finding led the authors to the conclusion that MucR regulates the expression of both genes required for the exopolysaccharide synthesis and motility in *S. meliloti*, thus coordinating two functions necessary at different stages of the symbiotic life cycle of this bacterium. In this study, Bahlawane et al. (2008b) showed an alignment of some sequences recognized by MucR in an unsuccessful attempt to find a consensus sequence.

Moreover, these studies suggested that MucR functions as a transcriptional repressor of genes involved in galactoglucan biosynthesis until this suppression is overcome by the antagonistic transcriptional activator ExpG (Bahlawane et al., 2008b). This provided another example of MucR working in concert with an antagonistic transcriptional activator to form a gene silencer/counter-silencer pair. Moreover, it was also postulated that the activity of this silencer/counter-silencer pair plays a crucial role in ensuring that important symbiosis genes are only expressed when *S. meliloti* and other rhizobia are in close contact with their plant hosts (Bahlawane et al., 2008b; Mueller & González, 2011; Udvardi & Poole, 2013). This proposition was further supported by the observations that—(a) the *nod* genes are strongly repressed by MucR in *S. meliloti* during in vitro cultivation (Mueller & González, 2011); (b) MucR has been shown to regulate nodulation genes in *Rhizobium etli* (Bittinger et al., 1997), *R. leguminosarum* (Janczarek & Skorupska, 2007) and *S. fredii* (Jiao et al., 2016); and (c) transcriptome analysis indicates that important symbiotic genes are dysregulated in *S. fredii mucR* mutants during plant infection (Acosta-Jurado et al., 2016; Jiao et al., 2016).

In order to test the importance of MucR expression in biofilm formation, the activity of *mucR* promoter in *S. meliloti* was examined in a nutritionally limiting medium, which promotes the transition from planktonic life to a sessile mode and elicits biofilm formation. The results showed that *mucR* expression is not induced under these conditions, suggesting that alterations in *mucR* expression are not required for biofilm formation (Rinaudi et al., 2010). It is important to note, however, that expression of the gene encoding the MucR homolog RosR in R. *leguminosarum* is regulated by phosphate, plant exudates, and carbon source availability (Janczarek & Skorupska, 2009, 2011) suggesting that the rhizobia may be able to adjust cellular MucR levels in response to biologically relevant stimuli during their symbiotic relationships with their plant hosts.

In *Sinorhizobium fredii*, two copies of *mucR* gene were identified: *mucR1* located on the chromosome and the *mucR2* on the symbiosis plasmid in a region showing the typical features of horizontal transfer (Jiao et al., 2016). The two encoded proteins, MucR1 and MucR2 show a high level of amino acid sequence identity, but a frameshift mutation in *mucR2* gene causes a disruption in the protein MucR2 of a conserved basic region (Acosta-Jurado et al., 2016; Jiao et al., 2016) known to be fundamental for the DNA binding activity of the MucR homologs Ros from *A. tumefaciens* and the Ml proteins from *Mesorhizobium loti* (Baglivo et al., 2009; Esposito et al., 2006). In *S. fredii*, MucR1 plays an essential role in nodulation as shown by a *mucR*1 defective strain becoming unable to establish an effective symbiosis with the host plant. In contrast, an *S. fredii mucR2* mutant did not show a symbiotic defect leading to the speculation that this result is due to the frameshift mutation in *mucR2*, which cannot fulfill the lack of *mucR1* gene in the mutant strain (Acosta-Jurado et al., 2016; Jiao et al., 2016).

The analysis of the *S. fredii mucR1* mutant bacteroid transcriptome demonstrated that several ion transporters were downregulated. Ions like iron, molybdenum, and sulfur are essential components of nitrogenase, thus the downregulation of ion transporters can impair nitrogen fixation, explaining the defect observed in nodulation when *mucR1* defective *S. fredii* strain is used (Jiao et al., 2016).

2.3 | MucR in *Brucella*

Brucella spp. are important pathogens in food animals and wildlife. Moreover, *Brucella* species are responsible for zoonosis spreading to humans and the capacity of these bacteria to resist killing by host macrophages plays a central role in their virulence (Roop et al., 2021). The *Brucella mucR* gene was originally identified in a genetic screen designed to detect genes required for the intracellular survival and replication of *B. melitensis* 16 M in a murine macrophage-like cell line (Wu et al., 2006). It was subsequently shown that MucR is essential for the wild-type virulence of *B. melitensis* (Arenas-Gamboa et al., 2011; Dong et al., 2013; Mirabella et al., 2013; Wu et al., 2006), *B. abortus* (Caswell et al., 2013), *B. canis* (Sun et al., 2021) and *B. ovis* (Tartilán-Choya et al., 2022) in the mouse model of chronic infection. Multiple genes linked to virulence including those involved in Type IV secretion, LPS biosynthesis, autotransporter adhesin biosynthesis, quorum sensing, and iron acquisition have been shown to be regulated by MucR (Caswell et al., 2013; Dong et al., 2013; Mirabella et al., 2013; Sun et al., 2021; Tartilán-Choya et al., 2022), and the nature of these regulatory links and their impact on *Brucella* virulence are currently an area of active investigation (Barton et al., 2023; Pirone et al., 2018). But it is important to note that the latter study provided evidence that MucR and the transcriptional activator MdrA have the capacity to function as a silencer/counter-silencer pair and differentially control the expression of the gene encoding the polar autotransporter adhesin BtaE, which is an important virulence determinant for *Brucella* (Bialer et al., 2020; Ruiz-Ranwez et al., 2013). This relationship is reminiscent of the role that MucR and ExpG play in coordinately regulating the expression of the genes encoding EPSII in *S. meliloti* (Bahlawane et al., 2008a), and in fact, MdrA and ExpG are homologous MarR-type regulators. More importantly, this relationship also resembles the competing regulatory roles that H-NS and H-NS-like proteins play with counter-silencers to ensure the proper temporal expression of virulence genes in other bacteria (Dorman & Dorman, 2018; Williams McMackin et al., 2019).

2.4 | MucR in *Caulobacter crescentus*

More recently MucR homologs, MucR1 and MucR2, have been also identified in *C. crescentus*.

In *C. crescentus* and evolutionary-related bacterial species, the cell cycle is mostly regulated by the cell cycle transcriptional regulator A (CtrA) (Brilli et al., 2010; Quon et al., 1996). This protein can activate or repress transcription and also inhibit DNA replication by binding the target sequence TTAA-N(7)-TTAA. CtrA undergoes proteolysis during G1 to S transition in the cell cycle and it is not considered a specific regulator of G1-phase transcription (Laub et al., 2007; Wu & Newton, 1997). In an attempt to find other factors determining the switch from S-phase to G1-phase, Viollier and coworkers (Fumeaux et al., 2014) demonstrated that MucR1/2, able to form heterodimer, function in a module together with CtrA and SciP, a protein containing a helix-turn-helix motif and accumulating in G1-phase (Gora et al., 2010; Tan et al., 2010), to regulate cell cycle progression. The authors reported that MucR1 and MucR2 have both negative and positive impacts on cell cycle genes and work in concert with CtrA and SciP in maintaining proper temporal progression through the cell cycle in *Caulobacter* (Fumeaux et al., 2014). Even if *C. crescentus* is not a pathogen, knowing the role of MucR in pathogenic and symbiotic bacteria, the authors proposed to consider MucR and its homologs not as a simply regulators of virulence and symbiosis genes, but as fundamental factors in the regulation of cell cycle transcription that allow virulence gene expression only in a specific cell cycle stage (Fumeaux et al., 2014).

MucR was also reported to cause DNA hypomethylation in *C. crescentus*. The bacterial genome is methylated by the DNA adenine methyltransferase CcrM and this epigenetic modification is involved in regulation of gene transcription, DNA replication, and repair (Laub et al., 2000; Marinus & Casadesus, 2009; Stephens et al., 1996). CcrM methylates position 6 of adenines in the recognized sequence 5′-GANTC-3′, introducing the methylation mark m6A. MucR can cover the CcrM target sequence, thus blocking the action of this DNA methylase and causing hypomethylation. It has been also shown that environmental conditions such as phosphate starvation promote methylation of the sites covered by MucR, thus demonstrating an ability to overcome the control system creating local hypomethylation during *C. crescentus* cell cycle (Ardissone et al., 2016).

3 | THE STRUCTURAL FEATURES OF MUCR AND ITS HOMOLOGS

The NMR structure of the DNA binding domain present in the MucR homolog Ros from *A. tumefaciens* was first solved in 2007 (Malgieri et al., 2007).

The structure of this domain reveals a large globular domain that folds in a ββββαα topology around a zinc ion and forms a 15 residues hydrophobic core. The metal is tetrahedrally coordinated by two cysteine and two histidine residues and plays a key structural role as the domain unfolds in its absence. This domain was identified as the prokaryotic zinc finger because of some similarities with the most common eukaryotic zinc finger, a smaller domain folding in a ββα topology, present in a large number of the eukaryotic sequence-specific transcription factors and responsible for DNA binding (Laity et al., 2001) (Figure 1).

Important structural features differentiate the prokaryotic zinc finger domain from its eukaryotic counterpart. The zinc coordination

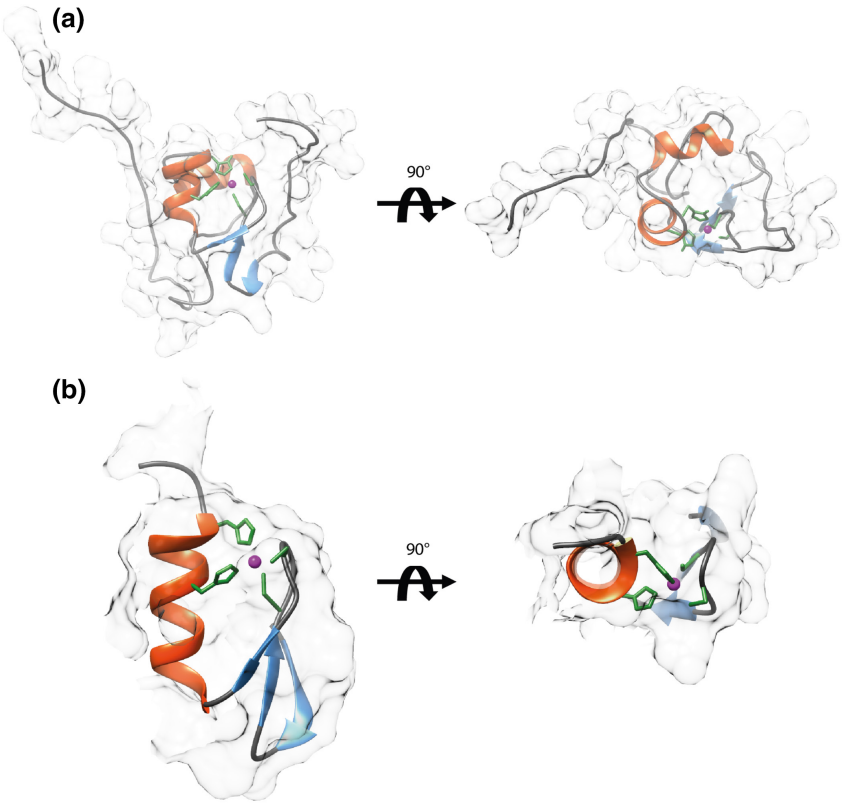

FIGURE 1 (a) The NMR solution structure of Ros87 (region spanning from the residue in position 56 to the last residue of the sequence in position 142) (Malgieri et al., 2007). (b) The first zinc finger domain (PDB ID: 2DRP) of the eukaryotic protein Tramtrack (Fairall et al., 1993). The zinc ion (violet) and the four coordinating side chains (green) are shown.

spheres in both domains are constituted by the thiolate sulfurs of the two cysteines residing on the β-hairpin and by the two histidines indole Nε nitrogens, that are located in the middle and at the C-terminus of the α-helix (α1). However, while this helix shows also a relative orientation with respect to the β-sheet similar to that found in the eukaryotic zinc finger domain, it is one turn shorter. Additionally, in Ros zinc-finger domain a second α-helix (α2) hooks the β-hairpin and the βββα region with an axis nearly orthogonal to α1 axis thus forming a 58 amino acids globular fold in which residues belonging to each of the secondary structure elements contribute to form the hydrophobic core. The N- and the C-terminal tails do not contribute to the overall structure and are mostly flexible.

An analysis of protein sequence data banks revealed that the family Ros/MucR comprises more than 300 members. Remarkably, while the sequence identity to Ros can be high in these proteins, the Cys_2His_2 zinc coordination sphere is not conserved in most of the members of this family that, on the contrary, shows different combinations of residues in the position occupied by the coordinating cysteines and histidines in Ros. Some of them show a substitution of the two cysteines, constituting the first and second zinc coordinating position, and of the histidine constituting the fourth coordinating position with other amino acid residues such as serine, aspartate, tyrosine, phenylalanine, leucine, and glycine (Figure 2) (Baglivo et al., 2009). These residues have never been found as zinc coordinating residues in eukaryotic zinc finger motifs and it is also hard to imagine them being able to bind zinc. Nonetheless, the residues that make up the Ros zinc finger hydrophobic core are highly conserved and the overall high sequence identity to Ros shown in Figure 2 suggests for all the members of this family a three-dimensional structure similar to that of Ros C-terminal DNA-binding domain.

The studies of these MucR/Ros homologs led to the conclusion that the presence of zinc is strictly related to the presence of the first coordinating cysteine. In fact, by replacing the first cysteine with a serine residue, the mutant protein could fold in the absence of zinc and retain the ability to bind DNA (Baglivo et al., 2009). Five MucR/Ros homologs from *M. loti* were studied showing that despite the heterogeneity of the zinc coordination sphere or the lack of the metal, they are all able to bind DNA and to recognize the Ros DNA target (Baglivo et al., 2009).

These findings indicate the prokaryotic zinc finger domain has evolved in different ways to functionally fold as it is capable to preserve the DNA binding activity either with the zinc ion bound through a highly variable set of coordinating residues or without zinc as well (Baglivo et al., 2009; D'Abrosca et al., 2016; Palmieri et al., 2013). Later, the structural characterization of the zinc-lacking DNA binding domain revealed that the domain substitutes the structural role of zinc with a network of hydrogen bonds and a larger hydrophobic core (Baglivo et al., 2014). Whether the activities of this class of proteins might be modulated by the available zinc concentrations remains to be investigated.

Interestingly, MucR homologs with different "Zn binding domains" are present in other α-proteobacteria in addition to *M. loti*. In *C. crescentus*, for instance, MucR1 shows a serine instead of the first zinc-coordinating cysteine and an aspartate instead of the second one (Figure 2), thus resembling the zinc-lacking protein Ml5 from *M. loti* (Baglivo et al., 2009, 2014), while MucR2 shows a coordination

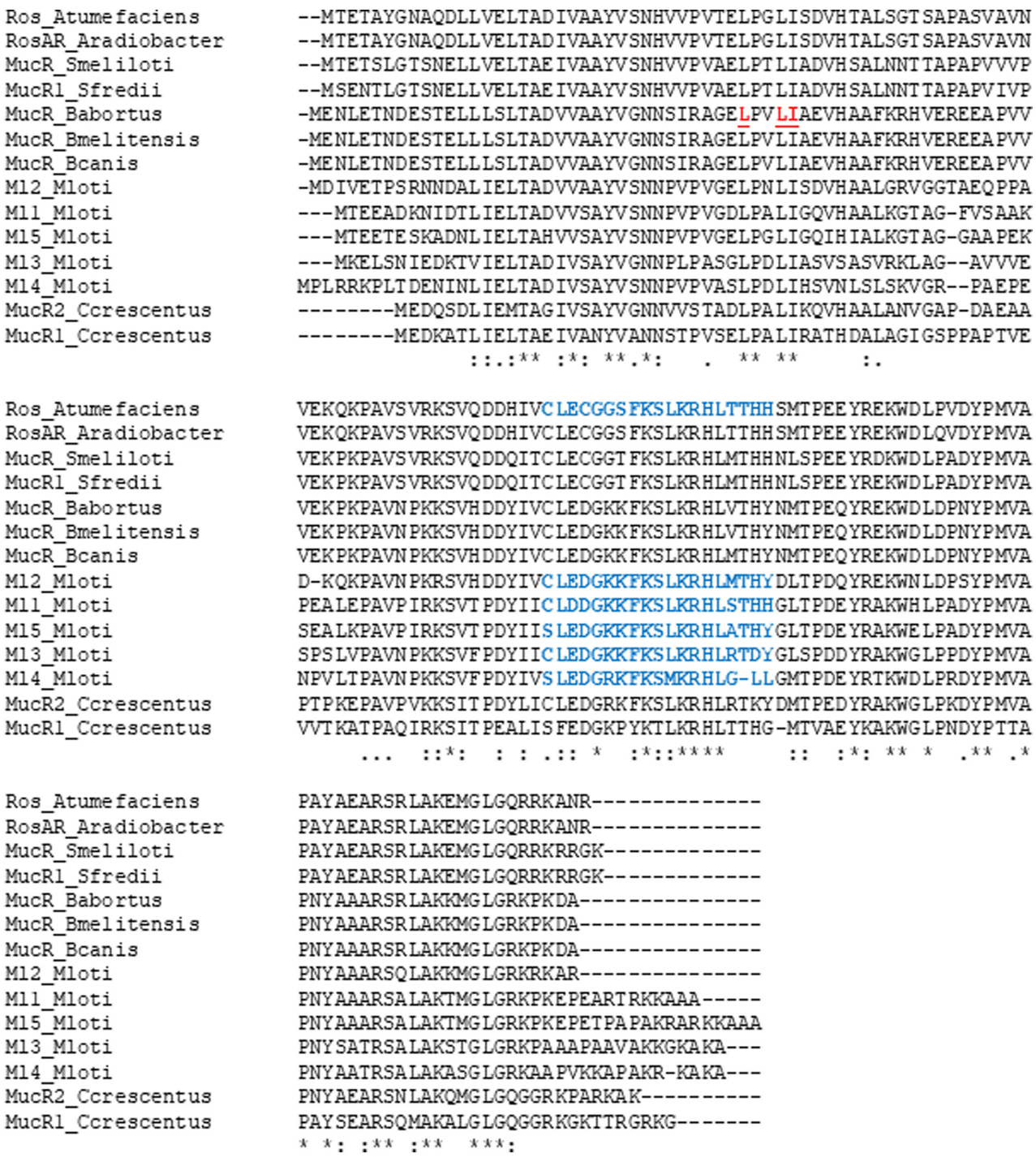

FIGURE 2 Sequences alignment of the functionally and/or structurally characterized MucR homologs. The amino acid residues identified as crucial for *B. abortus* MucR oligomerization (Pirone et al., 2018) are red and underlined in the protein sequence; regions involved in zinc coordination and the homologous zinc-lacking sequences of the DNA binding domains characterized by biochemical and structural analyses are in blue (Baglivo et al., 2009, 2014; Esposito et al., 2006; Malgieri et al., 2007).

sphere formed by CysAspHisAsp resembling the zinc-binding protein Ml3 from *M. loti* (Baglivo et al., 2009, 2014). MucR from *S. meliloti* and *S. fredii* shows the typical zinc coordination sphere (Cys_2His_2) of the prokaryotic zinc finger observed in Ros, while MucR from *B. abortus*, *B. melitensis*, and *B. canis* resembles Ml2 from *M. loti* presenting a $CysAspHis_2$ zinc coordination sphere. Further detailed structural studies of MucR proteins would be helpful to enlarge our knowledge regarding the structural features of the DNA-binding domain present in these proteins.

Recently, the ability to form oligomers has emerging as a fundamental structural feature for the regulatory function played by the members of this family of proteins. Indeed, while the deletion of the first 55 amino acids gives soluble monomeric proteins in all the studied members, the full-length proteins have been proven capable of

forming high-order oligomers (Baglivo et al., 2018; Pirone et al., 2018; Slapakova et al., 2023) indicating that the N-terminal portion of Ros/MucR proteins is an oligomerization domain. Molecular Dynamics simulations (D'Abrosca et al., 2020) have shown how the Ros N-terminal domain folds in two-antiparallel α-helices connected to the C-terminal DNA-binding domain by a flexible linker. The two helices define a second domain stabilized by hydrophobic interactions at the helices interfaces. The data collected indicate that the two protein domains define a sort of bilobed structure (Figure 3) in which each domain works as an independent unit with specific functions: the C-terminal zinc-binding domain is responsible for the DNA-binding activity while the N-terminal domain controls oligomerization.

The structural model of the MucR N-terminal domain dimer predicted by Alphafold2 shows that monomers can interact through the two α-helices longitudinally arranged in each monomer. The Alphafold2 predicted structural models of MucR tetramers and octamers show a circular shape of the structure with a flexible linker connecting the N-terminal domain to the C-terminal DNA binding domain (Shi et al., 2022).

4 | MUCR WORKS AS A HISTONE-LIKE NUCLEOID STRUCTURING (H-NS) PROTEIN

All the studies of the DNA-binding activity of MucR proteins and all the efforts to identify a DNA target sequence, which could help in detecting the direct MucR targeted promoters, revealed long degenerated AT-rich sequences and failed in finding a DNA consensus sequence. Starting in 2017, several analyses of the sequences recognized by MucR and its homologs led to the understanding that MucR proteins do not bind a specific DNA consensus sequence. Rather, these proteins show a clear preference for AT-rich DNA targets containing TA steps (Baglivo et al., 2018; Barton et al., 2023; Borriello et al., 2020; Jiao et al., 2021; Shi et al., 2022; Slapakova et al., 2023). The TA-step is an element of DNA that interrupts A-tracts, formed by three or more successive adenines, stiffening DNA and making the minor groove too narrow to allow the interaction with proteins (Rohs et al., 2009).

The analysis of the quaternary structure of MucR and its homologs also revealed that these proteins form high-order oligomers in solution through an oligomerization domain. Three residues were identified as responsible in MucR from *B. abortus* for oligomerization (Figure 2), which are located in the second putative α-helix present at the N-terminus of the protein (Pirone et al., 2018) (Figure 4). The three identified residues are conserved in all the MucR homologs studied so far (Figure 2) and this prompted the hypothesis that all these proteins oligomerize using the same domain and the same mechanism (Pirone et al., 2018; Shi et al., 2022; Slapakova et al., 2023). More importantly, the studies of Pirone et al. (2018) demonstrated that MucR oligomerization is essential for its regulatory activity in *Brucella*.

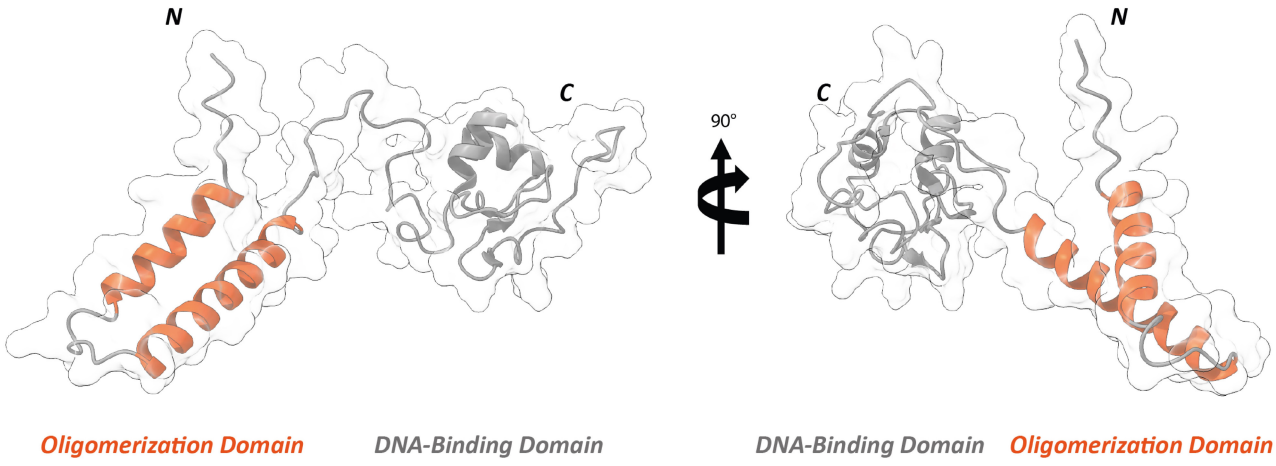

FIGURE 3 The proposed structure of the protein Ros full-length (D'Abrosca et al., 2020).

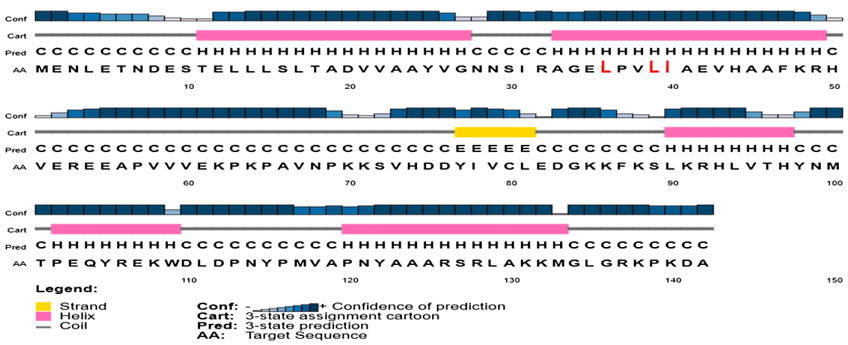

FIGURE 4 Secondary structure prediction of *B. abortus* MucR. The structural prediction has been obtained by using the PRESIPRED tool available at http://bioinf.cs.ucl.ac.uk/psipred/. The residues identified as crucial for *B. abortus* MucR oligomerization (Pirone et al., 2018) are indicated in red.

The modality adopted by MucR and its homologs to bind DNA recognizing AT-rich sequences containing TA-steps and contacting the minor groove, the ability to form high-order oligomers, the presence of an oligomerization domain at the N-terminus and a positive charged DNA-binding domain at the C-terminus led to the first hypothesis that these proteins might be acting in a manner analogous to that of the H-NS (Baglivo et al., 2018; Pirone et al., 2018). This was a relevant proposition because the vast majority of the α-proteobacteria lack H-NS homologs (Tendeng & Bertin, 2003).

H-NS and H-NS homologs were found to be present in many bacterial species (Ali et al., 2012), mostly belonging to β- and γ-proteobacteria. In some bacterial species, proteins that do not share a significant sequence homology with H-NS, but play the same role in structuring the nucleoid have been identified as H-NS-like proteins. Well-studied examples are MvaT and MvaU proteins in *Pseudomonas* (Castang & Dove, 2010; Tendeng et al., 2003; Vallet-Gely et al., 2005), Lsr2 in *Mycobacterium* (Gordon et al., 2008) and Rok in *Bacillus* (Erkelens et al., 2022). These proteins play a fundamental role in structuring the bacterial genome, condensing it, and globally repressing many genes. A proposed mechanism to explain H-NS gene repression implies that the condensed structure of the nucleoid makes promoters inaccessible to the transcription machinery (Dame et al., 2005; Dorman, 2007; Qin et al., 2019; Rashid & Dame, 2023).

H-NS and H-NS-like proteins have been referred to as "xenogeneic silencers" because they played the fundamental role in silencing genes acquired by Horizontal Gene Transfer (HGT), as typically pathogenic islands are, until environmental conditions required their expression. This activity prevents the unnecessary and energetically wasteful expression of genes that are only useful to the bacterium during specific stages of its lifecycle. When the H-NS silencing is not required, under particular environmental conditions, antagonistic transcriptional activators known as "counter-silencers" activate the required genes (Dorman, 2004; Dorman, 2007; Navarre et al., 2006, 2007). Notably, H-NS/counter-silencer pairs have been found to play vital roles in coordinating the proper temporal expression of virulence genes in many bacterial pathogens (Dorman & Dorman, 2018; Newman et al., 2018) and this function is strikingly similar to the role that MucR and antagonistic transcriptional activators like VirG, ExpG and MdrA have been proposed to play in coordinating virulence and symbiosis gene expression in *Agrobacterium*, *Sinorhizobium* and *Brucella* (Bahlawane et al., 2008a; Barton et al., 2023; Close et al., 1987).

H-NS silencing depends also on environmental conditions. Physicochemical conditions modulate H-NS activity and lead to a relieve of H-NS from the bacterial genome allowing gene expression (Dorman, 2004; Dorman, 2007; Qin et al., 2019; Rashid & Dame, 2023). Environmental stimuli affect Ros silencing in *A. tumefaciens* as the expression of Ros target genes is allowed only when phenolic compounds released by plants are sensed (Chou et al., 1998) and in *R. leguminosarum* phosphate and plant exudates regulate MucR levels (Janczarek & Skorupska, 2009, 2011) thus suggesting an effect of chemical conditions on MucR activity. It has been reported that *B. melitensis*, *B. abortus*, *B. canis mucR* mutants show a higher sensitivity to several environmental conditions (i.e., heat stress, iron limitation, oxidative, and saline stress) (Mirabella et al., 2013; Dong et al., 2013; Caswell et al., 2013; Sun et al., 2021.). Further investigations will better clarify the role of physico-chemical conditions on the activity of MucR and its homologs.

H-NS and H-NS-like proteins show a preference for AT-rich DNA sequences and contact the minor groove (Ding et al., 2015; Gordon et al., 2010) extending their presence on the genome by oligomerization (Dame et al., 2000; Lucchini et al., 2006; Navarre et al., 2006; Winardhi et al., 2012). The preference to bind AT-rich DNA sequences and the ability to oligomerize have been recently confirmed for MucR purified from *S. meliloti*, further supporting that MucR shows the typical features of an H-NS-like protein under its natural condition of expression (Slapakova et al., 2023), not only when overexpressed in *E. coli* and purified as a recombinant protein (Baglivo et al., 2018; Barton et al., 2023; Borriello et al., 2020).

Chromatin immunoprecipitation paired with sequencing (ChIP-seq) in *S. fredii* and in *B. abortus* have definitively confirmed the preferred in vivo MucR targets that are AT-rich DNA sequences containing TA-steps (Barton et al., 2023; Shi et al., 2022). ChIP-seq experiments have also shown that *B. abortus* MucR binds 26 of the 28 genomic islands thought to be acquired by HGT (Barton et al., 2023). These results, together with the analysis of the genes regulated by MucR in *S. fredii*, mostly acquired by HGT, have led to define MucR as a "xenogeneic silencer" (Jiao et al., 2021), by analogy with known H-NS and H-NS-like proteins. Bridging assays carried out with MucR from *S. fredii* have demonstrated the ability of the protein to bridge DNA in vitro (Figure 5), which is a typical feature of H-NS and H-NS-like proteins (Shi et al., 2022).

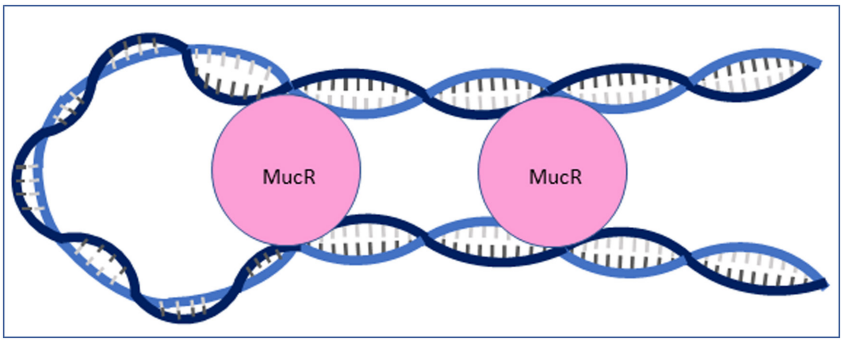

FIGURE 5 Cartoon model of DNA-bridging by MucR. MucR can bridge DNA (Shi et al., 2022). This activity might bend DNA and make the promoters inaccessible to the transcription machinery. The pink spheres represent MucR oligomers.

The observation that MucR can bridge DNA and the presence of extended binding regions seen for MucR within the promoters of regulated genes (Barton et al., 2023) suggest that MucR-mediated bridging within promoter regions of target genes is a likely mechanism of transcription regulation through direct occlusion of RNAP binding sites and/or occlusion of sites recognized by transcriptional activators. As seen with H-NS, RNAP entrapment is also a possibility within MucR-mediated loops. Further experiments mapping the direct binding regions of MucR within these extended ChIP peaks will be required to identify the mechanisms underlying transcriptional regulation and whether bridging alone is the mechanism used by MucR to silence genes and/or if other mechanisms implemented by other nucleoid-associated proteins are occurring within MucR-bound regions.

Complementation of *E. coli hns* defective strain by *S. fredii mucR* gene (Shi et al., 2022) and *B. abortus mucR* defective strain by *E. coli hns* (Barton et al., 2023) together with the experiments by chromosome conformation capture technique demonstrating that MucR can structure both chromosomes in *B. abortus* have provided further support for the classification of MucR as a new type of H-NS-like protein (Barton et al., 2023).

5 | CONCLUSION

MucR and its homologs have been studied for more than three decades. The fundamental role of MucR and its homologs in controlling the expression of genes crucial for virulence and symbiosis was immediately understood and the members of Ros/MucR protein family were considered for years as classical transcription regulators. The presence of a domain resembling the classical Cys2His2 eukaryotic zinc finger domain pushed to believe that the Ros/MucR prokaryotic protein family could work as the eukaryotic counterpart, which plays its role as a sequence-specific DNA-binding domain recognizing a consensus sequence present in the promoters of a discrete number of targeted genes. In the effort to find a consensus sequence recognized by MucR and its homologs and to identify the targeted promoters, some fundamental data suggesting that these proteins were not classical transcription factors escaped our notice. Footprinting experiments performed to identify the DNA region covered by Ros from *A. tumefaciens* could only detect a long AT-rich sequences, the ros box (Chou et al., 1998; D'Souza-Ault et al., 1993). The same result was obtained in the effort to identify the MucR DNA target in *S. meliloti* (Bahlawane et al., 2008a, 2008b; Bertram-Drogatz et al., 1998). This was the first indication that the Ros/MucR proteins did not work as the eukaryotic zinc finger. In fact, with exceptions such as the factor GAGA from *Drosophila melanogaster* that binds DNA by a single zinc finger domain and flanking basic regions (Omichinski et al., 1997), the eukaryotic zinc finger proteins work using two or more zinc finger domains to bind DNA with high affinity and recognize consensus sequences where three DNA bases are recognized by each zinc finger motif (Laity et al., 2001). Many other data suggested that MucR did not function as a classical transcription regulator: the presence of a region targeted by *S. meliloti* MucR far from the transcription starting site in its own promoter gene suggested that a DNA bending had to occur for impeding the transcription by RNA polymerase (Bertram-Drogatz et al., 1998); the observation that a high concentration of MucR was required to form the complex with DNA in EMSA suggested the presence of a multimer to bind DNA; long AT-rich DNA targets were required to obtain MucR-DNA complexes, which is not typical of a sequence specific transcription factor. These observations were all overlooked, while today they support the role of MucR in structuring the bacterial genome and acting as H-NS-like proteins bridging DNA (Barton et al., 2023). The involvement of this protein in cell cycle progression and in local DNA hypomethylation (Ardissone et al., 2016; Fumeaux et al., 2014) is also in line with the role of histone-like protein: a change of the structure of the bacterial genome might be required to activate a particular set of genes that can be repressed in S-phase and need to be activated for switching to G1 phase.

The study of MucR and its homologs have taught that every detail can tell us a story and place us on the right way to understand the role of biological molecules as proteins. In this particular case, the lack of sequence identity between MucR proteins and the already known nucleoid associated proteins (NAPs), the presence of a domain strongly resembling the eukaryotic zinc finger in Ros/MucR family members have made harder the identification of this new H-NS-like protein, which is a difficult task *per sè*, since the only observation of a large number of target genes or aspecific DNA sequence binding or modifications of the DNA structure upon protein-DNA interaction is not always enough to classify a protein as a NAP (Dorman et al., 2020).

Under this new light, many other questions to address arise. The H-NS and H-NS-like proteins form high-order oligomers in filamentous structure starting from dimers which bind nucleation sites (Dame et al., 2000, 2006; Qin et al., 2019). Further structural studies are required to clarify the structure of the high-order oligomers formed by MucR even if the absence of dimers or lower-order oligomers when MucR has been purified from *S. meliloti* naturally expressing the protein (Slapakova et al., 2023) suggests that this new N-NS-like protein needs to oligomerize before binding DNA and that the assembly of the high-order oligomer is very stable.

Studies of the DNA regions targeted by MucR will help to clarify whether the AT-rich sequences work as nucleation sites in the context of GC-rich regions, thus allowing MucR to extend its presence on the genome and to condense it. The mechanism adopted by MucR to repress target genes is not fully clarified yet.

Structuring bacterial genome is not a role played by H-NS and H-NS-like proteins alone as protein partners are required to obtain a condensed genome (Dorman, 2007; Singh et al., 2016). The investigation of MucR interactome will uncover the partners helping in structuring the nucleoid and MucR/counter silencers in the case these proteins interact with this new H-NS-like protein. The investigations of the role and the mechanism adopted by MucR/counter-silencers

will help to better understand the regulation of gene expression obtained by changing the structure of the bacterial genome, opening it, and making the promoters accessible to the transcription machinery. A proteomic analysis of bacteria defective for the *mucR* gene will reveal the effects of MucR on protein expression.

The investigation of the effects of physico-chemical changes on the bridging activity of MucR is compelling to understand the conditions and the mechanism underlying the activation of the MucR target genes.

Thus, after uncovering the role of MucR as a new type of H-NS-like protein, new directions for further studies are open and the interpretation of previous data in light of new findings will help to elucidate the remaining mysteries and contributions of MucR to the bacterial cell.

AUTHOR CONTRIBUTIONS

IB: Conceptualization, wrote the original draft, revised the draft, supervised. GM, RMRII: Helped in writing the draft. ISB, XW, VR, LP, EMP, PVP: Revised the original draft. All the authors read the final version of the article and approved it.

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CONFLICT OF INTEREST STATEMENT

The authors of this article declare to have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data reported in this manuscript are available in the cited publications.

ETHICS STATEMENT

The article respects all the etich issues.

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REFERENCES

Acosta-Jurado, S., Alias-Villegas, C., Navarro-Gómez, P., Zehner, S., Murdoch, P.D., Rodríguez-Carvajal, M.A. et al. (2016) The *Sinorhizobium fredii* HH103 MucR1 global regulator is connected with the nod regulon and is required for efficient symbiosis with *Lotus burttii* and *Glycine max* cv. Williams. *Molecular Plant-Microbe Interactions*, 29(9), 700–712. Available from: https://doi.org/10.1094/MPMI-06-16-0116-R

Akerley, B.J., Cotter, P.A. & Miller, J.F. (1995) Ectopic expression of the flagellar regulon alters development of the *Bordetella*-host interaction. *Cell*, 80(4), 611–620. Available from: https://doi.org/10.1016/0092-8674(95)90515-4

Ali, S.S., Xia, B., Liu, J. & Navarre, W.W. (2012) Silencing of foreign DNA in bacteria. *Current Opinion in Microbiology*, 15(2), 175–181. Available from: https://doi.org/10.1016/j.mib.2011.12.014

Ardissone, S., Redder, P., Russo, G., Frandi, A., Fumeaux, C., Patrignani, A. et al. (2016) Cell cycle constraints and environmental control of local DNA hypomethylation in α-proteobacteria. *PLoS Genetics*, 12(12), e1006499. Available from: https://doi.org/10.1371/journal.pgen.1006499

Arenas-Gamboa, A.M., Rice-Ficht, A.C., Kahl-McDonagh, M.M. & Ficht, T.A. (2011) Protective efficacy and safety of *Brucella melitensis* 16MΔ*mucR* against intraperitoneal and aerosol challenge in BALB/c mice. *Infection and Immunity*, 79(9), 3653–3658. Available from: https://doi.org/10.1128/IAI.05330-11 Erratum in: Infect Immun. 2011 Dec;79(12):5040.

Baglivo, I., Palmieri, M., Rivellino, A., Netti, F., Russo, L., Esposito, S. et al. (2014) Molecular strategies to replace the structural metal site in the prokaryotic zinc finger domain. *Biochimica et Biophysica Acta*, 1844(3), 497–504. Available from: https://doi.org/10.1016/j.bbapap.2013.12.019

Baglivo, I., Pirone, L., Malgieri, G., Fattorusso, R., Roop, R.M., II, Pedone, E.M. et al. (2018) MucR binds multiple target sites in the promoter of its own gene and is a heat-stable protein: is MucR a H-NS-like protein? *FEBS Open Bio*, 8(4), 711–718. Available from: https://doi.org/10.1002/2211-5463.12411

Baglivo, I., Russo, L., Esposito, S., Malgieri, G., Renda, M., Salluzzo, A. et al. (2009) The structural role of the zinc ion can be dispensable in prokaryotic zinc-finger domains. *Proceedings of the National Academy of Sciences of the United States of America*, 106(17), 6933–6938. Available from: https://doi.org/10.1073/pnas.0810003106

Bahlawane, C., Baumgarth, B., Serrania, J., Rüberg, S. & Becker, A. (2008a) Fine-tuning of galactoglucan biosynthesis in *Sinorhizobium meliloti* by differential WggR (ExpG)-, PhoB-, and MucR-dependent regulation of two promoters. *Journal of Bacteriology*, 190(10), 3456–3466. Available from: https://doi.org/10.1128/JB.00062-08

Bahlawane, C., McIntosh, M., Krol, E. & Becker, A. (2008b) *Sinorhizobium meliloti* regulator MucR couples exopolysaccharide synthesis and motility. *Molecular Plant-Microbe Interactions*, 21(11), 1498–1509. Available from: https://doi.org/10.1094/MPMI-21-11-1498. PMID: 18842098.

Balows, A., Trüper, H.G., Dworkin, M., Harder, W. & Schleifer, K.H. (1992) *The prokaryotes*. New York, NY: Springer-Verlag.

Barton, I.S., Ren, Z., Cribb, C.B., Pitzer, J.E., Baglivo, I., Martin, D.W. et al. (2023) *Brucella* MucR acts as an H-NS-like protein to silence virulence genes and structure the nucleoid. *MBio*, 14(6), e0220123. Available from: https://doi.org/10.1128/mbio.02201-23

Batut, J., Andersson, S.G. & O'Callaghan, D. (2004) The evolution of chronic infection strategies in the alpha-proteobacteria. *Nature Reviews. Microbiology*, 2(12), 933–945. Available from: https://doi.org/10.1038/nrmicro1044

Bertram-Drogatz, P.A., Quester, I., Becker, A. & Pühler, A. (1998) The *Sinorhizobium meliloti* MucR protein, which is essential for the production of high-molecular-weight succinoglycan exopolysaccharide, binds to short DNA regions upstream of exoH and exoY. *Molecular & General Genetics*, 257(4), 433–441. Available from: https://doi.org/10.1007/s004380050667. PMID: 9529524.

Bialer, M.G., Sycz, G., Muñoz González, F., Ferrero, M.C., Baldi, P.C. & Zorreguieta, A. (2020) Adhesins of *Brucella*: their roles in the interaction with the host. *Pathogens*, 9(11), 942. Available from: https://doi.org/10.3390/pathogens9110942

Bittinger, M.A., Milner, J.L., Saville, B.J. & Handelsman, J. (1997) *rosR*, a determinant of nodulation competitiveness in *Rhizobium etli*. *Molecular Plant-Microbe Interactions*, 10(2), 180–186. Available from: https://doi.org/10.1094/MPMI.1997.10.2.180

Borriello, G., Russo, V., Paradiso, R., Riccardi, M.G., Criscuolo, D., Verde, G. et al. (2020) Different impacts of MucR binding to the *babR* and *virB* promoters on gene expression in *Brucella abortus* 2308. *Biomolecules*, 10(5), 788. Available from: https://doi.org/10.3390/biom10050788

Brilli, M., Fondi, M., Fani, R., Mengoni, A., Ferri, L., Bazzicalupo, M. et al. (2010) The diversity and evolution of cell cycle regulation in alpha-proteobacteria: a comparative genomic analysis. *BMC Systems Biology*, 4, 52. Available from: https://doi.org/10.1186/1752-0509-4-52

Castang, S. & Dove, S.L. (2010) High-order oligomerization is required for the function of the H-NS family member MvaT in *Pseudomonas aeruginosa*. *Molecular Microbiology*, 78(4), 916–931. Available from: https://doi.org/10.1111/j.1365-2958.2010.07378.x. Epub 2010 Sep 24. PMID: 20815825; PMCID: PMC2978250.

Caswell, C.C., Elhassanny, A.E., Planchin, E.E., Roux, C.M., Weeks-Gorospe, J.N., Ficht, T.A. et al. (2013) Diverse genetic regulon of the virulence-associated transcriptional regulator MucR in *Brucella abortus* 2308. *Infection and Immunity*, 81(4), 1040–1051. Available from: https://doi.org/10.1128/IAI.01097-12. Epub 2013 Jan 14.

Chou, A.Y., Archdeacon, J. & Kado, C.I. (1998) *Agrobacterium* transcriptional regulator Ros is a prokaryotic zinc finger protein that regulates the plant oncogene *ipt*. *Proceedings of the National Academy of Sciences of the United States of America*, 95(9), 5293–5298. Available from: https://doi.org/10.1073/pnas.95.9.5293

Close, T.J., Rogowsky, P.M., Kado, C.I., Winans, S.C., Yanofsky, M.F. & Nester, E.W. (1987) Dual control of *Agrobacterium tumefaciens* Ti plasmid virulence genes. *Journal of Bacteriology*, 169(11), 5113–5118. Available from: https://doi.org/10.1128/jb.169.11.5113-5118.1987

Close, T.J., Tait, R.C. & Kado, C.I. (1985) Regulation of Ti plasmid virulence genes by a chromosomal locus of *Agrobacterium tumefaciens*. *Journal of Bacteriology*, 164, 774–781.

Collier, L., Balows, A. & Sussman, M. (1998) Topley and Wilson's microbiology and microbial infections, vol. 2. In: *Systematic Bacteriology*. London: Arnold.

Cooley, M.B., D'Souza, M.R. & Kado, C.I. (1991) The virC and virD operons of the *Agrobacterium* Ti plasmid are regulated by the *ros* chromosomal gene: analysis of the cloned *ros* gene. *Journal of Bacteriology*, 173(8), 2608–2616. Available from: https://doi.org/10.1128/jb.173.8.2608-2616.1991

D'Abrosca, G., Paladino, A., Baglivo, I., Russo, L., Sassano, M., Grazioso, R. et al. (2020) Structural insight of the full-length Ros protein: a prototype of the prokaryotic zinc-finger family. *Scientific Reports*, 10(1), 9283. Available from: https://doi.org/10.1038/s41598-020-66204-5

D'Abrosca, G., Russo, L., Palmieri, M., Baglivo, I., Netti, F., de Paola, I. et al. (2016) The (unusual) aspartic acid in the metal coordination sphere of the prokaryotic zinc finger domain. *Journal of Inorganic Biochemistry*, 161, 91–98. Available from: https://doi.org/10.1016/j.jinorgbio.2016.05.006

Dame, R.T., Luijsterburg, M.S., Krin, E., Bertin, P.N., Wagner, R. & Wuite, G.J. (2005) DNA bridging: a property shared among H-NS-like proteins. *Journal of Bacteriology*, 187, 1845–1848.

Dame, R.T., Noom, M.C. & Wuite, G.J. (2006) Bacterial chromatin organization by H-NS protein unravelled using dual DNA manipulation. *Nature*, 444(7117), 387–390. Available from: https://doi.org/10.1038/nature05283

Dame, R.T., Wyman, C. & Goosen, N. (2000) H-NS mediated compaction of DNA visualised by atomic force microscopy. *Nucleic Acids Research*, 28(18), 3504–3510. Available from: https://doi.org/10.1093/nar/28.18.3504

Dimitriu, T., Lotton, C., Bénard-Capelle, J., Misevic, D., Brown, S.P., Lindner, A.B. et al. (2014) Genetic information transfer promotes cooperation in bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 111(30), 11103–11108. Available from: https://doi.org/10.1073/pnas.1406840111

Ding, P., McFarland, K.A., Jin, S., Tong, G., Duan, B., Yang, A. et al. (2015) A novel AT-rich DNA recognition mechanism for bacterial xenogeneic silencer MvaT. *PLoS Pathogens*, 11(6), e1004967. Available from: https://doi.org/10.1371/journal.ppat.1004967

Dong, H., Liu, W., Peng, X., Jing, Z. & Wu, Q. (2013) The effects of MucR on expression of type IV secretion system, quorum sensing system and stress responses in *Brucella melitensis*. *Veterinary Microbiology*, 166(3–4), 535–542. Available from: https://doi.org/10.1016/j.vetmic.2013.06.023

Dorman, C.J. (2004) H-NS: a universal regulator for a dynamic genome. *Nature Reviews. Microbiology*, 2(5), 391–400. Available from: https://doi.org/10.1038/nrmicro883

Dorman, C.J. (2007) H-NS, the genome sentinel. *Nature Reviews. Microbiology*, 5(2), 157–161. Available from: https://doi.org/10.1038/nrmicro1598

Dorman, C.J., Schumacher, M.A., Bush, M.J., Brennan, R.G. & Buttner, M.J. (2020) When is a transcription factor a NAP? *Current Opinion in Microbiology*, 55, 26–33. Available from: https://doi.org/10.1016/j.mib.2020.01.019

Dorman, M.J. & Dorman, C.J. (2018) Regulatory hierarchies controlling virulence gene expression in *Shigella flexneri* and *Vibrio cholerae*. *Frontiers in Microbiology*, 9, 2686. Available from: https://doi.org/10.3389/fmicb.2018.02686

D'Souza-Ault, M.R., Cooley, M.B. & Kado, C.I. (1993) Analysis of the Ros repressor of *Agrobacterium* virC and virD operons: molecular intercommunication between plasmid and chromosomal genes. *Journal of Bacteriology*, 175(11), 3486–3490. Available from: https://doi.org/10.1128/jb.175.11.3486-3490.1993

Erkelens, A.M., Qin, L., van Erp, B., Miguel-Arribas, A., Abia, D., Keek, H.G.J. et al. (2022) The *B. subtilis* Rok protein is an atypical H-NS-like protein irresponsive to physico-chemical cues. *Nucleic Acids Research*, 50(21), 12166–12185. Available from: https://doi.org/10.1093/nar/gkac1064

Esposito, S., Baglivo, I., Malgieri, G., Russo, L., Zaccaro, L., D'Andrea, L.D. et al. (2006) A novel type of zinc finger DNA binding domain in the *Agrobacterium tumefaciens* transcriptional regulator Ros. *Biochemistry*, 45(34), 10394–10405. Available from: https://doi.org/10.1021/bi060697m

Fairall, L., Schwabe, J., Chapman, L., Finch, J.T. & Rhodes, D. (1993) The crystal structure of a two zinc-finger peptide reveals an extension to the rules for zinc-finger/DNA recognition. *Nature*, 366, 483–487. Available from: https://doi.org/10.1038/366483a0

Fernandez-Lopez, R., Ruiz, R., Del Campo, I., Gonzalez-Montes, L., Boer, D.R., de la Cruz, F. et al. (2022) Structural basis of direct and inverted DNA sequence repeat recognition by helix-turn-helix transcription factors. *Nucleic Acids Research*, 50(20), 11938–11947. Available from: https://doi.org/10.1093/nar/gkac1024

Fumeaux, C., Radhakrishnan, S.K., Ardissone, S., Théraulaz, L., Frandi, A., Martins, D. et al. (2014) Cell cycle transition from S-phase to G1 in *Caulobacter* is mediated by ancestral virulence regulators. *Nature Communications*, 5, 4081. Available from: https://doi.org/10.1038/ncomms5081

Gamsjaeger, R., Liew, C.K., Loughlin, F.E., Crossley, M. & Mackay, J.P. (2007) Sticky fingers: zinc-fingers as protein-recognition motifs. *Trends in Biochemical Sciences*, 32(2), 63–70. Available from: https://doi.org/10.1016/j.tibs.2006.12.007

Glazebrook, J. & Walker, G.C. (1989) A novel exopolysaccharide can function in place of the calcofluor-binding exopolysaccharide in nodulation of alfalfa by *Rhizobium meliloti*. *Cell*, 56(4), 661–672. Available from: https://doi.org/10.1016/0092-8674(89)90588-6

Gora, K.G., Tsokos, C.G., Chen, Y.E., Srinivasan, B.S., Perchuk, B.S. & Laub, M.T. (2010) A cell-type-specific protein-protein interaction modulates transcriptional activity of a master regulator in *Caulobacter*

crescentus. *Molecular Cell*, 39(3), 455–467. Available from: https://doi.org/10.1016/j.molcel.2010.06.024

Gordon, B.R., Imperial, R., Wang, L., Navarre, W.W. & Liu, J. (2008) Lsr2 of *Mycobacterium* represents a novel class of H-NS-like proteins. *Journal of Bacteriology*, 190(21), 7052–7059. Available from: https://doi.org/10.1128/JB.00733-08

Gordon, B.R., Li, Y., Wang, L., Sintsova, A., van Bakel, H., Tian, S. et al. (2010) Lsr2 is a nucleoid-associated protein that targets AT-rich sequences and virulence genes in *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America*, 107(11), 5154–5159. Available from: https://doi.org/10.1073/pnas.0913551107. Epub 2010 Jan 20. Erratum in: *Proc Natl Acad Sci U S A*. 2010 Oct 26;*107*(43), 18741.

Groisman, E.A. & Ochman, H. (1996) Pathogenicity islands: bacterial evolution in quantum leaps. *Cell*, 87(5), 791–794. Available from: https://doi.org/10.1016/s0092-8674(00)81985-6. PMID: 8945505.

Groisman, E.A. & Ochman, H. (1997) How *Salmonella* became a pathogen. *Trends in Microbiology*, 5(9), 343–349. Available from: https://doi.org/10.1016/S0966-842X(97)01099-8

Gupta, R.S. (2000) The phylogeny of proteobacteria: relationships to other eubacterial phyla and eukaryotes. *FEMS Microbiology Reviews*, 24(4), 367–402. Available from: https://doi.org/10.1111/j.1574-6976.2000.tb00547.x

Her, G.R., Glazebrook, J., Walker, G.C. & Reinhold, V.N. (1990) Structural studies of a novel exopolysaccharide produced by a mutant of *Rhizobium meliloti* strain Rm1021. *Carbohydrate Research*, 198, 305–312.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. & Stanley, T.W. (1994) *Bergey's manual of determinative bacteriology*. Baltimore, MD: Williams and Wilkins.

Isernia, C., Malgieri, G., Russo, L., D'Abrosca, G., Baglivo, I., Pedone, P.V. et al. (2020) Zinc Fingers. *Metal Ions in Life Sciences*, 20, 416–431. Available from: https://doi.org/10.1515/9783110589757-018

Janczarek, M. (2022) The Ros/MucR zinc-finger protein family in bacteria: structure and functions. *International Journal of Molecular Sciences*, 23(24), 15536. Available from: https://doi.org/10.3390/ijms232415536

Janczarek, M. & Skorupska, A. (2007) The *Rhizobium leguminosarum* bv. *Trifolii* RosR: transcriptional regulator involved in exopolysaccharide production. *Molecular Plant-Microbe Interactions*, 20(7), 867–881. Available from: https://doi.org/10.1094/MPMI-20-7-0867

Janczarek, M. & Skorupska, A. (2009) *Rhizobium leguminosarum* bv. *trifolii* rosR gene expression is regulated by catabolic repression. *FEMS Microbiology Letters*, 291(1), 112–119. Available from: https://doi.org/10.1111/j.1574-6968.2008.01443.x

Janczarek, M. & Skorupska, A. (2011) Modulation of rosR expression and exopolysaccharide production in *Rhizobium leguminosarum* bv. *trifolii* by phosphate and clover root exudates. *International Journal of Molecular Sciences*, 12(6), 4132–4155. Available from: https://doi.org/10.3390/ijms12064132

Jiao, J., Wu, L.J., Zhang, B., Hu, Y., Li, Y., Zhang, X.X. et al. (2016) MucR is required for transcriptional activation of conserved ion transporters to support nitrogen fixation of *Sinorhizobium fredii* in soybean nodules. *Molecular Plant-Microbe Interactions*, 29(5), 352–361. Available from: https://doi.org/10.1094/MPMI-01-16-0019-R

Jiao, J., Zhang, B., Li, M.L., Zhang, Z. & Tian, C.F. (2021) The zinc-finger bearing xenogeneic silencer MucR in α-proteobacteria balances adaptation and regulatory integrity. *The ISME Journal*, 16(3), 738–749. Available from: https://doi.org/10.1038/s41396-021-01118-2

Keller, M., Roxlau, A., Weng, W.M., Schmidt, M., Quandt, J., Niehaus, K. et al. (1995) Molecular analysis of the *Rhizobium meliloti mucR* gene regulating the biosynthesis of the exopolysaccharides succinoglycan and galactoglucan. *Molecular Plant-Microbe Interactions*, 8(2), 267–277. Available from: https://doi.org/10.1094/mpmi-8-0267

Klug, A. & Schwabe, J.W. (1995) Protein motifs 5. Zinc fingers. *The FASEB Journal*, 9(8), 597–604.

Laity, J.H., Lee, B.M. & Wright, P.E. (2001) Zinc finger proteins: new insights into structural and functional diversity. *Current Opinion in Structural Biology*, 11(1), 39–46. Available from: https://doi.org/10.1016/s0959-440x(00)00167-6

Laub, M.T., McAdams, H.H., Feldblyum, T., Fraser, C.M. & Shapiro, L. (2000) Global analysis of the genetic network controlling a bacterial cell cycle. *Science*, 290(5499), 2144–2148. Available from: https://doi.org/10.1126/science.290.5499.2144

Laub, M.T., Shapiro, L. & McAdams, H.H. (2007) Systems biology of *Caulobacter*. *Annual Review of Genetics*, 41, 429–441. Available from: https://doi.org/10.1146/annurev.genet.41.110306.130346

Lee, I.P.A., Eldakar, O.T., Gogarten, J.P. & Andam, C.P. (2022) Bacterial cooperation through horizontal gene transfer. *Trends in Ecology & Evolution*, 37(3), 223–232. Available from: https://doi.org/10.1016/j.tree.2021.11.006

Li, C., Wally, H., Miller, S.J. & Lu, C.D. (2009) The multifaceted proteins MvaT and MvaU, members of the H-NS family, control arginine metabolism, pyocyanin synthesis, and prophage activation in *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology*, 191(20), 6211–6218. Available from: https://doi.org/10.1128/JB.00888-09

López-García, P. & Moreira, D. (2020) Cultured asgard archaea shed light on Eukaryogenesis. *Cell*, 181(2), 232–235. Available from: https://doi.org/10.1016/j.cell.2020.03.058

Lucchini, S., Rowley, G., Goldberg, M.D., Hurd, D., Harrison, M. & Hinton, J.C. (2006) H-NS mediates the silencing of laterally acquired genes in bacteria. *PLoS Pathogens*, 2(8), e81. Available from: https://doi.org/10.1371/journal.ppat.0020081. Erratum in: *PLoS Pathog*. 2007 Mar;3(3):e38.

Malgieri, G., Russo, L., Esposito, S., Baglivo, I., Zaccaro, L., Pedone, E.M. et al. (2007) The prokaryotic Cys2His2 zinc-finger adopts a novel fold as revealed by the NMR structure of *Agrobacterium tumefaciens* Ros DNA-binding domain. *Proceedings of the National Academy of Sciences of the United States of America*, 104(44), 17341–17346. Available from: https://doi.org/10.1073/pnas.0706659104

Marinus, M.G. & Casadesus, J. (2009) Roles of DNA adenine methylation in host-pathogen interactions: mismatch repair, transcriptional regulation, and more. *FEMS Microbiology Reviews*, 33(3), 488–503. Available from: https://doi.org/10.1111/j.1574-6976.2008.00159.x

McGinty, S.É. & Rankin, D.J. (2012) The evolution of conflict resolution between plasmids and their bacterial hosts. *Evolution*, 66(5), 1662–1670. Available from: https://doi.org/10.1111/j.1558-5646.2011.01549.x

Mirabella, A., Terwagne, M., Zygmunt, M.S., Cloeckaert, A., De Bolle, X. & Letesson, J.J. (2013) *Brucella melitensis* MucR, an orthologue of *Sinorhizobium meliloti* MucR, is involved in resistance to oxidative, detergent, and saline stresses and cell envelope modifications. *Journal of Bacteriology*, 195(3), 453–465. Available from: https://doi.org/10.1128/JB.01336-12

Mueller, K. & González, J.E. (2011) Complex regulation of symbiotic functions is coordinated by MucR and quorum sensing in *Sinorhizobium meliloti*. *Journal of Bacteriology*, 193(2), 485–496. Available from: https://doi.org/10.1128/JB.01129-10

Murray, R.G.E., Brenner, D.J., Colwell, R.R., De Vos, P., Goodfellow, M., Grimont, P.A.D. et al. (1990) Report of the ad hoc committee on approaches to taxonomy within the Proteobacteria. *International Journal of Systematic Bacteriology*, 40, 213–215.

Navarre, W.W., McClelland, M., Libby, S.J. & Fang, F.C. (2007) Silencing of xenogeneic DNA by H-NS-facilitation of lateral gene transfer in bacteria by a defense system that recognizes foreign DNA. *Genes & Development*, 21(12), 1456–1471. Available from: https://doi.org/10.1101/gad.1543107 PMID: 17575047.

Navarre, W.W., Porwollik, S., Wang, Y., McClelland, M., Rosen, H., Libby, S.J. et al. (2006) Selective silencing of foreign DNA with low GC content by the H-NS protein in *Salmonella*. *Science*, 313(5784), 236–238. Available from: https://doi.org/10.1126/science.1128794

Netti, F., Malgieri, G., Esposito, S., Palmieri, M., Baglivo, I., Isernia, C. et al. (2013) An experimentally tested scenario for the structural evolution of eukaryotic Cys2His2 zinc fingers from eubacterial ros homologs. *Molecular Biology and Evolution*, 30(7), 1504–1513. Available from: https://doi.org/10.1093/molbev/mst068

Newman, S.L., Will, W.R., Libby, S.J. & Fang, F.C. (2018) The curli regulator CsgD mediates stationary phase counter-silencing of csgBA in *Salmonella* Typhimurium. *Molecular Microbiology*, 108(1), 101–114. Available from: https://doi.org/10.1111/mmi.13919

Omichinski, J.G., Pedone, P.V., Felsenfeld, G., Gronenborn, A.M. & Clore, G.M. (1997) The solution structure of a specific GAGA factor-DNA complex reveals a modular binding mode. *Nature Structural Biology*, 4(2), 122–132. Available from: https://doi.org/10.1038/nsb0297-122

Palmieri, M., Malgieri, G., Russo, L., Baglivo, I., Esposito, S., Netti, F. et al. (2013) Structural Zn(II) implies a switch from fully cooperative to partly downhill folding in highly homologous proteins. *Journal of the American Chemical Society*, 135(13), 5220–5228. Available from: https://doi.org/10.1021/ja4009562

Pirone, L., Pitzer, J.E., D'Abrosca, G., Fattorusso, R., Malgieri, G., Pedone, E.M. et al. (2018) Identifying the region responsible for *Brucella abortus* MucR higher-order oligomer formation and examining its role in gene regulation. *Scientific Reports*, 8(1), 17238. Available from: https://doi.org/10.1038/s41598-018-35432-1

Qin, L., Erkelens, A.M., Ben Bdira, F. & Dame, R.T. (2019) The architects of bacterial DNA bridges: a structurally and functionally conserved family of proteins. *Open Biology*, 9(12), 190223. Available from: https://doi.org/10.1098/rsob.190223

Quon, K.C., Marczynski, G.T. & Shapiro, L. (1996) Cell cycle control by an essential bacterial two-component signal transduction protein. *Cell*, 84(1), 83–93. Available from: https://doi.org/10.1016/s0092-8674(00)80995-2

Rashid, F.M. & Dame, R.T. (2023) Three-dimensional chromosome remodelling: the integral mechanism of transcription regulation in bacteria. *Molecular Microbiology*, 120(1), 60–70. Available from: https://doi.org/10.1111/mmi.15062

Reinhold, B.B., Chan, S.Y., Reuber, T.L., Marra, A., Walker, G.C. & Reinhold, V.N. (1994) Detailed structural characterization of succinoglycan, the major exopolysaccharide of *Rhizobium meliloti* Rm1021. *Journal of Bacteriology*, 176(7), 1997–2002. Available from: https://doi.org/10.1128/jb.176.7.1997-2002.1994

Reuber, T.L. & Walker, G.C. (1993) Biosynthesis of succinoglycan, a symbiotically important exopolysaccharide of *Rhizobium meliloti*. *Cell*, 74(2), 269–280. Available from: https://doi.org/10.1016/0092-8674(93)90418-p

Rinaudi, L.V., Sorroche, F., Zorreguieta, A. & Giordano, W. (2010) Analysis of the mucR gene regulating biosynthesis of exopolysaccharides: implications for biofilm formation in *Sinorhizobium meliloti* Rm1021. *FEMS Microbiology Letters*, 302(1), 15–21. Available from: https://doi.org/10.1111/j.1574-6968.2009.01826.x

Rohs, R., West, S.M., Sosinsky, A., Liu, P., Mann, R.S. & Honig, B. (2009) The role of DNA shape in protein-DNA recognition. *Nature*, 461(7268), 1248–1253. Available from: https://doi.org/10.1038/nature08473

Roop, R.M., 2nd, Barton, I.S., Hopersberger, D. & Martin, D.W. (2021) Uncovering the hidden credentials of *Brucella* virulence. *Microbiology and Molecular Biology Reviews*, 85(1), e00021-19. Available from: https://doi.org/10.1128/MMBR.00021-19

Ruiz-Ranwez, V., Posadas, D.M., Van der Henst, C., Estein, S.M., Arocena, G.M., Abdian, P.L. et al. (2013) BtaE, an adhesin that belongs to the trimeric autotransporter family, is required for full virulence and defines a specific adhesive pole of *Brucella suis*. *Infection and Immunity*, 81(3), 996–1007. Available from: https://doi.org/10.1128/IAI.01241-12

Sahota, G. & Stormo, G.D. (2010) Novel sequence-based method for identifying transcription factor binding sites in prokaryotic genomes. *Bioinformatics*, 26(21), 2672–2677. Available from: https://doi.org/10.1093/bioinformatics/btq501

Shi, W.T., Zhang, B., Li, M.L., Liu, K.H., Jiao, J. & Tian, C.F. (2022) The convergent xenogeneic silencer MucR predisposes α-proteobacteria to integrate AT-rich symbiosis genes. *Nucleic Acids Research*, 50(15), 8580–8598. Available from: https://doi.org/10.1093/nar/gkac664

Singh, K., Milstein, J.N. & Navarre, W.W. (2016) Xenogeneic silencing and its impact on bacterial genomes. *Annual Review of Microbiology*, 70, 199–213. Available from: https://doi.org/10.1146/annurev-micro-102215-095301

Slapakova, M., Sgambati, D., Pirone, L., Russo, V., D'Abrosca, G., Valletta, M. et al. (2023) MucR from *Sinorhizobium meliloti*: new insights into its DNA targets and its ability to Oligomerize. *International Journal of Molecular Sciences*, 24(19), 14702. Available from: https://doi.org/10.3390/ijms241914702

Stackebrandt, E., Murray, R.G.E. & Trüper, H.G. (1988) *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the 'purple bacteria and their relatives'. *International Journal of Systematic Bacteriology*, 38, 321–325.

Stephens, C., Reisenauer, A., Wright, R. & Shapiro, L. (1996) A cell cycle-regulated bacterial DNA methyltransferase is essential for viability. *Proceedings of the National Academy of Sciences of the United States of America*, 93(3), 1210–1214. Available from: https://doi.org/10.1073/pnas.93.3.1210

Sun, J., Dong, H., Peng, X., Liu, Y., Jiang, H., Feng, Y. et al. (2021) Deletion of the transcriptional regulator MucR in *Brucella canis* affects stress responses and bacterial virulence. *Frontiers in Veterinary Science*, 8, 650942. Available from: https://doi.org/10.3389/fvets.2021.650942

Tan, M.H., Kozdon, J.B., Shen, X., Shapiro, L. & McAdams, H.H. (2010) An essential transcription factor, SciP, enhances robustness of *Caulobacter* cell cycle regulation. *Proceedings of the National Academy of Sciences of the United States of America*, 107(44), 18985–18990. Available from: https://doi.org/10.1073/pnas.1014395107

Tartilán-Choya, B., Sidhu-Muñoz, R.S. & Vizcaíno, N. (2022) The transcriptional regulator MucR, but not its controlled acid-activated chaperone HdeA, is essential for virulence and modulates surface architecture and properties in *Brucella ovis* PA. *Frontiers in Veterinary Science*, 8, 814752. Available from: https://doi.org/10.3389/fvets.2021.814752

Tendeng, C. & Bertin, P.N. (2003) H-NS in gram-negative bacteria: a family of multifaceted proteins. *Trends in Microbiology*, 11(11), 511–518. Available from: https://doi.org/10.1016/j.tim.2003.09.005

Tendeng, C., Soutourina, O.A., Danchin, A. & Bertin, P.N. (2003) MvaT proteins in *Pseudomonas* spp.: a novel class of H-NS-like proteins. *Microbiology*, 149, 3047–3050. Available from: https://doi.org/10.1099/mic.0.C0125-0

Udvardi, M. & Poole, P.S. (2013) Transport and metabolism in legume-rhizobia symbioses. *Annual Review of Plant Biology*, 64, 781–805. Available from: https://doi.org/10.1146/annurev-arplant-050312-120235

Vallet-Gely, I., Donovan, K.E., Fang, R., Joung, J.K. & Dove, S.L. (2005) Repression of phase-variable cup gene expression by H-NS-like proteins in *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 11082–11087.

Wang, Z. & Wu, M. (2015) An integrated phylogenomic approach toward pinpointing the origin of mitochondria. *Scientific Reports*, 5, 7949. Available from: https://doi.org/10.1038/srep07949. PMID: 25609566; PMCID: PMC4302308.

Will, W.R., Navarre, W.W. & Fang, F.C. (2015) Integrated circuits: how transcriptional silencing and counter-silencing facilitate bacterial evolution. *Current Opinion in Microbiology*, 23, 8–13. Available from: https://doi.org/10.1016/j.mib.2014.10.005 Epub 2014 Nov 5.

Williams McMackin, E.A., Marsden, A.E. & Yahr, T.L. (2019) H-NS family members MvaT and MvaU regulate the *Pseudomonas*

aeruginosa type III secretion system. *Journal of Bacteriology*, 201(14), e00054-19. Available from: https://doi.org/10.1128/JB.00054-19

Winardhi, R.S., Fu, W., Castang, S., Li, Y., Dove, S.L. & Yan, J. (2012) Higher order oligomerization is required for H-NS family member MvaT to form gene-silencing nucleoprotein filament. *Nucleic Acids Research*, 40(18), 8942–8952. Available from: https://doi.org/10.1093/nar/gks669

Wu, J. & Newton, A. (1997) Regulation of the *Caulobacter* flagellar gene hierarchy; not just for motility. *Molecular Microbiology*, 24(2), 233–239. Available from: https://doi.org/10.1046/j.1365-2958.1997.3281691.x

Wu, Q., Pei, J., Turse, C. & Ficht, T.A. (2006) Mariner mutagenesis of *Brucella melitensis* reveals genes with previously uncharacterized roles in virulence and survival. *BMC Microbiology*, 6, 102. Available from: https://doi.org/10.1186/1471-2180-6-102

Zhan, H.J., Levery, S.B., Lee, C.C. & Leigh, J.A. (1989) A second exopolysaccharide of *Rhizobium meliloti* strain SU47 that can function in root nodule invasion. *Proceedings of the National Academy of Sciences of the United States of America*, 86(9), 3055–3059. Available from: https://doi.org/10.1073/pnas.86.9.3055

Zinder, S.H. (1998) Bacterial diversity. In: Balows, A. & Duerden, B.I. (Eds.) *Topley and Wilson's microbiology and microbial infections*, *Vol. 2*, *systematic bacteriology*. London: Arnold, pp. 125–147.

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