



Acetylation at Lysine 86 of *Escherichia coli* HU β Modulates the DNA-Binding Capability of the Protein

Victoria L. Barlow¹ and Yu-Hsuan Tsai^{1,2*}

¹ School of Chemistry, Cardiff University, Cardiff, United Kingdom, ² Institute of Molecular Physiology, Shenzhen Bay Laboratory, Shenzhen, China

OPEN ACCESS

Edited by:

Haiké Antelmann,
Freie Universität Berlin, Germany

Reviewed by:

Subhash Chandra Verma,
National Institutes of Health (NIH),
United States
Sumana Venkat,
University of Texas Southwestern
Medical Center, United States
Bruno Lima,
University of Minnesota Twin Cities,
United States

*Correspondence:

Yu-Hsuan Tsai
tsai.y-h@outlook.com

Specialty section:

This article was submitted to
Microbial Physiology and Metabolism,
a section of the journal
Frontiers in Microbiology

Received: 04 November 2021

Accepted: 24 December 2021

Published: 04 February 2022

Citation:

Barlow VL and Tsai Y-H (2022)
Acetylation at Lysine 86 of *Escherichia coli* HU β Modulates the DNA-Binding
Capability of the Protein.
Front. Microbiol. 12:809030.
doi: 10.3389/fmicb.2021.809030

DNA-binding protein HU is highly conserved in bacteria and has been implicated in a range of cellular processes and phenotypes. Like eukaryotic histones, HU is subjected to post-translational modifications. Specifically, acetylation of several lysine residues have been reported in both homologs of *Escherichia coli* HU. Here, we investigated the effect of acetylation at Lys67 and Lys86, located in the DNA binding-loop and interface of *E. coli* HU β , respectively. Using the technique of genetic code expansion, homogeneous HU β (K67ac) and HU β (K86ac) protein units were obtained. Acetylation at Lys86 seemed to have negligible effects on protein secondary structure and thermal stability. Nevertheless, we found that this site-specific acetylation can regulate DNA binding by the HU homodimer but not the heterodimer. Intriguingly, while Lys86 acetylation reduced the interaction of the HU homodimer with short double-stranded DNA containing a 2-nucleotide gap or nick, it enhanced the interaction with longer DNA fragments and had minimal effect on a short, fully complementary DNA fragment. These results demonstrate the complexity of post-translational modifications in functional regulation, as well as indicating the role of lysine acetylation in tuning bacterial gene transcription and epigenetic regulation.

Keywords: lysine acetylation, DNA-binding protein, HU, *Escherichia coli*, genetic code expansion

INTRODUCTION

Histone-like protein HU is a prevalent DNA-binding protein ubiquitous among bacterial species (Grove, 2011). This basic protein is highly conserved, consisting of an α -helical “body” and two β -sheets that are extended to β -ribbon “arms” interacting directly with DNA (Swinger et al., 2003). Through its interaction with DNA, HU is associated with bacterial phenotypes, including survival and virulence. For example, HU knockout in Gram-positive bacteria is lethal due to disruption in genome integrity (Micka and Marahiel, 1992; Bartels et al., 2001; Liu et al., 2008; Nguyen et al., 2009). HU can also modulate the pathogenicity of different bacteria through transcriptional regulation (Alberti-Segui et al., 2010; Koli et al., 2011; Mangan et al., 2011; Wang et al., 2014; Phan et al., 2015).

The majority of bacterial species produce one HU homolog, which forms homodimers (Grove, 2011). However, like other enterobacteria, *Escherichia coli* has two HU homologs: HU α and HU β , encoded by *hupA* and *hupB* genes, respectively. Consequently, three distinct dimeric forms of HU exist. HU α and HU β can each form homodimers (HU α_2 and HU β_2) alongside a heterodimer (HU $\alpha\beta$). The proportion of each dimer changes throughout the

isopropyl β -D-1-thiogalactopyranoside and 0.2% L-arabinose. Cultures were incubated at 20°C and 180 rpm for 16–18 h.

Cultures were centrifuged at 4500 \times g for 20 min at 4°C. The supernatant was discarded, and pellets were resuspended in 10 mL chilled lysis buffer (0.05 M Tris-HCl pH 8.0, 0.15 M NaCl, 0.01 M imidazole, 1 g/L lysozyme, 100 μ M PMSF) per 1 g cell pellet. Cells were sonicated (Sonics, Vibra-Cell™) on ice in bursts of 5-s ON and 15-s OFF using a 13 mm probe (Sonics) at 39% amplification until lysed. The lysate was then centrifuged at 30,000 \times g and 4°C for 20 min. The supernatant was collected and passed through a 0.44 μ m syringe filter, then combined with Ni-NTA resin equilibrated in lysis buffer and incubated at 4°C with gentle agitation for 1–2 h. The lysate/resin was poured into a gravity column, and the flow through was collected. The resin was washed with chilled wash buffer (0.05 M Tris-HCl pH 8.0, 0.15 M NaCl, 0.02 M imidazole) until no protein could be detected in the flow through by a NanoDrop One (ThermoFisher, #ND-ONE-W) measuring protein A₂₈₀ (1 Abs = 1 mg/mL). The protein was then eluted from the column in 1 column volume fractions using chilled elution buffer (0.05 M Tris-HCl pH 8.0, 0.15 M NaCl, 0.25 M imidazole) and analyzed by 20% SDS-PAGE.

Fractions containing protein at the estimated molecular weight were exchanged into potassium phosphate buffer (10 mM KPO, pH 7.0, 5% glycerol) using a HiPrep 26/10 desalting column (Cytiva, #17-5087-01) per manufacturer's instructions. The sample was then loaded onto a RESOURCE S column (Cytiva, #17-1180-01) and eluted in phosphate buffer with a 0 to 0.5 M NaCl gradient. In both instances, protein elution was monitored at 214 nm. Fractions containing a peak at 214 nm were analyzed by SDS-PAGE before being pooled and concentrated using a Vivaspin 20, 3000 MWCO PES (Sartorius, #VS2091). Protein was dialyzed using 3500 MWCO SnakeSkin dialysis tubing in at least 1000 times the protein sample volume of phosphate buffer at 4°C for 16–18 h. Protein concentration was determined by BCA assay. For storage at -80°C, final glycerol concentration was adjusted to 10%.

The molecular weight of all protein samples was analyzed using mass spectrometry by Cardiff University School of Chemistry Analytical Services. Mass spectra were acquired on an Waters Acquity H-Class UPLC system coupled to a Waters Synapt G2-Si quadrupole time of flight mass spectrometer with a Waters Acquity UPLC Protein C4 BEH column 300 Å, 1.7 μ m (2.1 \times 100 mm).

To produce the heterodimers, purified HU α_2 was combined with HU β_2 , HU β (K67ac)₂ or HU β (K86ac)₂ in a 1:1 molar ratio and incubated on ice for 5 min (Ramstein et al., 2003). The resulting heterodimers were confirmed *via* analytical size exclusion chromatography.

Analytical Size Exclusion Chromatography

Analytical size exclusion chromatography was performed with a high-performance liquid chromatography system (Agilent). A Bio SEC-3 column (Agilent) was equilibrated in PBS (10 mM NaPO pH 7.4, 154 mM NaCl). For generating the standard curve of molecular weights, Gel Filtration Standard (Bio-Rad,

#1511901) was diluted 1:10 in potassium phosphate buffer and 1 μ L of the diluted standard was injected into the column using an autosampler. PBS was passed through the column at a rate of 0.35 mL/min and the elution profile of the standards was analyzed at 214 nm. For analyses of HU protein, protein samples were prepared at 0.6 mg/mL and 20 μ L of sample was injected into the column using an autosampler. PBS was passed through the column at a rate of 0.35 mL/min and the elution profile of the proteins were analyzed at 214 nm.

For the standard curve, the log molecular weights of the protein standards were plotted against their retention time and a line of best fit generated. The equation of the line of best fit was then used to calculate an estimated molecular weight of the HU protein samples.

Electrophoretic Mobility Shift Assay

For electrophoretic mobility shift assays with 30 bp fragments, custom oligonucleotides (Merck) were purified by high pressure liquid chromatography and provided in TE buffer at 100 μ M concentration. Mixing individual oligonucleotides yielded three double-stranded DNA fragments: a 30-bp duplex with a 2 nucleotide gap, a 30-bp duplex with a nicked phosphate backbone, and a 30-bp complete duplex (Castaing et al., 1995). Annealing of the 30-bp fragments was ensured by heating combined oligonucleotides to 98°C for 5 min, then cooling to 4°C at a rate of 1°C/min.

Oligonucleotides used for constructing the 30-bp duplex with a 2-nucleotide gap: CCAACTTCCCTA ACCCAGCTGCGATCCGTA, TACGGATCGCAGC and GGTTAGGGAAGTTGG; for 30-bp duplex with a nicked phosphate backbone: CCAACTTCCCTAACCCAGCTGCGATCCGTA, TACGGATCGCAGC and TGGGTTAGGGAAGTTGG; for the 30-bp complete duplex: CCAACTTCCCTAACCCAGCTGCGATCCGTA and TACGGATCGCAGCTGGGTTAGGGAAGTTGG.

Each reaction contained 1 μ M of a 30 bp DNA fragment, varied concentrations of HU protein and binding buffer (30 mM Tris pH 7.5, 100 mM NaCl, 0.02% v/v Tween20, 0.5 mg/ml BSA) in a 10 μ L reaction volume and was incubated at 18°C for 10 min. After incubation, 2.5 μ L of GelPilot DNA Loading Dye (Qiagen, #239901) was added to the samples and the entire sample electrophoresed on a 10% non-denaturing acrylamide gel in TBE buffer (100 mM Tris-borate pH 8.3, 2 mM EDTA) polymerized with a final concentration of 0.2% ammonium persulfate and 0.1% tetramethylethylenediamine. Gels were electrophoresed on ice in TBE for 80 min before DNA was visualized using PAGE GelRed (Biotium, #41008) per manufacturer's instructions. Gels were imaged on a Bio-Rad ChemiDoc MP system.

For assays with longer DNA fragments, linearized plasmid, pCX eGFP (Perry et al., 1999; Liao et al., 2017) or pUC18 (Thermo Scientific, #SD0051), was produced through restriction enzyme digestion with *Hind*III (Thermo Scientific, #FD0504). A 1292-bp PCR fragment was obtained by PCR of the gene encoding maltose binding protein with primers TTTTGTTTAACTTTAAGAAGGAGATATACATATGAAAATA AAAACAGGTGCACGCATCC and CCTGAAAATA AAGATTCTCGCTAGCCCTTCCCTCGATCCCGAGGTTG.

The full DNA sequences of the 5500, 2700, and 1292 bp DNA fragments are provided in the **Supplementary Material**.

Each reaction contained 250 ng of DNA, varied concentrations of HU protein and binding buffer in a 15 μ L reaction volume and was incubated at 37°C for 1 h (Ghosh et al., 2016; Liao et al., 2017). After incubation, 3.75 μ L of GelPilot DNA Loading Dye was added to the samples and the entire sample electrophoresed on a 0.5% agarose gel containing SYBR Safe (ThermoFisher) in 1 \times TAE (40 mM Tris-acetate pH 8.0, 1 mM EDTA) buffer on ice. Gels were imaged on a Bio-Rad ChemiDoc MP system.

DNA Supercoiling Assay

DNA supercoiling assays were performed as described by Guo and Adhya (2007). Briefly, plasmid pCX eGFP was relaxed with *E. coli* topoisomerase I (New England Biolabs) according to manufacturer's instructions. Then, 100 ng of relaxed DNA was prepared in 1 \times topoisomerase buffer (TaKaRa Bio), 0.01% BSA and incubated with 0.5 μ g of HU β_2 or HU β (K86ac)₂ in a 10 μ L reaction. The reaction was incubated at 37°C for 30 min. Then, 7 units of calf thymus topoisomerase I (TaKaRa Bio) was added to each reaction and the reactions incubated at 37°C for 2 h. 10 μ g of proteinase K (Invitrogen) was added to each reaction, which were incubated at 37°C for 30 min. Then, 5 \times DNA loading dye (Qiagen, #239901) was added to each reaction and the samples were analyzed on 0.8% agarose in 1 \times TBE buffer and electrophoresed at 150 V for 90 min. Post-run, the gel was stained with SybrSafe (Invitrogen) according to manufacturer's instructions and imaged on a Bio-Rad ChemiDoc MP system.

Circular Dichroism Spectroscopy

These experiments were performed on an Applied PhotoPhysics Chirascan spectrometer using 5 μ M protein in deoxygenated potassium phosphate buffer (10 mM KPO, pH 7.0, 5% glycerol). Spectra were measured in triplicate between 200 and 300 nm in 1 mm quartz cuvettes under N₂ with a 50 nm/min scan speed, 0.5 nm data pitch, 1 nm bandwidth, and 0.5 s response time. To measure the thermal melting point, spectra were collected every 2°C as temperature increased from 4 to 84°C. The rate of temperature increase was 0.5°C/min with 300 s equilibration time at each temperature.

Experimental Design and Statistical Rationale

For electrophoretic mobility shift assay, a representative gel from triplicates was shown for each condition. Images of the other two independent repeats are provided in the **Supplementary Material**. Free DNA remaining in each condition was quantified using ImageLab (Bio-Rad) software with the band intensity of the control lane (DNA only) as the reference (100%). Mean and standard deviation of free DNA remaining calculated from the three experiments are presented. Tables presenting the raw values are provided in the **Supplementary Material**. Statistical significance between groups was analyzed using an independent two sample *t*-test with an alpha level of 0.05.

RESULTS

Identification of Lysine Residues of Which Acetylation May Disrupt Interaction With DNA

While the high-resolution crystal structure of *E. coli* HU β_2 is available (pdb: 4P3V), attempts to obtain the structures of HU β_2 or HU α_2 in a DNA-bound state have not yet been successful. Nevertheless, a DNA-bound structure of *Anabaena* HU (pdb: 1P51; Swinger et al., 2003), which shares 42% sequence identity and 71% sequence similarity to *E. coli* HU β , is available and was employed to generate a homology model using SWISS-MODEL (Biasini et al., 2014; Bienert et al., 2016). Based on the homology model, the β -ribbon arms (amino acid residues 56–73) and the C-terminal α -helix (amino acid residues 82–90) are involved in DNA binding (**Figure 1**), and this assertion is supported by previous biochemical studies of *E. coli* HU (Bhowmick et al., 2014; Hammel et al., 2016; Agapova et al., 2020; Thakur et al., 2021). A report of *E. coli* acetylome revealed acetylation at Lys3/9/18/67/86 of HU β (Castaño-Cerezo et al., 2014). As Lys67 (K67ac) and Lys86 (K86ac) are located on the β -ribbon arms and C-terminal α -helix, respectively, we decided to investigate if acetylation at either of these residues changes the properties and/or function of *E. coli* HU β .

Production of *E. coli* HU α , HU β , HU β (K67ac) and HU β (K86ac)

To investigate the effects of *E. coli* HU β acetylation, four variants of HU monomers, HU α , HU β , HU β (K67ac) and HU β (K86ac), were expressed recombinantly in *E. coli* and purified. Using these monomers, we were able to obtain their homodimers, as well as heterodimers of HU $\alpha\beta$, HU $\alpha\beta$ (K67ac) and HU $\alpha\beta$ (K86ac) since mixing of purified *E. coli* HU α_2 and HU β_2 in a 1:1 ratio *in vitro* leads to spontaneous rearrangement to afford the heterodimer (Ramstein et al., 2003).

To produce HU α , a plasmid containing *hupA* was constructed through PCR amplification of the genomic DNA of *E. coli* BL21(DE3), followed by Gibson assembly into a pBAD expression vector. A C-terminal 6xHis tag was appended at the same time to facilitate protein purification. The resulting construct, pBAD *hupA*-His6, was confirmed by sanger sequencing and transformed into *E. coli* BL21 AI for protein expression (**Figure 2A**). The expression cultures were purified by affinity and cation-exchange chromatography. The identity of the purified product was confirmed by mass spectrometry (**Figure 2A** and **Supplementary Figure 1**), where the observed peak (10,357.5 Da) is in close agreement with the calculated molecular weight (10,357.8 Da). Following the same protocol, *E. coli* HU β was expressed and purified (**Figure 2B**).

To produce the acetylated HU β , site-directed mutagenesis was used to mutate the codon corresponding to Lys67 or Lys86 to the amber codon (TAG) in pBAD *hupB*-His6, resulting in plasmids pBAD *hupB*(K67TAG)-His6 and pBAD *hupB*(K86TAG)-His6, respectively. Each construct was co-transformed with plasmid pCDF AckST (Liao et al., 2017), which expresses an orthogonal aminoacyl-tRNA synthetase/tRNA_{CUA}

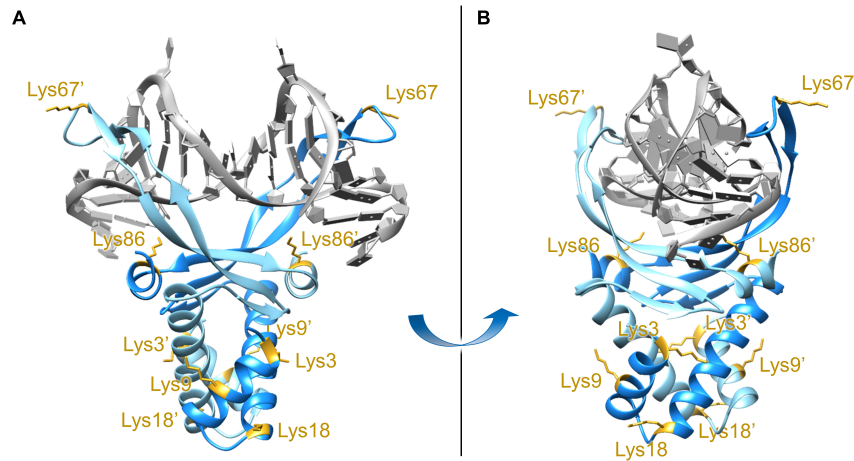


FIGURE 1 | Front view (A) and side view (B) of the DNA-bound *E. coli* HU β_2 homology model. The model was generated using SWISS-MODEL with the amino acid sequence of *E. coli* HU β_2 (Swiss-Prot/TrEMBL accession number P0ACF4) and PDB 1P51 (*Anabaena* HU) as the template. Lysine residues at position 3, 9, 18, 67, and 86 have been reported to be acetylated *in vivo* (Castaño-Cerezo et al., 2014) and are annotated in the two subunits. Lys67 and Lys86, located in the DNA-binding β -ribbon arms and interface, respectively, were chosen for further investigation.

pair (Neumann et al., 2009) for acetyl lysine incorporation in response to the amber codon, into *E. coli* BL21 AI. During protein expression, the media was supplemented with 5 mM acetyl lysine and 20 mM nicotinamide, a deacetylase inhibitor. Following the same purification procedure, HU β (K67ac) and HU β (K86ac) were obtained and confirmed by mass spectrometry (Figures 2C,D).

Effects of Acetylation on Binding to DNA

We performed the electrophoretic mobility shift assay to analyze DNA-binding capability of different *E. coli* HU dimers. In this assay, the protein is incubated with a DNA fragment. The mixture is then analyzed by electrophoresis to separate free DNA from DNA-protein complexes, which have reduced mobility in the gel.

We used a 30-bp double-stranded DNA fragment containing a 2-nt gap as the model substrate (Figure 3A). This DNA fragment was previously demonstrated to be a preferred substrate of *E. coli* HU over a fully complementary double-stranded DNA molecule (Castaing et al., 1995). In the assays, 1 μ M of the DNA fragment was incubated with different concentrations of HU dimers. Free DNA and HU-DNA complexes were resolved in polyacrylamide gel electrophoresis (Figure 4). We attributed the band migrating around the 100-bp DNA marker as a 1:1 HU dimer and DNA complex. The DNA-binding affinity was characterized by the amount of free, unbound DNA remaining in each condition. This was calculated by setting the intensity of the DNA only sample as the reference to quantify the relative amount of free DNA in the reactions containing HU dimers.

Complexes of the 2-nt gap DNA with HU β (K67ac) $_2$ and HU β (K86ac) $_2$ migrated slightly faster than that of HU β_2 , likely due to the presence of fewer positive charges in the acetylated proteins. While HU β (K67ac) $_2$ and HU β_2 displayed very similar DNA binding profiles (Supplementary Figure 2A), acetylation at Lys86 reduced the DNA binding affinity significantly ($p < 0.05$) as more than fourfold free DNA was observed in the presence of 4 μ M HU dimer. As HU $\alpha\beta$ heterodimer is the major species in

exponential and stationary phases of *E. coli* (Claret and Rouviere-Yaniv, 1997), we also reconstituted heterodimers of HU $\alpha\beta$, HU $\alpha\beta$ (K67ac), and HU $\alpha\beta$ (K86ac). All heterodimers showed higher affinity to the 2-nt gap DNA (Supplementary Figure 2B) compared to the homodimer (Figure 4A and Supplementary Figure 3), in agreement with previous reports (Castaing et al., 1995; Pinson et al., 1999). Intriguingly, acetylation at neither Lys67 nor Lys86 of HU β had a significant impact ($p > 0.15$) on DNA binding by the heterodimers.

To further investigate how acetylation at Lys86 of HU β affects binding with different types of DNA, a 30-bp double-stranded DNA containing a nicked phosphate backbone (Figure 3B) or the intact, fully complementary fragment (Figure 3C) was used as the substrate in the electrophoretic mobility shift assay. For the nicked DNA, again, no significant difference was observed between HU $\alpha\beta$ and HU $\alpha\beta$ (K86ac), while HU β (K86ac) had a lower affinity to the nicked DNA than that of HU β_2 (Figure 4B). For the fully complementary DNA, acetylation at Lys86 of HU β seemed to have no effect on DNA binding by either the hetero- or homodimer (Figure 4C). A similar effect was observed when a second 30 bp fully complementary DNA fragment with an alternative sequence was tested (Supplementary Figure 4).

Previously, we used long double-stranded DNA fragments (>3000 bp) from restriction digestion of plasmids as the substrates in electrophoretic mobility shift assays to investigate the effects of acetylation at Lys13 of *A. baumannii* HU (Liao et al., 2017). Thus, we wondered if increasing the length of fully complementary DNA from 30 bp would make any difference, and linear DNA fragments around 1300, 2700, and 5500 bp were employed as the substrates in electrophoretic mobility shift assays and analyzed by agarose gel electrophoresis (Figure 4D and Supplementary Figure 5). Again, the mobility profiles of HU β_2 and HU β (K67ac) $_2$ were similar, whereas more smeared bands were observed with samples incubated with HU β (K86ac) $_2$.

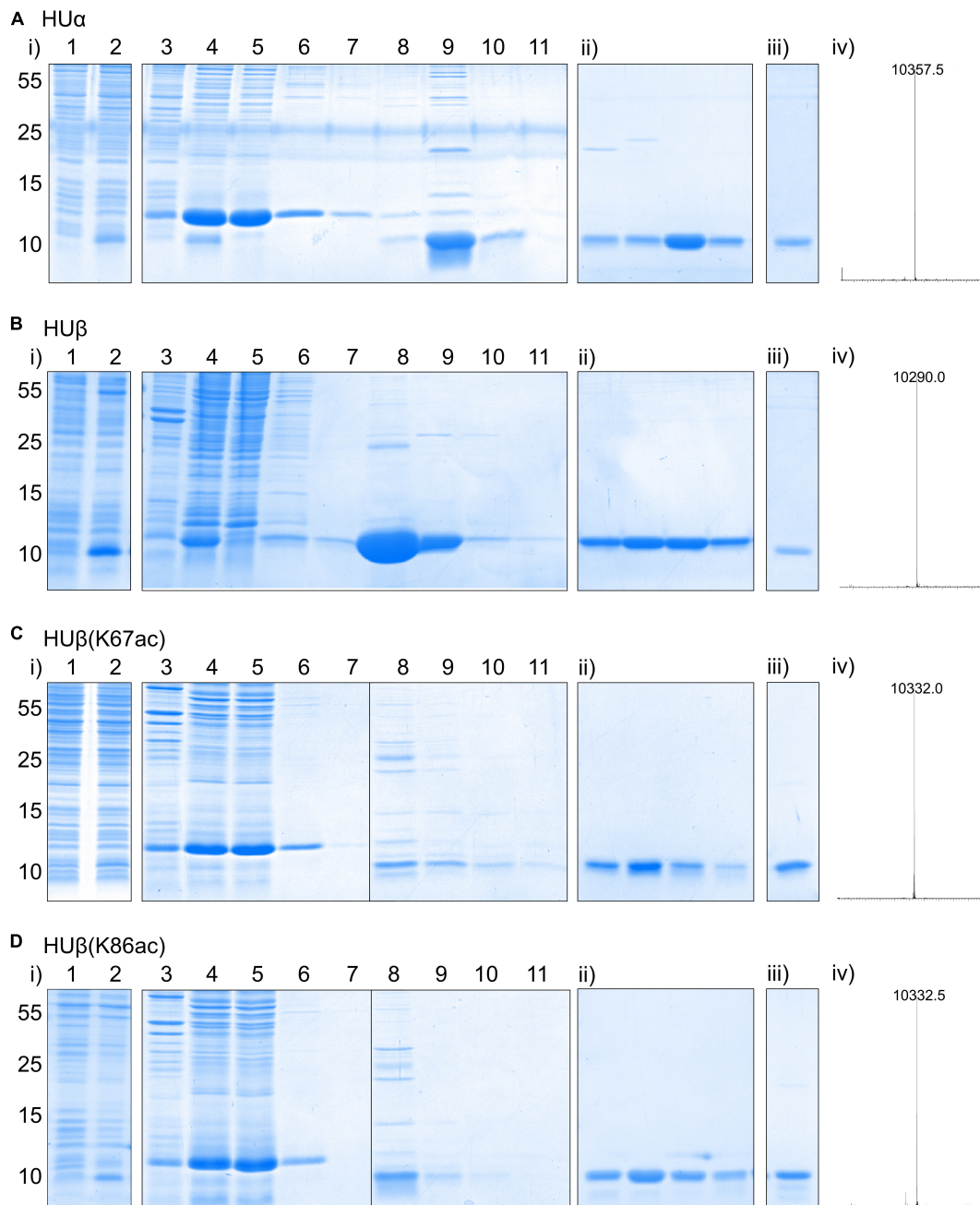
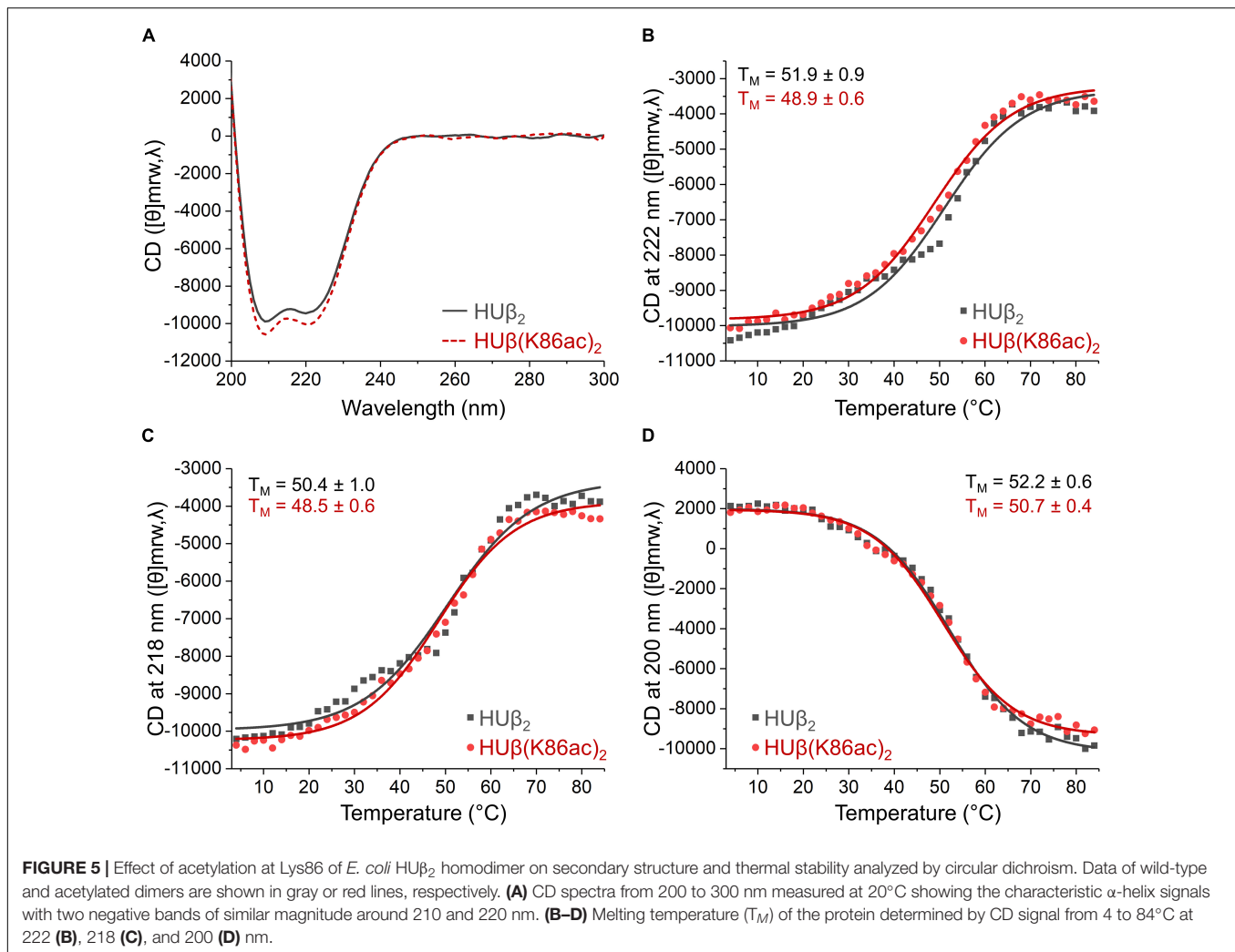


FIGURE 2 | Expression and purification of recombinant *E. coli* HU α (A), HU β (B), HU β (K67ac) (C), and HU β (K86ac) (D) from *E. coli* BL21-AI. See section "Materials and Methods" for the detailed procedure. In each panel, expression and nickel affinity purification results are shown in (i) with lane 1 = whole cell lysate before protein induction, lane 2 = whole cell lysate after protein induction for 16 h, lane 3 = insoluble fraction of cell lysate, lane 4 = soluble fraction of cell lysate, lane 5 = flow through from nickel affinity column, lane 6 = flow through from column wash fraction 1, lane 7 = flow through from column wash fraction 2, lane 8 = eluted protein fraction 1, lane 9 = eluted protein fraction 2, lane 10 = eluted protein fraction 3, lane 11 = eluted protein fraction 4. For (ii), purity of four protein fractions eluted from cation exchange chromatography using a NaCl gradient and absorption at 214 nm for detection was analyzed by SDS-PAGE. Results of the pooled purified protein for further studies analyzed by SDS-PAGE and mass spectrometry are shown in (iii) and (iv), respectively. Molecular weights are shown in Dalton (Da), and theoretical values for HU α , HU β , HU β (K67ac), and HU β (K86ac) are 10,357.5, 10,290.7, 10,332.7, and 10,332.7 Da, respectively. Deconvoluted ESI MS spectra are shown in the range of 5–15 kDa. The full-size spectra can be found in **Supplementary Figure 1**.

Surprisingly, the most mobile end of the smear was in line with free DNA, and the band extended past the most mobility-restricted DNA in the HU β_2 - or HU β (K67ac) $_2$ -containing

samples. A similar effect was observed when the HU variants were incubated with a 420 bp DNA fragment (**Supplementary Figure 4C**). These data suggest a greater level of retardation of



binding capacity is reduced, as it is in HU β_2 , will be critical for determining the extent of acetylation as a mechanism for modulating the DNA binding activity of HU *in vivo*, as HU α_2 predominates in the exponential phase and HU $\alpha\beta$ accounts for the majority of HU dimers in the stationary phase (Claret and Rouviere-Yaniv, 1997).

In assays with longer double-stranded DNA molecules (i.e., 1300, 3800, or 5500 bp), DNA incubated with HU β (K86ac) $_2$ consistently displayed decreased migration in the agarose gels and increased smearing, suggesting more HU dimers were bound to the DNA. Increased smearing may also highlight a difference in DNA bending or conformation, resulting in less uniformity of DNA fragments in the reactions and a more smeared band appearance. One plausible explanation for the stronger binding by HU β (K86ac) $_2$ is that long DNA molecules may have different interactions with HU β homodimers compared to 30-bp DNA fragments; it has been documented that interaction of HU with DNA varies depending on DNA length (Swinger et al., 2003; Hammel et al., 2016). Alternatively, long DNA molecules may have local secondary structures favored by HU β (K86ac) $_2$.

Overall, acetylation at Lys67 of HU β did not seem to affect DNA binding. The results were initially surprising as the residue is located in the β -arms that interact directly with DNA. However, Lys67 is not highly conserved (Grove and Saavedra, 2002; Kamashev et al., 2017); the negatively charged glutamic acid is often present at this position, suggesting that alteration to the charge of this side chain may not be critical to protein function. In contrast, Lys86 is highly conserved and has been reported as a key residue involved in HU-DNA interactions across bacterial species (Grove and Saavedra, 2002; Bhowmick et al., 2014; Kamashev et al., 2017). Our results bolster this assertion while also demonstrating that acetylation of this residue may be used to modulate DNA-binding capacity.

Neutralization of the positive charge at Lys86 can also be achieved by amino acid substitution. Indeed, replacement of Lys86 with alanine reduced the DNA binding capability of *E. coli* HU $\alpha\beta$ (Thakur et al., 2021). A similar effect was found with *Bacillus subtilis* HU, where K86A mutation reduced the DNA binding capacity to 20% of the wild-type protein (Köhler and Marahiel, 1998). In a HU homolog, K86Q also reduced the DNA binding affinity (Grove and Saavedra, 2002). Lys86, among other

residues, was also a target in the design of an effective inhibitor, which prevented interaction with DNA and reduced binding affinity of *M. tuberculosis* HU (Bhowmick et al., 2014).

In vivo multiple residues may be modified simultaneously. Therefore, Lys67 and Lys86 may be acetylated at the same time, giving a compounded effect on protein properties. Indeed, Thakur et al. (2021) investigated modifications of *E. coli* HU β and found when either Lys83 or Lys86 was replaced with alanine, or when the DNA-binding β -arms were deleted, HU could still bind DNA albeit with reduced affinity. However, when one residue was replaced with alanine and the DNA binding loop was deleted, no DNA binding capacity remained (Thakur et al., 2021). In this case, compromise of either site was tolerated but simultaneous modification of both sites rendered HU unable to bind DNA. Hence, it is possible that acetylation of both Lys67 and Lys86 may have a more pronounced effect on HU-DNA interaction.

HU is a truly versatile protein serving many different and sometimes contrasting functions in cells (Dame and Goosen, 2002; Grove, 2011; Stojkova et al., 2019). HU exhibits a minimum of two binding modes (Swinger et al., 2003; Koh et al., 2011; Hammel et al., 2016). For short, structurally deformed DNA fragments, HU binds the DNA with its arms and bends the DNA around its body. For native DNA, HU mainly interacts through its α -helical body and pairs of dimers interact across the DNA strand. Thus, it seems possible that the effect of acetylation may also have contrasting consequences depending on the type of DNA being interacted with and may therefore influence cellular processes in different ways. For example, acetylation of the protein could also be a mechanism of controlling the local concentration of HU in the cell. Deacetylating HU protein would increase the concentration in the cytosol by decreasing affinity for genomic and plasmid DNA, making more HU available to bind DNA damage repair intermediates. Clearly, considerably more work is needed to unveil the molecular basis of the varying interactions of HU with different types of DNA.

This work has provided a glimpse into how site-specific post-translational modification of *E. coli* HU β may regulate protein function. We have also demonstrated how genetic code expansion can be useful for studying site-specific post-translational modification (Chen and Tsai, 2021). There is of course considerably more to be done to uncover the effect of post-translational modification of HU from other bacterial species and to determine whether these modifications are involved in transcriptional regulation to modulate bacterial phenotypes, such as growth, pathogenicity and drug resistance. The *in vivo* effects of site-specific lysine acetylation can theoretically be studied through genetic incorporation of non-hydrolyzable acetyl lysine

analogs (Venkat et al., 2017; Zhang et al., 2018) although we have had very limited success in this approach. Alternatively, one can perform site-specific incorporation of acetyl lysine in deacetylase-knockout strains to obtain stoichiometrically acetylated proteins *in vivo* for functional studies. Unraveling the biological functions of molecular modifications deepens our understanding of the delicate controls regulating cellular physiology and can potentially uncover new antimicrobial targets.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Original files of CD data have been deposited to the Cardiff University data catalog at <http://doi.org/10.17035/d.2021.0143930703>. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

VB and Y-HT: conceptualization, formal analysis, methodology, and manuscript preparation. VB: data curation and investigation. Y-HT: funding acquisition, project administration, resources, and supervision. Both authors contributed to the article and approved the submitted version.

FUNDING

We were grateful for the financial support from the Engineering and Physical Sciences Research Council (2091711; Studentship to VB).

ACKNOWLEDGMENTS

We thank Sanjay Patel for the initial works on this project. We also thank Alexander Nödling and Ashish Radadiya for synthesizing acetyl lysine.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2021.809030/full#supplementary-material>

REFERENCES

- Agapova, Y. K., Altukhov, D. A., Timofeev, V. I., Stroylov, V. S., Mityanov, V. S., Korzhenevskiy, D. A., et al. (2020). Structure-based inhibitors targeting the alpha-helical domain of the *Spiroplasma melliferum* histone-like HU protein. *Sci. Rep.* 10:15128. doi: 10.1038/s41598-020-72113-4
- Alberti-Segui, C., Arndt, A., Cugini, C., Priyadarshini, R., and Davey, M. E. (2010). HU protein affects transcription of surface polysaccharide synthesis genes in *Porphyrromonas gingivalis*. *J. Bacteriol.* 192, 6217–6229. doi: 10.1128/JB.00106-10
- Bartels, F., Fernández, S., Holtel, A., Timmis, K. N., and de Lorenzo, V. C. (2001). The essential HupB and HupN proteins of *Pseudomonas putida* provide redundant and nonspecific DNA-bending functions. *J. Biol. Chem.* 276, 16641–16648. doi: 10.1074/jbc.M011295200
- Becker, N. A., Kahn, J. D., and James Maher, L. (2005). Bacterial repression loops require enhanced DNA flexibility. *J. Mol. Biol.* 349, 716–730. doi: 10.1016/j.jmb.2005.04.035

- Bensaid, A., Almeida, A., Drlica, K., and Rouviere-Yaniv, J. (1996). Cross-talk between topoisomerase I and HU in *Escherichia coli*. *J. Mol. Biol.* 256, 292–300. doi: 10.1006/jmbi.1996.0086
- Bhowmick, T., Ghosh, S., Dixit, K., Ganesan, V., Ramagopal, U. A., Dey, D., et al. (2014). Targeting *Mycobacterium tuberculosis* nucleoid-associated protein HU with structure-based inhibitors. *Nat. Commun.* 5:4124. doi: 10.1038/ncomms5124
- Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., et al. (2014). SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res.* 42, W252–W258.
- Bienert, S., Waterhouse, A., de Beer, T. A. P., Tauriello, G., Studer, G., Bordoli, L., et al. (2016). The SWISS-MODEL Repository—new features and functionality. *Nucleic Acids Res.* 45, D313–D319. doi: 10.1093/nar/gkw1132
- Broyles, S. S., and Pettijohn, D. E. (1986). Interaction of the *Escherichia coli* HU protein with DNA: evidence for formation of nucleosome-like structures with altered DNA helical pitch. *J. Mol. Biol.* 187, 47–60. doi: 10.1016/0022-2836(86)90405-5
- Castaing, B., Zelwer, C., Laval, J., and Boiteux, S. (1995). HU Protein of *Escherichia coli* binds specifically to DNA that contains single-strand breaks or gaps. *J. Biol. Chem.* 270, 10291–10296.
- Castano-Cerezo, S., Bernal, V., Post, H., Fuhrer, T., Cappadona, S., Sánchez-Díaz, N. C., et al. (2014). Protein acetylation affects acetate metabolism, motility and acid stress response in *Escherichia coli*. *Mol. Syst. Biol.* 10:762. doi: 10.15252/msb.20145227
- Chen, H., Venkat, S., McGuire, P., Gan, Q., and Fan, C. (2018). Recent development of genetic code expansion for posttranslational modification studies. *Molecules* 23:1662. doi: 10.3390/molecules23071662
- Chen, J., and Tsai, Y.-H. (2021). Applications of genetic code expansion in studying protein post-translational modification. *J. Mol. Biol.* doi: 10.1016/j.jmb.2021.167424
- Claret, L., and Rouviere-Yaniv, J. (1997). Variation in HU composition during growth of *Escherichia coli*: the heterodimer is required for long term survival. *J. Mol. Biol.* 273, 93–104. doi: 10.1006/jmbi.1997.1310
- Dame, R. T., and Goosen, N. (2002). HU: promoting or counteracting DNA compaction? *FEBS Lett.* 529, 151–156. doi: 10.1016/s0014-5793(02)03363-x
- Ghosh, S., Padmanabhan, B., Anand, C., and Nagaraja, V. (2016). Lysine acetylation of the *Mycobacterium tuberculosis* HU protein modulates its DNA binding and genome organization. *Mol. Microbiol.* 100, 577–588. doi: 10.1111/mmi.13339
- Grove, A. (2011). Functional evolution of bacterial histone-like HU proteins. *Curr. Issues Mol. Biol.* 13, 1–12.
- Grove, A., and Saavedra, T. C. (2002). The role of surface-exposed lysines in wrapping DNA about the bacterial histone-like protein HU. *Biochemistry* 41, 7597–7603. doi: 10.1021/bi016095e
- Guo, F., and Adhya, S. (2007). Spiral structure of *Escherichia coli* HU $\alpha\beta$ provides foundation for DNA supercoiling. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4309–4314.
- Hammel, M., Amlanjyoti, D., Reyes, F. E., Chen, J.-H., Parpana, R., Tang, H. Y. H., et al. (2016). HU multimerization shift controls nucleoid compaction. *Sci. Adv.* 2:e1600650. doi: 10.1126/sciadv.1600650
- Huang, L., Zhang, Z., and McMacken, R. (2021). Interaction of the *Escherichia coli* HU protein with various topological forms of DNA. *Biomolecules* 11:1724. doi: 10.3390/biom11111724
- Kamashev, D., Agapova, Y., Rastorguev, S., Talyzina, A. A., Boyko, K. M., Korzhenevskiy, D. A., et al. (2017). Comparison of histone-like HU protein DNA-binding properties and HU/IHF protein sequence alignment. *PLoS One* 12:e0188037. doi: 10.1371/journal.pone.0188037
- Kamashev, D., and Rouviere-Yaniv, J. (2000). The histone-like protein HU binds specifically to DNA recombination and repair intermediates. *EMBO J.* 19, 6527–6535. doi: 10.1093/emboj/19.23.6527
- Kar, S., Choi, E. J., Guo, F., Dimitriadis, E. K., Kotova, S. L., and Adhya, S. (2006). Right-handed DNA supercoiling by an octameric form of histone-like protein HU: MODULATION OF CELLULAR TRANSCRIPTION. *J. Biol. Chem.* 281, 40144–40153. doi: 10.1074/jbc.M605576200
- Koh, J., Shkel, I., Saecker, R. M., and Record, M. T. (2011). Nonspecific DNA binding and bending by HU $\alpha\beta$: interfaces of the three binding modes characterized by salt-dependent thermodynamics. *J. Mol. Biol.* 410, 241–267. doi: 10.1016/j.jmb.2011.04.001
- Köhler, P., and Marahiel, M. A. (1998). Mutational analysis of the nucleoid-associated protein HBSu of *Bacillus subtilis*. *Mol. Gen. Genet. MGG* 260, 487–491. doi: 10.1007/s004380050921
- Koli, P., Sudan, S., Fitzgerald, D., Adhya, S., Kar, S., and Finlay, B. B. (2011). Conversion of commensal *Escherichia coli* K-12 to an invasive form via expression of a mutant histone-like protein. *mBio* 2:e00182-11. doi: 10.1128/mBio.00182-11
- Liao, J.-H., Tsai, C.-H., Patel, S. G., Yang, J.-T., Tu, I.-F., Lo Cicero, M., et al. (2017). Acetylome of *Acinetobacter baumannii* SK17 reveals a highly-conserved modification of histone-like protein HU. *Front. Mol. Biosci.* 4:77. doi: 10.3389/fmolb.2017.00077
- Liu, D., Yumoto, H., Murakami, K., Hirota, K., Ono, T., Nagamune, H., et al. (2008). The essentiality and involvement of *Streptococcus intermedius* histone-like DNA-binding protein in bacterial viability and normal growth. *Mol. Microbiol.* 68, 1268–1282. doi: 10.1111/j.1365-2958.2008.06232.x
- Mangan, M. W., Lucchini, S., Ó Cróinín, T., Fitzgerald, S., Hinton, J. C. D., and Dorman, C. J. (2011). Nucleoid-associated protein HU controls three regulons that coordinate virulence, response to stress and general physiology in *Salmonella enterica* serovar Typhimurium. *Microbiology* 157, 1075–1087. doi: 10.1099/mic.0.046359-0
- Micka, B., and Marahiel, M. A. (1992). The DNA-binding protein HBSu is essential for normal growth and development in *Bacillus subtilis*. *Biochimie* 74, 641–650. doi: 10.1016/0300-9084(92)90136-3
- Neumann, H., Hancock, S. M., Buning, R., Routh, A., Chapman, L., Somers, J., et al. (2009). A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. *Mol. Cell* 36, 153–163. doi: 10.1016/j.molcel.2009.07.027
- Nguyen, H. H., De La Tour, C. B., Touelle, M., Vannier, F., Sommer, S., and Servant, P. (2009). The essential histone-like protein HU plays a major role in *Deinococcus radiodurans* nucleoid compaction. *Mol. Microbiol.* 73, 240–252. doi: 10.1111/j.1365-2958.2009.06766.x
- Nödling, A. R., Spear, L. A., Williams, T. L., Luk, L. Y. P., and Tsai, Y.-H. (2019). Using genetically incorporated unnatural amino acids to control protein functions in mammalian cells. *Essays Biochem.* 63, 237–266. doi: 10.1042/EBC20180042
- Oberto, J., Nabti, S., Jooste, V., Mignot, H., and Rouviere-Yaniv, J. (2009). The HU regulon is composed of genes responding to anaerobiosis, acid stress, high osmolarity and SOS induction. *PLoS One* 4:e4367. doi: 10.1371/journal.pone.0004367
- Perry, A. C. F., Wakayama, T., Kishikawa, H., Kasai, T., Okabe, M., Toyoda, Y., et al. (1999). Mammalian transgenesis by intracytoplasmic sperm injection. *Science* 284, 1180–1183. doi: 10.1126/science.284.5417.1180
- Petersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., et al. (2004). UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.* 25, 1605–1612. doi: 10.1002/jcc.20084
- Phan, N. Q., Uebanso, T., Shimohata, T., Nakahashi, M., Mawatari, K., Takahashi, A., et al. (2015). DNA-binding protein HU coordinates pathogenicity in *Vibrio parahaemolyticus*. *J. Bacteriol.* 197, 2958–2964. doi: 10.1128/JB.00306-15
- Pinson, V., Takahashi, M., and Rouviere-Yaniv, J. (1999). Differential binding of the *Escherichia coli* HU, homodimeric forms and heterodimeric form to linear, gapped and cruciform DNA. *J. Mol. Biol.* 287, 485–497. doi: 10.1006/jmbi.1999.2631
- Prieto, A. I., Kahramanoglou, C., Ali, R. M., Fraser, G. M., Seshasayee, A. S. N., and Luscombe, N. M. (2011). Genomic analysis of DNA binding and gene regulation by homologous nucleoid-associated proteins IHF and HU in *Escherichia coli* K12. *Nucleic Acids Res.* 40, 3524–3537. doi: 10.1093/nar/gkr1236
- Ramstein, J., Hervouet, N., Coste, F., Zelwer, C., Oberto, J., and Castaing, B. (2003). Evidence of a thermal unfolding dimeric intermediate for the *Escherichia coli* histone-like HU proteins: thermodynamics and structure. *J. Mol. Biol.* 331, 101–121. doi: 10.1016/s0022-2836(03)00725-3
- Rice, P. A., Yang, S.-W., Mizuuchi, K., and Nash, H. A. (1996). Crystal structure of an IHF-DNA complex: a protein-induced DNA U-turn. *Cell* 87, 1295–1306. doi: 10.1016/s0092-8674(00)81824-3
- Sakatos, A., Babunovic, G. H., Chase, M. R., Dills, A., Leszyk, J., Rosebrock, T., et al. (2018). Posttranslational modification of a histone-like protein regulates

- phenotypic resistance to isoniazid in mycobacteria. *Sci. Adv.* 4:eao1478. doi: 10.1126/sciadv.aao1478
- Stojkova, P., Spidlova, P., and Stulik, J. (2019). Nucleoid-associated protein HU: a lilliputian in gene regulation of bacterial virulence. *Front. Cell. Infect. Microbiol.* 9:159. doi: 10.3389/fcimb.2019.00159
- Stojkova, P., Spidlova, P., Lenco, J., Rehulkova, H., Kratka, L., and Stulik, J. (2018). HU protein is involved in intracellular growth and full virulence of *Francisella tularensis*. *Virulence* 9, 754–770. doi: 10.1080/21505594.2018.1441588
- Swinger, K. K., and Rice, P. A. (2004). IHF and HU: flexible architects of bent DNA. *Curr. Opin. Struct. Biol.* 14, 28–35. doi: 10.1016/j.sbi.2003.12.003
- Swinger, K. K., Lemberg, K. M., Zhang, Y., and Rice, P. A. (2003). Flexible DNA bending in HU–DNA cocystal structures. *EMBO J.* 22, 3749–3760. doi: 10.1093/emboj/cdg351
- Thakur, B., Arora, K., Gupta, A., and Guptasarma, P. (2021). The DNA-binding protein HU is a molecular glue that attaches bacteria to extracellular DNA in biofilms. *J. Biol. Chem.* 296:100532. doi: 10.1016/j.jbc.2021.100532
- Venkat, S., Nannapaneni, D. T., Gregory, C., Gan, Q., McIntosh, M., and Fan, C. (2017). Genetically encoding thioacetyl-lysine as a non-deacetylable analog of lysine acetylation in *Escherichia coli*. *FEBS Open Bio* 7, 1805–1814. doi: 10.1002/2211-5463.12320
- Wang, L., Xiao, J., Cui, S., Wang, Q., Wu, H., Liu, Q., et al. (2014). HU-induced polymorphous filamentation in fish pathogen *Edwardsiella tarda* leading to reduced invasion and virulence in zebrafish. *Vet. Microbiol.* 171, 165–174. doi: 10.1016/j.vetmic.2014.03.030
- Wang, W., Li, G.-W., Chen, C., Xie, X. S., and Zhuang, X. (2011). Chromosome organization by a nucleoid-associated protein in live bacteria. *Science* 333, 1445–1449. doi: 10.1126/science.1204697
- Zhang, F., Zhou, Q., Yang, G., An, L., Li, F., and Wang, J. (2018). A genetically encoded 19F NMR probe for lysine acetylation. *Chem. Comm.* 54, 3879–3882. doi: 10.1039/c7cc09825a
- Zhang, K., Zheng, S., Yang, J. S., Chen, Y., and Cheng, Z. (2013). Comprehensive profiling of protein lysine acetylation in *Escherichia coli*. *J. Proteome Res.* 12, 844–851. doi: 10.1021/pr300912q

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Barlow and Tsai. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.