A specific single-stranded DNA induces a distinct conformational change in the nucleoid-associated protein HU

Yuya Nishida\textsuperscript{a,b,*}, Teppei Ikeya\textsuperscript{c,d}, Tsutomu Mikawa\textsuperscript{a,c,e}, Jin Inoue\textsuperscript{c,d}, Yutaka Ito\textsuperscript{c,d}, Yasunori Shintani\textsuperscript{b}, Ryoji Masui\textsuperscript{a}\textsuperscript{f,g}, Seiki Kuramitsu\textsuperscript{a}\textsuperscript{u,g}, Seiji Takashima\textsuperscript{a}\textsuperscript{b,***}

\textsuperscript{a} Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita-shi, Osaka 565-0871, Japan
\textsuperscript{b} Department of Medical Biochemistry, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita-shi, Osaka 565-0871, Japan
\textsuperscript{c} Department of Chemistry, Graduate School of Science and Engineering, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji-shi, Tokyo 192-0373, Japan
\textsuperscript{d} CREST, JST, Saitama 332-0012, Japan
\textsuperscript{e} Division of Biology and Geosciences, Graduate School of Science, Osaka City University, 3-3-138 Sagimotou, Sumiyoshi-ku, Osaka-shi 558-8585, Japan
\textsuperscript{f} Laboratory for Biomolecular Structure and Dynamics, Cell Dynamics Research Core, RIKEN Quantitative Biology Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan
\textsuperscript{g} Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka-shi, Osaka 560-0043, Japan

A specific single-stranded DNA induces a distinct conformational change in the nucleoid-associated protein HU

Yuya Nishida\textsuperscript{a,b,*}, Teppei Ikeya\textsuperscript{c,d}, Tsutomu Mikawa\textsuperscript{a,c,e}, Jin Inoue\textsuperscript{c,d}, Yutaka Ito\textsuperscript{c,d}, Yasunori Shintani\textsuperscript{b}, Ryoji Masui\textsuperscript{a}\textsuperscript{f,g}, Seiki Kuramitsu\textsuperscript{a}\textsuperscript{u,g}, Seiji Takashima\textsuperscript{a}\textsuperscript{b,***}

\textsuperscript{a} Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita-shi, Osaka 565-0871, Japan
\textsuperscript{b} Department of Medical Biochemistry, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita-shi, Osaka 565-0871, Japan
\textsuperscript{c} Department of Chemistry, Graduate School of Science and Engineering, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji-shi, Tokyo 192-0373, Japan
\textsuperscript{d} CREST, JST, Saitama 332-0012, Japan
\textsuperscript{e} Division of Biology and Geosciences, Graduate School of Science, Osaka City University, 3-3-138 Sagimotou, Sumiyoshi-ku, Osaka-shi 558-8585, Japan
\textsuperscript{f} Laboratory for Biomolecular Structure and Dynamics, Cell Dynamics Research Core, RIKEN Quantitative Biology Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan
\textsuperscript{g} Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka-shi, Osaka 560-0043, Japan

ARTICLE INFO

Keywords:
- HU
- Nucleoid-associated protein
- Single-stranded DNA
- NMR
- Circular dichroism
- Thermus

ABSTRACT

In prokaryotic cells, genomic DNA forms an aggregated structure with various nucleoid-associated proteins (NAPs). The functions of genomic DNA are cooperatively modulated by NAPs, of which HU is considered to be one of the most important. HU binds double-stranded DNA (dsDNA) and serves as a structural modulator in the genome architecture. It plays important roles in diverse DNA functions, including replication, segregation, transcription and repair. Interestingly, it has been reported that HU also binds single-stranded DNA (ssDNA) regardless of sequence. However, structural analysis of HU with ssDNA has been lacking, and the functional relevance of this binding remains elusive.

In this study, we found that ssDNA induced a significant change in the secondary structure of Thermus thermophilus HU (TtHU), as observed by analysis of circular dichroism spectra. Notably, this change in secondary structure was sequence specific, because the complementary ssDNA or dsDNA did not induce the change. Structural analysis using nuclear magnetic resonance confirmed that TtHU and this ssDNA formed a unique structure, which was different from the previously reported structure of HU in complex with dsDNA. Our data suggest that TtHU undergoes a distinct structural change when it associates with ssDNA of a specific sequence and subsequently exerts a yet-to-be-defined function.

1. Introduction

In prokaryotic cells, genomic DNA forms an aggregated structure with various nucleoid-associated proteins (NAPs) [1]. NAPs have varied structures and hence diverse functions [2,3]. The functions of genomic DNA, such as replication, segregation, translation and repair, are related to its distinct structure, which is cooperatively modulated by NAPs [4–7].

HU (H protein from Escherichia coli U93) is the most conserved and the most abundantly expressed NAP [8–10]. In some bacteria, mutation in the HU gene and gene disruption of HU affect cell growth or is lethal [11,12]. These results have suggested that HU has a central role among NAPs. HU is a small protein consisting of approximately 90 amino acid residues and mainly exists as a dimer in solution [13]. It has been reported that the interaction between double-stranded DNA (dsDNA) and HU is non-specific [14,15]. The binding of HU leads to a bent and a negative supercoiling in the dsDNA structure [16,17]. Some structures of HU alone and in complex with dsDNA have been

---

**Abbreviations:** NAP, nucleoid-associated protein; TtHU, Thermus thermophilus HU; CD, circular dichroism; NMR, nuclear magnetic resonance; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; HSQC, heteronuclear single quantum coherence; SLBP, stem-loop binding protein.

* Corresponding author: Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita-shi, Osaka 565-0871, Japan.

** Corresponding author.

E-mail addresses: nishida@medbio.med.osaka-u.ac.jp (Y. Nishida), tikeya@tmu.ac.jp (T. Ikeya), mikawa@rikken.jp (T. Mikawa), jinoue@tmu.ac.jp (J. Inoue), ito-yutaka@tmu.ac.jp (Y. Ito), yshintani@medbio.med.osaka-u.ac.jp (Y. Shintani), rmasui@sci.osaka-cu.ac.jp (R. Masui), kuramitsu@bio.sci.osaka-u.ac.jp (S. Kuramitsu), takasima@cardiology.med.osaka-u.ac.jp (S. Takashima).

http://dx.doi.org/10.1016/j.bbrep.2016.09.014

Received 6 April 2016; Received in revised form 23 August 2016; Accepted 29 September 2016

Available online 11 October 2016

2405-5808/ © 2016 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
determined [18–20] and have shown that HU has two beta-arms and grips the dsDNA by engagement of the arms in the minor groove. HU also serves as an structural modulator of dsDNA architecture and plays important roles in DNA replication, segregation, repair and transcription [11,12,21–25].

Interestingly, it has been reported that HU can also bind single-stranded DNA (ssDNA), and this interaction is also non-specific [15,26,27]. ssDNA intermediates are created by DNA unwinding and serve as template for DNA replication or repair processes. However, little is known of the interaction between HU and ssDNA. Structural information has not been obtained, and the functional relevance of this binding remains elusive.

In this study, we used HU from Thermus thermophilus HB8 (ThU). To characterize the structure of ThU bound to ssDNA, we performed circular dichroism (CD) spectral analysis and nuclear magnetic resonance (NMR) spectral analysis. Our data suggest that ssDNA of a specific sequence induces a significant structural change in the secondary structure of ThU, which is different from the change shown previously in HU bound to dsDNA.

2. Materials and methods

2.1. Materials

The sequences of the chemically synthesized ssDNA (BEX Co., Ltd. or FASMAC Co., Ltd.) are described in Fig. 2L. dsDNA oligo AB and oligo CD were prepared by incubation of ssDNA oligos at 95 °C for 10 min, and the temperature was then decreased at a rate of 1 °C per min to anneal.

2.2. Purification of ThU

E. coli BL21(DE3) was transformed with ThU/pET-11a and grown at 37 °C in LB medium containing 50 µg/mL ampicillin. When the culture reached log phase, IPTG was added to 50 µg/mL. Cells were grown for 12 h after induction and harvested by centrifugation. Cells were suspended in 20 mM Tris–HCl (pH 7.8), 500 mM NaCl and 5 mM EDTA. The cells were disrupted by sonication and then heated at 70 °C for 20 min. After centrifugation at 22,500g for 1 h, the clear supernatant was loaded onto a Toyopearl SP-650 M column (Tosoh) equilibrated with 20 mM Tris–HCl (pH 7.8), 500 mM NaCl and 5 mM EDTA. The column was washed with the buffer and eluted with a gradient of 500–1500 mM NaCl in the buffer. The fractions containing ThU were detected by SDS-PAGE and concentrated using a Vivaspin 20 –10 K (MWCO 10,000 Da, GE healthcare) concentrator. The solution was then applied to a HiLoad 16/60 Superdex 75 column (Tosoh) equilibrated with 20 mM Tris–HCl (pH 7.8) and 2 M NaCl and eluted with the same buffer. Purified proteins were stored in 20 mM Tris–HCl (pH 7.8) and 150 mM NaCl at 4 °C.

2.3. Electrophoretic-mobility shift assay

Chemically synthesized oligo DNAs were incubated with various concentrations of ThU in 20 mM Tris–HCl (pH 7.5) and 100 mM KCl at 37 °C for 1 h. The mixtures were loaded onto a polyacrylamide gel and electrophoresed in TBE buffer (pH 8.2, 89 mM Tris-borate and 2 mM EDTA). The bands were visualized with GelRed (Wako) and UV irradiation.

2.4. Circular dichroism structural analysis

CD spectra were recorded with a JASCO-720W spectropolarimeter, with a 0.1 cm cuvette at 20 °C for 200–300 nm. In the titration analysis, 40 µM of DNA solution was added to 300 µL of 10 µM ThU solution (pH 7.2, 20 mM phosphate and 100 mM KCl). The effect of increasing volume due to titration was calculated after the experiment.

2.5. Purification of ThU for NMR

E. coli Rosetta2(DE3) was transformed with ThU/pET-11a and grown in M9 medium (0.6% Na2HPO4, 0.3% NaH2PO4, 0.05% NaCl, 0.1% NH4Cl, 0.2% glucose, 2 mM MgSO4·7H2O, 0.1 mM CaCl2·2H2O, 33 mM FeCl3·6H2O, and 50 µg/mL ampicillin, pH 7.2) containing [15N]NH4Cl and/or [13C]glucose as the sole nitrogen and/or carbon source for labelled 15N–ThU and/or 13C15N–ThU, respectively. The purification procedure for labelled ThU was the same as that for the unlabelled ThU.

2.6. NMR data collection

The NMR sample contained 1 mM ThU, 20 mM phosphate (pH 7.2) and 100 mM KCl. The NMR spectra were measured at 303 K by using a Bruker Avance III 600 MHz spectrometer equipped with a cryogenic TCI probe head. The sequence-specific backbone 1H N, 15N, 13Cα, 13Cβ, and 15N and side chain 13Cβ resonance assignments of 13C/15N-labelled ThU were obtained from CBCA(CO)NH, CBCANNH, HNCO, and HN(C)CA(CO) experiments [28,29]. Data were processed using CcpNmr Analysis [30].

2.7. NMR structural analysis of ThU’s DNA binding

The two-dimensional heteronuclear single quantum coherence (H2QC) spectra were acquired with 15N-labelled ThU in the presence of various concentrations of oligo A. In this study, a concentrated oligo A solution was added to a 0.1 mM 15N-labelled ThU solution to prevent changes in concentration.

2.8. Construction of a model structure of ThU

The model structure of ThU was constructed on the basis of a previous structure of HU from Staphylococcus aureus (PDB: 4QJU), using ROBETTA (http://robetta.bakerlab.org/).

3. Results

3.1. The effect of ssDNA on the secondary structure of ThU

Although it has previously been reported that HU can bind to ssDNA as well as dsDNA in a sequence-independent manner [14,15], little is known about the interaction between HU and ssDNA. In particular, structural analysis has been lacking.

In this study, we chose 2 sets of complementary ssDNAs that we usually use as controls for gel shift assays in our laboratories: partial oligonucleotides from beta-lactamase (oligos A and B, which are complementary to each other) and from beta-galactosidase (oligos C and D, also complementary). ThU bound to all those 4 ssDNAs as well as to their complementary dsDNAs (Fig. 1), in agreement with earlier reports [14,15,26].

Next, to evaluate the effect of ssDNA binding on the structure of ThU, we performed secondary structural analysis of ThU by using CD spectra. As shown in Fig. 2A (top, black line), the CD spectrum of ThU had a maximal negative signal at approximately 210–220 nm. This result was consistent with the structures of HU determined by X-ray crystallography, which showed that HU consists of an alpha-helix core [19,20]. We sequentially added various DNA solutions to the ThU solution and measured their CD spectra. The CD spectra of DNA without HU showed typical positive peaks at 220 nm and 280 nm and negative peaks at 210 nm and 250 nm (bottom spectra in all parts of Fig. 2). Interestingly, when oligo A was added to the ThU solution, the intensity of the negative peaks at approximately 210–220 nm decreased to one-third of the intensity before DNA addition, as shown in Fig. 2A. In contrast, when the complementary oligo B or double-stranded oligo AB was added, no significant spectral change was observed (Figs. 2B and 2C). Further, oligos C and D and the
double-stranded oligo CD did not cause a similar spectral change (Figs. 2D, 2E and 2F). We also performed the same experiments using 30 nt polydeoxyadenosine (poly dA) and 30 nt polydeoxythymidine (poly dT), which were non-self-structured oligonucleotides, but no significant change was observed (Fig. 2I).

Next, we tested several oligo DNAs with some variations in the sequence of oligo A by CD analysis. Whereas deletion of the 5’- or 3´-terminal stretches resulted in a partial loss of the effect of oligo A (Figs. 2G and 2I), complementary sequence-exchange almost completely cancelled the effect of oligo A (Figs. 2H and 2I), thus suggesting that oligo A has a unique sequence-specific effect on the CD spectrum with TtHU. These results led us to hypothesize that ssDNA of a specific sequence unfolds the secondary structure of TtHU, especially in the alpha-helical core, or induces changes in the conformation of TtHU. It is possible that the CD spectral change observed in this study was caused by the ssDNA. However, previously reported CD spectral changes of DNA due to both conformational changes of DNA and binding of proteins are smaller than the changes observed in this study [31,32].

The decrease in the CD spectrum was observed specifically for oligo A but not for oligo B or oligo AB, though TtHU bound similarly to all of them. Thus, it is unlikely that the decrease in the CD spectrum was caused by the aggregation of TtHU.

3.2. NMR structural analysis

To further investigate the effect of oligo A on the structure of TtHU, we performed NMR spectral analysis. By analysing the three-dimensional NMR spectra using TtHU alone, we successfully achieved sequential assignment for 91% of non-proline residues in TtHU (Fig. 3A). As shown in Fig. 3C, the unassigned residues were clustered on the beta-arms of TtHU, suggesting that those residues were less stable. This result was consistent with those of previous structural analyses showing flexibility of the beta-arms in HU [19,33].

To analyse the effect of oligo A on the structure of TtHU, we acquired the HSQC spectra of TtHU with and without oligo A. The intensity of the HSQC peaks with oligo A was decreased compared with the intensity without oligo A, thus suggesting that oligo A induced a significant change in the structure of TtHU. To identify the amino acid residues important for interaction with ssDNA, we sequentially added oligo A at low concentrations, from 0 µM to 20 µM, and acquired the HSQC spectra. Comparison of the HSQC spectra for different concentrations of oligo A revealed that almost all HSQC peaks for each amino acid residue decreased, but the extent varied. This result indicated that some residues were affected strongly by oligo A binding, whereas others were less affected.

To further elucidate the effect of oligo A on TtHU, the intensity of HSQC peaks after the titration of 10 µM DNA was normalized to the intensity before titration (Fig. 3B). Then, the amino acid residues with the relative intensity of less than 35% were plotted on the structure of TtHU constructed by homology modelling (Fig. 3C). These results show that the residues around the saddle region were strongly affected by oligo A binding. Interestingly, though the beta-arms of TtHU are reportedly important for dsDNA binding, the residues in red were not often observed around the arms in this study. Instead, ssDNA induced a strong effect on residues of the body, far from the beta-arms. These results suggest that TtHU bound to ssDNA of a specific sequence forms a distinct conformation from that of the complex with dsDNA.

4. Discussion

Few studies have focused on the interaction between HU and ssDNA, and no structural information on ssDNA-bound HU has
A specific ssDNA induced a change in the CD spectra of TtHU. CD spectra were observed in titration analyses with various DNAs. The titrant solutions were 40 µM of (A) oligo A, (B) oligo B, (C) oligo AB, (D) oligo C, (E) oligo D, (F) oligo CD, (G) oligo A (01-06) Del, or (H) oligo A (01-06) Com, as indicated above the parts. The sequences of the oligonucleotides are described in (I). The titrand solution was 10 µM HU (top) or buffer (bottom). The spectrum before titration is shown as a dark red line, and the colour is reduced with increasing concentration of DNA from 0 µM to 10 µM. The black line in (A) shows the results before titration. (I) The ratio of the CD value after titration ($\theta_{\text{after}}$) to the CD value before ($\theta_{\text{before}}$). The ratios were calculated by subtracting the value at 222 nm of HU solution as titrand from the CD value of the buffer solution.

### Table: Sequences and Ratios

| Name           | Sequence (5’ to 3’) | $\theta_{\text{after}}/\theta_{\text{before}}$ |
|----------------|--------------------|----------------------------------|
| oligo A (01–06) Del | AGT GCT GCA ATG ATA CCG CGA GAC | 79% |
| oligo A (25–30) Del | GGC CCC AGT GCT GCA ATG ATA CCG | 59% |
| oligo A (28–30) Del | GGC CCC AGT GCT GCA ATG ATA CCG CGA | 30% |
| oligo A (01–06) Com | CCG GGG AGT GCT GCA ATG ATA CCG CGA GAC | 97% |
| oligo A (13–18) Com | GGC CCC AGT CGT TAC ATA CCG CGA GAC | 94% |
| oligo A (25–30) Com | GGC CCC AGT GCT GCA ATG ATA CCG GCT CTG | 87% |
| oligo A (28–30) Com | GGC CCC AGT GCT GCA ATG ATA CCG CTG | 48% |
| oligo A          | GGC CCC AGT GCT GCA ATG ATA CCG CGA GAC | 33% |
| oligo B          | GTC TCG CGG TAT CAT TGC AGC ACT GGG GCC | 94% |
| oligo AB         | (dsDNA annealed oligo A and oligo B) | 98% |
| oligo C          | ATG ACA ACT AAA GCA ACA CCC AAA ACA | 104% |
| oligo D          | TGT TTT GGG TGT TGC TTT AGT TGT CAT | 102% |
| oligo CD         | (dsDNA annealed oligo A and oligo B) | 100% |
| poly dA          | AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA | 100% |
| poly dT          | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT | 102% |

Fig. 2. A specific ssDNA induced a change in the CD spectra of TtHU. CD spectra were observed in titration analyses with various DNAs. The titrant solutions were 40 µM of (A) oligo A, (B) oligo B, (C) oligo AB, (D) oligo C, (E) oligo D, (F) oligo CD, (G) oligo A (01–06) Del, or (H) oligo A (01–06) Com, as indicated above the parts. The sequences of the oligonucleotides are described in (I). The titrand solution was 10 µM HU (top) or buffer (bottom). The spectrum before titration is shown as a dark red line, and the colour is reduced with increasing concentration of DNA from 0 µM to 10 µM. The black line in (A) shows the results before titration. (I) The ratio of the CD value after titration ($\theta_{\text{after}}$) to the CD value before ($\theta_{\text{before}}$). The ratios were calculated by subtracting the value at 222 nm of HU solution as titrand from the CD value of the buffer solution.
previously been reported [15,26,27]. Here, we report that, in addition to the well-known structure with dsDNA, TtHU undergoes a distinct structural change when it associates with ssDNA of a specific sequence.

We supposed that the effect of the ssDNA (oligo A) depends on its structure, as nucleotide deletion did not cause the same effect as nucleotide exchange (for example, oligo A (25–30) Del vs oligo A (25–30) Com). Thus, we predicted the secondary structure of the oligo DNAs by using a program MaxExpect web server [34,35]. As shown in Fig. 4, Oligo A is predicted to have two small stem-loops which are bridged by short and nicked double-stranded DNA (dumbbell shape). Similar structures are also found in the predicted structures of oligo A (28–30) Del and oligo A (30) Del, both of which showed compatible effect on HU as oligo A did. The deletion or exchange in oligo A that lost the effect on HU are predicted to be destructive on the stem-loops in oligo A. For example, oligo A (25–30) Del is predicted to have two stem-loops and retains the effect on HU (59%), although oligo A (25–30) Com, which is predicted to have only a stem-loop and long double-stranded region, has little effect on HU. In the case of the DNAs which partially maintain the effect, the secondary structures might be mixed probably due to instability of the structure. Meanwhile, the DNAs, which did not have any effect on HU (Oligo C, D and poly dA), are predicted to have long single-stranded region which is distinct from the structure predicted for oligo A. Oligo B is predicted to have a similar structure with oligo A, although the loop size is slightly different. Despite of its similarity to oligo A in the predicted structure, the nucleotide base composition of oligo B is different from that of oligo A as it is complementary to oligo A. From these results, we suppose that the combination of structure and sequence differences might contribute to the specificity of the structural change of HU.

HU plays various important roles in DNA replication, segregation, repair and transcription [11,12,21–25], but the molecular details are still unclear. HU binds dsDNA in a sequence-independent manner and induces a bent and a negative supercoiling in the dsDNA structure, leading to the condensed structure of the nucleoid [8,15–17]. It has been suggested that the negative supercoiling induced by HU also resolves the distortion induced by replication and transcription, and thus HU indirectly promotes replication and transcription [4,16,17]. From our study, we presume that HU forms a distinct structure with untwisted ssDNA which forms some unique stem-loop structure with similarity to oligo A, as suggested by our results. In contrast to the indirect supercoiling-mediated effect of HU bound to dsDNA, such structure might directly help replication or transcription properly undergo. Stem-loop binding protein (SLBP) is one of the well-known proteins that recognize the stem-loop with a sequence and structure specificity [36,37]. As SLBP recognizes 26-nt stem-loop structure, our assumption that HU recognizes the unique stem-loop structure seems reasonable.

In conclusion, our data suggest that TtHU undergoes a distinct structural change when it associates with ssDNA of a specific sequence and a specific structure, thereby producing functional diversity. Thus far, structural research on HU has been based on the co-crystal structure of HU bound to dsDNA, and further structural analysis should provide novel insight into the diverse functions of HU with nucleic acids.
Acknowledgements

This research was supported by the Japan Agency for Medical Research and Development, AMED; grants-in-aid from the Ministry of Health, Labour, and Welfare-Japan; grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology-Japan; and grants-in-aid from the Japan Society for the Promotion of Science. This research was also supported by grants from the Takeda Science Foundation, Japan Heart Foundation, Mochida Memorial Foundation, Naito Foundation. We also gratefully acknowledge financial support from the Funding Program for the Core Research for Evolutional Science and Technology, CREST, from the Japan Science and Technology Agency, JST; Grant-in-Aid for the Challenging Exploratory Research (15K14494); Grants-in-Aid for the Scientific Research C (25440032 and 15K06979); and the Scientific Research on Innovative Areas (26102538 and 25120003) from the Japan Society for the Promotion of Science, JSPS. We thank Mr M. H. Dulay, Ms M. Cattus and Ms M. Tsujimura for their generous support.

Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.09.014.

References

[1] J. Kim, S.H. Yoshimura, K. Hirume, R.L. Ohiwa, A. Ishihama, K. Takeyasu, Fundamental structural units of the Escherichia coli nucleoid revealed by atomic force microscopy, Nucleic Acids Res. 32 (2004) 1982–1992. http://dx.doi.org/10.1093/nar/gkh5512.

[2] S.C. Dillon, C.J. Dorman, Bacterial nucleosid-associated proteins, nucleoid structure and gene expression, Nat. Rev. Microbiol. 8 (2010) 185–195. http://dx.doi.org/10.1038/nrmicro2261.

[3] J. Stavans, A. Oppenheim, DNA-protein interactions and bacterial chromosome architecture, Phys. Biol. 3 (2006) R1–10. http://dx.doi.org/10.1088/1478-3975/3/4/R01.

[4] R. Donczew, J. Zakrzeswka-Czerwińska, A. Zawiał-Pawlik, Beyond DnaA: the role of DNA topology and DNA methylation in bacterial replication initiation, J. Mol. Biol. 426 (2014) 2269–2282. http://dx.doi.org/10.1016/j.jmb.2014.04.009.

[5] C.J. Dorman, P. Deighan, Regulation of gene expression by histone-like proteins in bacteria, Curr. Opin. Genet. Dev. 13 (2003) 179–184. http://dx.doi.org/10.1016/S0959-437X(03)00025-X.

[6] D.F. Browning, D.C. Grainger, S.J. Busby, Effects of nucleoid-associated proteins on bacterial chromosome structure and gene expression, Curr. Opin. Microbiol. 13 (2010) 775–780. http://dx.doi.org/10.1016/j.mib.2010.09.013.

[7] C. Collier, C. Machón, G.S. Briggs, P. Soulitas, Unwinding of the DNA helix stimulates the endonuclease activity of Bacillus subtilis Nhe at AP sites, Nucleic Acids Res. 40 (2012) 739–750. http://dx.doi.org/10.1093/nar/gkr785.

[8] J. Rouvière-Yaniv, M. Yaniv, J.E. Germond, E. coli DNA binding protein HU forms nucleosome-like structure with circular double-stranded DNA, Cell 17 (1979) 265–274. http://dx.doi.org/10.1016/0092-8674(79)90152-1.

[9] K. Delica, J. Rouvière-Yaniv, Histonelike proteins of bacteria, Microbiol. Rev. 51 (1987) 301–319. http://dx.doi.org/10.1134/S0003683811060020.

[10] A. Grove, Functional evolution of bacterial histone-like HU proteins, Curr. Issues Mol. Biol. 13 (2011) 1–12 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3484776.

[11] A.M. Dri, J. Rouvière-Yaniv, P.L. Moreau, Inhibition of cell division in hupA hupB mutant bacteria lacking HU protein, J. Bacteriol. 173 (1991) 2852–2861 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC207866/.

[12] O. Huisman, M. Farrel, D. Girard, A. Jaffe, A. Toussaint, J. Rouvière-Yaniv, Multiple defects in Escherichia coli mutants lacking HU protein, J. Bacteriol. 171 (1989) 3704–3712 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC210114/.

[13] K. Giesler, H. Hoffmann-Berling, Proteins controlling the helical structure of DNA, Annu. Rev. Biochem. 50 (1981) 233–260. http://dx.doi.org/10.1146/annurev.bi.50.070181.001313.

[14] A.I. Prieto, C. Kahramanoglu, R.M. Ali, G.M. Fraser, A.S.N. Seshasayee, N.M. Luscombe, Genomic analysis of DNA binding and gene regulation by homologous nucleoid-associated proteins HIF and HU in Escherichia coli K12, Nucleic Acids Res. 40 (2012) 5524–5537. http://dx.doi.org/10.1093/nar/gkr1236.
[15] A.S. Krylov, Massive parallel analysis of the binding specificity of histone-like protein HU to single- and double-stranded DNA with generic oligodeoxyribonucleotide microchips, Nucleic Acids Res. 29 (2001) 2654–2660. http://dx.doi.org/10.1093/nar/29.12.2654.

[16] B. Kundukad, P. Cong, J.R.C. van der Maarel, P.S. Doyle, Time-dependent bending rigidity and helical twist of DNA by rearrangement of bound HU protein, Nucleic Acids Res 41 (2013) 8280–8288. http://dx.doi.org/10.1093/nar/gkt593.

[17] A. Mukherjee, A.O. Sokunbi, A. Grove, DNA protection by histone-like protein HU from the hyperthermophilic eubacterium Thermotoga maritima, Nucleic Acids Res. 36 (2008) 3956–3968. http://dx.doi.org/10.1093/nar/gkn348.

[18] T. Blomswinck, S. Ghosh, K. Dixit, V. Ganesan, U.A. Ramagopal, D. Dey, et al., Targeting Mycobacterium tuberculosis nucleoid-associated protein HU with structure-based inhibitors, Nat. Commun. 5 (2014) 4124. http://dx.doi.org/10.1038/ncomms5124.

[19] K.K. Swinger, K.M. Lemberg, Y. Zhang, P.A. Rice, Flexible DNA bending in HU–DNA octameric structures, EMBO J. 22 (2003) 3749–3760. http://dx.doi.org/10.1093/emboj/dcg351.

[20] I. Tanaka, K. Appelt, J. Dijk, S.W. White, K.S. Wilson, 3-Å resolution structure of a protein with histone-like properties in prokaryotes, Nature 310 (1984) 376–381. http://dx.doi.org/10.1038/310376a0.

[21] S. Rajesh, T. Sakamoto, M. Iwamoto-Sugai, T. Shibata, T. Kohno, Y. Ito, Ubiquitin binding interface mapping on yeast ubiquitin hydrolase by NMR chemical shift perturbation, Biochemistry 38 (1999) 9242–9253. http://dx.doi.org/10.1021/bi9903953.

[22] W.F. Vranken, W. Boucher, T.J. Stevens, R.H. Fogh, A. Pajon, M. Línas, et al., The CCPN data model for NMR spectroscopy: development of a software pipeline, Proteins 59 (2005) 687–696. http://dx.doi.org/10.1002/prot.20449.

[23] K. Nejedlý, J. Chládková, M. Vorlíckov, I. Hrabcová, J. Kypr, Mapping the B-A conformational transition along plasmid DNA, Nucleic Acids Res. 33 (2005) e5. http://dx.doi.org/10.1093/nar/gni008.

[24] V.I. Ivanov, L.E. Minchenkova, G. Burckhardt, E. Birch-Hirschfeld, H. Fritzsche, C. Zimmer, The detection of B-form/A-form junction in a deoxyribonucleotide duplex, Biophys. J. 71 (1996) 3344–3349. http://dx.doi.org/10.1016/S0006-3495(96)78927-9.

[25] H. Vis, M. Mariani, C.E. Vorgias, K.S. Wilson, R. Kaptein, R. Boelens, Solution structure of the HU protein from Bacillus stearothermophilus, J. Mol. Biol. 254 (1995) 692–703. http://dx.doi.org/10.1006/jmbi.1995.0648.

[26] D. Kamashev, A. Balandina, A.K. Mazur, P.B. Arimondo, J. Rouviere-Yaniv, HU loop binding protein, Nucleic Acids Res. 23 (1995) 6541–6552. http://dx.doi.org/10.1093/nar/23.22.6541.

[27] Z.J. Lu, J.W. Gloor, D.H. Mathews, Improved RNA secondary structure prediction by maximizing expected pair accuracy, RNA 15 (2009) 1805–1813. http://dx.doi.org/10.1021/nn903953.

[28] S. Bellaousov, J.S. Reuter, M.G. Seetin, D.H. Mathews, RNAstructure: web servers for RNA secondary structure prediction and analysis, Nucleic Acids Res. 41 (2013) W471–W474. http://dx.doi.org/10.1093/nar/gkt290.

[29] A.S. Williams, W.F. Marzluff, Z. Dominski, L. Tong, Structure of histon mRNA stem-loop, human stem-loop binding protein, and 3′ heExo ternary complex, Science 339 (2013) 318–321. http://dx.doi.org/10.1126/science.12371. http://dx.doi.org/10.1073/pnas.94.23.12366.

[30] A. Holck, K. Kleppe, Affinity of protein HU for different nucleic acids, FEBS Lett. 185 (1985) 121–124. http://dx.doi.org/10.1016/0014-5793(85)80753-5.