Journal of Microbiology (2015) Vol. 53, No. 4, pp. 250–255
DOI 10.1007/s12275-015-4681-8

Effect of promoter-upstream sequence on σ38-dependent stationary phase gene transcription

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(Received Dec 3, 2014 / Revised Jan 15, 2015 / Accepted Jan 26, 2015)

σ38 in *Escherichia coli* is required for expression of a subset of stationary phase genes. However, the promoter elements for σ38-dependent genes are virtually indistinguishable from that for σ70-dependent house-keeping genes. *hdeAB*p is a σ38-dependent promoter and *LEE5*p is a σ70-dependent promoter, but both are repressed by H-NS, a bacterial histone-like protein, which acts at promoter upstream sequence. We swapped the promoter upstream sequences of the two promoters and found that the σ dependency was switched. This was further verified using *lacUV5* core promoter. The results suggested that the determinant for σ38-dependent promoter lies in the promoter upstream sequence.

Keywords: *E. coli*, promoter specificity, RNA polymerase, *rpoS*, σ38

Introduction

The RNA polymerase holoenzyme (E) of *Escherichia coli* is composed of a core enzyme (2α, β, β') associated with one of seven σsubunits, which confers specificity on the holoenzyme for particular promoter sequences. Among the seven σ factors present in *E. coli*, σ70, a major σ factor, primarily controls gene transcription during the exponential phase of growth. The rest of σ factors, minor σ factors, regulate the expression of the subset of genes in response to various environmental stresses (Gross *et al.*, 1998; Ishihama, 2000). Thus, each minor σ factors should recognize and bind to different consensus promoter elements, -10 and -35 elements with the spacing between two elements, for selective gene transcription. Amongst minor σ factors, there is σ38, encoded by *rpoS* gene, responsible for the expression of a subset of stationary phase genes including those that confer resistance to various environmental stresses, such as nutrient starvation, heat, high salt, H_2O_2, UV irradiation, and others presumably to prolong survival during the non-growing stages of bacterial life (Battesti *et al.*, 2011). Most interestingly, however, the promoter elements for σ38-dependent genes are virtually indistinguishable from that for σ70-dependent house-keeping genes (Typas and Hengge, 2006). Moreover, σ38 reveals a high degree of amino acid sequence similarity with the σ70, especially in regions 2.4 and 4.2 that interact with the -10 and -35 promoter elements, respectively. Several different models have been proposed for σ38-specific gene transcription (Landini *et al.*, 2014). Promoter determinant for σ38 has been suggested to lie in the sequence near-10 element (Tanaka *et al.*, 1995; Wise *et al.*, 1996; Espinosa-Urgel *et al.*, 1996; Bordes *et al.*, 2000; Becker and Hengge-Aronis, 2001; Gaal *et al.*, 2001; Lee and Gralla, 2001) and also in the sequence at -35 element (Wise *et al.*, 1996; Rosenthal *et al.*, 2006). Another model suggested that A/T stretches in the spacer region of σ38-dependent promoter which often exhibit non-optimal spacer play role in σ38 selectivity (Typas and Hengge, 2006).

We have shown previously that *hdeAB*p in *E. coli* is a σ38-dependent stationary phase specific promoter since H-NS, a bacterial histone-like nucleoid-structuring protein (Dorman, 2009), represses the gene transcription only when it is transcribed by Eσ70 during exponential phase. This differential effect of H-NS was ascribed to the degree of DNA wrapping around two forms of RNP: Eσ70 binding to *hdeAB*p induces a sharp kink in the DNA, but E σ38 does not (Rivetti *et al.*, 1999; Shin *et al.*, 2005). In this case the H-NS bound initially to the AT tract centered at -118 (a nucleation site, Fig. 1) has been suggested to extend laterally by cooperatively recruiting H-NS molecules to the downstream sequence joined through DNA wrapping around Eσ70, which results in trapping of Eσ70 in a DNA loop. By contrast, DNA arms leaving Eσ38·*hdeAB*p are not close enough for the upstream bound H-NS to laterally oligomerize to the downstream DNA–no DNA looping. Thus, the H-NS-mediated repression would depend on the configuration of DNA wrapping around the RNP. *LEE5*p in enteropathogenic *E. coli* (EPEC) strain E2348/69 (Haack *et al.*, 2003) is not σ38-dependent but is also repressed by H-NS. In this case, H-NS binds to a cluster of A tracks centered at -138 (Bhat *et al.*, 2014) (Fig. 1), and spreads to a site at the promoter through the oligomerization of H-NS molecules (Shin *et al.*, 2012). At the promoter, the H-NS makes a specific contact with the carboxy terminal domain of the α subunit of RNP, resulting in a transcription repression. The mechanisms underlying transcription repression by H-NS of these two types of promoters are distinctive: *hdeAB*p by DNA looping and *LEE5*p by protein-protein contact with RNP. In this study, we swapped the promoter upstream DNA from -65 to -303 , Upstream Regulatory Element (URE), including H-NS action sites of two promoters and examined the transcription from the hybrid promoters by two forms of RNP *in vivo*, Eσ70 at exponential phase and Eσ38 at sta-

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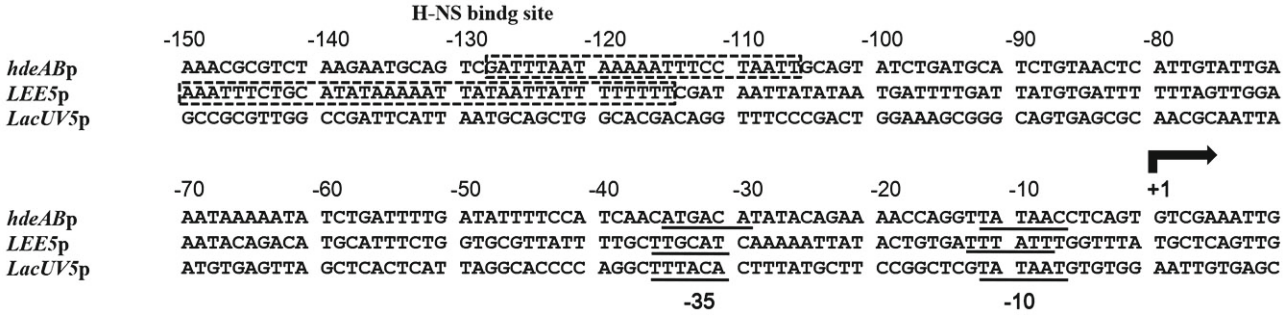

Fig. 1. Nucleotide sequence of *hdeABp*, *LEE5*p, and l*acUV5*p are shown. Transcription start site (+1), -10 and -35 elements are underlined. AT -rich H-NS binding sites are boxed with dashed lines.

tionary phase, in the presence or absence of H-NS. We observed by serendipity that the URE conferred promoter specificity on two forms of RNP. Consistently, a similar pattern of URE-dependent promoter selectivity was observed using *lacUV5* core promoter. In addition, it was noted that promoter strength of the hybrid promoters was also influenced by URE.

Materials and Methods

Strains

The *E. coli* strains used in this study are listed in Table 1. Bacterial strains were constructed by P1 transduction. Bacteriophage λ carrying test promoter::*lacZYA* gene fusion constructs were obtained by inserting the appropriate promoter fragments into the *Eco*RI and *Bam*HI sites of pRS415, followed by homologous recombination (Simons *et al.*, 1987).

Plasmids

*LEE5*p (-303 ~ +127) DNA was obtained by PCR amplification of EPEC strain E2348/69 chromosomal DNA; *hdeAB*p (-303 to +127) and *lacUV*5p DNA (-65 to +40) were obtained from MG1655 using the primers listed in Table 2. Hybrid promoter DNA was constructed by PCR amplification of each DNA fragments with overlapping ends, followed by PCR amplification of the mixture of two different corresponding DNA fragments using the third set of primers

Table 1. Strains and plasmid used in this study

Strains	Description	Reference
MG1655	Wild type	
CH 1018	Δ(arg-lac)U169	Shin *et al.* (2005)
LHJ 001	CH1018, Φ*hdeAB*p::*lacZYA* (-303 to +127)	This work
LHJ 002	LHJ001, Δ*hns*::*km*	This work
LHJ 003	LHJ001, Δ*rpoS*::*tet*	This work
LHJ 005	CH1018, Φ*LEE5*p::*lacZYA* (-303 to +127)	This work
LHJ 006	LHJ 005, Δ*hns*::*km*	This work
LHJ 007	LHJ 005, Δ*rpoS*::*tet*	This work
LHJ 009	CH1018, ΦURE$_{hdeAB}$-*LEE5*p ::*lacZYA* (-303 to -65 to +127)	This work
LHJ 010	LHJ 009, Δ*hns*::*km*	This work
LHJ 011	LHJ 009, Δ*rpoS*::*tet*	This work
LHJ 013	CH1018, ΦURE$_{Lee5}$ -*hdeAB*p::*lacZYA* (-303 to -65 to +127)	This work
LHJ 014	LHJ 013, Δ*hns*::*km*	This work
LHJ 015	LHJ 013, Δ*rpoS*::*tet*	This work
LHJ 017	CH1018, Φ*lacUV5*p::*lacZYA* (-120 to +40)	This work
LHJ 018	LHJ 017, Δ*hns*::*km*	This work
LHJ 019	LHJ 017, Δ*rpoS*::*tet*	This work
LHJ 021	CH1018, ΦURE$_{hdeAB}$ -*lacUV5*p::*lacZYA* (-303 to -65 to +40)	This work
LHJ 022	LHJ 021, Δ*hns*::*km*	This work
LHJ 023	LHJ 021, Δ*rpoS*::*tet*	This work
LHJ 024	CH1018, ΦURE$_{Lee5}$ -*lacUV5*p::*lacZYA* (-303 to -65 to +40)	This work
LHJ 025	LHJ 024, Δ*hns*::*km*	This work
LHJ 026	LHJ 024, Δ*rpoS*::*tet*	This work

Plasmid	Description	Reference
pRS415	*LacZ* fusion vector, Amp^r	Simons *et al.* (1987)

Table 2. PCR primers used to generate hybrid promoters

Primer (direction)	Sequence
*hdeAB*p -303 (F)	5′-GAG AAT TCG ATA AAC AGC AGC ACG GCA-3′
*hdeAB*p +127 (R)	5′-GAA GGA TCC TTT GCG CAT CCG CTG CAT T-3′
*Lee5*p -303 (F)	5′-GAG AAT TCA GTG ATA TCA AGG CTC TAA-3′
*Lee5*p +127 (R)	5′-GAA GGA TCC TGA TTG CCA TTT ACA TTA TT-3′
URE$_{hdeAB}$ -*Lee5* -65 (R)	5′-ATG CAT GTC TGT TTA TTT CAA TAC AAT GAG T-3′
URE$_{hdeAB}$ -*Lee5* -65 (F)	5′-TTG AAA TAA ACA GAC ATG CAT TTC TGG TGC-3′
URE$_{Lee5}$-*hdeAB* -65 (R)	5′-TCA GAT ATT TGT ATT TCC AAC TAA AAA ATC-3′
URE$_{Lee5}$-*hdeAB* -65 (F)	5′-TTG GAA ATA CAA ATA TCT GAT TTT GAT ATT-3′
lacUV5 -120 (F)	5′-GAG AAT TCG CAC GAC AGG TTT CCC GAC T-3′
lacUV5 +40 (R)	5′-GAA GGA TCC ATA GCT GTT TCC TGT GTG AA-3′
URE$_{hdeAB}$ -lacUV5 -65 (R)	5′-TGA GCT AAC TTT TAT TTC AAT ACA ATG AGT-3′
URE$_{Lee5}$-lacUV5 -65 (R)	5′-TGA GCT AAC TGT ATT TCC AAC TAA AAA ATC-3′
URE$_{hdeAB}$ -lacUV5 -65 (F)	TT G AAA TAA AAG TTA GCT CAC TCA TTA GGC-3′
URE$_{Lee5}$-lacUV5 -65 (F)	TTG GAA ATA CAG TTA GCT CAC TCA TTA GGC-3′

(Higuchi *et al.*, 1988). The various promoter DNA fragments were cloned in pRS415 as described above.

Growth conditions

E. coli carrying λ lysogens were grown in LB medium (Difco Laboratories, Becton Dickinson) containing 1% NaCl with vigorous aeration at 37°C. For solid support medium, 1.5% agar (Difco Laboratories) was included. Antibiotics (Sigma) were added at the following concentrations: ampicillin, 50 μg/ml; tetracycline, 15 μg/ml; kanamycin, 30 μg/ml.

β-Galactosidase assay

β-Galactosidase assays were performed as described by Miller (1972), using cells permeabilized with Koch's lysis solution (Putnam and Koch, 1975). *β*-Galactosidase-specific activity was expressed as Miller units ($A_{420/min/A600} \times 1000$). To measure *β*-galactosidase levels in bacteria at different stages of growth, overnight cultures were diluted 1:50 into LB and grown at 37°C until the cultures reached stationary phase. Samples were taken for enzyme assays at regular time intervals. Each strain was assayed in triplicate and average enzyme activities were plotted as a function of time.

Results and Discussion

*hdeAB*p (-303 to +127) was cloned in front of promoter-less *lacZYA* in pRS415 (Simons *et al.*, 1987), moved to λRS415, and placed in bacterial chromosome as an λ lysogen. λ lysogen was used to eliminate possible gene copy number changes. λ[ϕ*hdeAB*p-*lacZYA*] was moved to Hns$^-$ or RpoS$^-$ strain by P1 phage transduction. *E. coli* (MG1655) carrying λ[ϕ*hdeAB*p-*lacZYA*] were grown in LB medium overnight and diluted 50-fold into fresh LB and grown until the cultures entered stationary phase (Kim *et al.*, 2004). Promoter activities were determined by measuring β-galactosidase activities. In the wild type strain background, *hdeAB*p activity was induced (>70-fold) as culture entered stationary phase (after 4 h point) (Fig. 2A). In the RpoS$^-$ mutant strain background no such induction was observed. Whereas, a similar induction was observed in Hns$^-$ mutant strain background but the promoter activity in the exponential phase as well as stationary phase was elevated ~75-fold and ~2-fold, respectively. This pattern indicated that *hdeAB*p is a typical RpoS-dependent stationary phase promoter and is repressed during exponential phase by H-NS.

Subsequently, *LEE5*p (-303 ~ +127) was cloned in pRS415, and moved to λRS415, and placed in bacterial chromosome, as described for λ[ϕ*hdeAB*p-*lacZYA*]. *E. coli* carrying λ[ϕ*LEE5*p-*lacZYA*] were grown the same way and assayed for β-galactosidase activities (Fig. 2B). The *LEE5*p activities at the exponential and stationary phase were about the same throughout the growth phase. Consistently, *LEE5*p activity in RpoS$^-$ mutant strain background was the same as that in the wild type. However, *LEE5*p activity was elevated in Hns$^-$ mutant background ~4-fold at exponential phase and ~9-fold at stationary phase. These results indicated that *LEE5*p is not

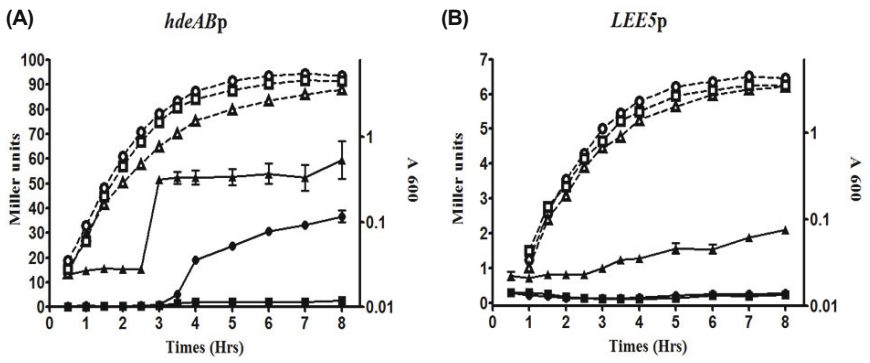

Fig. 2. ***E. coli*** **carrying (A) λ[ϕ*hdeAB*p-*lacZYA*] or (B) λ[ϕ*LEE5*p-*lacZYA*] in the wild-type background (circles), in the Hns$^-$ mutant background (triangles), and RpoS$^-$ mutant background (squares), were grown in LB until the cultures entered stationary phase and promoter activities at the indicated time were determined by β-galactosidase assay.** Dotted lines with open symbols show growth (A_{600}, *right* axis) of each bacterial strain. Solid lines with closed symbols are the β-galactosidase specific activities expressed in Miller Unit ($MU=A_{420}/min/mL/A_{600}$, *left* axis).

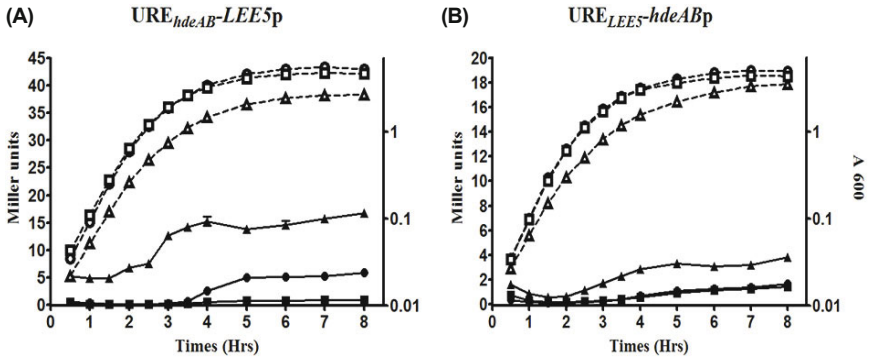

Fig. 3. ***E. coli*** **carrying (A) λ[ϕ URE$_{hdeAB}$-*LEE5*p-*lacZYA*] or (B) λ[ϕ URE$_{LEE5}$-*hdeAB*p-*lacZYA*] in the wild-type background (circles), in the Hns$^-$ mutant background (triangles), and RpoS$^-$ mutant background (squares), were grown in LB and the promoter activities were determined and presented as described in Fig. 2 legend.**

a stationary promoter but is repressed by H-NS at both exponential and stationary phase.

H-NS has been shown to act at a sequence centered at -118 in *hdeAB*p (Shin *et al*., 2005) and at a cluster of A tracks centered at -138 in *LEE5p* (Bhat *et al*., 2014). A set of hybrid promoters was constructed by swapping the sequence containing the URE (-65 to -303) between *hdeAB*p and *LEE5*p. The hybrid promoters, URE$_{hdeAB}$-*LEE5*p and URE$_{LEE5}$-*hdeAB*p, were constructed, cloned in pRS415, and moved to λRS415, and placed in bacterial chromosome as described previously. λ lysgenic *E. coli* carrying hybrid promoters were grown the same way in LB medium and assayed for β-galactosidase activities (Fig. 3). Most notably, URE$_{hdeAB}$-*LEE5*p behaved as *hdeAB*p (Fig. 3A): it was induced at the stationary phase (>30-fold) in rpoS-dependent manner and repressed about ~45-fold at the exponential phase and ~2.9-fold at the stationary phase by H-NS. Whereas, URE$_{LEE5}$-*hdeAB*p behaved as *LEE5*p (Fig. 3B): it was not induced at the stationary phase independent of *rpoS* but repressed ~2-fold at the exponential phase and ~4-fold at the stationary phase by H-NS. It was suggested that the element for RpoS-dependent stationary phase gene induction lies at the sequence upstream of core promoter. In addition, it should be noted that the activity of the hybrid promoters was influenced greatly by URE: *hdeAB*p activity was reduced by the presence of URE$_{LEE5}$ from >30 to ~3 MU while *LEE5*p activity increased by the presence of URE$_{hdeAB}$ from ~5 to ~15 MU.

To further validate above finding, URE sequences were placed upstream of *lacUV5*p at the position -65. The hybrid promoters, URE$_{hdeAB}$-*lacUV5*p and URE$_{LEE5}$-*lacUV5*p, were constructed, cloned in pRS415, moved to λRS415, placed in bacterial chromosome, assayed for promoter activities by determining β-galactosidase activities (Fig. 4). Wild type *lacUV5*p was not affected by H-NS or RpoS. Whereas, URE$_{hdeAB}$-*lacUV5*p behaved as *hdeAB*p (Fig. 4A): it was induced at the stationary phase (>6-fold) in *rpoS*-dependent manner and repressed about ~5-fold at the exponential phase and <2-fold at the stationary phase by H-NS. Whereas, URE$_{LEE5}$-*lacUV5*p behaved as *LEE5*p (Fig. 4B): it was virtually not induced at the stationary phase independent of *rpoS* but repressed ~2-fold by H-NS throughout the growth phase. It should be noted in the H-NS$^-$ mutant background that *lacUV5*p activities increased ~13-fold with URE$_{hdeAB}$ fusion and ~5-fold with URE$_{LEE5}$ fusion compared with the wild type promoter activity

Taken together the results suggested that the sequence upstream of -65 including H-NS action site plays a role in determining promoter specificity for σ*38*. Although -10 elements of *hdeAB*p is close to the canonical -10 element (5/6), the -35 elements deviates considerably (0/6) (Fig. 1), as suggested for characteristics of σ38-dependent promoters (Rosenthal *et al*., 2006; Typas and Hengge, 2006). Near consensus -10 element of σ38-dependent promoters has been suggested to compensate for an absence of a canonical-35 hexamer (Typas *et al*., 2007). A 'GCGG' motif has been suggested to play of role of an alternative -35 region for a subgroup of σ38-dependent promoters (Lee and Gralla, 2004), but no such motif is found in *hdeAB*p. The spacing between

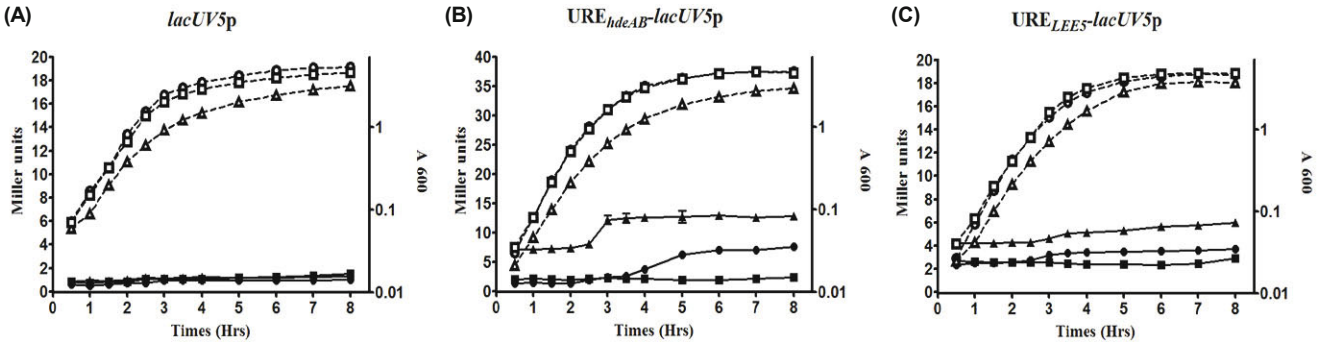

Fig. 4. ***E. coli*** **carrying (A) λ[ϕ *lacUV5p::ZYA*], (B) λ[ϕ URE$_{hdeAB}$-*lacUV5p::ZYA*] or (C) λ[ϕ URE$_{LEE5}$- *lacUV5p::ZYA*] in the wild-type background (circles), in the Hns$^-$ mutant background (triangles), and RpoS$^-$ mutant background (squares), were grown in LB and the promoter activities were determined and presented as described in Fig. 2 legend.**

-10 and -35 elements is canonical 17 bp and no notable sequence is found in the discriminator region downstream of the -10 element in *hdeAB*p, either. It has been reported that σ38 consensus CTATACT (from-13 to-7) differs from the σ70 consensus XTATAAT in the two underlined positions (Lee and Gralla, 2001). The -13C was suggested to prevent recognition by Eσ70. Thus, the promoter determinant for Eσ38 has been proposed to be at -13 position, although there are Eσ70-dependent promoters that carry C at this position (Becker and Hengge-Aronis, 2001; Gaal *et al*., 2001). DNA sequence of *hdeAB*p at the corresponding region is TTAT AAC with T at the -13 position.

Apart from promoter elements (Estrem *et al.*, 1998; Vicente *et al.*, 1991; Mitchell *et al.*, 2003; Shultzaberger *et al.*, 2007), the DNA geometry in the vicinity of the core promoter would be generally of prime importance (Geiselmann, 1997). Nucleoid-associated proteins often interact with DNA in a non-sequence-specific manner. In the case of H-NS, although a consensus binding sequence has been proposed based on the sequence found in *proU* in *E. coli*, TCGATAAATT (Lang *et al.*, 2007), it is known to bind preferentially to DNA containing curved regions. H-NS binding has been shown to induce DNA compaction and structural rearrangements (Dame, 2005; Luijsterburg *et al.*, 2006). H-NS regulates the transcription from *hdeAB*p through alteration of the DNA architecture in the vicinity of promoters by DNA looping (Shin *et al.*, 2005). DNA conformation and the exact geometry of bound transcription factors are crucial elements for the efficiency of many bacterial promoters (Plaskon and Wartell, 1987; Jauregui *et al.*, 2003; Olivares-Zavaleta *et al.*, 2006). We have shown that DNA would wrap around Eσ70 through a series of DNA kinks (Shin *et al.*, 2005). Consistently, unpaired bases at the promoter upstream of *hdeAB*p DNA were detected by $KMnO_4$ assay, indicative of DNA kinks (Shin *et al.*, 2005). Although further study is needed, we propose here that nucleotide sequence at URE would dictate DNA architecture at the region, which may influence the DNA conformation such that two forms of RNP, Eσ70, and Eσ38, differentially interact with the promoter elements. In addition, the URE influenced the promoter strength: URE_{hdeAB} does more than URE_{LEE5} when compared the *lacUV5* activities in the presence of heterologous upstream DNA fusion. It is unlikely due to upstream DNA itself, since no difference in promoter strength was noted with *hdeAB*p truncated at -134 and -44 *in vitro* with naked DNA (Shin *et al.*, 2005). Neither was *LEE5*p truncated at -231 and -44 (Shin *et al.*, 2012). HdeAB operon playing a role in acid protection is known to be inducible under acidic condition (Foster, 2004; Zhao and Houry, 2010) and also regulated by various transcription regulators in addition to RpoS and H-NS: GadX/GadW (Tramonti *et al.*, 2008), gcvA/B (Wilson *et al.*, 1995; Stauffer and Stauffer, 2012), MarR/SoxS (Schneiders *et al.*, 2004), Lrp and TorR (Ruiz *et al.*, 2008). It, therefore, may be possible that transcription activation by URE_{hdeAB} is ascribed to one or combination of these transcription activators. Unidentified elements in URE_{LEE5} may similarly influence the downstream core promoter activity.

Acknowledgements

This work was supported by NRF-2014R1A2A1A10051664. K.S.K was supported by Basic Science Research Program (NRF-2014R1A1A2004637) and J.H.J by Basic Science Research Program (NRF-2014R1A1A2006327).

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