Curr Microbiol (2014) 69:218–224
DOI 10.1007/s00284-014-0595-7

Integration Host Factor is Required for the Induction of Acid Resistance in *Escherichia coli*

Hongkai Bi · Changyi Zhang

Received: 8 February 2014 / Accepted: 5 March 2014 / Published online: 10 May 2014
© Springer Science+Business Media New York 2014

Abstract Integration host factor (IHF) is a heterodimeric histone-like DNA-binding protein that participates in many cellular functions. Many systems and global regulators of acid resistance (AR) under strongly acidic conditions have been reported, but the role of IHF has not been examined. In the present study, we report that IHF is necessary for the induction of AR in *Escherichia coli*. At acidic pH, a *ΔihfAΔifhB*-mutant strain was found to have significantly depressed levels of transcription of the arginine decarboxylase gene (*adiA*) and of translation of the lysine/cadaverine antiporter gene (*cadB*), when compared with wild-type strain. Thus, IHF induces the arginine- and lysine-dependent AR. These results indicate that in *E. coli*, by combined transcriptional and translational controls of gene expression, IHF activates expression of a specific set of genes required for survival at extremely acidic pH.

Introduction

Before orally ingested enteric pathogens such as *Escherichia coli* can reach their targeted mammalian host cells, they must first survive the extremely acidic conditions as they pass through the stomach (pH 2–4) on their way to the intestine. This is an extremely hostile environment, and thus *E. coli* contains multiple inducible systems for protection from acidic stress [11, 36].

Escherichia coli displays a high degree of acid resistance (AR) induction, classified as amino acid-dependent or amino acid-independent [9, 11, 18, 24]. The amino acid-independent system (AR1) is induced in cells grown to stationary phase in a moderately acidic medium, is suppressed by glucose, and is dependent on the RpoS sigma factor and the cyclic AMP receptor protein (Crp) [6]. The amino acid-dependent systems depend on the presence of specific amino acids. The glutamate-dependent system (AR2) requires two glutamate decarboxylases (GadA and GadB) plus GadC, an inner-membrane glutamate/γ-aminobutyrate antiporter [6, 15]. This is the most effective acid stress response pathway under extremely acid stress conditions, and over 20 proteins and 3 small noncoding RNAs have been identified in regulating the system [7, 14, 19, 28, 41, 47]. The complex regulation of AR2 is orchestrated by GadE, a global transcriptional activator [5, 23]. The AR3 system is arginine dependent and consists of the AdiA arginine decarboxylase and the AdiC arginine/agmatine antiporter [13]. It is induced by low pH and anaerobic conditions. AR4 is a lysine-dependent system that requires the CadA lysine decarboxylase and the CadB lysine/cadaverin antiporter [25, 43]. The expression of the *cadBA* operon is transcriptionally controlled by extracellular pH and lysine [30].

In addition to these structural enzymes and regulators, histone-like nucleoid-structuring proteins have also been reported to coordinate the AR systems in *E. coli*. H-NS was shown to regulate AR2 by inhibiting *gadA* and *gadX* transcription [12]. As a top-level regulator of AR with global influence, H-NS enhances degradation of *rpoS* mRNA [3] and represses expression of specific regulators of the amino acid-dependent AR systems [12, 19, 20]. More recently, another histone-like protein HU was shown to regulate expression of *adiA* and *adiC*, resulting in the induction of the arginine-dependent AR3 [2]. Hence, both H-NS and HU function as transcriptional regulators of AR induction.

H. Bi (✉) · C. Zhang
Department of Microbiology, University of Illinois, B103 Chemical and Life Sciences Laboratory, 601 South Goodwin Ave, Urbana, IL 61801, USA
e-mail: hkbi@illinois.edu

Table 1 Bacterial strains, plasmids and oligonucleotide primers used in this study

Strains	Genetype	Reference or source
W3110	λ⁻ F⁻ derived from wild type *E.coli* K-12	[16]
MC4100	F⁻ *araD*139 Δ(*argF*-lac)U169 *rpsL*150 *relA1 flhD*530 *deoC*1 *ptsF*25 *rbsR*	[37]
YK2920	YK1100 Δ82[*ihfA*]::Tn10 Δ3[*ihfB*]:: Cm^r	[45]
BH32	W3110 Δ82[*ihfA*]::Tn10	This study, W3110 × P1(YK2920)
BH33	W3110 Δ3[*ihfB*]::Cm^r	This study, W3110 × P1(YK2920)
BH36	W3110 Δ82[*ihfA*]::Tn10 Δ3[*ihfB*]:: Cm^r	This study, BH32 × P1(YK2920)
BHCB2	MC4100 Φ(*cadB-lacZ*) (p)	This study
BHCB34	BHAD2 Δ82[*ihfA*]::Tn10	This study, BHCB2 × P1(YK2920)
BHCB35	BHAD2 Δ3[*ihfB*]::Cm^r	This study, BHCB2 × P1(YK2920)
BHCB36	BHAD2 Δ82[*ihfA*]::Tn10, Δ3[*ihfB*]::Cm^r	This study, BHCB34 × P1(YK2920)
Plasmids		
pRS552	*ori* colE1 *lacZ* protein fusion vector, Km^r, Amp^r	[38]
pCadB975	*ori* colE1 Φ(*cadB-lacZ*) (p), Km^r, Amp^r	This study
Oligonucleotides	Sequence (5′–3′)	
cadL796	GCAC<u>GAATTC</u>CCAGAATTTACCTACGCGAG	
cadR179	ACTA<u>GGATCC</u>AGTCGGGCATATACATACGC	
gadA-f	AGTTCGAAATGGACCAGAAGCTGT	
gadA-r	GTCGATCCAGTTTTTATTGATCGAC	
gadB-f	GGAGTTTAAAATGGATAAGAAGCAAG	
gadB-r	TTGTCGATCCAGTTTTTGTTAATG	
gadC-f	GCGACTATCCGTTGGCTATG	
gadC-r	CCGTCCACTCAATTTCTGGT	
adiA-f	TAACTTCTCCCGCTTCAACC	
adiA-r	AATCAACCGCTTCGTCAATC	
adiC-f	TTCGGTGCAATTCAAAGTACC	
adiC-r	ATCGCGGTGGTAGAAAGTACA	
cadB-f	TCTTCTGTAATGGCGGCTTC	
cadB-r	CCTGGCCTACCAACATCATC	
cadA-f	CGGAGTCAAAGTGGATGGAT	
cadA-r	AAGAAACACCAAACGCAACC	
ybaS-f	ATCGCCTTAGAGTTGCATGG	
ybaS-r	TGTTTGTTCCGACTGGTTGA	
rplU-f	ACCGAGTAAGCGAAGGTCAG	
rplU-r	GACCGTGAGCAACAACTTCA	

The underlined italic sequences are the introduced restriction sites

In addition to H-NS and HU, *E. coli* possesses several other nucleoid architectural proteins that mimic the function of histones by assisting DNA folding and compaction. One of the best-characterized histone-like proteins is the integration host factor (IHF), a relatively abundant small DNA-bending protein [10]. IHF is a heterodimer which consists of α and β subunits, the products of the *ihfA* and *ihfB* genes [10, 29]. IHF, named for its role in phage λ integration [26], bends DNA by 160° [31] and facilitates the formation of nucleoprotein structures [42]. IHF binds to a specific DNA sequence and participates in a number of DNA activities including gene inversion, transposition, replication, partitioning of replicated DNA molecules, transfer of plasmids, and control of transcription [34, 39, 46].

IHF has been reported to act as a transcriptional regulator controlling the expressions of many specific operons [4, 8, 17]. IHF was also required for the induction of the

arginine decarboxylase gene (*adiA*) expression at acidic pH [33]. This led us to examine the potential role of IHF in other AR structural gene expression and AR induction. In this report, we propose that IHF regulates the induction of amino acid-dependent AR systems via both transcriptional and translational controls of gene expression.

Materials and Methods

Bacterial Strains and Growth Conditions

The *E. coli* K-12 strains used in this study are listed in Table 1. The strains were grown at 37 °C in Luria–Bertani (LB) medium or minimal EG medium [16] consisting of E medium containing 0.15 mM uracil, 0.05 mM thymine, and 0.4 % glucose. The medium pH was adjusted with HCl or KOH. When required, sodium ampicillin (100 μg/ml), tetracycline (15 μg/ml), kanamycin (25 μg/ml), or chloramphenicol (20 μg/ml) were added. Bacterial growth was monitored by measuring absorbance at 600 nm. The cell optical density at an absorbance of 0.3 was approximately 2×10^8 colony-forming units (CFU)/ml under our experimental conditions.

Acid Resistance (AR) Assay

AR was measured as described elsewhere [2]. Acid resistance is given as a percentage ratio of the number of surviving cells after acidic challenge at pH 2.5 to the number of cells before the challenge. When indicated, 1 mM glutamine, glutamate, arginine, or lysine was added to the EG medium for acidic adaptation and challenge.

RNA Isolation, cDNA Synthesis, and Quantitative Reverse Transcription (qRT)-PCR

RNA isolation and cDNA synthesis were carried out as described previously [2]. Gene expression was determined by qRT-PCR using Power SYBR Green PCR Master Mix (Applied Biosystems, CA) according to the manufacturer's protocol, which was performed as described previously [2]. The primer sets used are listed in Table 1. The mRNA levels relative to those of the wild type were calculated using the comparative critical threshold method ($2^{-\Delta\Delta CT}$), with *rplU* as an internal control gene as described previously [22].

Construction of the Chromosomal Translational Fusion and Other DNA Manipulations

The *cadB*::*lacZ* gene fusion was constructed on plasmids and then transferred in single copy to the chromosome of strain MC4100 by in vivo recombination as described previously [32]. A 975-bp *cadB* DNA fragment (from −796 to +179 relative to the translational start site) containing 59 codons was amplified by PCR using chromosomal DNA from strain MC4100 as template, primers cadL796 and cadR179, and Taq DNA polymerase (New England Biolabs, MA), using a Tm of 58 °C. The amplified DNA was digested with EcoRI and BamHI, and cloned into the vector pRS552 [38], yielding the translational fusion plasmids pCadB975, insert sequence of which was verified by sequencing. Strain MC4100 was subsequently transformed with the plasmid pCadB975; a transformant of strain MC4100 was infected with λ phage ARZ5 [32]; and the resulting lysate was used to transduce strain MC4100. Ampicillin-resistant, Lac^+ lysogens were isolated by plating onto LB plates containing ampicillin and X-gal (40 μg/ml). Phages were prepared from these lysogens and used to reinfect strain MC4100 at a multiplicity of infection of 0.01. The resulting strain BHCB2 was then screened using restriction enzyme analysis to confirm the presence of the *cadB*: :*lacZ* fusion. The lysogens (at least six isolates) were then screened for the presence of a single copy of the recombinant ARZ5 phage by measuring the activity of phage-encoded β-galactosidase and β-lactamase. The lysogens displaying the lowest enzyme levels were assumed to carry a single prophage.

Plasmid $CaCl_2$ transformation and phage P1 transduction to transfer mutations were performed according to standard procedures [35]

Western Blot Analysis

E. coli was cultured overnight in LB medium at 37 °C and then diluted 1:1,000 in EG medium with 5 mM lysine at pH 5.5 for acidic adaptation. After being anaerobically grown to the absorbance at 600 nm of 0.3, inside–out membrane vesicles were prepared, and Western blot analyses were carried out as described previously [40]. Rabbit polyclonal antibodies against CadB were kindly supplied by K. Igarashi. For Western blot analysis of CadB, membrane vesicles (20 μg protein) were separated by SDS–polyacrylamide gel electrophoresis on a 12 % acrylamide gel and transferred to a polyvinylidene fluoride membrane (Immobilon P, Millipore Bedford, MA). Antibodies were bound to proteins on the membrane. Staining was carried out with anti-rabbit IgG antibody conjugated with alkaline phosphatase. CadB protein was detected with ProtoBlot Western blot AP System (Promega).

β-Galactosidase Assay

β-galactosidase assays were performed as described previously [27]. Cells were cultured to log phase. The data

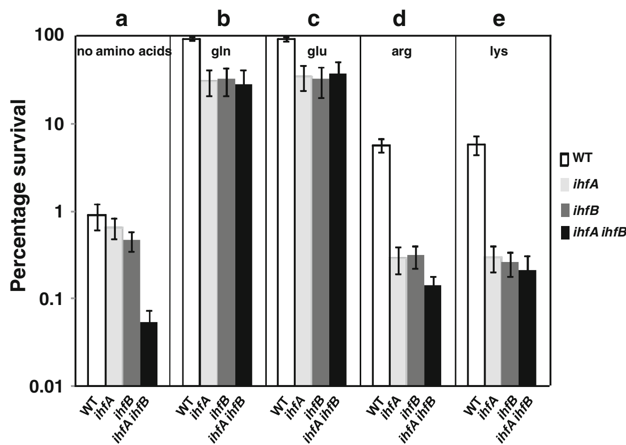

Fig. 1 The effect of *ihf* deletions on AR induction. The AR values for the wild type strainW3110 and its isogenic derivatives, BH32 (*ihfA*), BH33 (*ihfB*) and BH36 (*ihfAihfB*) were measured in EG medium after a 2 h acid challenge as described in Materials and Methods. No amino acids (**a**), Glutamine (gln) (**b**), glutamate (glu) (**c**), arginine (arg) (**d**) or lysine (lys) (**e**) was added at 1 mM as indicated in the figure. The means and standard deviations of duplicate determinations from at least three independent cultures are represented

were recorded in triplicate with more than three independent experiments.

Prediction of RNA Secondary Structure

The predicted secondary structure for full-length 5′ UTR of *cadB* was analyzed for minimal free energy and RNA secondary structure using the online MFOLD program prepared by M. Zuker [48].

Results

The Effects of *ihfA* and *ihfB* Deletions on the Induction of AR

Escherichia coli cells grown to stationary phase have been most used in the studies of AR induction, and such cells are more resistant to various stresses. To exclude the effect of IHF on stationary phase gene expression, cells growing exponentially were used in this study. The *ihf*-mutant strains were first cultured in EG medium at pH7.5 until the OD600 reached 0.3. Then, the cells were harvested by centrifugation, washed with EG medium at pH5.5, and suspended in the same medium at pH5.5. These cultures were adapted to the weakly acidic pH for 4 h to increase AR, and then survival at pH2.5 was measured.

Based on these data, only a small effect on the AR was observed in strains carrying *ihfA* or *ihfB* single deletion. However, survival of the strain lacking both IHFα and IHFβ subunits was 17-fold lower than that of the parent strain (Fig. 1a), indicating that IHF is required for the induction of AR.

The Role of IHF in the Amino Acid-Dependent Induction of AR

It is well established that glutamate, glutamine, arginine, and lysine increase AR [11, 21]. We next examined if IHF was required for the induction of amino acid-dependent AR (AR2, AR3 and AR4). The cells were adapted at pH 5.5, and then challenged at pH 2.5 in the presence of 1 mM of each amino acid followed by AR measurement. The glutamate- and glutamine-dependent AR inductions showed no significant effects upon deletion of the two *ihf* genes (Fig. 1b, c). However, the arginine-dependent and lysine-dependent survivals at pH2.5 were diminished by 18 to 41-fold by the deletion of single or double *ihf* genes (Fig. 1d, e), indicating that IHF has a role in the induction of arginine- and lysine-dependent AR.

The Effect of IHF Deletion on the Transcriptions of AR Structural Genes

The genes encoding amino acid decarboxylases and conjugate antiporters are known to play essential roles in the amino acid-dependent AR systems [21]. To test if IHF affects their expression, the mRNA levels of several genes were measured quantitatively by real-time PCR, and the relative levels of the *ΔihfAΔifhB* strain to those of the wild type strain were calculated as described in Materials and methods. No significant change caused by the IHF mutation was observed with *adiC*, whereas the *adiA* transcript level was nine-fold lower in the *ΔihfAΔifhB* strain than in the wild-type strain (Fig. 2), in agreement with previous *lacZ* transcriptional fusion data [33]. Thus, it seems that IHF regulates arginine-dependent AR3 induction by activating the transcription of the gene *adiA*, but not *adiC*. The levels of the *gadA*, *gadBC*, and *ybaS* (glutaminase A gene) transcripts were approximately two-fold higher in the *ΔihfAΔifhB* strain than those in the wild-type strain (Fig. 2). The *ybaS* gene was shown to be required for glutamine-dependent AR induction [2]. The slightly increased mRNA levels of *gadABC* and *ybaS* upon IHF deletion are insufficient to affect the induction of the glutamate- or glutamine-dependent AR systems.

More interestingly, transcription of *cadBA* also appeared to be somewhat increased by loss of IHF (Fig. 2). However, the relatively high level of *cadBA* mRNA in the *ΔihfAΔifhB* strain cannot account for low-survival ability of the mutant strain after acidic challenge in the presence of lysine.

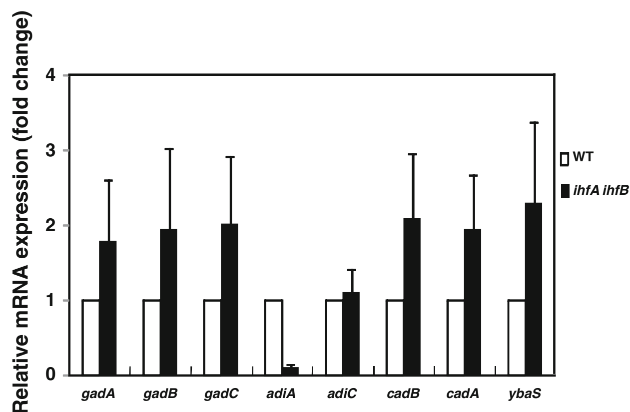

Fig. 2 The effect of IHF mutation on the transcription of AR induction genes. Strain W3110 (wild type) and BH36 (*ihfA ihfB*) were grown in EG medium at pH 5.5 for 4 h and harvested before acidic challenge (pH2.5). The EG medium was supplemented with 1 mM of glutamine, glutamate, arginine, or lysine to assess the transcriptional levels of *ybaS*; *gadA, B*, and *C*; *adiA* and *adiC*; or *cadBA*, respectively. Real-time PCR was performed, and the levels of Strain BH36 expression (*black bars*) relative to those of the wild type strain *(open bars)* are represented, as described in the Materials and Methods. The data are the averages ± standard deviations of three independent experiments

IHF Exerts Translational Control of CadB Expression

To determine if IHF induces lysine-dependent AR by control of the CadBA expression at acidic pH, we performed Western blot analysis to detect CadB expression. Under lysine induction conditions, the CadB protein levels were significantly lower in both the *ihf* single- and double-mutant strains compared with the wild-type strain (Fig. 3a). Lysine addition induced the CadB expression in the WT cell, consistent with a previous report [30]. To confirm the reduced CadB expression in the IHF- mutant cells, we constructed a translational fusion with *lacZ* as a reporter gene and measured the β-galactosidase activity to examine *cadB* translation. The level of β-galactosidase activity in the *ΔihfAΔifhB* strain was eight-fold lower than that in the wild-type strain at acidic pH with the addition of lysine. Similar fold decreases were observed in the *ihfA*- or *ihfB*-single- mutant cells (Fig. 3b), suggesting that IHF is essential for the translational control of *cadB* expression.

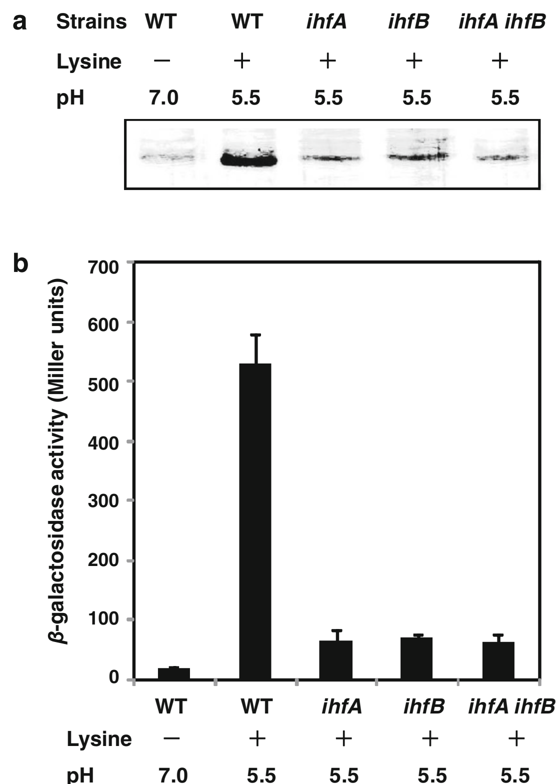

Fig. 3 The effect of IHF mutation on *cadB* translational expression. **a** Western blot analysis of CadB expression. BHCB2 (wild type), BHCB34 (*ihfA*), BHCB35 (*ihfB*) and BHCB36 (*ihfA ihfB*) were anaerobically grown with 5 mM lysine at pH 5.5 and western blot analysis was performed using 20 μg of protein of the inside-out membrane vesicles as described in the Materials and Methods. **b** The effect of *ihf* deletions on β-galactosidase expression from a chromosomal *cadB::lacZ* translational fusion. Cells were grown anaerobically as in Fig. 3a and β-galactosidase activity was measured. The data are the averages ± standard deviations of three independent experiments

Discussion

AR can help *E. coli* cells to overcome the challenge posed by extreme acidic environments. When they inevitably have to pass through the gastric acid in the stomach (pH 2.5), which serves as a natural antibiotic barrier, *E. coli* can survive in this hostile acidic condition. Therefore, understanding the complex regulatory mechanisms and pathways of AR response is crucial to developing strategies for clinical *E. coli* infection. In this study, we showed that IHF induces arginine- and lysine-dependent AR. To our knowledge, this is the first report showing the function of IHF in the control of AR induction.

It is generally considered that IHF functions as a transcriptional dual regulator and is involved in a variety of processes including transcription of certain promoters. However, we here reported that IHF also acts as a translational regulator. Our current data suggest that IHF induces the lysine-dependent AR by activating the *cadBA* translation at acidic pH. But it is not clear that the regulation by IHF is direct or indirect. Two possible mechanisms as to how IHF stimulates *cadB* translation can be proposed: either IHF modifies the RNA secondary structure to facilitate ribosome binding, or it modulates binding of other regulatory proteins as a chaperoning role, or both. Actually, the proposed secondary structure of 5′ UTR of

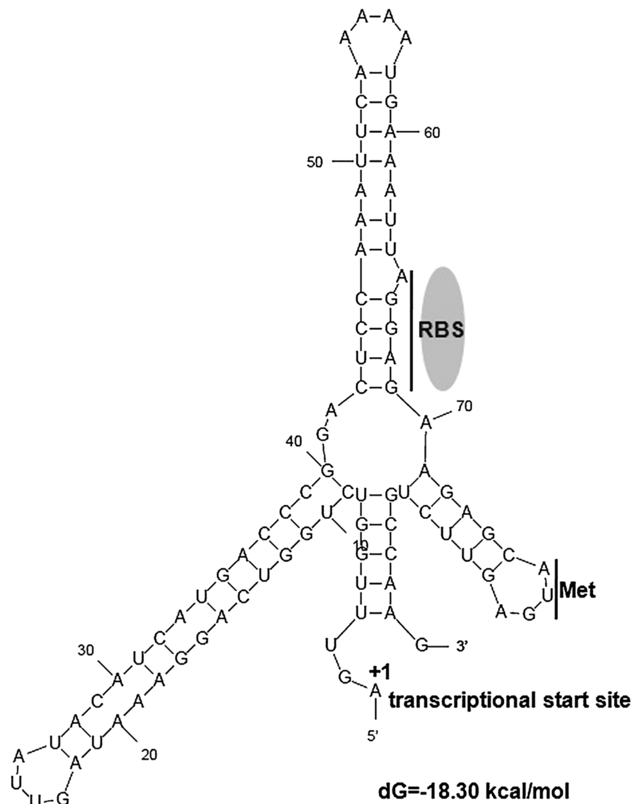

Fig. 4 Prediction of RNA secondary structure of the *cadB* 5′ UTR. The structure and ΔG_0 for 5′ UTR were displayed using the online MFOLD program [48], with the default settings and folding temperature at 37 °C. Designations: *+1*, transcriptional start site; *RBS*, ribosome binding site; *Met*, initiator methionine. The transcriptional start site was mapped by Watson N [44]

the *cadB* mRNA showed that a long mRNA stem–loop structure forms at the ribosome-binding site (Fig. 4). IHF could bind the *cadB* 5′ UTR to prevent the formation of RNA duplex structures that occlude the ribosome binding site of the *cadB* transcript. This question is currently being investigated. Thus, IHF might also be a RNA-binding protein that stimulates protein expression and affects cellular processes.

In this study, the IHF deletion decreased survival in medium without the addition of amino acids (Fig. 1a), suggesting that IHF regulates expression of other genes required for survival at an extremely low pH. We also reported previously that HU has a similar role in AR induction [2]. Actually, IHF and HU monomers share ~30 % sequence identity and are proposed to be related in both structure and function [1]. Furthermore, the HU/IHF family of proteins consists of orthologs that share significant sequence identity. Thus, our results support the importance of nucleoid-associated proteins function during environmental adaptation. Compounds that inhibit HU/IHF activity could be effective antimicrobials that are against multiple species of bacteria.

Acknowledgments The authors would like to thank Y. Kano and R.W. Simons for providing bacterial strains, and Dr. John Cronan for his valuable comments on the manuscript.

References

1. Becker NA, Kahn JD, Maher LJ 3rd (2007) Effects of nucleoid proteins on DNA repression loop formation in *Escherichia coli*. Nucleic Acids Res 35:3988–4000
2. Bi H, Sun L, Fukamachi T, Saito H, Kobayashi H (2009) HU participates in expression of a specific set of genes required for growth and survival at acidic pH in *Escherichia coli*. Curr Microbiol 58:443–448
3. Brescia CC, Kaw MK, Sledjeski DD (2004) The DNA binding protein H-NS binds to and alters the stability of RNA in vitro and in vivo. J Mol Biol 339:505–514
4. Cagle CA, Shearer JE, Summers AO (2011) Regulation of the integrase and cassette promoters of the class 1 integron by nucleoid-associated proteins. Microbiology 157:2841–2853
5. Castanie-Cornet MP, Cam K, Bastiat B, Cros A, Bordes P, Gutierrez C (2010) Acid stress response in *Escherichia coli*: mechanism of regulation of *gadA* transcription by RcsB and GadE. Nucleic Acids Res 38:3546–3554
6. Castanie-Cornet MP, Penfound TA, Smith D, Elliott JF, Foster JW (1999) Control of acid resistance in *Escherichia coli*. J Bacteriol 181:3525–3535
7. Castanie-Cornet MP, Treffandier H, Francez-Charlot A, Gutierrez C, Cam K (2007) The glutamate-dependent acid resistance system in *Escherichia coli*: essential and dual role of the His-Asp phosphorelay RcsCDB/AF. Microbiology 153:238–246
8. Devroede N, Huysveld N, Charlier D (2006) Mutational analysis of intervening sequences connecting the binding sites for integration host factor, PepA, PurR, and RNA polymerase in the control region of the *Escherichia coli carAB* operon, encoding carbamoylphosphate synthase. J Bacteriol 188:3236–3245
9. Diez-Gonzalez F, Karaibrahimoglu Y (2004) Comparison of the glutamate-, arginine- and lysine-dependent acid resistance systems in *Escherichia coli* O157:H7. J Appl Microbiol 96:1237–1244
10. Drlica K, Rouviere-Yaniv J (1987) Histonelike proteins of bacteria. Microbiol Rev 51:301–319
11. Foster JW (2004) *Escherichia coli* acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2:898–907
12. Giangrossi M, Zattoni S, Tramonti A, De Biase D, Falconi M (2005) Antagonistic role of H-NS and GadX in the regulation of the glutamate decarboxylase-dependent acid resistance system in *Escherichia coli*. J Biol Chem 280:21498–21505
13. Gong S, Richard H, Foster JW (2003) YjdE (AdiC) is the arginine:agmatine antiporter essential for arginine-dependent acid resistance in *Escherichia coli*. J Bacteriol 185:4402–4409
14. Hansen AM, Qiu Y, Yeh N, Blattner FR, Durfee T, Jin DJ (2005) SspA is required for acid resistance in stationary phase by downregulation of H-NS in *Escherichia coli*. Mol Microbiol 56:719–734
15. Hersh BM, Farooq FT, Barstad DN, Blankenhorn DL, Slonczewski JL (1996) A glutamate-dependent acid resistance gene in *Escherichia coli*. J Bacteriol 178:3978–3981
16. Jensen KF (1993) The *Escherichia coli* K-12 "wild types" W3110 and MG1655 have an rph frameshift mutation that leads to pyrimidine starvation due to low *pyrE* expression levels. J Bacteriol 175:3401–3407
17. Jeong JH, Kim HJ, Kim KH, Shin M, Hong Y, Rhee JH, Schneider TD, Choy HE (2012) An unusual feature associated

with LEE1 P1 promoters in enteropathogenic *Escherichia coli* (EPEC). Mol Microbiol 83:612–622
18. Kanjee U, Houry WA (2013) Mechanisms of acid resistance in *Escherichia coli*. Annu Rev Microbiol 67:65–81
19. Krin E, Danchin A, Soutourina O (2010) Decrypting the H-NS-dependent regulatory cascade of acid stress resistance in *Escherichia coli*. BMC Microbiol 10:273
20. Krin E, Danchin A, Soutourina O (2010) RcsB plays a central role in H-NS-dependent regulation of motility and acid stress resistance in *Escherichia coli*. Res Microbiol 161:363–371
21. Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW (1996) Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. Appl Environ Microbiol 62:3094–3100
22. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408
23. Ma Z, Gong S, Richard H, Tucker DL, Conway T, Foster JW (2003) GadE (YhiE) activates glutamate decarboxylase-dependent acid resistance in *Escherichia coli* K-12. Mol Microbiol 49:1309–1320
24. Mates AK, Sayed AK, Foster JW (2007) Products of the *Escherichia coli* acid fitness island attenuate metabolite stress at extremely low pH and mediate a cell density-dependent acid resistance. J Bacteriol 189:2759–2768
25. Meng SY, Bennett GN (1992) Nucleotide sequence of the *Escherichia coli cad* operon: a system for neutralization of low extracellular pH. J Bacteriol 174:2659–2669
26. Miller HI, Friedman DI (1980) An *E. coli* gene product required for lambda site-specific recombination. Cell 20:711–719
27. Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor
28. Mitra A, Fay PA, Morgan JK, Vendura KW, Versaggi SL, Riordan JT (2012) Sigma factor N, liaison to an *ntrC* and *rpoS* dependent regulatory pathway controlling acid resistance and the LEE in enterohemorrhagic *Escherichia coli*. PLoS ONE 7:e46288
29. Nash HA, Robertson CA, Flamm E, Weisberg RA, Miller HI (1987) Overproduction of *Escherichia coli* integration host factor, a protein with nonidentical subunits. J Bacteriol 169:4124–4127
30. Neely MN, Olson ER (1996) Kinetics of expression of the *Escherichia coli cad* operon as a function of pH and lysine. J Bacteriol 178:5522–5528
31. Rice PA, Yang S, Mizuuchi K, Nash HA (1996) Crystal structure of an IHF-DNA complex: a protein-induced DNA U-turn. Cell 87:1295–1306
32. Roland KL, Liu CG, Turnbough CL Jr (1988) Role of the ribosome in suppressing transcriptional termination at the *pyrBI* attenuator of *Escherichia coli* K-12. Proc Natl Acad Sci U S A 85:7149–7153
33. Rowbury RJ (1997) Regulatory components, including integration host factor, CysB and H-NS, that influence pH responses in *Escherichia coli*. Lett Appl Microbiol 24:319–328
34. Ryan VT, Grimwade JE, Camara JE, Crooke E, Leonard AC (2004) *Escherichia coli* prereplication complex assembly is regulated by dynamic interplay among Fis, IHF and DnaA. Mol Microbiol 51:1347–1359
35. Sambrook J, Russell DW (2006) The condensed protocols from Molecular cloning : a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
36. Seputiene V, Daugelavicius A, Suziedelis K, Suziedeliene E (2006) Acid response of exponentially growing *Escherichia coli* K-12. Microbiol Res 161:65–74
37. Silhavy TJ, Berman ML, Enquist LW, Cold Spring Harbor Laboratory (1984) Experiments with gene fusions. Cold Spring Harbor, Cold Spring Harbor Laboratory
38. Simons RW, Houman F, Kleckner N (1987) Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene 53:85–96
39. Sohanpal BK, Friar S, Roobol J, Plumbridge JA, Blomfield IC (2007) Multiple co-regulatory elements and IHF are necessary for the control of *fimB* expression in response to sialic acid and N-acetylglucosamine in *Escherichia coli* K-12. Mol Microbiol 63:1223–1236
40. Soksawatmaekhin W, Uemura T, Fukiwake N, Kashiwagi K, Igarashi K (2006) Identification of the cadaverine recognition site on the cadaverine-lysine antiporter CadB. J Biol Chem 281:29213–29220
41. Stincone A, Daudi N, Rahman AS, Antczak P, Henderson I, Cole J, Johnson MD, Lund P, Falciani F (2011) A systems biology approach sheds new light on *Escherichia coli* acid resistance. Nucleic Acids Res 39:7512–7528
42. Sugimura S, Crothers DM (2006) Stepwise binding and bending of DNA by *Escherichia coli* integration host factor. Proc Natl Acad Sci USA 103:18510–18514
43. Vazquez-Juarez RC, Kuriakose JA, Rasko DA, Ritchie JM, Kendall MM, Slater TM, Sinha M, Luxon BA, Popov VL, Waldor MK, Sperandio V, Torres AG (2008) CadA negatively regulates *Escherichia coli* O157:H7 adherence and intestinal colonization. Infect Immun 76:5072–5081
44. Watson N, Dunyak DS, Rosey EL, Slonczewski JL, Olson ER (1992) Identification of elements involved in transcriptional regulation of the *Escherichia coli cad* operon by external pH. J Bacteriol 174:530–540
45. Yasuzawa K, Hayashi N, Goshima N, Kohno K, Imamoto F, Kano Y (1992) Histone-like proteins are required for cell growth and constraint of supercoils in DNA. Gene 122:9–15
46. Yona-Nadler C, Umanski T, Aizawa S, Friedberg D, Rosenshine I (2003) Integration host factor (IHF) mediates repression of flagella in enteropathogenic and enterohaemorrhagic *Escherichia coli*. Microbiology 149:877–884
47. Zhao B, Houry WA (2010) Acid stress response in enteropathogenic gammaproteobacteria: an aptitude for survival. Biochem Cell Biol 88:301–314
48. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31:3406–3415