Evolution of Bacterial Global Modulators: Role of a Novel H-NS Paralogue in the Enteroaggregative Escherichia coli Strain 042

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ABSTRACT Bacterial genomes sometimes contain genes that code for homologues of global regulators, the function of which is unclear. In members of the family Enterobacteriaceae, cells express the global regulator H-NS and its paralogue StpA. In Escherichia coli, out of providing a molecular backup for H-NS, the role of StpA is poorly characterized. The enteroaggregative E. coli strain 042 carries, in addition to the hns and stpA genes, a third gene encoding an hns paralogue (hns2). We present in this paper information about its biological function. Transcriptomic analysis has shown that the H-NS2 protein targets a subset of the genes targeted by H-NS. Genes targeted by H-NS2 correspond mainly with horizontally transferred (HGT) genes and are also targeted by the Hha protein, a fine-tuner of H-NS activity. Compared with H-NS, H-NS2 expression levels are lower. In addition, H-NS2 expression exhibits specific features: it is sensitive to the growth temperature and to the nature of the culture medium. This novel H-NS paralogue is widespread within the Enterobacteriaceae.

IMPORTANCE Global regulators such as H-NS play key relevant roles enabling bacterial cells to adapt to a changing environment. H-NS modulates both core and horizontally transferred (HGT) genes, but the mechanism by which H-NS can differentially regulate these genes remains to be elucidated. There are several instances of bacterial cells carrying genes that encode homologues of the global regulators. The question is what the roles of these proteins are. We noticed that the enteroaggregative E. coli strain 042 carries a new hitherto uncharacterized copy of the hns gene. We decided to investigate why this pathogenic E. coli strain requires an extra H-NS paralogue, termed H-NS2. In our work, we show that H-NS2 displays specific expression and regulatory properties. H-NS2 targets a subset of H-NS-specific genes and may help to differentially modulate core and HGT genes by the H-NS cellular pool.

KEYWORDS EAEC, H-NS, gene regulation

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A novel H-NS paralogue in the EAEC strain 042.
xenogeneic silencer, silencing horizontally acquired DNA (7). Unwanted expression of horizontally transferred (HGT) DNA would result in fitness costs. Recent reports have shown that H-NS binding to AT-rich sequences results mainly in suppression of transcription from intragenic promoters. Spurious transcription sequesters RNA polymerase molecules, hence reducing bacterial cell fitness. H-NS preventing intragenic transcription of AT-rich DNA would therefore avoid fitness costs (10, 11). H-NS modulates the expression of not only HGT DNA but also of core genes (12). H-NS consists of three structural domains: (i) a C-terminal domain, responsible for DNA binding (13); (ii) an N-terminal domain, responsible for dimerization (14–17); and (iii) a central dimer-dimer interaction domain, responsible for multimer formation (18, 19). In addition to homodimer and multimer formation, H-NS monomers are capable of heteromeric interactions with, among other proteins, members of the Hha family (20). These proteins show structural mimicry with the H-NS N-terminal domain and fine-tune the regulatory activity of H-NS-like proteins (12, 20, 21, 22).

The simultaneous presence of additional copies of hns homologues in the same cell is a relevant feature of this regulatory system. The enterobacterial genomes carry an hns paralogue, the stpA gene (23). The StpA protein is overexpressed in hns mutants (24). In E. coli, stpA mutants do not show a clear phenotype, and it has been suggested that StpA provides a molecular backup for H-NS in E. coli. In contrast, it has been shown that StpA modulates the expression of a significant number of genes in Salmonella (25). hns orthologues are also encoded by genes in plasmids (26). The IncHI1 plasmid pSF-R27 carries the hns orthologue sfh. Unlike H-NS, Sfh displays growth phase-dependent regulation (27). In the Shigella flexneri 2a strain 2457T, it was shown that each of the three proteins H-NS, StpA, and Sfh could form heterodimers with the corresponding homologues, thus suggesting that these proteins can modulate each other’s activities (27). Further studies showed that expression of the Sfh protein in cells harboring plasmid pSF-R27 provides a stealth function, avoiding the fact that plasmid incorporation results in a fitness cost for the bacterial host (28). The uropathogenic E. coli strain 536 contains, in addition to the hns and stpA genes, a third H-NS paralogue, the product of the hfp gene (29). The main regulatory role of the Hfp protein was found to occur at temperatures outside the host (25°C).

We report in this work the identification and characterization of a novel chromosomally encoded hns paralogue in the enteroaggregative E. coli (EAEC) strain 042 (open reading frame [ORF] EC042_2834). We present in this work experimental data showing that this variant has a specific role in modulating a subset of the H-NS-silenced genes.

RESULTS

Identification of the H-NS paralogue ORF EC042_2834 in the genome of the EAEC strain 042. The H-NS parologue ORF EC042_2834 (from here on termed H-NS2) was identified in the annotated genome of E. coli strain 042 by performing a BLAST search (http://www.uniprot.org/blast/) using the amino acid sequence of the H-NS protein (UniProt accession no. D3H2L9) as the template. Figure S1 in the supplemental material shows the nucleotide and amino acid sequence alignments of H-NS and H-NS2.

Upon identification of this new hns paralogue, we decided to obtain hns, hns2, and hns hns2 mutant derivatives from wild-type (wt) EAEC strain 042 and then compared their growth rates at 37 and 25°C (Fig. 1A and B). At 37°C, the effect of the hns2 allele on the growth rate is negligible. Nevertheless, the negative impact on the growth rate at 37°C of the hns hns2 double mutant is higher than that of the hns mutant alone. At 25°C, the hns2 allele alone moderately reduces the growth rate, and when it is combined with the hns allele, it drastically reduces the growth rate. These data suggest that, when strain 042 grows at 37°C, H-NS2 functions might be fulfilled by H-NS, but H-NS function could be only partially replaced by the existing H-NS2 protein levels. At low temperatures, H-NS2 function cannot be completely replaced by H-NS. Depletion of both proteins renders cells unable to grow at 25°C. We also studied whether the hns mutation by the hns2 gene cloned in the vector pLG338-30 restores wt growth rate.
E. coli MG1655 hns cells harboring the recombinant plasmid pLG338-30hns2 show a growth rate at 37°C similar to that of wt cells (Fig. S2).

**Role of H-NS2 modulating gene expression in strain 042.** We next studied the modulatory role of this novel H-NS paralogue. To do this, we decided to compare the effects of the hns, hns2, and hns hns2 alleles on the transcriptome of strain 042. Taking into account the fact that several H-NS-modulated genes are comodulated by the Hha protein, we also studied the transcriptome of an hha mutant. As strain 042 contains two additional hha paralogues (hha2 and hha3), to include one of these paralogues (hha2) in the modulation of gene expression together with hha (30), a hha hha2 double mutant (considered an hha null mutant) was used instead of an hha single mutant. The complete results of transcriptome sequencing (RNA-seq) analyses are presented in Table S1. Taking into account the fact that the main role of H-NS or Hha is to silence gene expression, we analyzed in detail those genes that, compared with the wt strain, are upregulated in the different mutant genetic backgrounds (Table 1). The results obtained clearly show the existence of different groups of *E. coli* 042 genes with regard to Hha/H-NS/H-NS2 modulation: (i) genes upregulated in the hha null mutant that are also upregulated in the hns and hns2 mutants (shown in red in Table 1) (remarkably, most of these genes are not upregulated in the hns hns2 double mutant); (ii) genes that are highly upregulated in the hns and hns hns2 mutants but are only modestly upregulated in the hns2 mutant (shown in yellow in Table 1) (these genes are not upregulated in the hha null mutant); (iii) genes that are upregulated only in the hns mutant (shown in violet in Table 1). It is important to highlight that those genes showing the highest upregulation levels in the hns2 mutant overlap with those that are upregulated in the hha null mutant. Hence, it is apparent that the H-NS2 protein has a specific role in repressing genes that require Hha for efficient silencing. The functions of those genes modulated by H-NS without requiring Hha are known in many instances.

**FIG 1** Impact of the hns and hns2 alleles on growth of *E. coli* strain 042. (A and B) Growth kinetics of wild-type (WT) *E. coli* 042 and its hns, hns2, and hns hns2 mutant derivatives in LB medium at 37°C (A) and 25°C (B). The experiments were performed in three biological replicates. Values are means ± standard deviations (error bars) are shown.
and several of these genes belong to the core genome (i.e., hde or gad operon). In contrast, the functions of most of the genes that are targeted by H-NS/Hha are not known. Most likely, they are HGT genes. The reported RNA-Seq data were also validated by quantitative reverse transcription-PCR (qRT-PCR) (Fig. S3).

Loss of H-NS or H-NS/H-NS2 function results in increased resistance to acid shock in strain 042. To correlate transcriptomic data with phenotype, we examined whether some of the observed deregulatory effects of both hns alleles on specific genes or operons could be correlated with predictable physiological changes. Taking into account the fact that genes in both the gad and hde operons participate in the adaptation of bacterial cells to acid pH, we studied survival of the wt strain, the hns and hns2 single mutants, and the hns hns2 double mutant under conditions of acid shock. The results obtained show that both the hns single mutant and the hns hns2 double mutant are more resistant to acid shock (Fig. 2). These results are in accordance with the observed upregulation of gad and hde operons both in the hns single mutant and the hns hns2 double mutant.

Regulation of the expression of the H-NS2 protein. It has been recently shown that the plasmid-encoded AraC negative regulator Aar modulates expression of both H-NS and H-NS2 in E. coli strain 042 (31). We decided to investigate whether, in addition to Aar, environmental or physiological factors influence H-NS2 expression. The hns promoter is known to be activated under conditions of cold shock (32) and is subjected to autoregulation (33–35). Out of these conditions, the H-NS levels are similar in cells growing under different conditions and also in cells collected at different stages of the growth curve (29, 36, 37). We studied whether the expression pattern of this novel variant is similar to that of H-NS. To compare expression of both H-NS proteins in strain 042, we decided to introduce a FLAG epitope and thereafter immunodetect them in cell
extracts. Whereas the H-NS2-FLAG construct could be easily obtained, it was not possible to obtain the H-NS-FLAG construct. We assume that this modified H-NS variant is deleterious to 042 cells. We used the clone expressing H-NS2-FLAG to determine the effects of different growth conditions and physiological states on H-NS2 expression (Fig. 3). Compared with expression in rich medium (Luria broth [LB] medium), H-NS2 expression increases in cells growing in minimal medium and in Dulbecco modified Eagle medium (DMEM) (Fig. 3A). With respect to growth temperature, high temperature (37°C) increases H-NS2 expression in cells growing in LB medium (Fig. 3B). Western blotting data were also complemented with the results of qRT-PCR analysis of transcription of H-NS2, which confirmed the Western blotting data (Fig. S4). We analyzed H-NS expression by using H-NS-specific antibodies. As previously described (38), H-NS levels remained fairly constant in samples collected from cultures from strain 042.

![Graph showing survival to acid shock](image)

**FIG 2**  
**hns** and **hns hns2** alleles result in increased acid shock resistance. Survival to acid shock of *E. coli* strain 042 and its **hns**, **hns2**, and **hns hns2** mutant derivatives. Experiments were performed in three biological replicates. Values are means ± standard deviations (error bars) are shown.

![Western blot images](image)

**FIG 3**  
H-NS2-FLAG expression is upregulated in DMEM and M9 minimal medium and when cells enter the stationary growth phase. (A) Immunodetection of H-NS2-FLAG in cell extracts from *E. coli* strain 042 growing at 25°C and 37°C in LB, M9 minimal medium, and DMEM at the onset of the stationary phase (OD$_{600}$ of 2.0). (B) Immunodetection of H-NS2-FLAG in cell extracts from *E. coli* strain 042 growing in LB medium at 25°C and 37°C both at the exponential and early stationary growth phases (OD$_{600}$ of 0.4 and 2.0, respectively). Experiments were repeated three times. The results of a representative experiment are shown.
grown in the different conditions used (Fig. S5). We also determined H-NS2 expression in a strain 042 hns mutant in cells growing in LB medium at 25°C and 37°C. As expected, H-NS2 is overexpressed (Fig. 4).

We used qRT-PCR to compare transcription of H-NS and H-NS2 proteins. Cells were grown in LB medium until the beginning of stationary phase (optical density at 600 nm [OD600] of 2.0), and transcripts were quantified. Transcription of the hns gene is several orders of magnitude higher (more than 80-fold) than transcription of the hns2 gene.

Interaction of H-NS2 with other proteins. Taking into account the fact that H-NS interacts, among other proteins, with StpA and Hha, we also studied the interaction of H-NS2 with other proteins. To do this, pulldown experiments were performed using His-tagged H-NS2 protein with the tag at the N- or C-terminal end. Two proteins could be identified as interacting with H-NS-2: H-NS and the Lon protease (Fig. 5). These results do not rule out an interaction with Hha. As the cellular concentration of Hha is not high, experimental evidence for copurification of Hha with His-tagged H-NS requires Hha overproduction (39). To confirm H-NS2 interaction with Hha, pulldown experiments were performed with His-tagged Hha protein. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis of the fraction coeluting with Hha confirmed the presence of H-NS2 (Table S2). Because of the interaction of H-NS2 with Lon, we decided to ascertain whether, as shown for StpA (40), H-NS2 is sensitive to Lon-mediated proteolysis. In StpA, the amino acid residue at position 21 is F instead of C. In fact, the protease sensitivity would be lost after the mutation F21C (40). Interestingly, in H-NS2 protein, amino acid C is substituted by the hydrophobic amino acid L (Fig. 6). H-NS2 stability was measured in the wt strain and in hns and lon genetic backgrounds. No differences in stability could be found (Fig. S6).

Distribution of the hns2 gene within the Enterobacteriaceae. We also studied the distribution of H-NS2 among members of the family Enterobacteriaceae. To assess this,
we performed a BLAST search using the nucleotide sequence of the hns2 gene as the template. The results obtained (Fig. 7) show that this novel hns paralogue is distributed in different genera of the Enterobacteriaceae, including, in addition to Escherichia, Klebsiella and Salmonella. All strains carrying the hns2 gene also carried hns and stpA. The hns2 sequence was identical in all strains. Interestingly, the third paralogue, Hfp, of E. coli 536 is closely related to H-NS2. This is not the case for the plasmid-encoded Sfh and H-NSR27 (the H-NS protein encoded by the IncHI plasmid R27 gene) proteins. We also aligned H-NS, H-NS2, and StpA amino acid sequences (Fig. 6). Overall, the H-NS2 sequence shows higher similarity to the H-NS sequence (64.4%) than to the StpA sequence (52.99%). When the dimerization/oligomerization domain (amino acid residues 1 to 83) and DNA binding domain (amino acids 91 to 137) are compared, H-NS2 shows higher similarity to H-NS than StpA in the dimerization/oligomerization domain (62.65% versus 53.01%) but lower similarity in the DNA binding domain (64.44% versus 70.5%).

**DISCUSSION**

Several global regulators have been studied intensively in the last decades, and their biological role is well characterized. Nevertheless, this is not the case for their corresponding paralogues and orthologues, whose function remains obscure in many cases. Previous reports have shown that the cellular H-NS pool can include, in addition to the H-NS protein and its paralogue StpA, at least a third paralogue. This was reported for

![Fig 5](image-url)
Shigella flexneri strain 2a 2457T, which harbors the pSF-R27 plasmid that expresses the Sfh protein (41), and for the uropathogenic E. coli strain 536, which expresses the Hfp protein (29). We show in this report that a new H-NS paralogue is expressed, among other enterobacterial strains, by EAEC strain 042. As expected from the already established biological role, the H-NS paralogues StpA or Hfp (23, 29), H-NS2 provides a molecular backup for H-NS. This can be shown when growth of wt 042 cells, hns or hns2 single mutants, and hns hns2 double mutant derivatives in LB medium is compared. The combination of hns and hns2 alleles has a stronger impact in the growth rate than the hns allele alone.

A relevant difference between H-NS and H-NS2 is the set of genes targeted by these proteins. H-NS targets a large set of genes in strain 042, including both core genes and HGT genes. The latter set of genes is also modulated by Hha-like proteins, as shown in the transcriptomic analysis of the hha null mutant. Most of the genes showing high-level upregulation in an hns mutant (some of these genes are core genome genes) are only modestly upregulated in an hns2 mutant and do not require comodulation by Hha. Examples are the genes belonging to the gad, mat, and hde operons. Interestingly, the most upregulated genes in an hns2 mutant are the genes that are also the most upregulated in the hha null mutant. Hence, H-NS or H-NS2 modulates, with Hha, several E. coli 042 genes, most of them of unknown function and likely of HGT origin. In contrast, several core genes are mainly modulated by H-NS without the requirement for Hha. H-NS2 targeting a subset of the H-NS-modulated genes is also supported by the fact that some of the genes showing significant upregulation in an hns mutant show wt expression levels in the hns2 mutant. When considering recent findings showing that a main role of H-NS is to silence transcription that occurs from intragenic promoters in AT-rich HGT DNA (10, 11), it can be hypothesized that H-NS2 participates in these processes in strain 042. It is also remarkable that, whereas most of the H-NS-modulated genes that are not targeted by Hha show the highest upregulation in a hns hns2 double mutant, most of the Hha-modulated genes (which are comodulated by H-NS/H-NS2) show a wt regulatory pattern in that hns hns2 mutant. In such a genetic background,
the stpA gene is strongly upregulated. We hypothesize that high levels of StpA would occur when H-NS and H-NS2 are not available (avoiding unwanted upregulation of the set of genes modulated by the H-NS/Hha system).

H-NS2 preferentially targeting HGT genes is reminiscent of the role of the H-NS protein encoded by the IncHI plasmid R27 gene (H-NSR27)(12). H-NSR27 specifically silenced HGT genes, as Hha did. It is apparent that a global modulator needs to develop specific mechanisms to discriminate between different sets of genes to be regulated (i.e., HGT and core DNA). In the H-NS model, variants such as the plasmid-encoded H-NSR27 or the chromosomally encoded H-NS2 proteins appear to have evolved mainly to recognize structural domains of only a subset of the H-NS-modulated genes.

In addition to the already reported Aar-dependent modulation of H-NS2 (31), we show here that, unlike H-NS, H-NS2 shows temperature- and nutrient-dependent regulation. With respect to temperature, H-NS2 expression is higher at 37°C than at 25°C. This is consistent with H-NS2 modulating expression of strain 042 virulence determinants. With respect to nutrient concentration, it is apparent that a low growth rate (i.e., growth in mineral medium) results in higher H-NS2 expression. This may be correlated with growth within the host, conditions leading to reduced growth rates. Comparing hns and hns2 transcription showed that hns2 transcription is significantly lower than hns transcription. Because we were unable to obtain an H-NS-FLAG construct (most likely because the recombinant protein was deleterious), we could not directly compare H-NS and H-NS2 protein levels. Nevertheless, the significant difference in transcription predicts that H-NS2 levels would be significantly lower than H-NS levels. These data suggest that, whereas H-NS contributes simultaneously to chromosome architecture and gene silencing, H-NS2 displays only regulatory functions. The molecular mechanism by which proteins such as H-NS2 in vivo preferentially target some of

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FIG 7 Evolutionary relationships of H-NS, StpA, and third H-NS paralogues in different members of Enterobacteriaceae. The dendrogram shows the relationships between the different proteins. Enterobacterial strains that contain a chromosomal copy of the hns2 gene are presented. The amino acid sequences used from the following species are shown in the dendrogram (the NCBI accession numbers are shown in parentheses): Klebsiella aerogenes strain CAV1320 (AKK82112.1), Klebsiella michiganensis HK02PL1 (AHW87442.1), Escherichia coli strain K-15KW01 (ANR82355.1), Escherichia coli strain SaT040 (AML15501.1), Klebsiella pneumoniae subsp. aerogenes strain KPNH29 (AIX70716.1), Escherichia coli PA458 (ASW60759.1), Salmonella enterica serotype Enteritidis strain 74-1357 (ATT16728.1), Escherichia coli 042-HNS (CBG35667), H-NS2 (CBG35667), and StpA (CBG35697), Escherichia coli MG1655 H-NS (NP_417155.1) and StpA (NP_417155.1), R27 plasmid H-NS (NP_058377.1), Shigella flexneri 2a 5th (AAN38840.1), Vibrio cholerae AM-192226 Vich (ZP_04963444.1), and Escherichia coli 536 Hfp (ABG69928). The evolutionary history was inferred using the neighbor-joining method, and evolutionary analyses were conducted in MEGA7 (50). The bar shows 0.050 nucleotide substitutions per position.
the H-NS-regulated genes and not others remains to be elucidated. Band shift assays with different DNA fragments corresponding to the regulatory region of both subsets of H-NS-regulated genes did not show that H-NS2 preferentially binds to any of them, either in the presence or absence of the Hha protein (our unpublished results). In addition, no significant differences regarding curvature, AT percentage, or presence/absence of consensus H-NS binding sites could be identified by our in silico analysis of these DNA sequences (our unpublished data).

In vitro interaction of H-NS2 with Lon protease predicted that, as has been shown for StpA (40), H-NS2 may be subjected to Lon-mediated proteolysis. This assumption was reinforced by the fact that the cysteine 21 present in H-NS is replaced by the hydrophobic residues leucine in StpA and phenylalanine in H-NS2. Leucine 21 is required for StpA sensitivity to proteolytic cleavage (40). Nevertheless, testing of H-NS2 stability, both in the wt and in hns and lon mutants, did not provide evidence for H-NS2 being unstable. Whether H-NS2–Lon interaction is an artifact or indicates H-NS2 proteolytic degradation under conditions not tested by us remains to be elucidated.

According to the available information, the third H-NS paralogues share properties such as the following. (i) Their regulatory pattern can be different from that of H-NS. (ii) Their expression levels are in some instances lower than that of H-NS. (iii) They may expand and fine-tune the regulatory features of the H-NS system either by forming heteromeric complexes with H-NS/STM or by recognizing only a subset of the H-NS-modulated genes (i.e., the genes modulated by the Hha family of proteins). The fact that all strains shown to possess the hns2 gene also possess the hns and stpA genes suggests specific functions for the H-NS2 protein that are different from those of StpA. H-NS2 shows a higher degree of similarity to H-NS than to StpA. Interestingly, when considering the H-NS2 dimerization/oligomerization domain and DNA binding domain, the former shows a higher degree of similarity to H-NS than that of StpA, but the latter does not. This suggests H-NS2 showing dimerization/oligomerization properties more similar to those of H-NS, and differing more significantly in its DNA binding proteins. This can be correlated with the fact that several of the H-NS-targeted genes are not targeted by H-NS2. All strains found containing the hns2 gene that have been characterized so far are virulent isolates displaying multiple antibiotic resistance phenotypes (42, 43). Further characterization of the role of the hns2 gene in virulence regulation may contribute to developing specific strategies to combat infections caused by these strains.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. All bacterial strains used in this work are listed in Table S3 in the supplemental material. Cultures were usually grown in Luria broth (LB) medium (10 g NaCl, 10 g tryptone, and 5 g yeast extract [all per liter]). Cultures were also grown in M9 minimal medium (44) and Dulbecco modified Eagle medium (DMEM) (Gibco) supplemented with 0.45% glucose with vigorous shaking at 200 rpm (Innova 3100 water bath shaker; New Brunswick Scientific). The following antibiotics were used at the concentrations indicated: kanamycin (50 μg ml⁻¹), carbenicillin (100 μg ml⁻¹).

Plasmid construction. In order to perform complementation experiments, the hns2 gene (open reading frame [ORF] EC042_2834) of E. coli 042 strain was cloned into the pLG338-30 vector. Primers hns2 pLG338 ECORI fw 5 (fw stands for forward) and hns2 pLG338 BamHI rev 3 (rev stands for reverse) (see Table S4 for sequences) were used to PCR amplify the hns2 gene using Phusion Hot Start II DNA polymerase (Thermo Fisher Scientific). The PCR fragment was purified using GeneJET PCR purification kit (Thermo Scientific) and digested with EcoRI and BamHI restriction enzymes (Thermo Scientific). Ligation was performed in pLG338-30 digested with the same restriction enzymes and treated with alkaline phosphatase. The resulting plasmid (pLG338-30hns2) was transformed into E. coli DH5α cells and selected in the presence of carbenicillin. For confirmation of correct in-frame insertion of the hns2 gene, primers pLG338 EB Fw (Fw stands for forward) and pLG338 EB Rv (Rv stands for reverse) (see Table S4 for sequences) were used for sequencing.

Genetic manipulations. All enzymes used to perform standard molecular and genetic procedures were used according to the manufacturer’s recommendations. To introduce plasmids in E. coli, bacterial cells were grown until an optical density at 600 nm (OD₆₀₀) of 0.6 was reached. Cells were then washed several times with 10% glycerol, and the respective plasmids were introduced by electroporation using an Eppendorf gene pulser (Electroporator 2510).

Mutant derivatives lacking the hns and hns2 genes in enteropathogenic E. coli (EAEC) strain 042 were obtained by the λ Red recombinant method (45). Briefly, the kanamycin antibiotic resistance cassette of
plasmid pKD4 was amplified using oligonucleotides Hns042P1 and Hns042P2 and oligonucleotides 2834P1 and 2834P2 for hns and hns2 deletions, respectively (see Table S4 for sequences). DNA templates were treated with DpnI (Thermo Fisher Scientific) following the manufacturer's recommendations and then purified and electroporated to the competent cells. Mutants were selected on LB plates containing the appropriate selection marker (kanamycin in this case), and the successful deletion of the gene was confirmed by PCR using the KT primer (kanamycin resistance; Kmr) in combination with specific primers located in the remaining gene sequence in the bacterial chromosome (see Table S4 for the sequence).

If necessary, the antibiotic resistance determinant was eliminated by transforming the mutant strain with plasmid pCP20 and subsequent incubation at 42°C for two or more passages as reported previously (45). The pCP20 plasmid carries the gene encoding the Flp recombinase that catalyzes recombination between the FRT sites flanking the kanamycin cassette (45). The double deletions were obtained by combining one previous deletion with another deletion associated with an antibiotic resistance cassette.

A chromosomal insertion of FLAG sequence into the hns2 gene was obtained by a modification of the Red recombinant method, as previously described (46). The antibiotic resistance determinant of plasmid pSU811 was amplified using oligonucleotides 28340423XP1 and 28340423XP2 (see Table S4 for sequences). Mutants were selected on LB plates containing kanamycin, and successful FLAG insertion was confirmed by PCR using the oligonucleotide KT (kanamycin resistance; Kmr) in combination with specific oligonucleotides located in the remaining gene sequence nearby (28343XP1UP and 28343XP2DOWN, see Table S4 for sequences). The chromosomal fusion H-NS2-FLAG was constructed in the parental strain E. coli 042 and in the isogenic Δhns mutant, generating 042hns-hns2-Flag.

**SDS-PAGE and Western blotting.** Whole-cell protein extracts (pelleted cells resuspended in A50 buffer) were separated on a 15% SDS-polyacrylamide gel. To detect specific proteins, they were transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad) by semidy electrotransfer. Prior to the Western blotting procedure, the total protein content of whole-cell extracts was checked by Coomassie blue staining.

For immunodetection of H-NS2-FLAG protein, a primary monoclonal anti-FLAG (Sigma) was used. To immunodetect H-NS native protein, a primary polyclonal anti-H-NS was used. Detection of primary antibodies bound to the specific proteins analyzed was performed with a horseradish peroxidase (HRP)-conjugated secondary antibody. The detection reagent used was ECL Prime Western blotting (GE Healthcare). Detection and the visualization of the chemiluminescent bands corresponding to the proteins being studied were performed using Molecular Imager ChemiDoc XRS system and Quantity One software (Bio-Rad).

**His tagging and pulldown experiment.** For overexpression of the H-NS2 protein, the hns2 gene was cloned into the aLICator LIC cloning and expression vector (Thermo Fisher Scientific), following the manufacturer's recommendations. The hns2 gene was amplified by PCR using the Phusion Hot Start II DNA polymerase (Thermo Scientific) in combination with oligonucleotides (hns2_plate51NT fw and hns2_plate51NT rev) for N-terminal cloning and oligonucleotides (hns2_plate31CT fw and hns2_plate31 CT rev) for C-terminal cloning. PCR products were purified using the GeneJET PCR purification kit (Thermo Scientific), and DNA concentration and quality were measured using a Nano-Drop 1000 instrument (Thermo Fisher Scientific). DNA ligations to pLATE vectors were performed following the manufacturer’s recommendations, generating plasmids pLATE51-6His-H-NS2 and pLATE31H-NS2-6His, respectively. His-tagged Hha (His-Hha) protein was purified as described (39). Pulldown experiments were performed using isopropyl-β-D-thiogalactoside (IPTG)-induced E. coli BL21(DE3) Δhns cells containing plasmids pLATE31H-N52-6His, pLATE51H-6His-H-NS2, and pET15bHisHha (Table S3). Cells were resuspended in A50 buffer (20 mM HEPES, 100 mM KCl, 5 mM MgCl₂, and 50 mM imidazole), lysed by using a French press (three times at 800 l/min), and centrifuged. Supernatant-free cells containing overexpressed His-tagged H-NS2 and His-Hha proteins were purified using Ni²⁺-agarose resin (Qiagen). Briefly, Ni²⁺-aggerose resin was washed five times with A50 buffer. Then, cell-free supernatant and Ni²⁺-agarose resin were mixed together and allowed to interact overnight at 4°C. After two washing steps of the resin with A50 buffer, His-tagged H-NS2 variants were eluted with the same buffer supplemented with 200 mM imidazole. Eluted proteins were analyzed by SDS-PAGE and stained with Coomassie blue to check the correct purification of the proteins. Afterward, the elution fractions were mixed with E. coli 042 total protein extract. Again, His-tagged H-NS2 and His-Hha proteins were newly purified. The proteins that copurified with H-NS2 and Hha were identified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

**In vivo H-NS2 protein stability.** The intracellular stability of H-NS2-FLAG was evaluated as previously described (47). Protein stability was monitored after inhibition of protein synthesis by the addition of chloramphenicol to the corresponding bacterial cultures. Chloramphenicol (100 mg/ml) was added to bacterial cultures grown to an OD₆₀₀ of 2.0 in LB medium at 37°C to a final concentration of 25 μg/ml. After antibiotic addition, samples were removed at the indicated time intervals and whole protein extracts were analyzed by Western blotting.

**Protein identification (LC-MS/MS).** Protein identification was performed at Proteomic Platform (Barcelona Science Park, Barcelona, Spain). Briefly, proteins were manually digested with trypsin (sequencing grade modified; Promega) in the gel. The excised band was washed sequentially with NH₄HCO₃ (25 mM) and acetonitrile (ACN). Proteins were reduced and alkylated by treatment with 20 mM dithiothreitol (DTT) solution for 60 min at 60°C, followed by treatment with a 50 mM solution of iodoacetamide for 30 min at room temperature, respectively. After sequential washings with buffer and acetonitrile, the proteins were digested overnight at 37°C with 200 ng of trypsin. Tryptic peptides were identified using a Q-Exactive mass spectrometer (Thermo Fisher Scientific), and the data were analyzed using the Proteome Discoverer software (Thermo Fisher Scientific). The database search was performed against the *Escherichia coli* genome (Gene ID: 1117 (Strain: K12)). The peptide-matching algorithm was set to the MS/MS ion fragments to identify the proteins present in the samples.
extracted from the gel matrix with 10% formic acid and acetonitrile; the extracts were pooled and dried in a vacuum centrifuge. The dried peptide mixture was analyzed in a nanoAcquity liquid chromatography column (Waters) coupled to an LTQ-Orbitrap Velos (Thermo Fisher Scientific) mass spectrometer. The tryptic digest was resuspended in 1% formic acid (FA) solution, and an aliquot was injected for chromatographic separation. Peptides were trapped on a Symmetry C18 trap column (5 μm; 180 μm by 20 mm; Waters) and separated using a C18 reverse-phase capillary column (ACQUITY UPLC BEH column; 130 Å, 1.7 μm, 75 μm by 250 mm; Waters). The gradient used for the elution of the peptides was 1 to 40% solvent B in 30 min, followed by a gradient from 40% to 60% in 5 min (solvent A is 0.1% FA; solvent B is 100% ACN and 0.1% FA), with a 250 nl min⁻¹ flow rate. Eluted peptides were subjected to electrospray ionization with an emitter needle (New Objective PicoTip; Scientific Instrument Services, Inc.) with an applied voltage of 2,000 V. Peptide masses (m/z; 300 to 1,700) were analyzed in data-dependent mode where a full-scan MS was acquired in the Orbitrap mass spectrometer with a resolution of 60,000 full width at half maximum (FWHM) at an m/z of 400. Up to the 15 most abundant peptides (minimum intensity of 500 counts) were selected from each MS scan and then fragmented in the linear ion trap using collision-induced dissociation (CID) (38% normalized collision energy) with helium as the collision gas. The scan time settings were as follows: 250 ms (1 microscan) for full MS and 120 ms for MSn. Generated .raw data files were collected with Thermo Xcalibur (v.2.2). The .raw file obtained in the mass spectrometry analysis was used to search against a database containing all entries for Enterobacteriaceae present in the public database UniProt (v.13/2/2017). A database containing common laboratory contaminant proteins was added to this database. The software used was Thermo Proteome Discoverer (v.1.4.1.14) with Sequest HT as the search engine. Both a target and a decoy database were searched in order to obtain a false-discovery rate (FDR), and thus estimate the number of incorrect peptide-spectrum matches that exceed a given threshold. The search results were visualized in Proteome Discoverer (v.1.4.1.14) and exported to Excel as a list of identified proteins.

**RNA-Seq.** RNA extraction, DNase treatment, and evaluation of RNA quality and cDNA libraries for Illumina sequencing were performed by Vertis Biotechnologie AG, Freising-Weihenstephan, Germany. Total RNA was isolated from the cell pellets using a bead mill and the mirVana RNA isolation kit (Ambion) including DNase treatment. The total RNA preparations were examined by capillary electrophoresis. From the total RNA samples, RNA molecules were depleted using the Ribozero rRNA removal kit for bacteria (Illumina). From the rRNA-depleted RNA samples, first-strand cDNA was synthesized using an N6 randomized primer. After fragmentation, the Illumina TruSeq sequencing adapters were ligated in a strand-specific manner to the 5’ and 3’ ends of the cDNA fragments. The cDNA was finally amplified by PCR (15 PCR cycles) using a proofreading enzyme. For Illumina sequencing, cDNA libraries were pooled in a 25:1 ratio. The library pool was fractionated in the size range of 250 to 500 bp using a differential clean-up with the Agencourt AMPure kit. The cDNA pool was sequenced on an Illumina NextSeq 500 system using 75-bp read length. For single-end sequencing, we used an Illumina NextSeq 500 system and a MID 150 kit with a single 75-bp read length. Base calling was performed online during the sequencing procedure with the Real-Time Analysis (RTA) software version 2.4.11 and System Suite version 2.1.2.1. Illumina sequencing instruments generate per-cycle BCL base call files as primary sequencing output in the bcl2 format. Conversion of the bcl2 file to gzipped fastq files was performed using the bcl2fastq Script v. 2.180.12 provided by Illumina. Quality and adapter trimming was performed with the CLC Genomics Workbench 9.0 software package using the “Trim Sequences” tool with standard parameters. Mapping of the trimmed reads to the reference sequences was also performed with the CLC Genomics Workbench 9.0 using the “Map Reads to Reference” tool with standard parameters. For quantification of gene expression (read counting), the alignments generated with the Genomics Workbench were exported in BAM format. Read counting was then performed with the FeatureCounts v. 1.5.0-p1 program using the following parameters and settings: level, meta-feature level; paired-end, no; strand specific, yes; multimapping reads, counted (as fractions); multioverlapping reads, not counted; overlapping bases, 30; read orientations, fr.

**Quantitative reverse transcription-PCR (qRT-PCR).** Total RNA was isolated from bacterial pellets using the Tripure isolation reagent (Roche) according to the manufacturer’s recommendations. Potential traces of DNA were removed by digestion with DNase I (TurbRNA-free; Ambion), according to the manufacturer’s instructions. RNA concentration and RNA quality were measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). For cDNA synthesis, 1 μg of total RNA isolated previously was reverse transcribed to generate cDNA using the High-capacity cDNA reverse-transcription kit (Applied Biosystems) according to the manufacturer’s protocol. All samples within an experiment were reverse transcribed at the same time, and the resulting cDNA was diluted 1:100 in nuclease-free water and stored in aliquots at −80°C until used. As a control, parallel samples were run in which reverse transcriptase was omitted from the reaction mixture. Real-time PCR was conducted using Maxima SYBR green/ROX qPCR master mix (2X) (Thermo Scientific) and the ABI Prism 7700 sequence detection system (Applied Biosystems). Specific oligonucleotides complementary to the genes of interest were designed using primer3 software (see Table S4 for sequence). Relative quantification of gene expression of mutants versus wild-type (wt) strain was performed using the comparative threshold cycle (ΔΔCT) method (48). The relative amount of target cDNA was normalized using the gapA gene as an internal reference standard. Fold change values referring to relative expression of target genes in mutant strains versus the wt strain were calculated by dividing the ΔCT (difference between the C_T values for the target gene and the internal reference standard gapA gene) obtained for the different mutant strains versus the wt strain.

**Acid shock assay.** The acid shock assay was performed as described previously (49). Briefly, bacterial cultures were grown in LB medium to early stationary phase (OD600 of 2.0) and subjected to acid stress.
by adding gradually 6 N HCl to cultures until a pH of 3.2 was reached. The pH values were monitored by pH measurements on a separate culture. After acid addition, the cultures were shaken at 37°C for 30 min, 1 h, and 2 h. At the corresponding time intervals, cells were serially diluted in 0.9% NaCl and then plated on LB agar plates for colony counting.

**H-NS2 phylogeny.** hns paralogues were identified by performing a BLAST search ([blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi)) using the nucleotide sequence of the hns gene from E. coli 042 strain (ORF EC042_1292) as the template. Then, a phylogenetic tree with H-NS, StpA, and third H-NS paralogues were constructed using neighbor joining as a clustering method conducted in MEGA7 (50).

**Accession number(s).** The RNA sequencing reads have been deposited in the Gene Expression Omnibus (GEO) Sequence Read Archive of the National Center for Biotechnology Information (GSE105133) under accession numbers GSM2822965, GSM2822966, GSM2822967, GSM2822968, and GSM2822969.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at [https://doi.org/10.1128/mSystems.00220-17](https://doi.org/10.1128/mSystems.00220-17).

**FIG S1,** TIF file, 0.2 MB.

**FIG S2,** TIF file, 0.3 MB.

**FIG S3,** TIF file, 0.1 MB.

**FIG S4,** TIF file, 1.7 MB.

**FIG S5,** TIF file, 0.1 MB.

**FIG S6,** TIF file, 0.2 MB.

**TABLE S1,** XLS file, 2 MB.

**TABLE S2,** DOC file, 0.05 MB.

**TABLE S3,** DOC file, 0.1 MB.

**TABLE S4,** DOC file, 0.1 MB.

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**REFERENCES**

1. Maurelli AT, Sansonetti PJ. 1988. Identification of a chromosomal gene controlling temperature-regulated expression of Shigella virulence. Proc Natl Acad Sci U S A 85:2820–2824. [https://doi.org/10.1073/pnas.85.8.2820](https://doi.org/10.1073/pnas.85.8.2820).

2. Göransson M, Söndén B, Nilsson P, Dagberg B, Forsman K, Emanuelsson K, Uhlin BE. 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature 344:682–685. [https://doi.org/10.1038/344682a0](https://doi.org/10.1038/344682a0).

3. Higgins CF, Dorman CJ, Stirling DA, Waddell L, Booth IR, May G, Bremer E. 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. Cell 52:569–584. [https://doi.org/10.1016/0092-8674(88)90470-9](https://doi.org/10.1016/0092-8674(88)90470-9).

4. Dorman CJ. 2007. H-NS, the genome sentinel. Nat Rev Microbiol 5:157–161. [https://doi.org/10.1038/nrmicro1598](https://doi.org/10.1038/nrmicro1598).

5. Grainger DC, Hurd D, Goldberg MD, Busby SJW. 2006. Association of nucleoid proteins with coding and non-coding segments of the Escherichia coli genome. Nucleic Acids Res 34:4642–4652. [https://doi.org/10.1093/nar/gkl542](https://doi.org/10.1093/nar/gkl542).

6. Lucchini S, Rowley G, Goldberg MD, Hurd D, Harrison M, Hinton JCD. 2006. H-NS mediates the silencing of laterally acquired genes in bacteria. PLoS Pathog 2:e81. [https://doi.org/10.1371/journal.ppat.0020081](https://doi.org/10.1371/journal.ppat.0020081).

7. Navarre WW, Porovnik S, Wang Y, McClelland M, Rosen H, Libby SJ, Fang FC. 2006. Selective silencing of foreign DNA with low GC content by the H-NS protein in *Salmonella*. Science 313:236–238. [https://doi.org/10.1126/science.1128794](https://doi.org/10.1126/science.1128794).

8. Oshima T, Ishikawa S, Kurokawa K, Alba H, Ogasawara N. 2006. Escherichia coli histone-like protein H-NS preferentially binds to horizontally acquired DNA in association with RNA polymerase. DNA Res 13:141–153. [https://doi.org/10.1093/dnares/dsi009](https://doi.org/10.1093/dnares/dsi009).

9. Kahramanoglu C, Seshasayee ASN, Prieto AI, Ibbersson D, Schmidt S, Zimmermann J, Benes V, Fraser GM, Luscombe NM. 2011. Direct and indirect effects of H-NS and Fis on global gene expression control in *Escherichia coli*. Nucleic Acids Res 39:2073–2091. [https://doi.org/10.1093/nar/gkt934](https://doi.org/10.1093/nar/gkt934).

10. Singh SS, Singh N, Bonocora RP, Fitzgerald DM, Wade JT, Grainger DC. 2014. Widespread suppression of intragenic transcription initiation by H-NS. Genes Dev 28:214–219. [https://doi.org/10.1101/gad.234336.1113](https://doi.org/10.1101/gad.234336.1113).

11. Lamberte LE, Baniulyte G, Singh SS, Stringer AM, Bonocora RP, Stracy M, Kapanidis AN, Wade JT, Grainger DC. 2017. Horizontally acquired AT-rich genes in *Escherichia coli* cause toxicity by sequestering RNA polymerase. Nat Microbiol 2:16249. [https://doi.org/10.1038/nmicrobiol.2016.249](https://doi.org/10.1038/nmicrobiol.2016.249).

12. Baños RC, Vivero A, Aznar S, García J, Pons M, Madrid C, Juárez A. 2009. Differential regulation of horizontally acquired and core genome genes by the bacterial modulator H-NS. PLoS Genet 5:e1000513. [https://doi.org/10.1371/journal.pgen.1000513](https://doi.org/10.1371/journal.pgen.1000513).

13. Shindo H, Iwaki T, Ueda K, Kurumizaka H, Ueguchi C, Mizuno T, Morikawa S, Nakamura H, Kuboniwa H. 1995. Solution structure of the DNA binding domain of a nucleoid-associated protein, H-NS, from *Escherichia coli*. J Mol Biol 234:36–46. [https://doi.org/10.1016/0022-2836(95)80079-O](https://doi.org/10.1016/0022-2836(95)80079-O).

14. Bloch V, Yang Y, Margate E, Chavanieu A, Augé MT, Robert B, Arold S, Arold ST. 2003. Crystal structure of the N-terminal dimerization domain of a nucleoid-associated protein, H-NS, from *Escherichia coli*. J Mol Biol 334:179–185. [https://doi.org/10.1016/j.jmb.2003.09.051](https://doi.org/10.1016/j.jmb.2003.09.051).
16. Esposito D, Petrovic A, Harris R, Ono S, Eclestone JF, Mbabaali A, Haq I, Higgins CF, Hinton JCD, Driscoll PC, Ladbury JE. 2002. H-NS oligomerization domain structure reveals the mechanism for high order self-association of the intact protein. J Mol Biol 324:841–850. https://doi.org/10.1006/jmbi.2002.2836(02)01141-5.

17. Ueguchi C, Suzuki T, Yoshida T, Tanaka K, Mizuno T. 1996. Systematic mutational analysis revealing the functional domain organization of *Escherichia coli* nucleoid protein H-NS. J Mol Biol 263:149–162. https://doi.org/10.1006/jmbi.1996.0566.

18. Arold ST, Leonard PG, Parkinson GN, Ladbury JE. 2010. H-NS forms a superhelical protein scaffold for DNA condensation. Proc Natl Acad Sci USA 107:15728–15732. https://doi.org/10.1073/pnas.1009661017.

19. Leonard PG, Ono S, Gor J, Perkins SJ, Ladbury JE. 2009. Investigation of the self-association and hetero-interaction interactions of H-NS and StPa from Enterobacteria. Mol Microbiol 73:165–179. https://doi.org/10.1111/j.1365-2958.2009.06754.x.

20. Madrid C, Balsalobre C, García J, Juárez A. 2007. The novel Hha/YmaO family of nucleoid-associated proteins: use of structural mimicry to modulate the activity of the H-NS family of proteins. Mol Microbiol 63:7–14. https://doi.org/10.1111/j.1365-2958.2006.05497.x.

21. García J, Madrid C, Juárez A, Pons M. 2006. New roles for key residues in helices H1 and H2 of the *Escherichia coli* H-NS N-terminal domain: H-NS dimer stabilization and Hha binding. J Mol Biol 359:679–689. https://doi.org/10.1016/j.jmb.2006.03.059.

22. Ali SS, Whitney JC, Stevenson J, Robinson H, Howell PL, Navarre WW. 1997. *Escherichia coli* hns is a repressor of its own transcription. Mol Gen Genet 236:171–178. https://doi.org/10.1007/BF00277109.

23. Arold ST, Leonard PG, Parkinson GN, Ladbury JE. 2010. H-NS forms a superhelical protein scaffold for DNA condensation. Proc Natl Acad Sci USA 107:15728–15732. https://doi.org/10.1073/pnas.1009661017.

24. Alejandro A, Miralles A, Pascual C, Feu J. 2009. The H-NS-like nucleoid-structuring protein Sfh, StpA and H-NS nucleoid-structuring proteins of *Shigella flexneri* mid 80:32–44.

25. Lucchini S, McDermott P, Thompson A, Hinton JCD. 2002. H-NS oligomerization domain structure reveals the mechanism for high order self-association of the intact protein. J Mol Biol 324:841–850. https://doi.org/10.1006/jmbi.2002.2836(02)01141-5.

26. Atlung T, Ingmer H. 1997. H-NS: a modulator of environmentally regulated gene expression. Mol Microbiol 63:7–14. https://doi.org/10.1111/j.1365-2958.1995.mmi_18010101.x.

27. Deighan P, Beloin C, Dorman CJ. 2003. Three-way interactions among H-NS, Hha and H-NS. Mol Gen Genet 263:349–358. https://doi.org/10.1007/s00438-003-0897-0.

28. Shintani M, Suzuki-Minakuchi C, Nojiri H. 2015. Nucleoid-associated histone-like proteins in *Escherichia coli*. Mol Microbiol 73:165–179. https://doi.org/10.1111/j.1365-2958.2015.12477.x.

29. Santiago AE, Yan MB, Hazen TH, Sauder B, Meza-Segura M, Rasko DA, Prieto et al. 2011.1111/j.1365-2958.2011.06929.x.

30. Prieto A, Urcola I, Blanco I, Dahlbæ A, Muiens M, Quiró P, Palenzuela L, Chakraborty T. 2016. Tracking bacterial virulence: global modulators as indicators. Sci Rep 6:35973. https://doi.org/10.1038/srep35973.

31. Santiago AE, Yan MB, Hazen TH, Sauder B, Meza-Segura M, Rasko DA, Prieto et al. 2011.1111/j.1365-2958.2011.06929.x.

32. Atlung T, Ingmer H. 1997. H-NS: a modulator of environmentally regulated gene expression. Mol Microbiol 63:7–14. https://doi.org/10.1111/j.1365-2958.1995.mmi_18010101.x.

33. Prieto A, Urcola I, Blanco I, Dahlbæ A, Muiens M, Quiró P, Palenzuela L, Chakraborty T, Huttener M, Juárez A. 2016. Tracking bacterial virulence: global modulators as indicators. Sci Rep 6:35973. https://doi.org/10.1038/srep35973.

34. Atlung T, Ingmer H. 1997. H-NS: a modulator of environmentally regulated gene expression. Mol Microbiol 63:7–14. https://doi.org/10.1111/j.1365-2958.1995.mmi_18010101.x.

35. Atlung T, Ingmer H. 1997. H-NS: a modulator of environmentally regulated gene expression. Mol Microbiol 63:7–14. https://doi.org/10.1111/j.1365-2958.1995.mmi_18010101.x.

36. Atlung T, Ingmer H. 1997. H-NS: a modulator of environmentally regulated gene expression. Mol Microbiol 63:7–14. https://doi.org/10.1111/j.1365-2958.1995.mmi_18010101.x.

37. Free A, Dorman CJ. 1999. Differential protease-mediated turnover of H-NS and StPa revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*. Proc Natl Acad Sci USA 106:10776–10781. https://doi.org/10.1073/pnas.99.136576.

38. Free A, Dorman CJ. 1999. Differential protease-mediated turnover of H-NS and StPa revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*. Proc Natl Acad Sci USA 106:10776–10781. https://doi.org/10.1073/pnas.99.136576.

39. Free A, Dorman CJ. 1999. Differential protease-mediated turnover of H-NS and StPa revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*. Proc Natl Acad Sci USA 106:10776–10781. https://doi.org/10.1073/pnas.99.136576.

40. Johansson J, Uhlin BE. 1999. Differential protease-mediated turnover of H-NS and StPa revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*. Proc Natl Acad Sci USA 106:10776–10781. https://doi.org/10.1073/pnas.99.136576.