JOURNAL OF BACTERIOLOGY, Nov. 1997, p. 6618–6625 Vol. 179, No. 21
0021-9193/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Promoter-Specific Repression of *fimB* Expression by the *Escherichia coli* Nucleoid-Associated Protein H-NS

GINA M. DONATO, MICHAEL J. LELIVELT, AND THOMAS H. KAWULA*

Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599

Received 15 May 1997/Accepted 21 August 1997

The H-NS protein is a major component of the *Escherichia coli* nucleoid. Mutations in *hns*, the gene encoding H-NS, have pleiotropic effects on the cell altering both the expression of a variety of unlinked genes and the inversion rate of the DNA element containing the *fimA* promoter. We investigated the interaction between H-NS and *fimB*, the gene encoding the bidirectional recombinase that catalyzes *fimA* promoter flipping. In β-galactosidase assays, we found that *fimB* expression increased approximately fivefold in an *hns2-tetR* insertion mutant. In gel mobility shift assays with purified H-NS, we have also shown that H-NS bound directly and cooperatively to the *fimB* promoter region with greater affinity than for any other known H-NS-regulated gene. Furthermore, this high-affinity interaction resulted in a promoter-specific inhibition of *fimB* transcription. The addition of purified H-NS to an in vitro transcription system yielded a fivefold or greater reduction in *fimB*-specific mRNA production. However, the marked increase in cellular FimB levels in the absence of H-NS was not the primary cause of the mutant rapid inversion phenotype. These results are discussed in regard to both H-NS as a transcriptional repressor of *fimB* expression and its role in regulating type 1 pilus promoter inversion.

H-NS is an approximately 15.4-kDa nucleoid-associated *Escherichia coli* protein involved in bacterial chromatin condensation (12, 49, 50). The *hns* gene is autoregulated (16, 54), and its expression is induced three- to fourfold upon cold shock (5, 30). The H-NS protein binds tightly to double-stranded DNA (dsDNA) as a homodimer (15, 49). H-NS does not bind DNA in a strict sequence-specific manner (46), yet it preferentially binds curved DNA (43, 51, 57). Most importantly, H-NS is a global regulator of a variety of unrelated genes in *E. coli* (2, 31, 58) as well as being involved in virulence expression in pathogenic *Shigella* (35), *Salmonella* (21), and enteroinvasive *E. coli* (7) strains. In most instances, such as with *proU* (22, 36), the *bgl* operon (8, 22), and the *pap* locus (20), mutations in *hns* cause derepressed gene expression. However, H-NS also acts as a positive regulator for *lrp* (33) and flagellum biosynthesis genes (3). Two prevailing models exist to explain the role that H-NS plays in modulating gene expression. In the first, H-NS takes an active role by directly binding to DNA and inhibiting transcription (55). In the other, H-NS acts passively in a purely structural role by inducing changes in DNA supercoiling or chromosomal topology (23, 25, 53).

Type 1 pilus expression is predominately controlled at the transcriptional level by the inversion of a 314-bp DNA element containing the promoter of *fimA*, the gene encoding the major pilus structural subunit (1, 13). Piliation is subject to phase variation whereby in the "ON" orientation, the promoter initiates transcription, FimA monomers are synthesized, assembled, and translocated to the bacterial cell surface. Conversely, when the promoter element is in the "OFF" orientation, *fimA* transcription cannot occur and the bacteria are nonpiliated. Two different *hns* mutations (*hns-1* and *hns2-tetR*) each cause a 100-fold increase in *fimA* promoter inversion without affecting reporter plasmid superhelical density. This mutant phenotype is exhibited only at lower temperatures (30°C) in an *hns-1* strain but is temperature independent (30 to 42°C) in an *hns2-tetR* strain (28).

FimB and FimE have been shown both genetically and biochemically to be the bi- and unidirectional invertases, respectively (18, 37, 38), required for flipping the *fim* switch in the chromosome. Strains exhibiting the rapid inversion phenotype in *hns* mutant backgrounds lacked FimE. In these *hns* mutant strains FimB is the only recombinase present. Therefore, it is plausible that H-NS may act indirectly to alter *fimA* promoter inversion rates by controlling *fimB* expression. If H-NS normally represses *fimB* transcription, then in the absence of H-NS, *fimB* expression would increase. Elevated cellular FimB levels could subsequently lead to an increase in FimB-driven *fimA* promoter recombination events, resulting in rapid DNA inversion. In this study, we show that H-NS regulates FimB levels in the cell, but this regulation is not the sole cause for increased DNA flipping observed in *hns* mutant allele backgrounds. We also provide evidence that H-NS has an independent relationship with *fimB*, avidly and directly binding to the promoter region and inhibiting transcription.

MATERIALS AND METHODS

Bacterial strains, plasmids, phage, media, genetic techniques, and enzyme assays. The bacterial strains, plasmids, and phage used in this study are listed in Table 1. Media consisted of Luria-Bertani (LB) broth, LB agar, or MacConkey agar (Difco, Detroit, Mich.). When used, antibiotics were added to a final concentration of 100 μg (ampicillin), 20 μg (tetracycline), 40 μg (kanamycin), 50 μg (streptomycin), or 50 μg (spectinomycin) per ml of medium. Restriction and other DNA-modifying enzymes were used as instructed by the manufacturers (Gibco-BRL, Gaithersburg, Md., and New England Biolabs, Beverly, Mass.). Generalized transductions using P1 *vir* were carried out as previously described (39). β-Galactosidase assays were performed as described elsewhere (39) on strains grown in minimal media.

RNase protection assays. Total RNA was isolated and RNase protection assays were performed as described elsewhere (32). Plasmid pML22 (Table 1) served as the template for in vitro-transcribed ^{32}P-labeled *hns* antisense probe. Labeled RNA-RNA hybridized duplexes were separated on nondenaturing polyacrylamide gels and quantitated with a PhosphorImager (model 425F; Molecular

* Corresponding author. Mailing address: Department of Microbiology and Immunology, CB#7290, University of North Carolina School of Medicine, Chapel Hill, NC 27599. Phone: (919) 966-9699. Fax: (919) 962-8103. E-mail: kawula@med.unc.edu.

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

TABLE 1. Bacteria, plasmids, and bacteriophage used

Strain, plasmid, or phage	Description	Reference or source
Bacteria		
AAE261A	MG1655 Δ*lacZYA fimB-lacZYA*	4
AL88	Same as AAE261A except *hns*$^+$ linked to *tetR*	P1 transduction from THK60
AL90	Same as AAE261A except *hns2-tetR*	P1 transduction from THK62
AL92	Same as AAE261A except *hns-1* linked to *tetR*	P1 transduction from THK64
AL106	Same as ORN185 except *fimB-tetR*	P1 transduction from ORN203
THK30	Same as ORN185 except *hns2-tetR*	28
THK32	Same as THK63 except Φ(*fimA′-lacZYA-kan*)	28
THK38	*thr-1 leuB thi-1* Δ(*argF-lac*)*U169 malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA2 supE44 hns*$^+$ linked to *tetR*	27
THK63	Same as THK38 except *hns-1* linked to *tetR*	27
THK60	Same as THK38 except Φ(*proU′-lacZYA-kan*)	27
THK62	Same as THK60 except *hns2-tetR*	27
THK64	Same as THK60 except *hns-1* linked to *tetR*	27
ORN185	Same as THK38 except Φ(*fimA′-lacZYA-kan*) Tet^s *fimE* (insertion sequence element)	28
ORN203	Same as ORN185 except *fimE*$^+$ *hns-1 fimB-tet*	Gift from Paul Orndorff
NEC26	BL21(DE3) Δ*fim*	18
Plasmids and phage		
pGEM4Z	Cloning vector with opposing T7 and SP6 promoters	Promega
pKK223-3	Expression vector containing *tac* promoter	Pharmacia Biotech
pUC19	Cloning vector	Promega
pHP45Ω	Vector containing Ω fragment (Sm^r Spc^r)	45
pSH2	Contains entire *fim* operon	41
pTHK113	pBR322 *hns*$^+$	28
pML22	pGEM4Z with 175-bp amplified *hns* fragment from pTHK113	This study (Fig. 2)
pIB378	pET11 *fimB*$^+$	18
pFIMB14	pUC19 with 1.5-kb amplified *fimB* fragment from pSH2	This study (Table 3)
pYANK1	pFIMB14 with 2-kb *Sma*I fragment from pHP45Ω	This study (Fig. 5)
pTACB	pKK223-3 with 190-bp amplified *fimB* fragment from pYANK1	This study (Fig. 5)
P1	*vir*	Laboratory collection

Dynamics, Sunnyvale, Calif.) and ImageQuant version 3.3 software (Molecular Dynamics).

H-NS purification. H-NS was purified by the protocol of Dersch et al. (9). Briefly, a large-scale *E. coli* culture harboring *hns* on a multicopy plasmid (pTHK113) was grown at 37°C, centrifuged to harvest the cells, and broken via a French pressure cell. The cell lysate was clarified by ultracentrifugation and subjected to two differential precipitations with 40 and 60% ammonium sulfate. The resulting sample was passed through a dsDNA-cellulose column, and H-NS was eluted with a 500 mM NaCl buffer. Fractions were analyzed on sodium dodecyl sulfate (SDS)–15% polyacrylamide gels stained with Coomassie brilliant blue. Fractions containing H-NS were pooled, dialyzed, confirmed by Western analysis, and stored in aliquots. Protein concentrations were measured with a Bio-Rad Dc protein assay kit (Bio-Rad, Hercules, Calif.).

Gel mobility shift assays. DNA substrates were generated by PCR amplification from pSH2 and column purified with Wizard PCR preps (Promega, Madison, Wis.). Fragments encompassing the *fimB* promoter region and upstream repeats are the same as those depicted in Fig. 5A. PCR primers were generated from sequence determined by Schwan et al. (48) and GenBank accession no. Z37500. A constant amount of DNA was added to various amounts of purified H-NS lacking contaminating DNase in a binding buffer consisting of 40 mM Tris-Cl (pH 8.0), 40 mM $MgCl_2$, 100 mM KCl, and 10% glycerol. The reaction mixtures were incubated for 20 min at room temperature, electrophoresed on a 1% agarose gel in 1× Tris-acetate-EDTA, and stained with ethidium bromide.

In vitro transcription assays. Plasmid pYANK1, which contains *fimB* under the control of its own promoter and upstream repeats, was made by cloning a 2-kb *Sma*I Ω fragment from pHP45Ω into the unique *Cla*I site of *fimB* in pFIMB14 (Table 1). Plasmid pTACB, which contains *fimB* under the transcriptional control of the *tac* promoter, was generated by cloning a 190-bp *fimB* PCR product from pYANK1 into the *Eco*RI-*Hin*dIII sites of pKK223-3 (Table 1). The single-round in vitro transcription assays were performed basically as previously described (55). Supercoiled DNA was column purified (Qiagen, Chatsworth, Calif.) and incubated with various amounts of H-NS in a reaction mixture containing 50 mM Tris-Cl (pH 7.4), 14 mM $MgCl_2$, 100 mM KCl, 5 mM dithiothreitol, and 40 μg of acetylated bovine serum albumin per ml. The reaction mixture was preincubated for 20 min at 37°C before 25 U of RNase inhibitor (Ambion, Austin, Tex.) and 2 U of *E. coli* RNA polymerase (Boehringer Mannheim, Indianapolis, Ind.) were added and then further incubated for 1 h at 37°C to form a transcriptional open complex. RNA synthesis was initiated by adding a mixture of nucleoside triphosphates (Gibco-BRL) and 20 μCi of [α-^{32}P]UTP (800 Ci/mmol; Amersham, Arlington Heights, Ill.), and the mixture was incubated for another 3 min. Remaining DNA template was degraded by adding 2 U of RNase-free DNase I (Ambion) and incubating the mixture for another 15 min at 37°C. The reaction was stopped and cleaned by extraction with acid-phenol–chloroform pH 4.7 (5:1; Ambion) and chloroform-isoamyl alcohol (5prime→3prime, Boulder, Colo.). Transcripts were ethanol precipitated with 5 μg of yeast RNA as a carrier (Ambion), dried, and resuspended in 20 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF). Samples were heated at 80°C for 3 to 5 min and run on 5% polyacrylamide–8 M urea denaturing gels in 1× Tris-borate-EDTA. The gels were subsequently dried and exposed to a phosphorimaging screen. Transcripts were detected and quantitated by a Molecular Dynamics Storm 860 PhosphorImager and ImageQuant version 4.2 software (Molecular Dynamics).

Protein preparation and Western blotting. Strains were grown with the appropriate antibiotics at 37°C overnight, centrifuged to harvest the cells, and resuspended in 5 ml of sonication buffer (300 mM NaCl, 50 mM NaH_2PO_4 [pH 7.8]). Bacteria were treated with 1 mg of lysozyme per ml and sonicated on ice to lyse. The sonicate was centrifuged, and soluble proteins in the supernatant were concentrated through a Centricon-10 column as instructed by the manufacturer (Amicon, Beverly, Mass.). Protein concentrations were measured with a Bio-Rad Dc protein assay kit. FimB extract, NEC26(pIB378), was a gift from David Gally (18) and was used at a 1:100 dilution.

Protein lysates (500 μg of each) were solubilized and then electrophoresed on a 10 to 20% denaturing gradient gel (Jule Biotechnologies Inc., New Haven, Conn.) at 20 mA. Proteins were transferred to nitrocellulose (Schleicher & Schuell, Keene, N.H.) in a Hoefer TE22 unit (Hoefer Scientific Instruments, San Francisco, Calif.) overnight at 20 mA. The membrane was probed with FimB antiserum provided by David Gally (47) and goat anti-rabbit immunoglobulin-horseradish peroxidase secondary antibody conjugate (Sigma, St. Louis, Mo.). The antigen-antibody complexes were detected with the SuperSignal chemiluminescent substrate (Pierce, Rockford, Ill.).

RESULTS

Effect of *hns* lesions on *fimB* expression. FimB and FimE are recombinases that catalyze the inversion of the *fimA* promoter

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

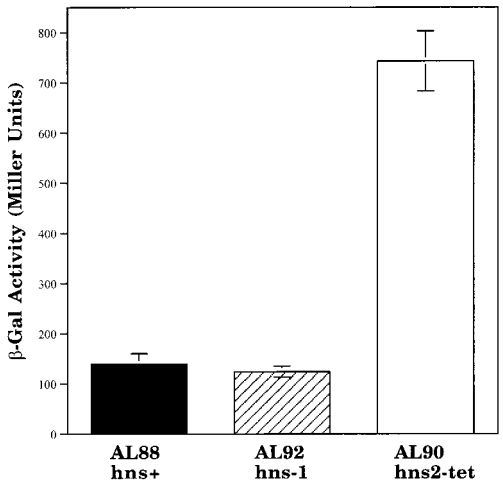

FIG. 1. Effects of *hns* alleles on *fimB* expression. All strains containing a *fimB-lacZYA* fusion and the indicated *hns* allele were grown to mid-log phase at 30°C and assayed for β-galactosidase activity (39). Data represent the averages of three independent experiments, each done in triplicate.

in the chromosome (18, 29, 37, 38). In strains lacking *fimE*, *fimA* promoter inversion is catalyzed solely by the bidirectional FimB recombinase. We have shown that *hns* mutations increase *fimA* promoter inversion 100-fold in *fimE* mutant strains (28). The effect of an *hns2-tetR* mutation on inversion is independent of temperature. However, the *hns-1* mutation is cold sensitive (28) and presents a mutant phenotype only at 30°C. It has been postulated (28, 40) that H-NS may affect inversion rates indirectly by influencing *fimB* expression. To determine whether H-NS alters *fimB* expression, we delivered three *hns* alleles (*hns*$^+$, *hns-1*, and *hns2-tetR*) via P1 transductions into a single-copy chromosomal *fimB-lacZ* fusion strain (Table 1) and measured β-galactosidase activity (39) (Fig. 1). At 30°C, *fimB* expression was fivefold higher in the *hns2-tetR* insertion mutant than in the wild-type strain. Interestingly, the promoter point mutation *hns-1* had no effect on *fimB-lacZ* (Fig. 1). We concluded that *fimB* expression was significantly induced in the absence of H-NS.

The *hns-1* mutation confers a reduction in mRNA levels relative to the wild-type strain. The *hns-1* mutant allele contains a single T-to-G transversion in the −35 region of the *hns* promoter (28). Since the mutation does not alter the *hns* coding sequence and its effect on inversion can be alleviated by increasing the copy number of the *hns-1* clone in *trans* (28), we hypothesized that this point mutation bestowed a decreased level of H-NS in the cell. To test this hypothesis, an RNA antisense probe derived from pML22 was used to detect *hns* mRNA transcripts from *hns*$^+$ (THK38) and *hns-1* (THK63) background strains in RNase protection assays (Fig. 2). As predicted, the *hns-1* allele resulted in an approximately 50% decrease in *hns*-specific mRNA levels compared to a wild-type strain at 30°C. There is also a corresponding decrease in H-NS protein made by a strain carrying this mutation (our unpublished data).

H-NS directly binds the *fimB* promoter region. The effects of the *hns-1* and *hns2-tetR* lesions on *fimB* expression suggested to us that H-NS may have a high-affinity binding capacity for *fimB*. The *hns-1* allele conferred 50% less *hns* mRNA and subsequently less H-NS protein in the cell compared to wild-type levels (Fig. 2). However, this large decrease in cellular H-NS amounts had no effect on *fimB* expression. In β-galactosidase assays, *fimB* was still basally expressed and not derepressed in an *hns-1* background (Fig. 1). One way this observation could be explained is if H-NS bound tightly to *fimB*. High-affinity H-NS binding sites on *fimB* could compensate for having one-half as much H-NS in the cell. Thus, at lowered H-NS levels as exhibited in an *hns-1* background, there would still be enough protein to repress *fimB* expression because of the tight binding between H-NS and *fimB* DNA. To study a possible H-NS–*fimB* interaction, we investigated the binding of purified H-NS to portions of the *fimB* promoter region in gel mobility shift assays.

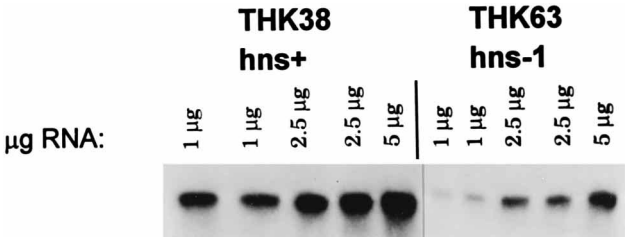

FIG. 2. Comparison of *hns* mRNA levels. Total RNA isolated from THK38 (*hns*$^+$) and THK63 (*hns-1*) grown at 30°C was hybridized to a ^{32}P-labeled *hns* antisense probe and separated on a nondenaturing polyacrylamide gel. Duplexes were quantitated with a PhosphorImager.

H-NS was purified to homogeneity (Fig. 3), and increasing amounts were incubated separately with two contiguous DNA fragments of the *fimB* promoter region. These fragments are the same as those depicted in Fig. 5A. The *fimB* promoter is a 464-bp DNA segment encompassing the two putative promoters and all sequence up to the translational start (ATG) (48). The second piece of DNA is directly 5′ of the promoters and contains 457 bp including two sets of direct repeats, herein termed upstream repeats (URs), implicated by Schwan et al. as possible protein binding sites (48). Several conclusions can be drawn from this binding assay (Fig. 4). First, H-NS bound to both the UR region (lanes 5 to 7) and the *fimB* promoter (lanes 11 to 14), as shown by slower-migrating complexes. Second, in agreement with previous work (46), H-NS exhibited

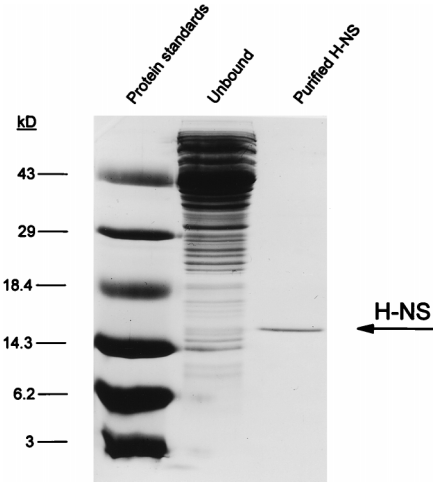

FIG. 3. H-NS purification analysis. Samples were run on an SDS–15% polyacrylamide gel and stained with Coomassie brilliant blue. Lane 1, protein standards with sizes indicated to the left; lane 2, flowthrough that did not bind to the dsDNA-cellulose column; lane 3, purified H-NS that was bound and eluted off of the column, pooled, and dialyzed. Protein in lane 3 served as the source of purified H-NS in all subsequent experiments in this study.

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

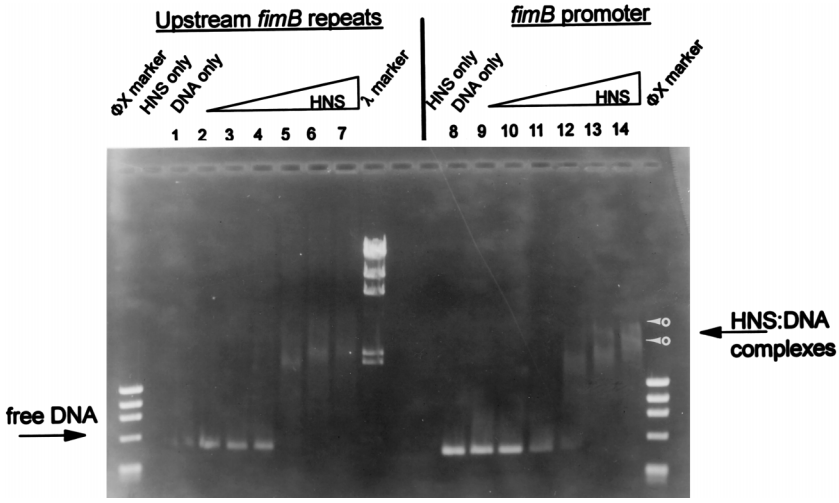

FIG. 4. Gel mobility shift assays with H-NS and fragments of the *fimB* promoter region. H-NS–DNA complexes were electrophoresed on a 1% agarose gel and stained with ethidium bromide. The left side of the gel represents complexes with the distal 5′ UR region, and the right side represents binding to the *fimB* promoter. Each lane contains a constant amount of DNA (0.67 pM for UR and 1.13 pM for *fimB* promoter) with increasing amounts of H-NS. Lanes 1 and 8, DNA only; lanes 2 and 9, 1.3 pM H-NS; lanes 3 and 10, 13 pM H-NS; lanes 4 and 11, 39 pM H-NS; lanes 5 and 12, 65 pM H-NS; lanes 6 and 13, 91 pM H-NS; lanes 7 and 14, 130 pM H-NS. Free DNA and complexes are indicated by arrows; multiple shifted bands within a single lane are indicated by arrowheads with open circles.

a cooperative mode of binding to both substrates. This cooperative binding was exemplified when a less than twofold increase in protein concentration changed the DNA substrate profile from 100% free to mostly H-NS bound (compare lanes 4 and 5). Also, as more H-NS was added to subsequent lanes, the protein-DNA complex became increasingly retarded within the gel. The decrease in intensity of fluorescence between free and bound DNA (compare lanes 4 and 5) was probably a consequence of H-NS binding and obscuring ethidium bromide intercalating sites on the DNA. A disappearance of visible free DNA without an equal intensity of complexed DNA is a typical result in ethidium bromide-stained agarose gel mobility shift assays (34, 53, 56).

However, there were also differences between H-NS binding patterns with each substrate. In particular, H-NS showed a higher affinity for the *fimB* promoter than for the URs by binding at a lower concentration. The first signs of binding to the *fimB* promoter occurred in lane 11 at a remarkably low 35:1 molar ratio of H-NS to DNA. Another interesting observation was that multiple shifted bands were visible within the same lane (lanes 13 and 14) as the H-NS concentration was increased. This ladder effect is usually indicative of multiple specific binding sites on the DNA fragment. We concluded from these experiments that (i) H-NS bound cooperatively and directly to both the core *fimB* promoter region and upstream AT-rich sequences, (ii) binding to the promoter may have been tighter than binding to the URs, (iii) there were two possible specific H-NS binding domains within the promoter region, and (iv) this H-NS–*fimB* promoter interaction may result in *fimB* repression.

H-NS represses transcription of *fimB* in vitro. We sought to determine whether the direct binding of H-NS to a DNA segment carrying the *fimB* promoter element and proximal sequences had any effect on *fimB* expression. It is clear that the ability to bind directly to or near the core region could enable H-NS to repress *fimB* expression (6). To determine the role of H-NS at the *fimB* promoter, we undertook an in vitro transcriptional analysis with several different templates (Fig. 5A). We used only circular supercoiled DNA plasmid templates in an attempt to accurately reflect in vivo conditions and to account for the observation that some H-NS-regulated genes have been proposed to be sensitive to changes in DNA topology (10, 23–26, 53).

Plasmid pYANK1 was generated by cloning a 1.5-kb insert containing the entire *fimB* coding sequence with its putative promoters and upstream repeats into pUC19. A 2-kb Ω fragment (45) which is flanked by inverted repeats with transcriptional and translational termination signals was also inserted into *fimB* to shorten *fimB*-specific transcript size and minimize nonspecific vector-based transcripts. Thus, mRNA from pYANK1 represents transcription of *fimB* under the control of its own promoter. Figure 5B shows the results of an in vitro transcription assay with pYANK1 as the input DNA template and increasing H-NS concentrations (0 to 585 pM). The fold decreases in expression between individual reactions and reactions with no H-NS added, as quantitated by a PhosphorImager, are summarized in Table 2. There are several salient points to be made based on these data. The addition of 97.5 pM pure H-NS yielded the first modest but detectable and reproducible 1.3-fold decrease in *fimB* transcription (lane 3). At higher H-NS concentrations, this decrease dropped to approximately fivefold (lane 4), which represents an 80% inhibition of transcription. With the addition of even more H-NS, *fimB* mRNA was virtually undetectable (lanes 5 and 6). It is interesting that two transcripts were consistently observed from pYANK1, and both were equally affected by H-NS. Their estimated sizes of 350 (P1) and 490 (P2) nucleotides correspond to *fimB*-specific mRNAs identified by previous primer extension studies (48). These data clearly support the fact that *fimB* contains two active promoters which are both transcriptionally repressed by H-NS.

To assess the specificity of this H-NS-controlled transcriptional inhibition of *fimB*, we performed a promoter swap. In previous studies, it has been shown that the *tac* promoter is relatively *hns* independent (55, 56). Thus, we cloned a 190-bp fragment initiating from the *fimB* translational start (ATG) into pKK223-3. In the resulting plasmid, pTACB (Fig. 5A), *fimB* was under the transcriptional control of P_{tac} and vector readthrough was blocked by the strong *rrnB* ribosomal terminators (T_1T_2) downstream. In contrast to pYANK1, when *fimB*

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

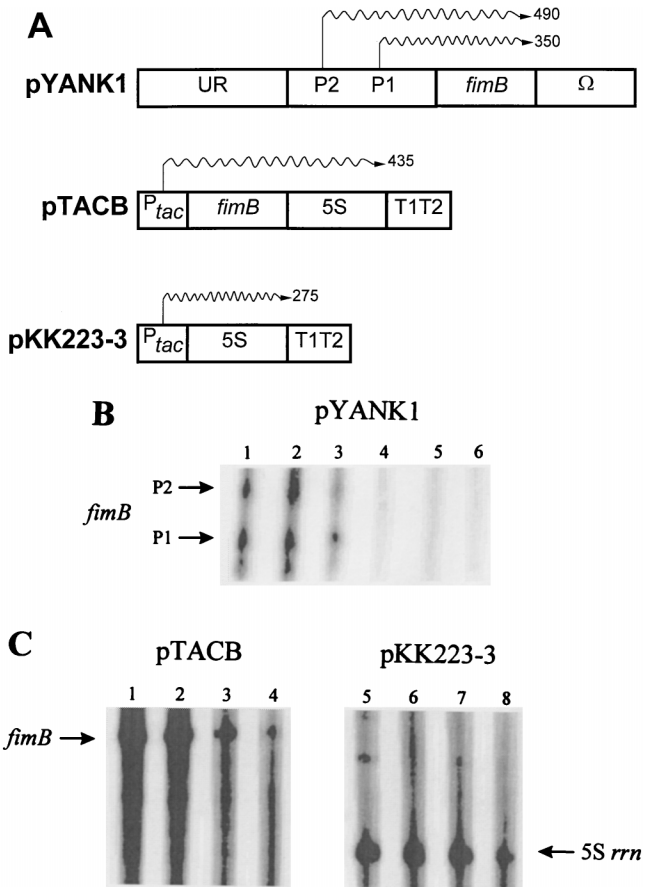

FIG. 5. In vitro transcription analysis. (A) Schematic diagram of promoter fragments used in these experiments. Plasmid names are indicated to the left. Transcripts with estimated sizes are indicated by wavy lines. (B) Phosphorimage of a single-round in vitro transcription assay with pYANK1 and H-NS. Supercoiled plasmid DNA (0.15 pM) was added to increasing amounts of H-NS. Lanes 1 to 6 contain 0, 48.75, 97.5, 195, 390, and 585 pM H-NS, respectively. *fimB* transcripts from both promoters, P1 and P2, are indicated by arrows to the left. (C) Phosphorimage of a single-round in vitro transcription assay with pTACB or pKK223-3 and H-NS. Supercoiled plasmid template (0.15 pM each) was added to increasing amounts of H-NS. Lanes 1 and 5, 0 pM H-NS; lanes 2 and 6, 97.5 pM H-NS; lanes 3 and 7, 195 pM H-NS; lanes 4 and 8, 390 pM H-NS. Transcripts for each gene are indicated by arrows.

expression was driven by the *tac* promoter, transcription was unaffected by H-NS until high concentrations were added (Fig. 5C, lanes 3 and 4). Even at the maximum amount of H-NS added (390 pM), the fold reduction in *fimB* expression was only 1.7 (Table 2). As a control, we also measured transcription from the vector pKK223-3 alone. In addition to the *tac* promoter, this vector contains the 5S rRNA gene, which was transcriptionally unaffected by H-NS (Fig. 5C) as evidenced by the minuscule reduction in transcription at the maximal H-NS concentration (lane 8). We concluded from these experiments (Fig. 5 and Table 2) that H-NS functions as a direct transcriptional repressor of *fimB* expression specific for the natural *fimB* promoter and/or upstream sequences.

Increased cellular FimB levels do not mimic the effect of an *hns* mutation on inversion. Since *fimB* expression is increased (reference 40 and this study) and *fimA* promoter inversion rates are rapid in the absence of H-NS (28), it is possible that the mechanism by which H-NS regulates *fimA* promoter inversion is through altering FimB levels. To directly test this possibility, we added *fimB* in *trans* to a strain with a wild-type *hns*

TABLE 2. Quantitation of *fimB* and 5S mRNA transcriptional repression depicted in Fig. 5B and C

Plasmid	Fold decrease in transcription[a] with H-NS concn (pM) of:		
	97.5	195	390
pYANK1	1.3	5	TR[b]
pTACB	1	1.2	1.7
pKK223-3	1	1	1.2

[a] Determined by comparing transcript pixel volumes between reactions with the indicated H-NS amounts and reactions with no H-NS. For example, a one-fold decrease reflects no difference in pixel values, twofold represents a 50% reduction, etc. Each number represents an average of three independent experiments quantitated on a PhosphorImager.
[b] TR, total repression (no visible *fimB* mRNA).

background and assayed for the effect on inversion. In strains carrying a *fimA'-lacZYA-kan* fusion, inversion is detectable phenotypically on lactose MacConkey plates. In wild-type strains, individual colonies are either red (Lac^+) or white (Lac^-). These colors represent colony populations with the *fimA* promoter orientation predominately ON or OFF, respectively. However, colonies from *hns* mutant strains are uniformly pink, consisting of approximately equal numbers of ON- and OFF-oriented individual cells (28). If H-NS is working solely through *fimB* expression to affect inversion rates, then higher cellular FimB amounts should mirror an *hns* lesion, i.e., rapid *fimA* promoter inversion (pink colony phenotype).

We used pFIMB14 as a source of exogenous *fimB* (Table 1). This plasmid is the parental clone of pYANK1 containing the entire *fimB* coding, promoter, and upstream repeat sequences but lacking the Ω fragment. This high-copy-number plasmid carrying *fimB* complemented a *fimB* knockout strain (AL106) and expressed a full-length protein product (data not shown), thus ensuring its biological activity. To confirm that the addition of cloned *fimB* in *trans* did indeed lead to elevated FimB levels in the cell, we performed a Western blot analysis on equal amounts of cell lysates probed with FimB antiserum (Fig. 6). AL106 served as an inactivated *fimB* negative control. This mutant strain contains a *tet* cassette inserted into *fimB*. As a positive control, we used a FimB extract which has previously been demonstrated to possess FimB activity in vitro (18). It was evident that when *fimB* was provided in *trans* to a wild-type background strain, the amount of FimB in the cell substantially increased (lane 4). FimB expression from the wild-type strain alone (ORN185) was not detectable in this assay (lane 3). This

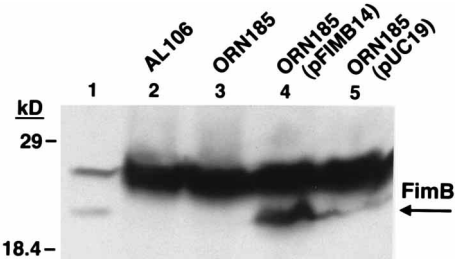

FIG. 6. Western blot of FimB expression. Lysates were run on an SDS–10 to 20% polyacrylamide gradient gel, transferred to nitrocellulose, and probed with FimB antiserum (47). Lane 1, control FimB extract, NEC26(pIB378), diluted 1:100; lane 2, *fimB* knockout; lane 3, wild-type strain; lane 4, wild-type strain with *fimB* clone in *trans*; lane 5, wild-type strain with vector alone in *trans*; lanes 2 to 5, 500 μg of protein lysates. Protein standard sizes are indicated by lines to the left; full-length FimB is indicated by the arrow to the right.

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

TABLE 3. Effect of additional *fimB* in *trans* on *fimA* promoter inversion

Strain	Inversion phenotype[a]
ORN185 (*hns*$^+$)	Red and white (normal)
THK30 (*hns2-tetR*)	Pink (rapid)
THK32[b] (*hns-1*)	Pink (rapid)
ORN185(pUC19)	Red and white
ORN185(pFIMB14)	Red and white
ORN185(pSH2)	Red and white

[a] All strains carry *fimA'-lacZYA-kan* fusions and were grown at 37°C on lactose MacConkey indicator medium.
[b] THK32 was grown at 30°C due to the cold sensitivity of the *hns-1* allele (28).

result was not surprising since FimB represents only 2 to 5% of the total soluble protein in the cell (18).

When plated on indicator media, wild-type *E. coli* strain ORN185 produced red and white colonies, whereas a rapid inversion frequency represented by pink colonies was seen in both *hns* lesion backgrounds (Table 3). When any of the experimental plasmids were transformed into the *hns*$^+$ background strain (ORN185), there was no effect on inversion. Thus, the addition of *fimB* in *trans* alone (pFIMB14) or in combination with the entire *fim* operon (pSH2) did not cause a detectable increase in *fimA* promoter inversion. We concluded from these experiments that in a FimE-independent inversion system, an excess of cellular FimB did not mimic the effect of an *hns* mutation.

DISCUSSION

One of the many systems in which H-NS plays a regulatory role is the expression of *E. coli* type 1 piliation. We have previously shown that *fimA* promoter inversion frequencies are increased 100-fold (28) in strains containing either an *hns* promoter point (*hns-1*) or an insertion (*hns2-tetR*) mutation. We envisage two ways H-NS may be acting to influence inversion: (i) directly at the invertible element (IE) containing the *fimA* promoter or (ii) indirectly through the gene for the bidirectional recombinase, *fimB* (18, 29, 37, 38). In the first model, wild-type levels of H-NS could bind to DNA sequences within the IE, to sequences on either side of the IE, or to 9-bp inverted repeats flanking the IE to hinder flipping or preclude the binding of activators such as integration host factor (11, 14) and Lrp (4, 19) or the recombinases (FimB and FimE). In the latter model, H-NS may affect DNA inversion indirectly by altering levels of FimB in the cell. Since FimB is the bidirectional recombinase for the *fim* switch and the only recombinase present in many strains, increased FimB could potentially increase the chromosomal recombination events of the *fimA* promoter. Addressing the indirect model, in this study we showed that *fimB* expression increased over fivefold in a strain lacking H-NS but was unaffected in a strain containing approximately one-half the normal amount of the protein. The discrepancy between our results and previous data reporting a 20-fold increase in *fimB* expression in an *hns* mutant strain (40) is probably due to the fact that Olsen and Klemm (40) used a plasmid-based system whereas our β-galactosidase assays were performed with a more relevant, in vivo-like single-copy chromosomally located *fimB-lacZ* fusion.

Two lines of evidence presented here suggest that this increase in *fimB* expression was not the sole cause of the rapid-inversion phenotype witnessed in an *hns* mutant background. First, mimicking the loss of H-NS by supplying *fimB* exogenously on a plasmid in *trans* did not alter the normal inversion phenotype. That is, excess FimB in the cell did not cause a faster-flipping IE in a wild-type *hns* background. Second, an *hns* lesion, *hns-1*, which causes a rapid switch rate (28) did not affect *fimB* expression. Additionally, we have isolated *hns* point mutations that dramatically increase *fimB* expression (up to 25-fold) but have no effect on inversion (unpublished data). Thus, H-NS does not regulate inversion strictly through modulating the levels of the FimB recombinase.

Previously we characterized *hns-1* as a promoter point mutation (28). Since the H-NS protein produced by this mutant allele is not altered, we hypothesized that the mutant phenotypes observed in this strain were due to decreased *hns* mRNA and subsequently decreased protein levels. Our RNase protection analysis confirmed that there was indeed a 50% decrease in *hns-1* mRNA levels relative to the wild-type *hns* allele at 30°C. Although this *hns* mutation confers less H-NS protein in the cell, this reduced concentration did not affect *fimB* expression. This result suggested the possibility that H-NS had a high-affinity binding capacity for *fimB*. If H-NS could bind tightly to *fimB* regulatory regions, then one-half as much H-NS present in an *hns-1* strain could still conceivably repress transcription. This observation is quite novel since it is the first instance in which this *hns-1* mutation did not mimic *hns2-tetR* in regard to the derepression of gene expression. Both *proU* and *bgl* are derepressed in the presence of either lesion, albeit to different levels (27).

To study the H-NS–*fimB* interaction, we purified H-NS and two separate fragments of the *fimB* promoter region and showed that H-NS bound directly to both segments in gel mobility shift assays. The purity of our H-NS preparation was particularly important due to the nature of our purification scheme involving a final dsDNA-cellulose column chromatography step. Others have suggested that H-NS may act indirectly by binding and stabilizing other protein-DNA interactions (17). If this were the case at *fimB*, then other eluted DNA-binding proteins would be prime candidates for directly binding *fimB*. However, due to the extreme purity of our H-NS sample and the fact that we were able to detect H-NS in the H-NS–*fimB* promoter complex (data not shown), we are confident that all observed shifts in DNA mobility were due solely to the presence of H-NS alone.

While analyzing the gel shifts, we were struck by a number of interesting points. It was evident that H-NS bound both the *fimB* promoter and the UR region (48) at concentrations that did not shift a synthetic control noncurved DNA fragment (data not shown). Binding appeared to be cooperative, as suggested by Rimsky and Spassky (46), whereby a small change in H-NS concentration led to a drastic difference in DNA mobility. This result suggests that H-NS may first bind tightly to DNA and then form H-NS–H-NS interactions as protein concentrations are increased. Though the data are not definitive, it also appeared that H-NS may bind to at least two separate sites within the *fimB* promoter, as illustrated by multiple complexes within one reaction lane. This binding pattern is similar to that for the *rrnB* P1 promoter region, where H-NS has been shown through several different footprinting techniques to interact with three DNA domains (52). Most notable, though, were the very low amounts of H-NS needed to first form a protein-DNA complex. We needed only a 35:1 H-NS-to-DNA molar ratio to shift the *fimB* promoter fragment. To the best of our knowledge, this is the highest affinity binding capacity that H-NS has exhibited for any DNA substrate yet. The H-NS affinity for *fimB* is approximately 10^4- to 10^5-fold higher than the binding of H-NS to *proU* or *rrnB* DNA (34, 52) and about 3- to 10-fold higher than binding to an ideal synthetic curved substrate (56, 59). In the past, researchers have had to add exorbitant

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

amounts of H-NS in order to visualize a DNA-binding ability. Here we demonstrate the specificity that H-NS has for the *fimB* promoter region by the addition of very little protein. Taking into account that H-NS acts as a dimer (15, 49), a 35:1 molar ratio of total H-NS to DNA may represent only a 17.5:1 ratio of functional H-NS to DNA.

Due to the tightness of the interaction between H-NS and the *fimB* promoter sequences, we surmised that this interaction was the cause of decreased *fimB* expression in wild-type cells. Our in vitro transcription analyses confirmed the biological effect that H-NS had on *fimB*: direct, promoter-specific repression. The negligible effect that H-NS had on a control plasmid, pKK223-3, carrying the *tac* promoter but no *fimB* sequence could be accounted for by the weak binding affinity that H-NS has exhibited for P_{tac} at very high concentrations (52). Thus, at a saturating amount of H-NS protein, it was not surprising that there was a low level of 5S mRNA inhibition. A modest increase in repression was quantitated when assays were done with pTACB. The only difference between this plasmid and pKK223-3 is the presence of 190 bp of *fimB* coding sequence. It is possible, and not unprecedented, that H-NS binds downstream of the transcriptional start site and still affects transcription. This has been demonstrated in the *proU* system of *E. coli* and *Salmonella typhimurium* (34, 43), where H-NS binds within *proV*, the first gene of the operon, and alters gene expression. However, the greatest repression in our in vitro transcription assays was observed when H-NS was added to pYANK1, a supercoiled template harboring *fimB* under the control of its natural promoter and upstream 5′ repeat sequences. Since the promoter sequences are the only difference between pYANK1 and pTACB, it is likely that H-NS requires the recognition and binding of the *fimB* promoter in order to specifically repress *fimB* transcription. This binding is the mode of operation of H-NS in regard to *rrnB* P1 inhibition (52). In this system, H-NS binds to three sites, one directly over the RNA polymerase binding site and two upstream of the core region to block transcription initiation. The apparent differences in H-NS/DNA molar ratios needed to see an effect between the gel mobility shift and in vitro transcription assays are probably due to the nature of each assay. There are inherent differences in procedure, sensitivity, buffers, and especially input template DNA (linear versus supercoiled) between the two. It is also reasonable that less protein may be needed to visualize an initial binding to DNA than to exert an effect on transcription.

We envision two ways in which H-NS could alter *fimB* expression based on the in vitro studies. In the simpler model, the high-capacity binding between H-NS and the core *fimB* promoter may preclude RNA polymerase from binding and forming an efficient transcription initiation complex. In an alternate though not mutually exclusive model, the binding of H-NS upstream of the promoter may aid in blocking transcription. Many intrinsically curved sequences in the *E. coli* chromosome are found upstream of promoters (51), and H-NS is known to have an affinity for curved DNA (43, 57). Thus, H-NS may act as a negative regulator by steric hindrance (44). By binding to the UR, H-NS could bend the DNA in such a way as to change the −10 and −35 spacing or disrupt the RNA polymerase-DNA promoter interactions. In either case, H-NS is not acting as a classical repressor since we do not believe that it recognizes a specific consensus sequence. However, we also do not think that H-NS binds randomly to *fimB* promoter sequences to exert an effect. This is most evident in the necessity of providing the *fimB* natural promoter rather than the *tac* promoter in order to observe transcriptional repression. Instead, it is possible that H-NS recognizes a specific DNA feature. This feature could be a specific conformation such as an intrinsic curvature or a stretch of AT base pairing. Tracts of AT base pairing are abundant in the *fimB* promoter and UR regions and has been implicated before (34) as a requirement for the H-NS effect on gene expression. These concepts do not exclude the role other factors, such as DNA topology, may play in H-NS-mediated regulation.

Through combined in vitro and in vivo assays, we have identified a gene, *fimB*, in which H-NS acts as a promoter-specific transcriptional repressor. Further work will continue to determine the affinity that H-NS has for the *fimB* promoter region, to distinguish the exact domains necessary for repression, and to determine whether H-NS acts to antagonize yet unidentified activators of *fimB* expression.

ACKNOWLEDGMENTS

We thank Ian Blomfield for *fimB-lacZYA* strain AAE261A, Paul Orndorff for strain ORN203, Rolf Wagner for helpful discussions on optimal conditions for H-NS, and members of the Kawula lab for their patience and support. We offer a special note of thanks to David Gally for providing us with a FimB extract, NEC26(pIB378), unpublished materials (FimB antiserum), and numerous helpful discussions.

This work was supported by grant R01 AI34176 from the National Institutes of Health.

REFERENCES

1. **Abraham, J. M., C. S. Freitag, J. R. Clements, and B. I. Eisenstein.** 1985. An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**5724–5727.
2. **Bertin, P., P. Lejeune, C. Laurent-Winter, and A. Danchin.** 1990. Mutations in *bglY*, the structural gene for the DNA-binding protein H1, affect expression of several *Escherichia coli* genes. Biochimie **72:**889–891.
3. **Bertin, P., E. Terao, E. H. Lee, P. Lejeune, C. Colson, A. Danchin, and E. Collatz.** 1994. The H-NS protein is involved in the biogenesis of flagella in *Escherichia coli*. J. Bacteriol. **176:**5537–5540.
4. **Blomfield, I. C., P. J. Calie, K. J. Eberhardt, M. S. McClain, and B. I. Eisenstein.** 1993. Lrp stimulates phase variation of type 1 fimbriation in *Escherichia coli* K-12. J. Bacteriol. **175:**27–36.
5. **Brandi, A., C. L. Pon, and C. O. Gualerzi.** 1994. Interaction of the main cold shock protein CS7.4 (CspA) of *Escherichia coli* with the promoter region of *hns*. Biochimie **76:**1090–1098.
6. **Collado-Vides, J., B. Magasanik, and J. D. Gralla.** 1991. Control site location and transcriptional regulation in *Escherichia coli*. Microbiol. Rev. **55:** 371–394.
7. **Colonna, B., M. Casalino, P. A. Fradiani, C. Zagaglia, S. Naitza, L. Leoni, G. Prosseda, A. Coppo, P. Ghelardini, and M. Nicoletti.** 1995. H-NS regulation of virulence gene expression in enteroinvasive *Escherichia coli* harboring the virulence plasmid integrated into the host chromosome. J. Bacteriol. **177:** 4703–4712.
8. **DeFez, R., and M. DeFelice.** 1981. Cryptic operon for β-glucosidase metabolism in *Escherichia coli* K-12: genetic evidence for a regulatory protein. Genetics **97:**11–25.
9. **Dersch, P., K. Schmidt, and E. Bremer.** 1993. Synthesis of the *Escherichia coli* K-12 nucleoid-associated DNA-binding protein H-NS is subjected to growth-phase control and autoregulation. Mol. Microbiol. **8:**875–889.
10. **Dorman, C. J., N. N. Bhriain, and C. F. Higgins.** 1990. DNA supercoiling and environmental regulation of virulence gene expression in *Shigella flexneri*. Nature **344:**789–792.
11. **Dorman, C. J., and C. F. Higgins.** 1987. Fimbrial phase variation in *Escherichia coli*: dependence on integration host factor and homologies with other site-specific recombinases. J. Bacteriol. **169:**3840–3843.
12. **Durrenberger, M., A. LaTeana, G. Citro, F. Venanzi, C. O. Gualerzi, and C. L. Pon.** 1991. *Escherichia coli* DNA-binding protein H-NS is localized in the nucleoid. Res. Microbiol. **142:**373–380.
13. **Eisenstein, B. I.** 1981. Phase variation of type 1 fimbriae in *Escherichia coli* is under transcriptional control. Science **214:**337–339.
14. **Eisenstein, B. I., D. S. Sweet, V. Vaughn, and D. I. Friedman.** 1987. Integration host factor is required for the DNA inversion that controls phase variation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84:**6506–6510.
15. **Falconi, M., M. T. Gualtieri, A. LaTeana, M. A. Losso, and C. L. Pon.** 1988. Proteins from the prokaryotic nucleoid: primary and quaternary structure of the 15-kD *Escherichia coli* DNA-binding protein H-NS. Mol. Microbiol. **2:**323–329.
16. **Falconi, M., N. P. Higgins, R. Spurio, C. L. Pon, and C. O. Gualerzi.** 1993. Expression of the gene encoding the major bacterial nucleoid protein H-NS is subject to transcriptional autorepression. Mol. Microbiol. **10:**273–282.
17. **Falconi, M., V. McGovern, C. Gualerzi, D. Hillyand, and N. P. Higgins.** 1991.

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest

Mutations altering chromosomal protein H-NS induce mini-Mu transposition. New Biol. **3:**615–625.
18. **Gally, D. L., J. Leathart, and I. C. Blomfield.** 1996. Interaction of FimB and FimE with the *fim* switch that controls the phase variation of type 1 fimbriae in *Escherichia coli* K-12. Mol. Microbiol. **21:**725–738.
19. **Gally, D. L., T. J. Rucker, and I. C. Blomfield.** 1994. The leucine responsive regulatory protein binds to the *fim* switch to control phase variation of type 1 fimbrial expression in *Escherichia coli* K-12. J. Bacteriol. **176:**5665–5672.
20. **Goransson, M., B. Sonden, P. Nilsson, B. Dagberg, D. Forsman, K. Emanuelsson, and B. E. Uhlin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature (London) **344:**682–685.
21. **Harrison, J. A., D. Pickard, C. F. Higgins, A. Khan, S. N. Chatfield, T. Ali, C. J. Dorman, C. E. Hormaeche, and G. Dougan.** 1994. Role of *hns* in the virulence phenotype of pathogenic salmonellae. Mol. Microbiol. **13:**133–140.
22. **Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, L. R. Booth, G. May, and E. Bremer.** 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. Cell **52:**569–584.
23. **Higgins, C. F., J. C. D. Hinton, C. S. J. Hulton, T. Owen-Hughes, G. D. Pavitt, and A. Seirafi.** 1990. Protein H1: a role for chromatin structure in the regulation of bacterial gene expression and virulence? Mol. Microbiol. **4:** 2007–2012.
24. **Hinton, J. C. D., D. S. Santos, A. Seirafi, C. S. J. Hulton, G. D. Pavitt, and C. F. Higgins.** 1992. Expression and mutational analysis of the nucleoid-associated protein H-NS of *Salmonella typhimurium*. Mol. Microbiol. **6:**2327–2337.
25. **Hulton, C. S. J., A. Seirafi, J. C. D. Hinton, J. M. Sidebotham, L. Waddell, G. D. Pavitt, T. Owen-Hughes, A. Spassky, H. Buc, and C. F. Higgins.** 1990. Histone-like protein H1(H-NS), DNA supercoiling, and gene expression in bacteria. Cell **63:**631–642.
26. **Jordi, B. J. A. M., T. A. Owen-Hughes, C. S. J. Hulton, and C. F. Higgins.** 1995. DNA twist, flexibility and transcription of the osmoregulated *proU* promoter of *Salmonella typhimurium*. EMBO J. **14:**5690–5700.
27. **Kawula, T. H., and M. J. Lelivelt.** 1994. Mutations in a gene encoding a new Hsp70 suppress rapid DNA inversion and *bgl* activation, but not *proU* derepression, in *hns-1* mutant *Escherichia coli*. J. Bacteriol. **176:**610–619.
28. **Kawula, T. H., and P. E. Orndorff.** 1991. Rapid site-specific DNA inversion in *Escherichia coli* mutants lacking the histonelike protein H-NS. J. Bacteriol. **173:**4116–4123.
29. **Klemm, P.** 1986. Two regulatory *fim* genes, *fimB* and *fimE* control the phase variation of type 1 fimbriae in *Escherichia coli*. EMBO J. **5:**1389–1393.
30. **LaTeana, A., A. Brandi, M. Falconi, R. Spurio, C. L. Pon, and C. O. Gualerzi.** 1991. Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS. Proc. Natl. Acad. Sci. USA **88:**10907–10911.
31. **Laurent-Winter, C., P. Lejeune, and A. Danchin.** 1995. The *Escherichia coli* DNA-binding protein H-NS is one of the first proteins to be synthesized after nutritional upshift. Res. Microbiol. **146:**5–16.
32. **Lelivelt, M. J., and T. H. Kawula.** 1995. Hsc66, an Hsp70 homolog in *Escherichia coli*, is induced by cold shock but not by heat shock. J. Bacteriol. **177:**4900–4907.
33. **Levinthal, M., P. Lejeune, and A. Danchin.** 1994. The H-NS protein modulates the activation of the *ilvIH* operon of *Escherichia coli* K-12 by LRP, the leucine regulatory protein. Mol. Gen. Genet. **242:**736–743.
34. **Lucht, J. M., P. Dersch, B. Kempf, and E. Bremer.** 1994. Interactions of the nucleoid-associated DNA-binding protein H-NS with the regulatory region of the osmotically controlled *proU* operon of *Escherichia coli*. J. Biol. Chem. **269:**6578–6586.
35. **Maurelli, A. T., and P. J. Sansonetti.** 1988. Identification of a chromosomal gene controlling temperature-regulated expression of *Shigella* virulence. Proc. Natl. Acad. Sci. USA **85:**2820–2824.
36. **May, G., P. Dersch, M. Haardt, A. Middendorf, and E. Bremer.** 1990. The *osmZ* (*bglY*) gene encodes the DNA-binding protein H-NS (H1a), a component of the *Escherichia coli* K-12 nucleoid. Mol. Gen. Genet. **224:**81–90.
37. **McClain, M. S., I. C. Blomfield, K. J. Eberhardt, and B. I. Eisenstein.** 1993. Inversion-independent phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **175:**4335–4344.
38. **McClain, M. S., I. C. Blomfield, and B. I. Eisenstein.** 1991. Roles of *fimB* and *fimE* in site-specific DNA inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **173:**5308–5314.
39. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Olsen, P. B., and P. Klemm.** 1994. Localization of promoters in the *fim* gene cluster and the effect of H-NS on the transcription of *fimB* and *fimE*. FEMS Microbiol. Lett. **116:**95–100.
41. **Orndorff, P. E., and S. Falkow.** 1984. Identification and characterization of a gene product that regulates type 1 piliation in *Escherichia coli*. J. Bacteriol. **160:**61–66.
42. **Orndorff, P. E., P. A. Spears, D. Schauer, and S. Falkow.** 1985. Two modes of control of *pilA*, the gene encoding type 1 pilin in *Escherichia coli*. J. Bacteriol. **164:**321–330.
43. **Owen-Hughes, T. A., G. D. Pavitt, D. S. Santos, J. M. Sidebotham, C. S. J. Hulton, J. C. D. Hinton, and C. F. Higgins.** 1992. The chromatin-associated protein H-NS interacts with curved DNA to influence DNA topology and gene expression. Cell **71:**255–265.
44. **Perez-Martin, J., F. Rojo, and V. deLorenzo.** 1994. Promoters responsive to DNA bending: a common theme in prokaryotic gene expression. Microbiol. Rev. **58:**268–290.
45. **Prentki, P., and H. M. Krisch.** 1984. *In vitro* insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
46. **Rimsky, S., and A. Spassky.** 1990. Sequence determinants for H1 binding on *Escherichia coli lac* and *gal* promoters. Biochemistry **29:**3765–3771.
47. **Ritter, A., D. L. Gally, P. B. Olsen, A. Friedrich, U. Dobrindt, P. Klemm, and J. Hacker.** The Pai-associated *leuX* specific tRNA5Leu affects type 1 fimbriation in pathogenic *Escherichia coli* by control of FimB recombinase expression. Mol. Microbiol., in press.
48. **Schwan, W. R., H. S. Seifert, and J. L. Duncan.** 1994. Analysis of the *fimB* promoter region involved in type 1 pilus phase variation in *Escherichia coli*. Mol. Gen. Genet. **242:**623–630.
49. **Spassky, A., S. Rimsky, H. Garreau, and H. Buc.** 1984. H1a, an *E. coli* DNA-binding protein which accumulates in stationary phase, strongly compacts DNA *in vitro*. Nucleic Acids Res. **12:**5321–5340.
50. **Spurio, R., M. Durrenberger, M. Falconi, A. LaTeana, C. L. Pon, and C. O. Gualerzi.** 1992. Lethal overproduction of the *Escherichia coli* nucleoid protein H-NS: ultramicroscopic and molecular autopsy. Mol. Gen. Genet. **231:** 201–211.
51. **Tanaka, K., S. Muramatsu, H. Yamada, and T. Mizuno.** 1991. Systematic characterization of curved DNA segments randomly cloned from *Escherichia coli* and their functional significance. Mol. Gen. Genet. **226:**367–376.
52. **Tippner, D., H. Afflerbach, C. Bradaczek, and R. Wagner.** 1994. Evidence for a regulatory function of the histone-like *Escherichia coli* protein H-NS in ribosomal RNA synthesis. Mol. Microbiol. **11:**589–604.
53. **Tupper, A. E., T. A. Owen-Hughes, D. W. Ussery, D. S. Santos, D. J. P. Ferguson, J. M. Sidebotham, J. C. D. Hinton, and C. F. Higgins.** 1994. The chromatin-associated protein H-NS alters DNA topology *in vitro*. EMBO J. **13:**258–268.
54. **Ueguchi, C., M. Kakeda, and T. Mizuno.** 1993. Autoregulatory expression of the *Escherichia coli hns* gene encoding a nucleoid protein: H-NS functions as a repressor of its own transcription. Mol. Gen. Genet. **236:**171–178.
55. **Ueguchi, C., and T. Mizuno.** 1993. The *Escherichia coli* nucleoid protein H-NS functions directly as a transcriptional repressor. EMBO J. **12:**1039–1046.
56. **Ueguchi, C., T. Suzuki, T. Yoshida, K. Tanaka, and T. Mizuno.** 1996. Systematic mutational analysis revealing the functional domain organization of *Escherichia coli* nucleoid protein H-NS. J. Mol. Biol. **263:**149–162.
57. **Yamada, H., S. Muramatsu, and T. Mizuno.** 1990. An *Escherichia coli* protein that preferentially binds to sharply curved DNA. J. Biochem. **108:**420–425.
58. **Yamada, H., T. Yoshida, K. Tanaka, C. Sasakawa, and T. Mizuno.** 1991. Molecular analysis of the *Escherichia coli hns* gene encoding a DNA-binding protein, which preferentially recognizes curved DNA sequences. Mol. Gen. Genet. **230:**332–336.
59. **Zuber, F., D. Kotlarz, S. Rimsky, and H. Buc.** 1994. Modulated expression of promoters containing upstream curved DNA sequences by the *Escherichia coli* nucleoid protein H-NS. Mol. Microbiol. **12:**221–240.

Downloaded from http://jb.asm.org/ on July 16, 2020 by guest