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Plasmids Bearing *hfq* and the *hns*-Like Gene *stpA* Complement *hns* Mutants in Modulating Arginine Decarboxylase Gene Expression in *Escherichia coli*

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Biodegradative arginine decarboxylase is inducible by acid and is derepressed in an *hns* mutant. Several plasmids from an *Escherichia coli* library that could complement the *hns* phenotype were characterized and placed into groups. One group includes plasmids that contain the *hns* gene and are considered true complements. Another group was found to carry the *hfq* gene, which encodes the host factor HF-1 for bacteriophage Q_β replication. Plasmids of the third group contain inserts that mapped at 60.2 min on the *E. coli* chromosome. We identified an open reading frame (*stpA*) with a deduced amino acid sequence showing more than 60% identity with the sequences of H-NS proteins from several species as being responsible for the *hns* complementing phenotype of the third group.

In prokaryotic organisms, several proteins sharing the characteristics of being abundant, small, basic, and binding DNA nonspecifically have been found to be associated with the nucleoid and been named histone-like proteins after the eukaryotic histones (26). These include HU, H-NS (H1 or H1a), integration host factor, and Fis. Among them, H-NS is relatively neutral and functions as a homodimer. It is encoded by *hns* at 27 min on the *Escherichia coli* chromosome, has 136 amino acid residues with an apparent molecular mass of 16 kDa (23, 28), and can affect DNA supercoiling (7, 13, 19). H-NS binds double-stranded DNA nonspecifically but seems to prefer curved DNA structures (22, 35). It exerts transcriptional repression on its own expression (8, 35), an effect antagonized by Fis, another histone-like protein (8). The general importance of H-NS in the regulation of gene expression is demonstrated by the fact that the expression patterns of a number of otherwise unrelated genes are affected in *hns* mutant strains (3, 11, 12, 19, 27, 32). Among the genes affected by *hns* mutation are *adi* and *cadA*, the structural genes for the acid-induced biodegradative arginine and lysine decarboxylases, respectively (21, 27, 29). In this report, we describe the characterization of several plasmids that complement the effect of an *hns* mutation on *adi* gene expression.

Complementation of an *hns* mutant phenotype by plasmids carrying genes other than *hns*. It had been previously described that mutations in *hns*, the structural gene for an *E. coli* histone-like protein, H-NS, can cause derepressed expression of the acid-induced arginine decarboxylase gene (*adi*) at a noninducing pH (pH 8.0) (27). A series of plasmids that could complement the derepressing effect of *hns* mutations on *adi* expression were isolated from an *E. coli* plasmid library as described previously (27). Strains and plasmids used in this study are described in Table 1. Most of the plasmids contained a functional *hns* gene (27), but others did not, indicating that these plasmids could complement the *hns* phenotype by providing the mutant cells with some product other than H-NS. Among the latter are plasmids that were isolated by their ability to complement mutant GNB725 (*adi*::*lac hns-25*::Tn*5*), namely, p25T1, p25T33, p25T56, p25T91, p25T95, and p25T129. Restriction enzyme mapping identified them as four different plasmids (Fig. 1). Their *hns*-complementing phenotypes were demonstrated by their abilities to lower the derepressed β-galactosidase synthesis in *adi*::*lac hns* strains (Table 2). The β-galactosidase values of cultures grown at pH 8.0 are reduced 4- to 15-fold. A wild-type *hns* gene, on the other hand, could reduce the β-galactosidase production of *adi*::*lac hns* strains 20- to 40-fold at pH 8.0 and 3- to 50-fold at pH 5.5 (27).

On the basis of the restriction map alignments and hybridization experiments using probes derived from the plasmid segments shown in Fig. 1, these p25T plasmids could be placed into three groups: p25T33, which mapped to 73.8 to 73.9 min on the *E. coli* chromosome; p25T91, which mapped to 60.2 to 60.3 min and contains *stpA*; and p25T1 and p25T56, which mapped to 94.8 to 94.9 min and contain the gene *hfq* (Fig. 1).

Characterization of *hfq* as the *hns*-complementing gene on plasmids p25T1 and p25T56. Plaque-lift screening of the miniset *E. coli* library (18), using nick-translated p25T56 DNA as a probe, showed a positive signal at phage 3A1 (serial no. 652). Southern hybridization experiments on restriction enzyme-digested DNAs from phage 3A1 and plasmids p25T1 and p25T56 showed that they carry a common DNA segment. Thus, the inserts on plasmids p25T1 and p25T56 mapped to the phage clone 3A1 which is located at 94.6 to 94.95 min on the *E. coli* chromosome.

Self-ligation of the larger *Pvu*II fragment of p25T1 resulted in a derivative, p25T1BV, which retained the phenotypic activity of p25T1 when assayed in different host strains (Table 3). Since p25T1 and p25T56 have similar phenotypes (Fig. 1), we assumed that the phenotype of p25T56 is also caused by the ~1.3-kb *Bam*HI-*Pvu*II fragment of the insert.

Examination of the restriction map of phage 3A1 and its alignment on the *E. coli* chromosome showed that the *Bam*HI-*Pvu*II fragment mapped near 94.8 min on the chromosome. The DNA sequences of two adjacent genes had been reported in this region: *miaA* (5) and *hfq* (16). Since there is a *Bam*HI site within the coding sequence of *miaA* and there is only one *Bam*HI site at the end of the insert on either p25T1 or p25T56, our plasmids could contain only part of the *miaA* gene but could contain a complete *hfq* gene.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
Strains		
71-18	*supE thi* Δ(*lac-proAB*) F′(*proAB lacI*^{q} *lacZ*ΔM15)	6
MC4100	F$^-$ *araD139* (*argF-lac*)*U169 rpsL150 relA flb-5301 ptsF25 deoC1*	4
GNB7145K	MC4100 *adi*::Mu dI1734 (Km^r *lac*)	1
GNB725	GNB7145K *hns-25*::Tn*5* (Cm^r)	27
TX2761	NU426 [W3110 *sup*(Am)] *hfq2*::Ω (Km^r)	33
TX2822	NU426 [W3110 *sup*(Am)] *hfq1*::Ω (Km^r)	33
Plasmids		
pBR322 *E. coli* library	Ap^r Tc^s	27
pEMBL8$^+$	Ap^r	6
p25T1	pBR322 *hfq*$^+$	This study
p25T56	pBR322 *hfq*$^+$	This study
p25T1BV	Derivative of p25T1; *hfq*$^+$ Ap^r	This study
pPcrQ	*hfq* PCR fragment cloned at the *Sma*I site of pEMBL8$^+$; Ap^r	This study
p25T91	pBR322 *stpA*$^+$	This study
pK20T141	pBR322 *stpA*$^+$	This study
pStpA	720-bp *stpA* PCR fragment cloned into the *Sma*I site of pEMBL8$^+$; Ap^r	This study
pXS06	430-bp *Eco*RI fragment from p25T91 cloned into pEMBL8$^+$	This study
pXS09	710-bp *Eco*RI fragment from p25T91 cloned into pEMBL8$^+$	This study
pXS15	1.26-kb *Eco*RI fragment from p25T91 cloned into pEMBL8$^+$	This study
pXSK3	p25T1BV with the *Kpn*I site in *hfq* disrupted and religated (clone 3)	This study
pXSK12	p25T1BV with the *Kpn*I site in *hfq* disrupted and religated (clone 12)	This study
pXS16	p25T1BV with the DHFR[a] gene inserted in the *Kpn*I site in *hfq*	This study

[a] DHFR, dihydrofolate reductase.

A PCR amplification was carried out on chromosomal DNA from MC4100, using two primers designed according to the published *hfq* DNA sequence (5′ ATGAATTCCATTCGTT GCGTGGGTTATCGC 3′ [5′-primer] and 5′ ATGGATC CAGTACCGCCTGCTCACCA 3′ [3′-primer] [16]) so that only the *hfq* structural gene plus adjacent upstream and downstream sequences would be amplified. The expected fragment (740 bp) was observed, and the PCR fragment amplified from MC4100 chromosomal DNA was cloned into the *Sma*I site on the phagemid pEMBL8$^+$ (6), resulting in the plasmid pPcrQ. When transformed into the *adi*::*lac* fusion strains, pPcrQ demonstrated the phenotype of plasmids p25T1 and p25T56 (Table 3).

We further confirmed that *hfq* was indeed the gene responsible for the observed *hns*-complementing phenotype. Plasmid p25T1BV was digested with *Kpn*I, a unique restriction enzyme site within the *hfq* structural gene. DNA encoding a type II dihydrofolate reductase specifying trimethoprim resistance from pMT100 (31) was inserted to yield plasmid pX516 (Ap^r Tp^r). Plasmids pXSK3 and pXSK12 (Ap^r Tp^s) were formed from religation without the Tp^r fragment and retained the *hns*-complementary phenotype, while pXS16 lost this property (Table 3). The regions around the original *Kpn*I site on the three plasmids were sequenced as described elsewhere (20), and sequence deletions at the junction were found. The two plasmids which retained *hns*-complementing activity, pXSK3 and pXSK12, had 15- and 8-bp deletions, resulting in peptides of 98 and 79 amino acid residues, respectively. The peptides produced are shown in Fig. 2. The fact that plasmids pXSK3 and pXSK12 retained the *hns*-complementing phenotype of wild-type *hfq* and that plasmid pXS16 (a foreign protein fusion) lost the *hns*-complementation phenotype indicates that the C-terminal 26 amino acid residues of HF-1 (the product of the *hfq* gene) are dispensable for the *hns*-complementation function, but the formation of certain foreign peptide fusions in the C-terminal region can interfere with HF-1's normal function. A Maxicell experiment (25) was conducted to detect the protein products encoded by the three plasmids (data not shown). The observed sizes of the mutant HF-1 proteins from pXSK3 and pXSK12 were as predicted from the sequence data shown in Fig. 2. Plasmid pXS16 produced a minor band of ~10 kDa and a major product of smaller size, indicating that the peptide might be unstable.

The *hns*-complementing phenotype of HF-1 seems to be

TABLE 2. *hns*-complementing phenotypes of p25T plasmids

Plasmid	β-Galactosidase activity (Miller units) in[a]:								
	GNB7145K		GNB723		GNB725		GNB729		GNB88
	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0	pH 5.5	pH 8.0
None	2	380	90	590	70	350	95	340	3,000
p25T1	2	94	13	270	10	170	8	250	2,700
p25T56	2	150	20	350	13	280	15	250	2,500
p25T33	2	400	18	660	18	400	17	330	2,400
p25T91	2	300	6	440	7	470	6	300	530

[a] GNB723, GNB725, and GNB729 are *hns* mutants of GNB7145K. GNB88 is an *hns* mutant of the *cad*::*lac* strain GNB8385K (27). β-Galactosidase assays were performed as described previously (27). The relative β-galactosidase activities of repeated assays were within a standard deviation of 15%.

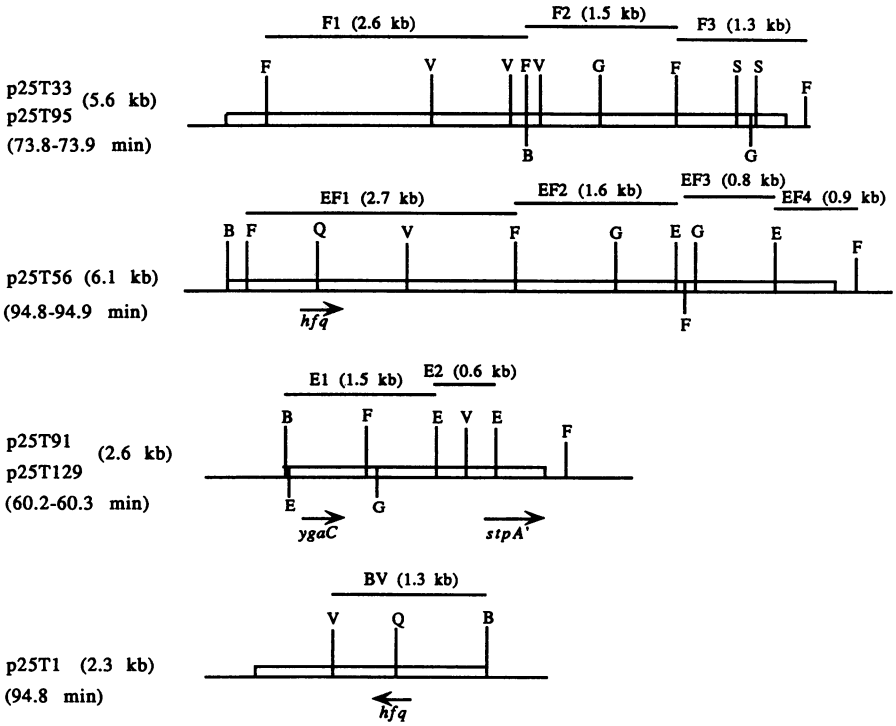

FIG. 1. Restriction enzyme maps of GNB725-complementing plasmids. Open boxes, DNA insertions in the plasmids. The size of the insert (as determined by restriction mapping) and the chromosomal location of the insert (as determined by Southern hybridization experiments with the miniset λ phage library [18]) are indicated for each plasmid. Sequences from the vector pBR322 are represented by horizontal lines, and the *Eco*RV site on the vector is shown to indicate the direction of the cloned insert. Two plasmid pairs (plasmids p25T33 and p25T95 and plasmids p25T91 and p25T129) were found to have identical restriction maps. The positions and transcription directions of the genes that are mentioned in the text are indicated (arrows). The probes used in hybridization experiments are shown above each restriction map. Restriction enzymes: B, *Bam*HI; E, *Eco*RI; F, *Eco*RV; G, *Bgl*I; Q, *Kpn*I; S, *Pst*I; V, *Pvu*II.

specific to *adi* expression, since the *hfq*$^+$-containing plasmids did not complement the effect of an *hns* mutation (derepression) on the expression of another acid-induced gene, *cadA* (Table 2).

HF-1, encoded by *hfq* at 94.8 min on the *E. coli* chromosome, was originally identified as being required for the synthesis of minus-strand RNA complementary to phage Q_β RNA (16). HF-1 was found to be a small (11.6 kDa), basic (pI 7.91), heat-stable, abundant (30,000 to 60,000 molecules per cell), RNA-binding protein which is associated with ribosomes and functions as a hexamer (2, 16, 17). When HF-1 protein was purified from Q_β-infected *E. coli* cells, it was found to be contaminated with the histone-like protein H-NS throughout the purification procedures, until the two were separated by reverse-phase high-pressure liquid chromatography (16). This has led to the suggestion that these two proteins might interact with each other. Another explanation for the copurification is that these two proteins have very similar physicochemical properties upon which the purification procedures are based. The possibility that these two proteins might interact with each other suggests a possible means for the functional complementation we observed. In our study, we found that overproduction of HF-1 from an *hfq* gene on a multicopy plasmid could counteract the effect of *hns* mutations (*hns-25*, *hns-23*, and *hns-29* alleles) on the expression of the biodegradative arginine decarboxylase gene, *adi*. This function of HF-1 might or might not need interaction with H-NS, since our *hns* alleles could still produce truncated or hybrid H-NS proteins. This has previously been reported with other *hns*::Tn derivatives (14).

Recently, an *hfq* defective mutant was constructed by transposon insertion at the promoter-proximal end of the structural gene (*hfq1*::Ω), and it was shown to have pleiotropic effects on cell physiology (33). The *hfq1*::Ω mutant exhibited defects in cell growth, osmosensitivity, cell shape and size, sensitivity to UV light, and plasmid supercoiling. In addition, this *hfq* mutant allele affected the osmosensitivity phenotype of the *osmZ205*::Tn*10* allele of *hns* and the expression of the cryptic operon *bgl*, and it counteracted the effect of an *hns-37*::Tn*10* allele on *bgl* expression. Another insertional mutant, *hfq2*::Ω, however, displayed a phenotype similar to the wild type (33). The transposon in the *hfq2*::Ω mutant was inserted at the *Kpn*I site within the *hfq* gene, a mutation which was considered to be a promoter-distal disruption of *hfq* that did not cause loss of function. The near-wild-type phenotype of the *hfq2*::Ω strain is consistent with the results of our attempts to disrupt the plasmid-borne *hfq* gene at the *Kpn*I site.

When either of two *hfq* insertional mutations, the promoter-proximal *hfq1*::Ω from strain TX2822 or the promoter-distal *hfq2*::Ω from TX2761 (33), was introduced into an *adi*::*lac* fusion strain, no effect on the β-galactosidase activity, i.e., on *adi* gene expression, was observed in exponential-phase cultures. This suggests that under normal physiological conditions (in an *hns*$^+$ background and when HF-1 is produced from the chromosomal copy of the *hfq* gene), HF-1 is not involved in *adi* gene regulation.

Characterization of *stpA* as the *hns*-complementing gene on plasmid p25T91. The introduction of plasmid p25T91 into GNB7145K *hns* strains caused a 10-fold reduction in the

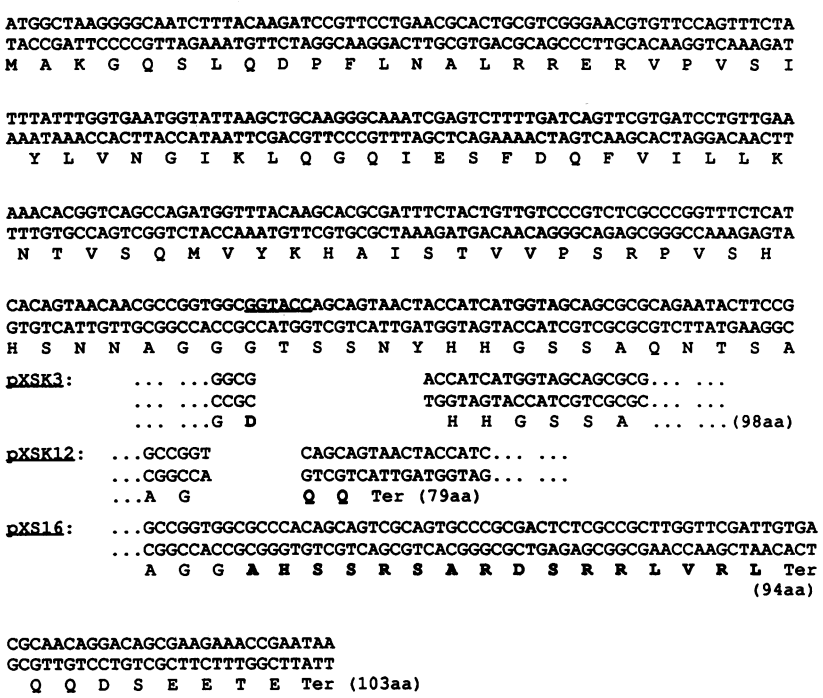

FIG. 2. DNA sequences at the ligation and religation junctions on plasmids pXSK3, pXSK12, and pXS16. The sequences are aligned with the portion of the wild-type *hfq* coding sequence which is underlined. The remainder of the *hfq* coding sequence as obtained by Kajitani and Ishihama is also shown (16). Deduced amino acid sequences are shown below the DNA sequences. Amino acid residues that are different from the wild-type sequences as a result of the DNA manipulations are boldfaced, and the nucleotide sequences that are identical to the sequence of the wild-type gene are represented by dots. Translation termination codons (Ter) and the total length of each peptide in amino acid (aa) residues are indicated.

production of β-galactosidase from cultures grown at pH 8.0 (Table 2). In this case the plasmid, and others bearing *stpA*, also reduced expression of the *cad* operon in strains containing *hns* mutations (Table 2), although not as efficiently as plasmids bearing *hns* (27). When ^{32}P-labeled p25T91 DNA was used to probe a Gene Mapping Membrane (Takara Shuzo Co., Ltd.) containing the miniset recombinant λ phage library of *E. coli* originally established by Kohara et al. (18), two overlapping phages, 10H9 (serial no. 444), located at 60.1 to 60.4 min, and 8G10 (serial no. 445), located at 60.2 to 60.6 min, hybridized specifically to the probe. The restriction map of p25T91 defined the location of the insert as 60.2 min. The insert on p25T91 and related segments of other plasmids (e.g., pK20T141) were sequenced to define the gene responsible for the observed *trans*-acting phenotype (Fig. 3), and two open reading frames were found.

A homology search with the two deduced peptide sequences was conducted. No significant match was found for the 105-residue peptide (YgaC). The 134-residue peptide was found to have high (>65%) similarity with H-NS proteins from several organisms, and the identity was about 60%. As an example, the homology between this peptide sequence and the H-NS protein sequence of *E. coli* is shown in Fig. 4. A region of >900 bp including this coding region was previously sequenced by Zhang and Belfort, and they named this gene *stpA* (36). However, no functional characterization of this region was reported.

In order to characterize *stpA*, a primer extension experiment was carried out to determine the 5′ end of the transcript. The *stpA* transcript begins at the A at position 1588 (+1 in Fig. 3). In support of this, comparison of the upstream *stpA* sequence with the consensus *E. coli* promoter sequences using the program MacTargsearch (10) revealed a promoter site at an appropriate distance from +1 (−10 and −35 in Fig. 3). Also, 12 nucleotides downstream of the termination codon, there is a palindromic structure indicative of a weak transcription terminator (arrows in Fig. 3).

A PCR experiment was conducted to show that *stpA* is the gene responsible for the observed *hns*-complementing phenotype of p25T91 and pK20T141. A 720-bp fragment was amplified from purified MC4100 chromosomal DNA by using a 5′

TABLE 3. Effects of the *hfq* plasmid p25T1 and its derivatives on β-galactosidase expression in *adi*::*lac* fusion strains[a]

Plasmid	β-Galactosidase activity in[b]:			
	GNB7145K[c]		GNB725[d]	
	pH 8.0	pH 5.5	pH 8.0	pH 5.5
None	4.9 ± 0.3	343 ± 41	84 ± 4	649 ± 75
pBR322[e]	5.1 ± 0.4	676 ± 57	63 ± 2	798 ± 60
p25T1	5.6 ± 0.7	143 ± 6	13 ± 2.5	110 ± 4
p25T1BV	5.9 ± 0.8	119 ± 15	9 ± 1.5	94 ± 4
pXSK3	5.2 ± 0.3	160 ± 13	11 ± 1.5	82 ± 15
pXSK12	6.5 ± 0.7	174 ± 5	12 ± 1.5	117 ± 18
pXS16	7.3 ± 0.2	493 ± 17	56 ± 6	613 ± 40
pPcrQ	5.0 ± 1	22 ± 1	8 ± 0.6	37 ± 6.4

[a] The plasmids were transformed into either host strain. The transformants were then assayed for β-galactosidase production expressed from the *lac* fusion as described elsewhere (27). The pXS16 transformant was *hfq* negative; the others were *hfq*$^+$.

[b] The data are averages and standard deviations for at least three independent assays.

[c] *adi*::*lac*.

[d] *adi*::*lac hns-25*.

[e] Vector.

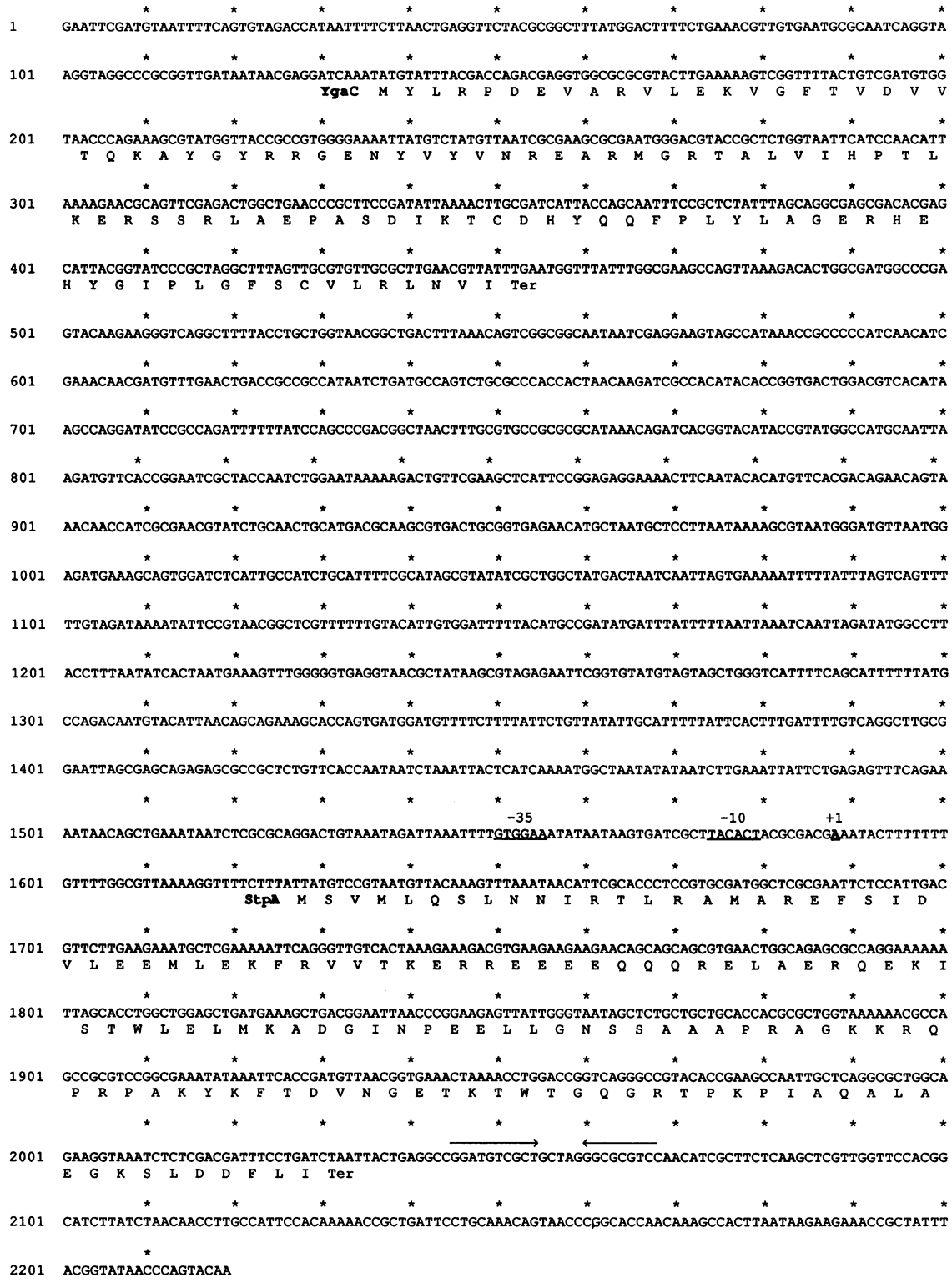

FIG. 3. Complete nucleotide sequence of the *ygaC stpA* region. Amino acid sequences of YgaC and StpA are presented below the nucleotide sequence. The promoter sequence of the *stpA* gene (−10 and −35), the 5′ end of its transcript (+1), as determined by primer extension, and the palindromic structure downstream of *stpA* (arrows) are indicated.

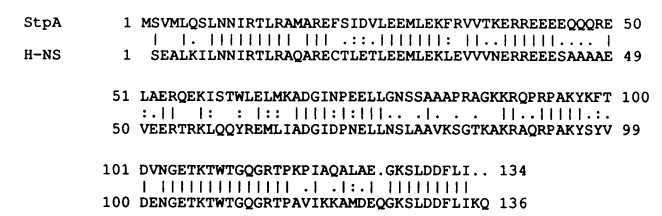

FIG. 4. Comparison of the StpA amino acid sequence with that of the *E. coli* DNA-binding protein H-NS. Vertical bars, colons, and dots, identical, homologous, and semihomologous residues, respectively. The sequences of these two peptides have 67% similarity.

primer corresponding to bp 1385 to 1405 and a 3′ primer corresponding to bp 2083 to 2105 (Fig. 3), and the fragment was cloned into the *Sma*I site of phagemid pEMBL8$^+$ (6) to produce a PCR clone, pStpA. β-Galactosidase assays of pStpA transformants showed that this plasmid retained the *hns*-complementary phenotype of the two larger plasmids p25T91 and pK20T141 (Table 4).

We further showed that *stpA* codes for a protein in a Maxicell experiment (25) (data not shown). Major products were observed for the 31-kDa β-lactamase, the 15.2-kDa StpA protein product from pStpA or pK20T141, and a 12- and a 16-kDa (StpA′) peptides encoded by p25T91. Sequencing and Maxicell experiments indicated that p25T91 produced a protein, StpA′, which is 10 amino acids longer than StpA, because of the absence of the normal termination codon, but apparently this did not affect its ability to complement the effect of an *hns* mutation on *adi* expression. The intensity of the StpA protein band relative to that of the β-lactamase band on the gel from Maxicell analysis suggests that StpA is probably expressed at a substantial level in the cell, likely because of the high activity of the *stpA* promoter. Since *stpA* has an appropriate transcription signal, produces a detectable transcript and protein product, and is associated with a detectable phenotype, we conclude that *stpA* is a functional gene coding for an H-NS-like protein in *E. coli* K-12.

The other 12-kDa peptide produced from p25T91, YgaC, designated according to convention (24), is very likely encoded by *ygaC*, which codes for a peptide of 105 amino acid residues with a deduced molecular mass of 12.1 kDa.

StpA has a net charge of +1 and is basic (pI ~9); thus, it seems to fit the definition of a histone-like protein. It is widely accepted that H-NS is identical to H1a, the most basic of the three components of H1 isolated from *E. coli* when analyzed by two-dimensional gel electrophoresis (28). The other two components of H1 (H1b and H1c) have pIs between 7 and 7.5, as estimated from gel electrophoresis work. Therefore, even though H-NS and StpA have the same apparent molecular weight, StpA may not correspond to one of the other two H1 components. When the charges and isoelectrical points of the H-NS proteins with which StpA has high sequence homology were examined by using the computer software program from the Genetics Computer Group, Inc., they seemed to have lower calculated pIs than those reported from experimental measurement.

A number of growth conditions and proteins that have been shown to affect the expression of *adi*, including acidic pH and anaerobiosis (1, 21, 29), H-NS (27), and integration host factor (30), are all able to influence DNA topology (9, 12, 15, 33). The Gene *stpA* resembles the histone-like protein gene family, and its protein product could hinder the formation of the transcriptional initiation complex at the *adi* promoter, either through direct interference by binding DNA or indirectly through effects on local DNA supercoiling. The ability of *stpA* to function in complementing the phenotypes of *hns* mutants in expression of the *cad* operon distinguishes it from *hfq* (which does not) and further suggests that StpA may act in a fashion similar to that of H-NS.

Nucleotide sequence accession number. The nucleotide sequence shown in Fig. 3 has been submitted to the GenBank database under accession number UO7823.

TABLE 4. *hns*-complementary phenotype of *stpA*$^+$ plasmids on *adi* gene expression[a]

Plasmid	β-Galactosidase activity in[b]:		
	GNB7145K[c] (pH 5.5)	GNB725[d]	
		pH 8.0	pH 5.5
None	466 ± 18	132 ± 9	1,632 ± 12
pBR322	526 ± 67	117 ± 3	1,532 ± 202
p25T91	224 ± 20	18 ± 1	569 ± 94
pK20T141	215 ± 23	17 ± 2	401 ± 68
pStpA	180 ± 37	19 ± 5	215 ± 31

[a] The plasmids were transformed into the indicated host strains, and β-galactosidase production of the *adi*::*lac* fusion strains was assayed as described elsewhere (27).

[b] The data are averages and standard deviations for at least three independent assays.

[c] *adi*::*lac*.

[d] *adi*::*lac hns-25*.

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REFERENCES

1. **Auger, E. A., K. E. Redding, T. Plumb, L. C. Childs, S.-Y. Meng, and G. N. Bennett.** 1989. Construction of *lac* fusions to the inducible arginine- and lysine decarboxylase genes of *Escherichia coli* K-12. Mol. Microbiol. **3**:609–620.
2. **Barrera, I., D. Schuppli, J. M. Sogo, and H. Weber.** 1993. Different mechanisms of recognition of bacteriophage Q beta plus and minus strand RNAs by Q beta replicase. J. Mol. Biol. **232**:512–521.
3. **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.
4. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
5. **Connolly, D., and M. E. Winkler.** 1991. Structure of *Escherichia coli* K-12 *miaA* and characterization of the mutator phenotype caused by *maiA* insertion mutations. J. Bacteriol. **173**:1711–1721.
6. **Dente, L., G. Cesareni, and R. Cortese.** 1983. pEMBL: a new family of single stranded plasmids. Nucleic Acids Res. **11**:1645–1655.
7. **Durrenberger, M., A. La Teana, 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.
8. **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.
9. **Freundlich, M., N. Ramani, E. Mathew, A. Sirko, and P. Tsui.** 1992. The role of integration host factor in gene expression in *Escherichia coli*. Mol. Microbiol. **6:**2557–2563.
10. **Goodrich, J. A., M. L. Schwartz, and W. R. McClure.** 1990. Searching for and predicting the activity of sites for DNA binding proteins: compilation and analysis of the binding sites for *Escherichia coli* integration host factor (IHF). Nucleic Acids Res. **18:**4993–5000.
11. **Göransson, M., B. Sorden, P. Nilsson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature (London) **344:**682–685.
12. **Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. 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.
13. **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.
14. **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.
15. **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.
16. **Kajitani, M., and A. Ishihama.** 1991. Identification and sequence determination of the host factor gene for bacteriophage Q_β. Nucleic Acids Res. **19:**1063–1066.
17. **Kajitani, M., A. Kato, A. Wada, Y. Inokuchi, and A. Ishihama.** 1994. Regulation of the *Escherichia coli hfq* gene encoding the host factor for phage Q_β. J. Bacteriol. **176:**531–534.
18. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
19. **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.
20. **Meng, S.-Y., and G. N. Bennett.** 1992. Nucleotide sequence of the *Escherichia coli cad* operon: a system for neutralization of low external pH. J. Bacteriol. **174:**2659–2669.
21. **Meng, S.-Y., and G. N. Bennett.** 1992. Regulation of the *Escherichia coli cad* operon: location of a site required for pH induction. J. Bacteriol. **174:**2670–2678.
22. **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.
23. **Pon, C. L., A. C. Raffaele, and C. O. Gualerzi.** 1988. Identification, cloning, nucleotide sequence and chromosomal map location of *hns*, the structural gene for *Escherichia coli* DNA-binding protein H-NS. Mol. Gen. Genet. **212:**199–202.
24. **Rudd, K. E.** 1993. Maps, genes, sequences, and computers: an *Escherichia coli* case study. ASM News **59:**335–341.
25. **Sancar, A., R. P. Wharton, S. Seltzer, B. M. Kacinski, N. B. Clark, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148:**45–62.
26. **Schmid, M. B.** 1990. More than just "histone-like" proteins. Cell **63:**451–453.
27. **Shi, X., B. C. Waasdorp, and G. N. Bennett.** 1993. Modulation of acid-induced amino acid decarboxylase gene expression by *hns* in *Escherichia coli*. J. Bacteriol. **175:**1182–1186.
28. **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.
29. **Stim, K. P., and G. N. Bennett.** 1993. Nucleotide sequence of the *adi* gene, which encodes the biodegradative acid-induced arginine decarboxylase of *Escherichia coli*. J. Bacteriol. **175:**1221–1234.
30. **Stim, K. P., M. Rives, B. Waasdorp, and G. Bennett.** 1993. Investigation of the role of integration host factor (IHF) in the regulation of the biodegradative arginine and lysine decarboxylases of *Escherichia coli*, p. 218. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. Atlanta, Ga.
31. **Swift, G., B. J. McCarthy, and F. Heffron.** 1981. DNA sequence of a plasmid-encoded dihydrofolate reductase. Mol. Gen. Genet. **181:**441–447.
32. **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.
33. **Tsui, H.-C. T., H.-C. E. Leung, and M. E. Winkler.** 1994. Broadly pleiotropic effects of an *Escherichia coli* K-12 *hfq* insertion mutation on cellular processes mediated by DNA topology. Mol. Microbiol. **13:**35–49.
34. **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.
35. **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.
36. **Zhang, A., and M. Belfort.** 1992. Nucleotide sequence of a newly-identified *Escherichia coli* gene, *stpA*, encoding an H-NS-like protein. Nucleic Acids Res. **20:**6735.