# Differential Dependence of StpA on H-NS in Autoregulation of *stpA* and in Regulation of *bgl*

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StpA has functional similarity to its homologue, the nucleoid structuring protein H-NS. It binds to AT-rich, planar, bent DNA and constrains DNA supercoils. In addition, StpA acts as an RNA chaperone. StpA and H-NS also form heterodimers. However, cellular levels of StpA are low due to repression of *stpA* by H-NS and negative autoregulation. Here we show that effective (30-fold) repression of *stpA* transcription requires a downstream regulator element located within the *stpA* coding region. In addition, we show that StpA represses *stpA* threefold in an *hns* null mutant. In contrast, repression of the *bgl* operon, another H-NS-repressed system, is not achieved by StpA alone. It becomes StpA dependent in the presence of a fusion protein encompassing the N-terminal 37 amino acids of H-NS, which comprise the core of the dimerization domain. StpA also effectively complements H-NS-I119T, a mutant defective in specific DNA binding, in repression of the *bgl* operon. Thus, StpA complements H-NS proteins defective in DNA binding to repress *bgl*, while in autoregulation of *stpA* it acts autonomously, indicating a difference in the mechanisms of repression.

The 15.3-kDa StpA protein of *Escherichia coli* is 58% identical to H-NS, a pleiotropic repressor and nucleoid structuring protein (14, 44). StpA binds to AT-rich, planar, bent DNA and constrains supercoils, as does its homologue H-NS, but with a higher affinity (24, 38). In addition to these features which are functionally similar to those of H-NS, StpA is an RNA chaperone. StpA was initially identified as a suppressor of a T4 *td* intron mutant defective in splicing, where *stpA* is carried on a multicopy plasmid (44, 45). StpA catalyzes annealing of the mutant *td* intron RNA and promotes its self-splicing (45). In addition, StpA binds to MicF, a small regulatory RNA, resulting in destabilization of MicF (7). MicF is known to interact with the *ompF* RNA, triggering its degradation and thus resulting in downregulation of the OmpF outer membrane porin (8).

Genetic and biochemical data demonstrated that StpA and H-NS form heterodimers (21, 22, 40). Heterodimer formation might be important in the modulation of H-NS activity by StpA, and heterodimers may have specific functions. Furthermore, StpA was found to be unstable in *hns* mutants, while it is stable in the wild type, which demonstrated that heterodimer formation with H-NS protects StpA from degradation by Lon (21, 22). The cellular level of StpA is significantly lower than that of its abundant homologue, H-NS, most likely due to repression of *stpA* by H-NS (37, 46). In *hns* mutants, StpA negatively autoregulates transcription of *stpA* (37, 46). Furthermore, *stpA* expression is temperature regulated, activated during exponential growth, regulated by Lrp, and induced by osmotic stress (16, 37).

Overexpression of StpA in *hns* mutants can restore the repression of several H-NS-repressed systems, such as *proU*, *bgl*, the synthetic 5A6A *galP1* promoter, and *hns* (21, 40, 46). Likewise, chromosomally encoded StpA partially compensates for

positive regulation of translation of *malT* by H-NS (20). Therefore, StpA is considered a molecular backup of H-NS (46). However, StpA may have distinct functions from those of H-NS, since StpA has unique features, mutation of *stpA* has no effect on repression by H-NS, and complementation of *hns* mutants occurs only partially and often requires artificially high levels of StpA (16). Furthermore, in several studies of complementation of *hns* mutants by StpA, *hns* mutants with insertions or replacements of the *hns* coding sequence were used, which may express truncated H-NS proteins (for example, see references 20 and 46). For example, H-NS proteins with truncation of the C-terminal DNA-binding domain are sufficient to render repression of *bgl* StpA dependently, indicating that StpA acts as an adapter to target H-NS mutants defective in DNA binding to *bgl* (17, 18, 29, 39).

Here we addressed the regulation of *stpA* and *bgl* by H-NS and by StpA. We found that repression of *stpA* by H-NS is significantly more effective when a downstream sequence encompassing the *stpA* coding region is present. In addition, StpA autoregulates *stpA*, with threefold repression, in an *hns* null mutant, i.e., independent of H-NS. In contrast, repression of *bgl* by StpA occurs only in the presence of the N-terminal H-NS protein domain or an H-NS-I119T mutant, i.e., in the presence of mutant H-NS proteins defective in specific DNA binding. This supports the model that StpA complements such H-NS mutants to repress *bgl* and may indicate that dimerization and/or oligomerization by H-NS is required for repression of *bgl* by StpA or that StpA nucleates (or targets) the defective H-NS proteins to *bgl*.

## MATERIALS AND METHODS

**Strains and plasmids.** The genotypes of the *E. coli* strains and the relevant structures of the plasmids used for this study are listed in Table 1. All experiments were performed using isogenic *E. coli* K-12 CSH50 (28) derivatives (Table 1). Transductions were performed using phage T4*GT7* (41). Integration of *lacZ* reporter constructs into the chromosomal phage lambda attachment site *attB* was performed as described previously (10, 12). Briefly, strain S541 or mutant derivatives of this strain harboring the temperature-sensitive, integrase-expressing

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TABLE 1. *E. coli* K-12 strains and plasmids used for this study

| Strain or plasmid | Genotype or structure[a] | Construction[b] or reference |
|---|---|---|
| **Strains** | | |
| CSH50 | *bgl*° Δ(*lac-pro*) *ara thi* | 27 |
| M182 | Δ(*lacIPOZYA*)74 *galU galK strA* | 9 |
| PD32 | MC4100 *hns*-206::*amp* | 46 |
| S159 | M182 *stpA*::*tet* | 46 |
| S160 | M182 *hns*::*kan* | 46 |
| S541 | Δ*bgl-AC11* Δ*lacZ-Y217 ara thi* (derived from CSH50) | 12 |
| S614 | S541 *hns*::*amp* | × T4*GT7* (PD32) |
| S2303 | S541 *hns*::*kan* | × T4*GT7* (S160) |
| S1195 | S541 *attB*::[Spec$^r$ $P_{lacUV5}$ *bglG*(*orf*) (+95 to 971) *lacZ*] | × pKESD49 (13) |
| S1258 | S1195 *hns*::*amp* | 13 |
| S2323 | S1195 *hns*::*kan* | × T4*GT7* (S160) |
| S1371 | S1195 *stpA*::*tet* | × T4*GT7* (S159) |
| S1767 | S1371 *hns*::*amp* | × T4*GT7* (PD32) |
| S2476 | S1371 *hns*::*kan* | × T4*GT7* (S160) |
| S1213 | S541 *attB*::[Spec$^r$ $P_{bgl}$ (+25) *lacZ*] | × pKEKB30 (13) |
| S1471 | S1213 *hns*::*amp* | × T4*GT7* (PD32) (13) |
| S2442 | S1213 *hns*::*kan* | × T4*GT7* (S160) |
| S1437 | S1213 *stpA*::*tet* | × T4*GT7* (S159) |
| S3158 | S1437 *hns*::*kan* | × T4*GT7* (S160) |
| S2217 | S541 *attB*::[Spec$^r$ *stpA* (−540 to +466) *lacZ*] | × pKES121 |
| S2262 | S2217 *hns*::*amp* | × T4*GT7* (PD32) |
| S2519 | S2217 *hns*::*kan* | × T4*GT7* (S160) |
| S2221 | S541 *attB*::[Spec$^r$ $P_{stpA}$ (−540 to +41) *lacZ*] | × pKES123 |
| S2266 | S2221 *hns*::*amp* | × T4*GT7* (PD32) |
| S2523 | S2221 *hns*::*kan* | × T4*GT7* (S160) |
| S2258 | S2221 *stpA*::*tet* | × T4*GT7* (S159) |
| S2951 | S2258 *hns*::*amp* | × T4*GT7* (PD32) |
| S2604 | S2258 *hns*::*kan* | × T4*GT7* (S160) |
| S2509 | S541 *attB*::[Spec$^r$ $P_{stpA}$ *stpA*(*orf*) (−540 to +466) *lacZ*] | × pKETW3 |
| S2844 | S2509 *hns*::*amp* | × T4*GT7* (PD32) |
| S2554 | S2509 *hns*::*kan* | × T4*GT7* (S160) |
| S2561 | S2509 *stpA*::*tet* | × T4*GT7* (S159) |
| S2953 | S2561 *hns*::*amp* | × T4*GT7* (PD32) |
| S2633 | S2561 *hns*::*kan* | × T4*GT7* (S160) |
| S3215 | S2509 Δ*hns*::*kan*$_{KD4}$ | This work |
| S2513 | S541 *attB*::[Spec$^r$ $P_{stpA}$ (−540 to +98) *lacZ*] | × pKETW5 |
| S2848 | S2513 *hns*::*amp* | × T4*GT7* (PD32) |
| S2557 | S2513 *hns*::*kan* | × T4*GT7* (S160) |
| S2565 | S2513 *stpA*::*tet* | × T4*GT7* (S159) |
| S2955 | S2565 *hns*::*amp* | × T4*GT7* (PD32) |
| S2637 | S2565 *hns*::*kan* | × T4*GT7* (S160) |
| S2741 | S541 *attB*::[Spec$^r$ $P_{lacUV5}$ *stpA*(*orf*) (+1 to +466) *lacZ*] | × pKETW17 |
| S2850 | S2741 *hns*::*amp* | × T4*GT7* (PD32) |
| S2770 | S2741 *hns*::*kan* | × T4*GT7* (S160) |
| S2766 | S2741 *stpA*::*tet* | × T4*GT7* (S159) |
| S2957 | S2766 *hns*::*amp* | × T4*GT7* (PD32) |
| S2774 | S2766 *hns*::*kan* | × T4*GT7* (S160) |
| **Plasmids** | | |
| pLG | pSC101 *neo* | 40 |
| pLG-HNS | *hns* in pLG | 40 |
| pLG-HNS | *hns*-L26P in pLG | 40 |
| pLG-HNS | *hns*-E53G-T55P in pLG | 40 |
| pLG-HNS | *hns*-I119T in pLG | 40 |
| pKETW18 | pSC101 *cat* | This work |
| pKETW20 | *hns* wt with *hns* promoter in pKETW18 | This work |
| pKETW21 | *hns*-L26P with *hns* promoter in pKETW18 | This work |
| pKETW22 | *hns*-E53G-T55P with *hns* promoter in pKETW18 | This work |
| pKETW23 | *hns*-I119T with *hns* promoter in pKETW18 | This work |
| pKETW15 | *lacI*$^q$ *lacO3*-Ptac-*lacO*, multiple cloning site in pACYC, *cat* | This work |
| pKETW13 | *hns*-His6 in pKETW15 | This work |
| pKETW14 | *hns*::*amp*-His6 in pKETW15 | This work |
| pKETW7 | *hnsKN*-His6 in pKETW15 | This work |
| pKETW8 | *hns*::*kan*-His6 in pKETW15 | This work |
| pKEM30 | *lacI*$^q$ *lacO3*-Ptac-*lacO*, multiple cloning site, HA tag in pACYC, *neo* | This work |
| pKETW10 | *hnsKN*-HA under control of *lacI*$^q$ *tac* in pKEM30 | This work |
| pKEM51 | *lacI*$^q$ *tac hns*-HA in pSC101 Cm (pKETW18) | This work |

[a] For the *lacZ* reporter constructs integrated into *attB*, the relevant structure is given. Positions of cloned fragments and fusions are given in brackets, e.g., $P_{bgl}$ (+25) indicates that the *lacZ* gene was fused downstream of base pair +25 relative to the transcription start of the *bgl* promoter, and *stpA*(*orf*) (+1 to +466) indicates that a *stpA* fragment encompassing positions +1 to +466 relative to the transcription start and carrying an ATG-to-ATA mutation of the translation start was cloned. The *lacUV5* promoter encompasses a fragment from positions −40 to +1 relative to the transcription start and thus lacks *lacO*. Compiled sequences of all plasmids are available upon request. For other details, see references 11–13, 26, and 35. Δ*hns*::*kan*$_{KD4}$ was constructed as described in Material and Methods.

[b] Transductions and integrations into *attB* were performed as described in Materials and Methods.

plasmid pLDR8 (10) were transformed with religated originless BamHI fragments carrying *attP*, a spectinomycin resistance cassette, and the respective *lacZ* reporter gene fusion (Table 1). Integrants were selected on LB-spectinomycin plates at 42°C. Independent colonies were tested by PCR to verify the integration of a monomer into *attB* and the integrity of the *lacZ* fusion construct. Two independent clones were used in the enzyme assays. Strain S3215 (S2509 Δ*hns*::*kan*$_{KD4}$), which has an exact deletion of *hns* (including the start codon and last codon), was constructed as described previously (6), using primers S665 (TCTATTATTACCTCAACAAACCACCCCAATATAAGTTTGAGATTACT ACAgtgtaggctggagctgcttcg) and S672 (AAATCCCGCCGCTGGCGGGATTTT AAGCAAGTGCAATCTACAAAAGATTAcatatgaatatcctccttagttcctattcc). The sequence that is complementary to the template pKD3 or pKD4 for PCR is in lowercase letters.

Plasmids were constructed according to standard techniques (1, 34). All *lacZ* reporter fusions were derived from a plasmid that carries a pACYC177 origin, the *neo* gene (4), *attP*, and an omegon-spectinomycin resistance cassette (33) as described previously (12). Site-specific mutations and fusions of *stpA* or *bgl* to *lacZ* were constructed by PCR. Plasmids pKETW20 to pKETW23 for expression of the dominant-negative *hns* mutants were constructed by PCR amplification of fragments encompassing *hns* and *hns* mutants, using the pLG plasmid series (40) as the template and primers S601 (GCGTCGACTTATTGCTTGATCAGGAA ATCGTC) and S602 (ACTCTAGATCCTTACATTCCTGGCTATTGCA). The PCR fragments were subjected to restriction digestion with XbaI and SalI (restriction sites are indicated by underlining), and the fragments were cloned into XbaI- and SalI-digested pKETW20, which is a pSC101-derived vector conferring chloramphenicol resistance. Plasmids pKETW13, pKETW14, pKETW8, and pKETW7 were constructed by PCR amplification of *hns*, *hns-206*::*amp*, *hns*::*kan*, and *hnsKN* (i.e., the 5′ open reading frame of *hns*::*kan*). The PCR fragments encompass 26 bp upstream of the ATG codon. At the 3′ end of the coding sequence, six histidine codons (CAT) and a stop codon (TGA) were added. The PCR fragments were digested with the restriction enzyme BglII or BamHI and cloned into plasmid pKETW15. Plasmid pKETW15 is a pACYC-derived vector that confers chloramphenicol resistance and carries a *lacI*$^q$ *tacOP* cassette followed by a multiple cloning site. Plasmids pKEM51 and pKETW10 were constructed similarly by PCR amplification of *hns* or *hnsKN* and cloning of the PCR fragments. In these plasmids, expression is driven by *tacOP*, and the *hns* or *hnsKN* open reading frame is fused to the *lacZ* Shine-Dalgarno sequence. At the C terminus of the open reading frame, a sequence (TACCCATACGATGTTC CAGATTACGCTtaa) coding for a hemagglutinin (HA) tag followed by a stop codon (lowercase) was added in frame. All regions of plasmids that were derived from PCR fragments were sequenced. The relevant structures of the plasmids are shown schematically in the figures and in Table 1. Details of constructions and compiled sequences of the plasmids are available upon request. Media and plates were used as described previously (12). Antibiotics were added to final concentrations of 25 μg/ml (kanamycin), 50 μg/ml (ampicillin), 15 μg/ml (chloramphenicol), 50 μg/ml (spectinomycin), and 12.5 μg/ml (tetracycline) where necessary.

**Determination of β-galactosidase activity.** Enzyme activities were determined for cultures grown to exponential phase (optical density at 600 nm [OD$_{600}$] of 0.5) in LB medium (Difco). LB medium was used because *hns* mutants grow poorly in minimal medium. The cultures were inoculated from fresh overnight cultures and harvested after growth at 37°C to an OD$_{600}$ of 0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a concentration of 1 mM, where indicated. β-Galactosidase assays were performed as described previously (27). The enzyme activities were determined from at least three independent cultures, and standard deviations were <10%.

**Western blotting and immunodetection of H-NS and StpA.** Cultures were grown in LB medium at 37°C to an OD$_{600}$ of 0.5. For expression of HA-tagged H-NS, IPTG was added to a final concentration of 1 mM, and the growth of cultures was stopped on ice after 2 h of induction. For StpA quantification, chloramphenicol was added to stop translation. Samples were taken just prior to chloramphenicol addition as well as 40, 80, and 120 min after its addition. The cells were harvested by centrifugation and resuspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (25) at a concentration of 0.1 OD$_{600}$ unit per 40 μl sample buffer. Five microliters (for detection of H-NS–HA) or 2 μl (for quantification of StpA) of cell suspension was separated by SDS-PAGE (18%), using an SE600 16-cm gel electrophoresis unit (GE Healthcare). The gel was blotted onto a 0.45-μm-pore-size polyvinylidene difluoride transfer membrane, using a TE70 semidry blotting apparatus (GE Healthcare). The blot was handled using a standard Western blotting protocol (22). For H-NS detection, monoclonal rat antiserum directed against the HA tag (Roche Diagnostics) was used as the primary antibody, at a concentration of 0.2 μg/ml. Horseradish peroxidase-coupled anti-rat antibody and an

enhanced chemiluminescence kit (GE Healthcare) were used for visualization according to the manufacturer's recommendations. For quantification of StpA, a monoclonal rabbit antiserum directed against StpA was used as the primary antibody, as described previously (5). Alexa fluor 680-conjugated goat anti-rabbit immunoglobulin G (IgG) (heavy plus light chains; Molecular Probes) was used as the secondary antibody at a concentration of 0.5 μg/ml. Visualization and quantification were performed using an Odyssey imaging system (Li-Cor Biosciences) according to the manufacturer's instructions.

## RESULTS

**Regulation of *stpA* by H-NS and StpA.** The *stpA* gene is known to be repressed by H-NS and by StpA, as shown by *stpA* promoter-*lacZ* fusions and Northern analyses (16, 37, 46). To examine the transcriptional regulation of *stpA* by H-NS and StpA in more detail, we constructed a series of *stpA*-*lacZ* reporter constructs (Fig. 1). In these reporter constructs, *stpA* fragments which encompass the *stpA* promoter and the *stpA* coding region (from positions −540 to +466 relative to the transcription start [16, 37]) (Fig. 1A and B) or the promoter only (from positions −540 to +41 and +540 to +98) (Fig. 1C and D) were fused to *lacZ*. In addition, the *stpA* open reading frame was inserted between a constitutive promoter (*lacUV5*) and the *lacZ* gene (Fig. 1E). In this *lacUV5-stpA*(*orf*)-*lacZ* construct, translation of *stpA* is prevented by mutation of the start codon from ATG to ATA [indicated by *stpA*(*orf*)]. The *stpA*-*lacZ* reporter constructs were integrated into the chromosome at the phage lambda *attB* attachment site, and their expression was tested in the wild type, in *stpA* and *hns* single mutants, and in *stpA hns* double mutants grown in LB medium to the mid-exponential growth phase (OD$_{600}$ of 0.5).

The *stpA*-*lacZ* reporter carrying the *stpA* promoter and the *stpA* coding region (from positions −540 to +466) fused to *lacZ* expressed 65 units of β-galactosidase activity in the wild type (Fig. 1A, bar a). In the *hns*::*kan* mutant, the expression level increased threefold, to 183 units (Fig. 1A, compare bars c and a). Surprisingly, the expression level directed by this construct was significantly higher (371 units) in the *hns*::*amp* mutant (Fig. 1A, compare bars e and c).

The *stpA* promoter-*stpA*-*lacZ* fusion encompasses the sequence from positions −540 to +466 relative to the transcription start. A fusion encompassing a *stpA* fragment from −300 to +466 gave similar results (data not shown). Since this *stpA*-*lacZ* fusion encodes StpA, its expression was not tested in *hns stpA* double mutants. To address autoregulation by StpA, we constructed a derivative in which the translation initiation codon of *stpA* was mutated to ATA (Fig. 1B). The expression level directed by this *stpA* promoter-*stpA*(*orf*)-*lacZ* fusion was significantly lower: in the wild type, only 3 units of β-galactosidase activity was detected (Fig. 1B, bar a). This low level of expression suggests polarity within the nontranslated *stpA* gene. The introduction of an *stpA*::*tet* allele into the wild-type strain had no effect, as 3 units of β-galactosidase activity was detected, as seen in the wild type (Fig. 1B, compare bars a and b). In the *hns*::*kan* mutant, expression increased ∼6-fold, to 17 units (Fig. 1B, bar c), while in the *hns*::*amp* mutant the expression level increased ∼12-fold, to 35 units (Fig. 1B, bar e). However, in the *hns*::*kan stpA*::*tet* and *hns*::*amp stpA*::*tet* double mutants, expression increased to similar values, 89 and 88 units, respectively, i.e., 30-fold higher than that of the wild type (Fig. 1B, bars d and f). These data show that H-NS is sufficient
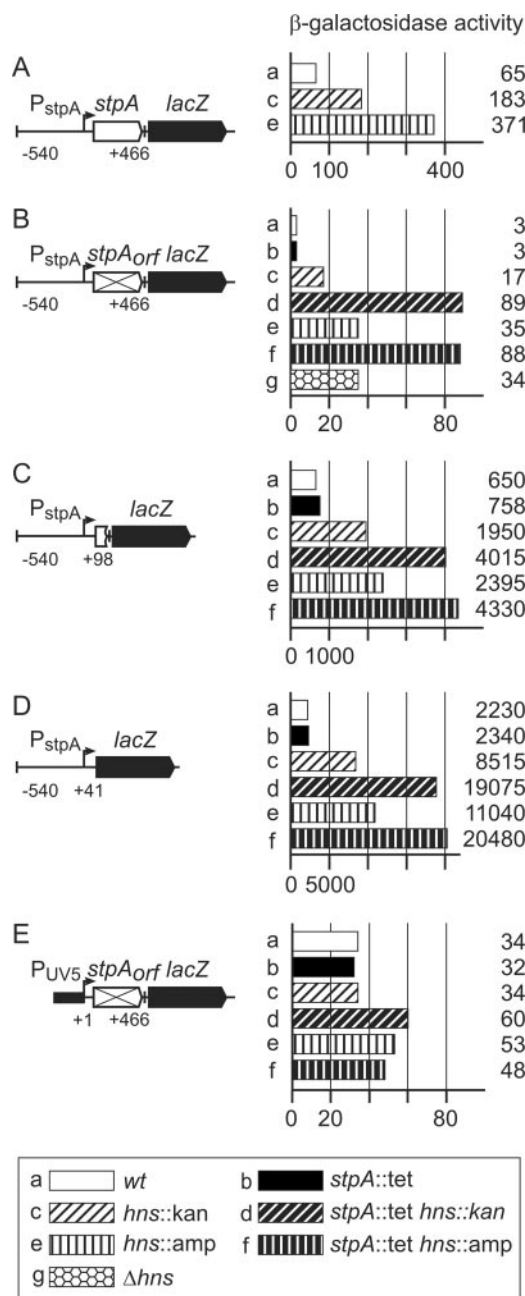
FIG. 1. Regulation of *stpA-lacZ* fusions by H-NS and StpA. The expression level directed by *stpA-lacZ* fusions integrated into the chromosome was determined for the wild type (a) and for *stpA::tet* (b), *hns::kan* (c), *hns::kan stpA::tet* (d), *hns::amp* (e), *hns::amp stpA::tet* (f), and Δ*hns-kan*$_{KD4}$ (g) mutants (see legend in figure). The positions of the *stpA* fragments are given relative to the transcription start (16, 37). The *lacZ* gene carries its native ribosome binding site, and the *lacUV5* promoter, which lacks *lacO*, encompasses positions −40 to +1 relative to the transcription start. *stpA*$_{orf}$ indicates that the *stpA* mRNA cannot be translated due to a mutation of the ATG start codon of *stpA* to ATA. β-Galactosidase activities were determined for cultures grown in LB to an OD$_{600}$ of 0.5.

for repression of *stpA* and that chromosomally encoded StpA causes a threefold (in the *hns::amp* mutant) to sixfold (in the *hns::kan* mutant) repression of *stpA*. Maximal expression of *stpA* was obtained in the *hns stpA* double mutants only.

**Difference between *hns::amp* and *hns::kan* alleles.** The *stpA* promoter-*stpA-lacZ* fusion (Fig. 1A) and the *stpA* promoter-*stpA*(*orf*)-*lacZ* fusion (Fig. 1B) were expressed at twofold higher levels in the *hns::amp* mutant than in the *hns::kan* mutant (Fig. 1A and B, compare bars e and c). The *hns::amp* allele carries an ampicillin resistance cassette inserted at the single HpaI site of H-NS (Fig. 2) (9). The *hns::kan* allele carries a replacement of the *hns* internal HpaI-PvuII fragment with a kanamycin resistance cassette (Fig. 2) (42). In both of these alleles, the 37 N-terminal codons of *hns* remain intact (9, 42). The N-terminal 37 amino acids (aa) of the gene product encompass the first two alpha helices and one-half of the third alpha helix of the dimerization domain (3, 15). Translation of the 5′ end of *hns::amp* would stop after five additional codons (Fig. 2), while translation of the 5′ end of *hns::kan* could result in a 70-amino-acid peptide that carries the 37 N-terminal amino acids of H-NS and 33 additional amino acids (Fig. 2). This *hns::kan* fusion open reading frame and its putatively encoded protein are called *hnsKN* and H-NS$_{KN}$, respectively (also see below).

To further analyze the difference in regulation of *stpA* in *hns::amp* and *hns::kan* mutant strains, we constructed a Δ*hns::kan*$_{pKD4}$ mutant, in which the *hns* coding region was precisely deleted and replaced by a kanamycin resistance cassette (6). The expression of the *stpA* promoter-*stpA*(*orf*)-*lacZ* fusion, which directs 3 units of β-galactosidase activity in the wild type (Fig. 1B, bar a), increased to 34 units in the Δ*hns::kan*$_{pKD4}$ mutant, i.e., to an identical level as that in the *hns::amp* mutant (Fig. 1B, compare bars g and e). This result demonstrates that the *hns::amp* allele resembles a null allele and that *hns::kan* is not a null allele. Thus, *hns::kan* mutants may express the N-terminal H-NS peptide H-NS$_{KN}$, which may be active in repression of *stpA* in concert with StpA (addressed below).

**Repression of *stpA* requires upstream and downstream sequences.** The level of regulation using the *stpA* promoter-*stpA*(*orf*)-*lacZ* reporter is high (30-fold). It was shown for *proU* (23, 31, 32) and later also for other H-NS-repressed systems, including *bgl* (13, 36), *hilA* (30), and *eltAB* (43), that downstream sequences are required for full repression by H-NS. To address whether downstream sequences are likewise important in the repression of *stpA*, we constructed two *stpA* promoter-*lacZ* fusions. One of these fusions carries the promoter up to position +98 relative to the transcription start (Fig. 1C) and is thus similar to a fusion used before (37). The second *stpA* promoter-*lacZ* fusion carries the *lacZ* gene fused at position +41 relative to the transcription start. This fusion does not include the *stpA* start codon ATG (mapping at positions +42 to +44) (Fig. 1C). The longer promoter construct directed 650 units of β-galactosidase activity in the wild type (Fig. 1C, bar a). The introduction of an *stpA::tet* allele again had very little effect (758 units) (Fig. 1C, bar b). In the *hns::kan* mutant, the activity increased to 1,950 units (Fig. 1C, bar c), and in the *hns::amp* mutant it increased approximately threefold, to 2,395 units (Fig. 1C, bar e). In the *hns::kan stpA::tet* and *hns::amp stpA::tet* double mutants, 4,016 and 4,330 units of β-galactosidase, respectively, were expressed (Fig. 1C, bars d and f). Thus, H-NS and StpA together repress the promoter sixfold (Fig. 1C, compare bar a to bars d and f). StpA represses the promoter
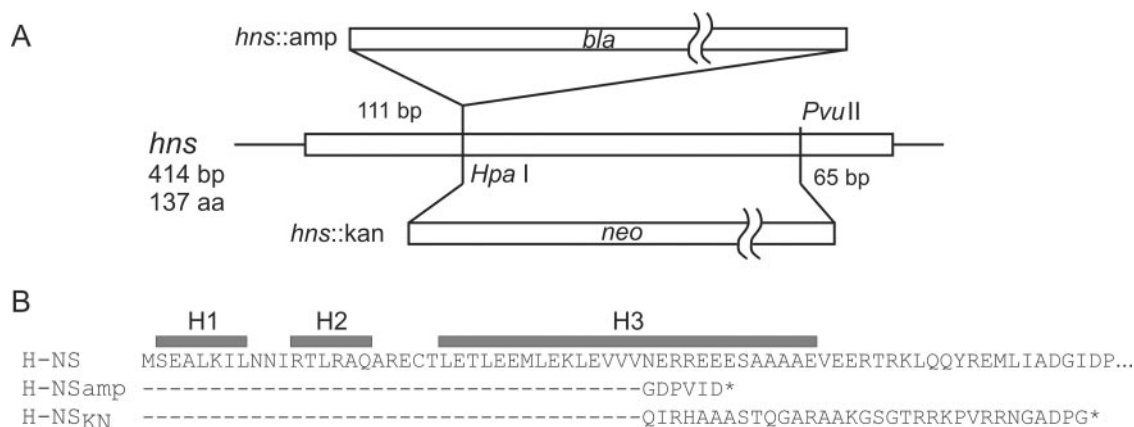
FIG. 2. Structures and coding capacities of the *hns*::*amp* and *hns*::*kan* alleles. (A) The *hns*::*amp* allele carries an insert of an ampicillin cassette at the HpaI site (9). The *hns*::*kan* allele carries a deletion of the *hns* internal HpaI-PvuII fragment, which is replaced by a kanamycin cassette (42, 46). (B) Fusions of the inserts to *hns* were sequenced, and the sequences and coding capacities of the alleles are given. Also indicated are the extents of the three N-terminal alpha helices, which form the core of the dimerization domain (3, 15). The putative peptide encoded by the 5′ end of *hns*::*kan* is called H-NS$_{KN}$.

twofold in the absence of H-NS (Fig. 1C, compare bars d and f to bars c and e).

The shorter (fused at position +41) *stpA* promoter-*lacZ* fusion directed an even higher level of β-galactosidase activity (2,230 units) in the wild type (Fig. 1D, bar a). The mutation of *stpA* again had no effect (2,340 units) (Fig. 1D, bar b). In the *hns*::*kan* and *hns*::*amp* mutants, expression increased four- and fivefold (to 8,515 and 11,040 units), respectively (Fig. 1D, bars c and e). In the *hns*::*kan stpA*::*tet* and *hns*::*amp stpA*::*tet* double mutants, the expression increased to 19,075 and 20,480 units, respectively (Fig. 1D, bars d and f). The repression of the *stpA* promoter (+41)-*lacZ* fusion by H-NS and StpA together was eightfold (Fig. 1D, compare bar a to bars d and f). Repression by StpA in the absence of H-NS was twofold (Fig. 1D, compare bars d and f to bars c and e). Interestingly, there is hardly any difference between the *hns*::*amp* and *hns*::*kan* alleles in the case of *stpA* promoter-*lacZ* fusions. In these fusions, the *stpA* promoter extends from positions −540 to +41 or +98. Shorter *stpA* promoter constructs that extend from positions −300 to +41 or +98 gave similar results (data not shown).

To analyze whether downstream sequences alone confer regulation by H-NS and/or StpA, the *stpA* coding region [*stpA*(*orf*)] (including positions +1 to +466 relative to the transcription start) was inserted between the constitutive *lacUV5* promoter and the *lacZ* gene (Fig. 1E). The expression levels directed by this construct were rather low in the wild type and the *stpA*::*tet* mutant (34 units and 32 units, respectively) (Fig. 1E, bars a and b). In the *hns*::*amp* mutant, the expression level increased approximately twofold, to 53 units (Fig. 1E, bar e), while in the *hns*::*kan* mutant only the wild-type level of β-galactosidase activity (34 units) was detected (Fig. 1E, bar c). In the *hns*::*amp stpA*::*tet* and *hns*::*kan stpA*::*tet* double mutants, the expression level increased to 48 and 60 units, respectively (Fig. 1E, bars f and d). Thus, the presence of the downstream sequences alone results in a rather low, possibly significant level of repression by H-NS and StpA.

Taken together, these data show that H-NS is necessary and sufficient for maximal repression of *stpA*, while StpA moderately represses *stpA* in the absence of H-NS. The data also show that downstream sequences contribute to repression by H-NS and StpA, as repression was 30-fold when *lacZ* was fused downstream of *stpA*(*orf*), while it was only 6- to 8-fold in the mutants carrying *stpA* promoter-*lacZ* fusions. Furthermore, the data indicate that the *stpA* gene exerts a strong polar effect, since the expression levels directed by the *stpA* promoter-*lacZ* fusions were 10- to 30-fold higher than those obtained when *lacZ* was fused downstream of *stpA*. The polarity was enhanced when translation of *stpA* was prevented, which resulted in an additional 20-fold reduction of expression. The difference between the *hns*::*amp* and *hns*::*kan* alleles, which was more pronounced with the reporter constructs carrying the downstream regulatory element, is presumably attributable to a small fusion protein, H-NS$_{KN}$, encoded by the *hns*::*kan* allele (also see below). In addition, it should be noted that the growth of the *hns*::*amp stpA*::*tet* double mutant was more retarded than that of the *hns*::*kan stpA*::*tet* double mutant.

**Role of StpA in regulation of *bgl*.** It was shown previously that StpA contributes to silencing of *bgl* in *hns* mutant backgrounds which express N-terminal fragments of the H-NS protein (17, 18). These H-NS fragments encompass amino acids 1 to 91 and 1 to 93 and include the complete dimerization and oligomerization domain (14) and a region of H-NS (aa 39 to 60) which is necessary for stabilization of StpA (3, 15, 21). It has also been shown that the requirement of these truncated H-NS mutants for complementation by StpA depends on their level of expression (17). In addition, we have shown that repression of *bgl* by H-NS involves two H-NS binding sites (13), as shown earlier for *proU* (31, 32). In *bgl*, one of the H-NS binding sites is located upstream of the promoter, while the second site is located approximately 600 to 700 bp downstream of the transcription start site (13) (Fig. 3, top panel).

To analyze whether StpA can affect repression of *bgl* at both the upstream and downstream sites, we used two *bgl-lacZ* reporter constructs that are integrated in the chromosome at the *attB* site (11–13). One reporter is specific for repression via the upstream site and carries the *bgl* promoter fused to the *lacZ* gene at position +25 relative to the transcription start (Fig. 3A). A second reporter is specific for repression via the down-

FIG. 3. Regulation of *bgl-lacZ* fusions by H-NS and StpA. At the top, a schematic view of the *bgl* locus is given, including the positions of H-NS binding regions upstream of the promoter, the catabolite regulator protein (CRP) binding site, and 600 to 700 bp downstream mapping within *bglG*. t1 indicates a transcriptional terminator in the leader of the operon, where BglG mediates antitermination depending on the availability of β-glucosides to the cell (19). The expression levels directed by chromosomal *bgl-lacZ* fusions specific for repression via the upstream silencer (A) or the downstream silencer (B) were determined for the wild type and for *hns* and *stpA* single and double mutants (see legend in the figure). Strains used were S1213 and derivatives (A) and S1195 and derivatives (B). n.d., not determined.

stream site and carries a fragment downstream of *bgl* (from positions +95 to +971 relative to the transcription start). This downstream fragment, encompassing the nontranslatable coding region of *bglG*, is inserted between the constitutive *lacUV5* promoter and the *lacZ* gene (Fig. 3B).

The *bgl* promoter-*lacZ* fusion directed 83 units of β-galactosidase activity in the wild type and 81 units in the *stpA* mutant (Fig. 3A, bars a and b). In the *hns*::*kan* mutant, only 138 units of activity were detected (Fig. 3A, bar c), while in the *hns*::*amp* mutant expression increased threefold (to 218 units) compared to that in the wild type (Fig. 3A, compare bars a and e), as reported previously (13). Likewise, in the *hns*::*kan stpA*::*tet* double mutant, expression increased threefold compared to that in the wild type (249 units) (Fig. 3A, compare bars a and d). Similar results were obtained for the *bgl* reporter construct specific for repression via the downstream site (Fig. 3B). Expression increased fivefold, from 115 units in the wild type to 591 units in the *hns*::*amp* mutant (Fig. 3B, compare bars a and e) (13). The expression level in the *hns*::*kan* mutant (321 units) was again lower than that in the *hns*::*amp* mutant (590 units) (Fig. 3B, compare bars e and c). The introduction of the *stpA*::*tet* allele had no effect in the wild type (Fig. 3B, compare bars a and b) or in the *hns*::*amp* mutant (Fig. 3B, compare bars

f and e). However, in the *hns*::*kan* mutant, the introduction of the *stpA*::*tet* allele resulted in a twofold increase, from 321 to 600 units of β-galactosidase activity (Fig. 2B, compare bars c and d). These data show that StpA has no effect on upstream or downstream silencing of *bgl* in the wild type or the *hns*::*amp* mutant. In the *hns*::*kan* mutant, StpA regulates *bgl* approximately twofold, via both the upstream and downstream sites.

***hns*::*kan* encodes a 70-aa fusion protein, H-NS_KN, comprising 37 aa of the H-NS N terminus.** The derepression level of *stpA* as well as of *bgl* was higher in the *hns*::*amp* mutant than in the *hns*::*kan* mutant. However, derepression levels were identical in the *hns*::*amp stpA*::*tet* and *hns*::*kan stpA*::*tet* double mutants (Fig. 1 and 3). This demonstrates that the *hns*::*amp* and *hns*::*kan* mutants, which are structurally different (Fig. 2), are also functionally different and that one of them may express a fragment that is functional together with StpA.

To analyze the difference between the two *hns* alleles, complementation analyses were performed. To this end, the wild-type *hns* gene, the *hns*::*amp* allele, and the *hns*::*kan* allele were cloned into pACYC-derived plasmids under the control of the *tac* promoter (Fig. 4). Six histidine codons were added to the 3′ end of the *hns* coding region in all plasmids, and all plasmids carry a *lacI*^q gene upstream of the *tac* promoter. In addition, a plasmid was constructed which could express a His-tagged version of the putative H-NS_KN fusion protein of 70 amino acids (Fig. 2). All of these plasmids were used for transformation of the wild type as well as of single and double *hns* and *stpA* mutants carrying the *bgl-lacZ* reporter specific for repression via the downstream site (Fig. 4).

Transformation of the empty vector (pKETW15) had no effect on the levels of β-galactosidase activity (compare Fig. 3B and 4A). Partial derepression was obtained in the *hns*::*kan* mutant (Fig. 4A, bar c), and complete derepression was obtained in the *hns*::*amp* single mutant (Fig. 4A, bar e) as well as in the *hns*::*amp stpA*::*tet* and *hns*::*kan stpA*::*tet* double mutants (Fig. 4A, bars d and f). In transformants carrying the plasmid (pKETW13) encoding wild-type H-NS, strong repression occurred in all strain backgrounds, with expression levels ranging from 30 to 53 units (Fig. 4B). In transformants carrying the plasmid (pKETW14) encoding the *hns*::*amp* allele, expression was not altered. Full repression occurred in the wild type and the *stpA* mutant (73 and 71 units, respectively) (Fig. 4C, bars a and b), partial repression occurred in the *hns*::*kan* mutant (281 units) (Fig. 4C, bar c), and complete derepression occurred in the *hns*::*kan stpA*::*tet* double mutant (677 units) (Fig. 4, bar d). Thus, *hns*::*amp* does not complement. However, complementation was obtained in transformants containing plasmids carrying the *hns*::*kan* allele (pKETW8) or *hnsKN* (pKETW7), which encodes the putative H-NS_KN fusion peptide (Fig. 4D and E). As expected for the wild type and the *stpA*::*tet* mutant, full repression was measured (Fig. 4D and E, compare bars a and b). Strikingly, in the *hns*::*amp* background, complementation with *hns*::*kan* and *hnsKN* resulted in partial repression (225 and 328 units, respectively) (compare Fig. 4D and E, bars e, with Fig. 4A, bar e). In the *hns*::*amp stpA*::*tet* double mutant, *hns*::*kan* and *hnsKN* had no effect, as expected (compare Fig. 4D and E, bars f, with Fig. 4A, bar f). These data suggest that the *hns*::*kan* allele does indeed encode a fusion protein, H-NS_KN, comprised of 37 N-terminal amino acids of H-NS and
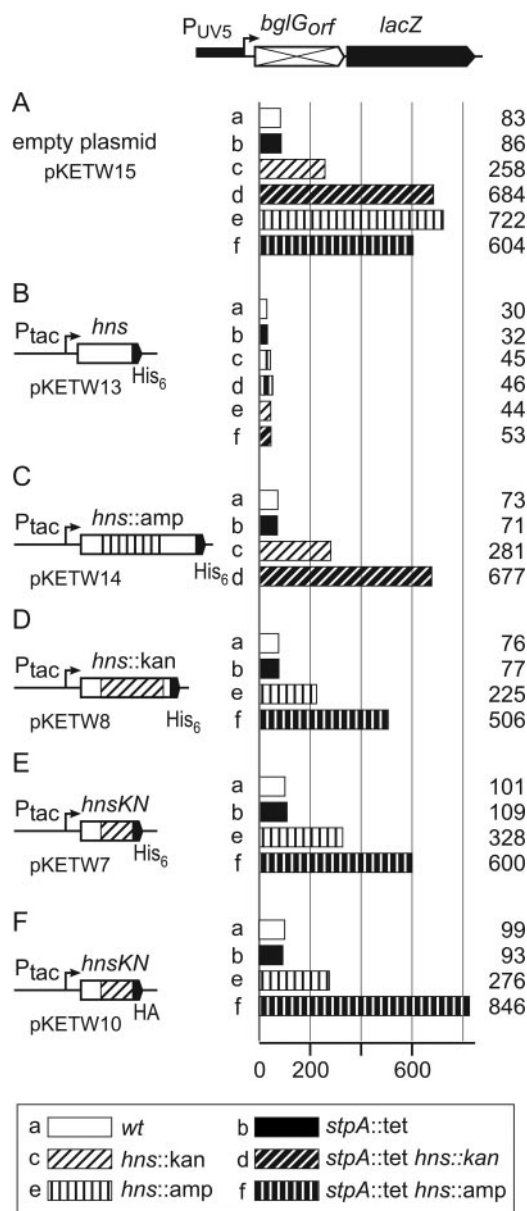
FIG. 4. Complementation shows that *hns::kan* is not a null allele. Strains carrying the chromosomal *bgl-lacZ* reporter specific for repression via the downstream site by H-NS and StpA were transformed with a control plasmid (pKETW15) (A) or with plasmids encoding wild-type *hns* (pKETW13) (B), *hns::amp* (pKETW14) (C), *hns::kan* (pKETW8) (D), and *hnsKN* (the 5′ end of *hns::kan*; pKETW7) (E) under the control of the *tac* promoter. All plasmids code for C-terminal His$_6$-tagged H-NS variants. In addition, plasmid pKETW10, encoding an HA-tagged version of H-NS$_{KN}$, (F) was used. The plasmids also carry the *lacI*$^q$ gene, which is present upstream of the *tac* promoter. Strains that carry the *lacUV5* promoter-*bglG(orf)-lacZ* reporter include S1195 (wild type) and *stpA* and *hns* derivatives (Table 1).
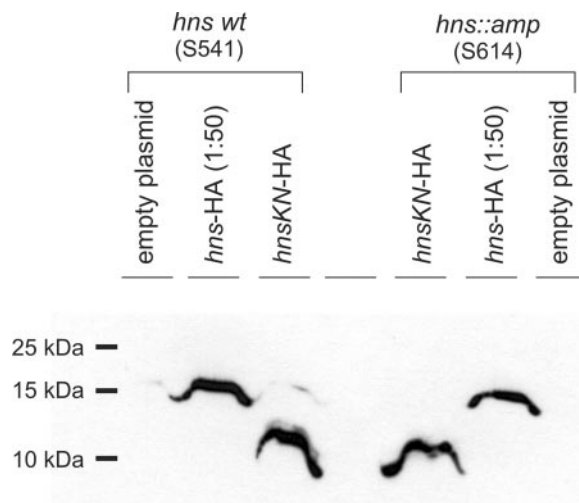


FIG. 5. Western blot for immunodetection of the *hns::kan*-encoded fusion peptide H-NS$_{KN}$. Transformants of strains S541 (wild type) and S614 (*hns::amp*) with plasmids pKEM30 (empty vector), pKEM51 (*hns*-HA), and pKETW10 (*hnsKN*-HA) were grown in LB at 37°C to an OD$_{600}$ of 0.5. IPTG was added to a final concentration of 1 mM, cultures were stopped on ice after 2 h of induction, cells were harvested, and the expressed proteins were analyzed by Western blotting using an HA-specific antibody as described in Materials and Methods. To compensate for very weak expression of the H-NS$_{KN}$–HA fusion peptide, a 1:50 dilution of the H-NS–HA wild-type fusion was loaded.

nally HA-tagged H-NS protein and a pACYC-derived plasmid for expression of the *hnsKN* allele, which codes for the H-NS$_{KN}$ fusion peptide with a C-terminal HA tag. In these plasmids, expression is directed by the *tac* promoter, and the plasmids carry the *lacI*$^q$ gene. The HA-tagged versions were used because a Western analysis using the pSC101-derived plasmids encoding the His-tagged H-NS$_{KN}$ variant, which were used in the complementation analysis, was not sensitive enough (data not shown). We confirmed that the plasmid encoding H-NS$_{KN}$–HA has the same activity in complementation as the plasmid encoding the His-tagged variant of H-NS$_{KN}$ (Fig. 4F).

For Western blot analysis, the transformants were grown in LB medium with the respective antibiotics for selection of the plasmids, and expression was induced with 1 mM IPTG for 2 h prior to harvesting of the cells. Cell lysates were separated in 18% SDS-PAGE gels, blotted, and probed with HA-specific antibodies. Induction of HA-tagged *hns* and *hnsKN* revealed proteins of the expected sizes of 16.976 kDa for H-NS–HA and 9.107 kDa for H-NS$_{KN}$–HA (Fig. 5). Expression of the H-NS$_{KN}$–HA-tagged fusion protein was significantly lower (50 times) than that of the wild-type H-NS–HA protein, for which a 1:50 dilution was loaded. No difference was observed between the wild type and the *hns::amp* mutant by use of this ectopic expression system (Fig. 5). These data show that the *hns::kan* allele is not a null allele, and they support the conclusion that weak expression of an N-terminal H-NS 37-amino-acid fragment as part of a fusion protein is sufficient to render the expression of *bgl* StpA dependent.

Furthermore, we analyzed whether StpA stability is altered in the *hns::kan* mutant. StpA is a substrate of the protease Lon, but it is stable in wild-type cells due to the formation of heterodimers with H-NS (22). The steady-state amount and sta-
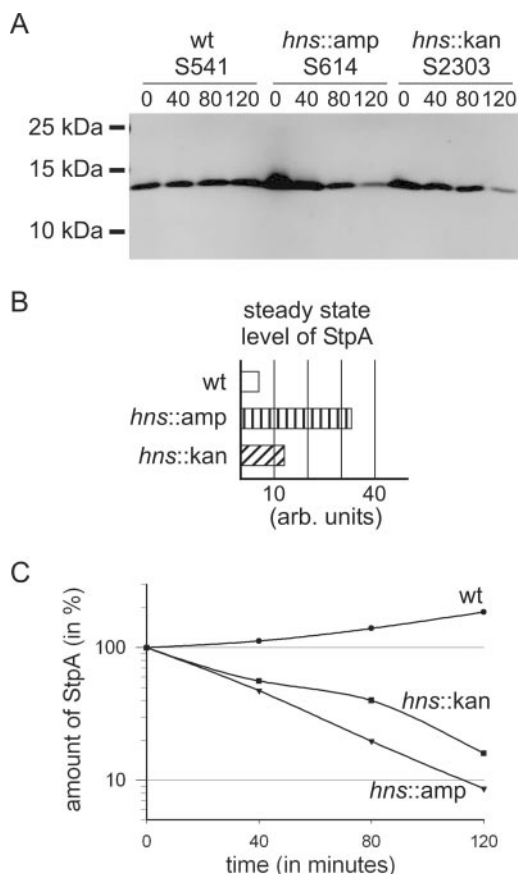
33 additional amino acids. This fusion protein, together with StpA, causes partial repression of *bgl*.

Next, the expression of the proposed N-terminal H-NS$_{KN}$ fusion protein was confirmed by Western blot analysis (Fig. 5). To this end, the wild type and an *hns::amp* mutant were transformed with a pSC101-derived plasmid encoding a C-termi-

FIG. 6. StpA is unstable in the *hns*::*kan* mutant expressing the H-NS$_{KN}$ fusion protein. Wild-type *E. coli* and *hns*::*amp* and *hns*::*kan* mutants were grown in LB. At mid-exponential phase (OD$_{600}$, 0.5), chloramphenicol was added to stop translation. Samples were taken at 0, 40, 80, and 120 min, separated by SDS-PAGE, and analyzed by Western blotting using StpA-specific antibodies. Quantification of the Western blot was done with an Odyssey imaging system (Li-Cor Biosciences). (A) Representative Western blot. (B) Steady-state amounts of StpA, determined by quantification of the signals at time zero (in arbitrary units). (C) Relative stability of StpA, as determined by signal intensities obtained at 40, 80, and 120 min relative to the intensity of the signal at time zero (normalized to 100%).

bility of StpA were determined in the wild type, in the *hns*::*amp* mutant, and in the *hns*::*kan* mutant (which expresses the N-terminal H-NS$_{KN}$ fusion protein). Cells were grown in LB medium to an OD$_{600}$ of 0.5, and then protein synthesis was inhibited by the addition of chloramphenicol (Fig. 6) or spectinomycin (not shown). Samples were taken just prior to chloramphenicol addition and 40, 80, and 120 min after the addition of chloramphenicol. Cell lysates were separated by SDS-PAGE, blotted, and analyzed with StpA-specific antibodies that do not cross-react with H-NS (37) by quantitative Western analysis (Fig. 6A). The steady-state levels (Fig. 6B) were determined for samples taken prior to chloramphenicol addition (time zero in Fig. 6A). The steady-state level of StpA was lowest in the wild type. In the *hns*::*amp* mutant, the steady-state level was increased fourfold compared to that in the wild type, and in the *hns*::*kan* mutant it was increased twofold compared to that in the wild type (Fig. 6B). This is in agreement with the increased expression rate of *stpA* in the *hns*::*amp*

and *hns*::*kan* mutants (Fig. 1). The stability of StpA was determined by analysis of the decrease in the amount of StpA 40, 80, and 120 min after chloramphenicol addition relative to the steady-state amount at time zero (Fig. 6A and B). In the wild type, StpA was stable, and the relative amount of StpA increased slightly after inhibition of translation, as shown before (37). In contrast, StpA was unstable in the *hns*::*amp* mutant and in the *hns*::*kan* mutant. These data show that stabilization of StpA and its functional cooperation with a fragment of the N-terminal H-NS dimerization domain can be separated.

**Regulation of *bgl* and *stpA* by dominant-negative H-NS mutants.** To further address the interdependence of StpA and truncated or mutated H-NS proteins in the repression of *stpA* and *bgl*, dominant-negative mutants of H-NS defective in dimerization/oligomerization or specific DNA binding were used (2, 40). Three H-NS mutants were chosen, including H-NS-L26P, H-NS-E53G/T55P, and H-NS-I119T. H-NS-L26P and H-NS-E53G/T55P carry mutations in the dimerization/oligomerization domain. These two proteins specifically bind to H-NS sites; however, they do not form extended assemblies, and no higher-order oligomers are detected in cross-links (2). In contrast, H-NS-I119T binds DNA and forms higher-order assemblies but does not specifically recognize H-NS binding sites (2).

Low-copy-number pSC101-derived plasmids encoding either wild-type H-NS or one of the H-NS mutants were used to transform the wild type, the *hns* mutants, and the *hns stpA* double mutants carrying the *bgl-lacZ* reporter construct specific for repression via the downstream site (Fig. 7, left column) or the *stpA* promoter-*stpA*(*orf*)-*lacZ* reporter (Fig. 7, right column). Analysis of these dominant-negative mutants in the *hns*::*amp stpA*::*tet* double mutant by using the pLG plasmid series pLG-HNS (wild-type H-NS), pLG-HNS-L26P, and pLG-HNS-E53G/T55P (40) turned out to be problematic due to severe growth defects and concomitant variations in the β-galactosidase assay. Therefore, we analyzed these dominant-negative H-NS mutants in the wild type, the *hns*::*kan* mutant, and the *hns*::*kan stpA*::*tet* double mutant by using a set of pSC101-derived plasmids encoding wild-type or mutant H-NS proteins that confer chloramphenicol resistance (Table 1). The dominant-negative mutant protein H-NS-I119T was also analyzed in the *hns*::*amp* and *hns*::*amp stpA*::*tet* mutants (Fig. 7E).

In transformants carrying a plasmid encoding wild-type H-NS, the *bgl* and *stpA* reporter constructs were repressed in all strain backgrounds, as expected (Fig. 7A). When H-NS-L26P and H-NS-E53G/T55P were provided in *trans*, expression of *bgl* and the *stpA* reporter increased roughly 1.5-fold in the wild type, confirming the dominant-negative activity of these mutants (compare Fig. 7B and C to Fig. 7A). In the presence of H-NS-L26P, expression was rather high in the *hns*::*kan* mutant as well as in the double mutant, indicating that H-NS-L26P is inactive in repression and that StpA cannot complement this mutant (Fig. 7B). Expression of H-NS-E53G/T55P gave intermediate results, indicating that this protein has residual activity in repression of *bgl* and of *stpA* and that StpA could complement this mutant (Fig. 7C).

An unexpected result was obtained upon expression of H-NS-I119T in *trans* (Fig. 7D and E). This protein led to more effective repression of *bgl* (~30 units) than did native H-NS (~80 units) in the wild type and the *hns*::*kan* and *hns*::*amp*
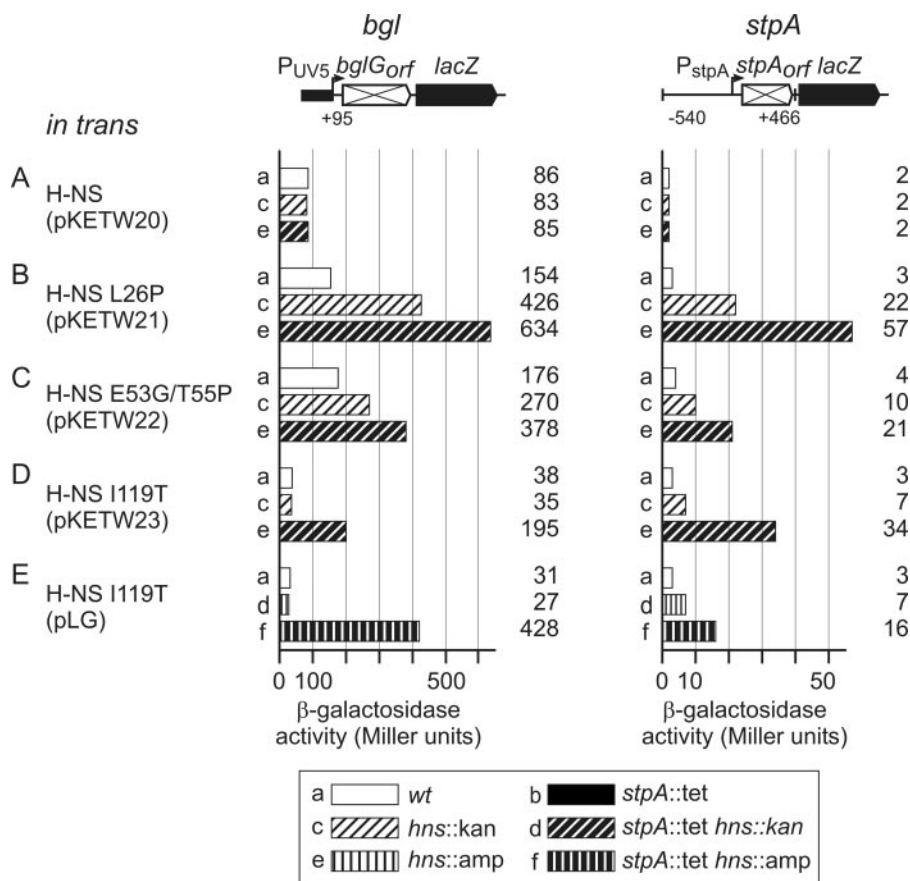
FIG. 7. H-NS-I119T, a dominant-negative mutant of H-NS, specifically represses *bgl* together with StpA. A chromosomal *bgl-lacZ* fusion specific for repression of *bgl* by the downstream site (*bgl*) and a chromosomal *stpA-lacZ* fusion (*stpA*) were used to determine the dependence of dominant-negative mutants of H-NS on StpA. Strain S1195 (wild type) and its derivatives (*bgl*) and strain S2741 and its derivatives were transformed with pSC101-derived plasmids encoding wild-type H-NS (A), H-NS-L26P (B), HNS-E53G/T55P (C), and HNS-I119T (D and E). On these plasmids, expression of *hns* is directed by its own promoter. pSC101-derived plasmids pKETW20 to pKETW23 were used for analysis of *hns*::*kan* and *hns*::*kan stpA*::*tet* strains (A to D). The pLG-H-NS-I119T (40) plasmid was used for analysis of the *hns*::*amp* and *hns*::*amp stpA*::*tet* mutants (E).

single mutants (compare the *bgl* results in Fig. 7A, bars a and c, with those in Fig. 7D, bars a and c, and Fig. 7E, bars a and e). Transformation of both the *hns*::*amp stpA* and *hns*::*kan stpA* double mutants with the H-NS-I119T-encoding plasmid yielded very few colonies, and cultures of these colonies grew very poorly. However, the β-galactosidase assays with these transformants were reproducible. Expression increased to 195 units in the *hns*::*kan stpA* double mutant and to 430 units in the *hns*::*amp stpA*::*tet* double mutant (compare the *bgl* results in Fig. 7D, bar e, with those in Fig. 7E, bar f). These data show that H-NS-I119T does not repress *bgl* in the absence of StpA. Highly effective repression by H-NS-I119T in *stpA*+ strains demonstrated that StpA effectively complements H-NS-I119T in repression of *bgl*. In contrast, the results using the *stpA* promoter-*stpA*(*orf*)-*lacZ* reporter for analysis of the dominant-negative H-NS-I119T mutant were different from those with the *bgl* reporter (Fig. 7D and E, compare *bgl* and *stpA* results). When H-NS-I119T was provided in *trans*, the expression of the *stpA-lacZ* reporter increased slightly (1.5-fold) in the wild type, which is in agreement with the dominant-negative activity of H-NS-I119T, as this mutant was initially identified (40). Ex-

pression increased further in the *stpA* mutant background with H-NS-I119T provided in *trans* and was highest in the *stpA hns* double mutants (Fig. 7D, bar d, and Fig. 7E, bar f [*stpA* results]). Thus, StpA effectively complements H-NS-I119T in repression of *bgl* but not in repression of *stpA* (Fig. 7D and E, compare *bgl* and *stpA* results).

## DISCUSSION

We have shown that repression of the *stpA* gene by H-NS involves a downstream regulatory element mapping within the *stpA* coding region. In the presence of this element, repression of transcription, as monitored using a *stpA-lacZ* fusion, is 30-fold, while repression is only 6- to 8-fold in the absence of the downstream element. In addition, we found that in an *hns* null mutant, StpA represses its gene threefold. In contrast, StpA does not repress the *bgl* operon in an *hns* null mutant. Repression of *bgl* becomes StpA dependent in the presence of H-NS mutants defective in (specific) DNA binding. These data indicate a difference in the mechanisms of repression of *stpA* and *bgl* by StpA.

The specificity of regulation of *stpA* by H-NS is high (30-fold) in the presence of a downstream regulatory element (Fig. 1). This suggests that H-NS and StpA bind within the *stpA* coding region. In agreement with this, DNA structure prediction using the Bend-It web tool (http://hydra.icgeb.trieste.it/~kristian/dna/) indicates an AT-rich planar bend within the *stpA* coding region centered at approximately position +130. Upstream of the promoter, a strong planar bend at position −300 relative to the transcription start was predicted. According to the present model of repression by H-NS (14), H-NS binds as a dimer to AT-rich and bent nucleation sites and then forms extended nucleoprotein complexes on the DNA. These H-NS–DNA filaments can trap RNA polymerase at a promoter by zipping the DNA strands located upstream and downstream of the promoter together (14). By consideration of this model, our data suggest that H-NS may bind upstream and downstream to the *stpA* promoter to form such a stable nucleoprotein complex, resulting in repression of the *stpA* promoter. Effective repression of other genes also requires a downstream regulatory element, as first shown for *proU* and later shown for other systems, including *bgl*, *hilA*, and *eltAB* (11, 23, 30–32, 36, 43). Results of Northern and Western analyses similarly suggest a high level of regulation of *stpA* by H-NS (16, 37, 46). Autorepression of *stpA* in Δ*hns* or *hns*::*amp* null mutants is likewise more effective (threefold) in the presence of the *stpA* downstream regulatory element than in its absence (twofold). This may indicate that StpA acts similarly to H-NS. It is also possible that the RNA chaperone StpA binds to its mRNA. In addition, autorepression by StpA is enhanced in an *hns*::*kan* mutant carrying a 70-amino-acid fusion protein, H-NS$_{KN}$ (Fig. 4), encompassing the 37 N-terminal amino acids of H-NS, which include the first two alpha helices and half of the third alpha helix that form the core of the dimerization domain (3, 15). This H-NS$_{KN}$ fusion protein might stabilize the repressing nucleoprotein complex formed by StpA. These results and the finding that repression of *bgl* in *hns*::*kan* mutants (encoding H-NS$_{KN}$) is StpA dependent demonstrate the importance of using *hns* null mutants in the analysis of StpA. Since in several studies *hns* mutants that may express H-NS fragments were used (for example, see references 20 and 46), autoregulation of *stpA* may be a first example of H-NS-independent regulation of transcription by chromosomally encoded StpA.

For repression of *bgl*, it was shown before that StpA complements truncated H-NS proteins comprising the N-terminal dimerization/oligomerization domain, including amino acids 1 to 91 (or 93) (17, 18, 21). High levels of such truncated H-NS proteins repress *bgl* independently of StpA (17, 29). Here we show that the H-NS$_{KN}$ protein encompassing the 37 N-terminal amino acids of H-NS only is sufficient to render *bgl* expression StpA dependent. StpA remains unstable in this background, in agreement with the result that stabilization of StpA by H-NS requires H-NS amino acids 39 to 60 (21). Furthermore, we have shown that StpA together with H-NS-I119T represses *bgl* more effectively than does wild-type H-NS, although H-NS-I119T is dominant negative in repression of *proU* (40). H-NS-I119T is also dominant negative in repression of *stpA*, and StpA only moderately complements H-NS-I119T in repression of *stpA* (Fig. 7). H-NS-I119T is defective in specific DNA binding but functional in forming extended nucleoprotein complexes (2). Taken together, these data indicate that in repression of *bgl*, StpA may very specifically nucleate (or target) the defective H-NS-I119T protein to the DNA, while in repression of *proU* or *stpA* the StpA protein is less specific. This difference in regulation of *bgl* versus *stpA* and *proU* by H-NS-I119T and StpA may further imply that growth conditions which induce *stpA* may allow the maintenance of strong repression of *bgl*, even if H-NS is less active under such conditions.

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