Regulation of the *yjjQ-bglJ* Operon, Encoding LuxR-Type Transcription Factors, and the Divergent *yjjP* Gene by H-NS and LeuO[▽]†

Thomas Stratmann, S. Madhusudan, and Karin Schnetz*

Institut für Genetik, Universität zu Köln, Zülpicher Str. 47, 50674 Köln, Germany

The *yjjQ* and *bglJ* genes encode LuxR-type transcription factors conserved in several enterobacterial species. YjjQ is a potential virulence factor in avian pathogenic *Escherichia coli*. BglJ counteracts the silencing of the *bgl* (β-glucoside) operon by H-NS in *E. coli* K-12. Here we show that *yjjQ* and *bglJ* form an operon carried by *E. coli* K-12, whose expression is repressed by the histone-like nucleoid structuring (H-NS) protein. The LysR-type transcription factor LeuO counteracts this repression. Furthermore, the *yjjP* gene, encoding a membrane protein of unknown function and located upstream in divergent orientation to the *yjjQ-bglJ* operon, is likewise repressed by H-NS. Mapping of the promoters as well as the H-NS and LeuO binding sites within the 555-bp intergenic region revealed that H-NS binds to the center of the AT-rich regulatory region and distal to the divergent promoters. LeuO sites map to the center and to positions distal to the *yjjQ* promoters, while one LeuO binding site overlaps with the divergent *yjjP* promoter. This latter LeuO site is required for full derepression of the *yjjQ* promoters. The arrangement of regulatory sites suggests that LeuO restructures the nucleoprotein complex formed by H-NS. Furthermore, the data support the conclusion that LeuO, whose expression is likewise repressed by H-NS and which is a virulence factor in *Salmonella enterica*, is a master regulator that among other loci, also controls the *yjjQ-bglJ* operon and thus indirectly the presumptive targets of YjjQ and BglJ.

The enterobacterial histone-like nucleoid structuring (H-NS) protein is an abundant global repressor that affects many genes and cellular processes (16, 18, 45). H-NS controls genes related to pathogenicity and stress responses, and H-NS represses genes acquired by horizontal gene transfer, including pathogenicity islands (24, 25, 36, 46, 48). H-NS binds to AT-rich DNA with weak specificity and forms extended nucleoprotein complexes that when located close to a promoter, repress transcription (16, 51). The formation of the repressing nucleoprotein complex is initiated by H-NS dimers binding to nucleation sites, followed by their extending laterally along the DNA, a process that has been best characterized for the *Escherichia coli proU* operon (2, 3, 28, 37). The formation of the nucleoprotein complex can involve DNA loop formation and trapping of RNA polymerase at the promoter (9, 10, 57). DNA loop formation is likely to be based on the formation of DNA–H-NS–DNA bridges by the binding of single H-NS dimers to two DNA double helixes within the nucleoprotein complex (10, 17, 38).

Silencing by H-NS is presumably regulated gene specifically, although physiological parameters such as temperature, osmolarity, and DNA supercoiling have been postulated to pleiotropically modulate H-NS activity (45). Silencing can be relieved by the binding of specific transcription factors that disrupt or change the structure of the repressing nucleoprotein complex, by the temperature-dependent alteration of the DNA

structure, and by other changes in the physiological conditions that may change DNA structure and DNA supercoiling at specific loci (16, 45, 55). Furthermore, repression by the binding of H-NS within transcription units can be affected by the promoter activity (44).

LeuO is a LysR-type regulator which is a virulence factor in *Salmonella enterica* and is required for biofilm formation by *Vibrio cholerae* (29, 42, 58). In *Salmonella enterica* and in *E. coli*, LeuO is one of the transcription factors that counteracts H-NS-mediated repression of specific loci (4, 22, 39, 60). First, the *leuO* gene itself is repressed by H-NS and is positively autoregulated (5, 6, 26). Relief of repression of the *leuO* gene, which is located upstream and in a divergent orientation to the *leuABCD* leucine synthesis operon, is complex. Binding of LeuO presumably delimits the spread of H-NS into the promoter and thus causes activation (4, 6, 7). In addition, the expression of *leuO* is coupled to the expression of the *ilvIH* operon located downstream of *leuO*. Transcriptional coupling presumably involves LeuO-induced DNA looping by LeuO binding to sites upstream of the *leuO* promoter and downstream of the *leuO* coding region (7), as well as a transcription-induced change in DNA topology (20, 21). Other systems in which LeuO activates expression include the H-NS-repressed *E. coli bgl* (aryl-β,D-glucoside) operon (60), the H-NS-repressed *Salmonella enterica* serovar Typhi *ompS1* gene, and the *ompS2* gene encoding outer-membrane proteins and pathogenicity determinants (12, 22, 52). Furthermore, LeuO positively regulates the *Yersinia enterocolitica rovA* gene, encoding a MarR-type transcriptional regulator of the *inv* gene, which encodes the major adherence factor invasin (30). LeuO also represses the *cadC* and *dsrA* genes in *E. coli*. CadC is a positive regulator of the H-NS-repressed *cadBA* locus, encoding an acid-inducible lysine decarboxylase required in the acid stress

* Corresponding author. Mailing address: Institut für Genetik, Universität zu Köln, Zülpicher Str. 47, 50674 Köln, Germany. Phone: 49-221-4703815. Fax: 49-221-4705185. E-mail: schnetz@uni-koeln.de.
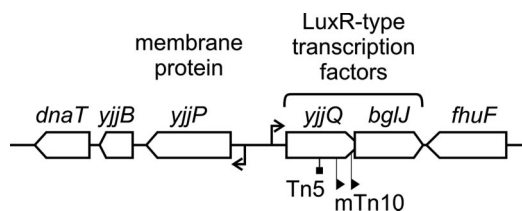
FIG. 1. Organization of the *yjjP-yjjQ-bglJ* locus mapping at 99 min of the *E. coli* K-12 genome in between *yjjB* (encoding a conserved inner membrane protein) and *fhuF* (encoding a ferric iron reductase protein). The *yjjQ* and *bglJ* genes encode LuxR-type transcription factors. A *yjjQ*::Tn*5* insertion mutation attenuates the virulence of APEC (33), while mini-Tn*10* insertions upstream of *bglJ*, causing the constitutive expression of *bglJ*, relieve the silencing of the *bgl* operon by H-NS in *E. coli* K-12 (23, 39). The *yjjP* gene encodes a membrane protein of unknown function (8).

response (56). DsrA is a small regulatory RNA that regulates *rpoS* and *hns* translation at a low temperature (26, 31, 32, 50). Thus, LeuO, which counteracts the H-NS-mediated repression of several loci, indirectly controls H-NS and RpoS synthesis under specific conditions.

Here we analyzed the regulation of the *yjjP-yjjQ-bglJ* locus (Fig. 1), which is present in the enterobacterial species *E. coli* (including the *Shigella* spp.) and *S. enterica*. The *yjjQ* and *bglJ* genes, encoding LuxR-type transcription factors, are arranged in tandem with overlapping open reading frames and may form an operon. The *yjjP* gene, which encodes a membrane protein of unknown function, is mapped to a position upstream of the *yjjQ* gene and in a divergent orientation. (8). In avian pathogenic *E. coli* (APEC), the virulence of a mutant with a Tn*5* insertion that disrupted *yjjQ* was found to be attenuated (33). Furthermore, the *yjjQ* mutants were negatively selected in one of two independent screens for long-term systemic infection by *Salmonella enterica* serovar Typhimurium in mouse (29). In *E. coli* K-12, mini-Tn*10* insertions that cause constitutive expression of *bglJ* relieve H-NS-mediated repression of the *bgl* operon (23). Here we show that *yjjQ* and *bglJ* are expressed as an operon, which is repressed by H-NS and activated by LeuO. The divergent *yjjP* gene is likewise repressed by H-NS. Mapping of the divergent promoters and regulatory sites revealed that H-NS binds at the center of the extended intergenic region and distal to the divergent promoters. Similarly, LeuO binds to three regions, including a site at the center, a site distal to the *yjjQ* promoters, and with the highest affinity, at a site overlapping the *yjjP* promoter. This last site is required for the complete activation of the *yjjQ* promoter(s) by LeuO. The complex arrangement of divergent promoters and binding sites for H-NS and LeuO is in agreement with a mechanism of regulation where binding of LeuO and possible LeuO-induced DNA looping restructure the H-NS nucleoprotein complex and delimit spreading of H-NS to the *yjjQ* promoter(s).

MATERIALS AND METHODS

Strains and plasmids. The genotypes of the *E. coli* strains and the relevant structures of the plasmids are given in Table 1. Transductions were performed using phage T4*GT7* (61). Integration of the *lacZ* reporter constructs into the chromosomal phage lambda attachment site *attB* was performed as described previously (13, 14). The *attB* site was used as described previously for the integration of similar *lacZ* reporter fusions used in the analysis of the H-NS-mediated regulation of *bgl* and *proU* (44), and chromatin immunoprecipitation

with microarray analysis (ChIP-on-chip) analysis suggested that H-NS does not bind next to *attB* (24). The Δ*hns*::*kan*$_{KD4}$ deletion mutants, in which the complete coding region of *hns* was deleted, were described previously (44). The Δ(*yjjP-yjjQ-bglJ*)::*cat*$_{KD3}$ deletion mutant was constructed by using primers S783 and S676 as described previously (11). The sequences of oligonucleotides are given in Table S2 in the supplemental material.

Plasmids (shown in Table S1 in the supplemental material) were constructed according to standard techniques (1, 53). All *lacZ* reporter fusions are descendants of the plasmid pKES15, which carries the pACYC177 origin, the *neo* gene, the *attP* gene, and an omegon-spectinomycin resistance cassette (14). Site-specific mutations and fusions to *lacZ* were constructed using restriction fragments or PCR fragments. All regions of plasmids that were derived from PCR fragments were sequenced. The relevant structures of the plasmids are shown schematically in the figures in which they are used and are given in Table S1 in the supplemental material. Details for the constructions and the compiled sequences of the plasmids are available upon request. Media and plates were used as described previously (14, 41). The final concentrations of the antibiotics added were 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 activities. The β-galactosidase assays were performed as described previously (41). Cultures in LB medium were inoculated from fresh overnight cultures to an optical density at 600 nm (OD$_{600}$) of 0.1 and harvested after growth at 37°C to an OD$_{600}$ of 0.5. Isopropyl-β,D-thiogalactoside (IPTG) was added to the overnight and the exponential cultures to a concentration of 1 mM for the induction of *leuO* expression (carried on the plasmid pKEDR13). The enzyme activities were determined from at least three independent cultures, and standard deviations were less than 15%.

Northern blotting and primer extension. RNA was isolated from cultures grown in LB medium to an OD$_{600}$ of 0.5, using an SV total RNA isolation system (Promega). For Northern blotting analyses, 10 μg of RNA was separated by using denaturing acrylamide gels (6% acrylamide/bisacrylamide, 19:1; 7 M urea; 0.9× Tris-borate-EDTA [TBE]) next to an RNA marker (high-range RNA ruler ladder; Fermentas), and blotted onto a Hybond N+ nylon membrane (GE-Healthcare). The membranes were baked for 2 h at 80°C (1) and then stained with methylene blue (0.03% methylene blue in 0.3 M Na-acetate [pH 5.2]) to identify the marker bands and to verify uniform loading and transfer of the RNA. To generate the *yjjQ* riboprobe, a PCR fragment that carries a terminal T7 promoter sequence was amplified using primers S529 and S843 (see Table S2 in the supplemental material). Then, the riboprobe was synthesized using 0.2 pmol of the PCR fragment in 20 μl of 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 5 μM UTP, [α-^{32}P]UTP (50 μCi, 800 Ci/mmol), T7 RNA polymerase transcription buffer, and 10 units of T7 RNA polymerase according to the instructions of the supplier (Fermentas). Unincorporated nucleotides were removed by passing the samples over Sephadex-G50 nick columns (GE-Healthcare), and the labeling efficiency was determined by counting 1 μl of the final eluate of 400 μl. The membranes were incubated for 3 h at 65°C with hybridization solution (5× SSPE [1× SSPE is 0.18 M NaCl, 10 mM NaH$_2$PO$_4$, and 1 mM EDTA {pH 7.7}], 5× Denhardt solution, 50% [wt/vol] formamide, 0.5% [wt/vol]), sodium dodecyl sulfate, and 72 μg of denatured herring sperm DNA, as described previously (1), and then hybridized for 16 h at 65°C with the probes added to hybridization solution and washed as described previously (1). The membranes were exposed to X-ray film or to a phosphorimager plate and then scanned, using a Typhoon imager (GE-Healthcare).

For primer extensions, 5 pmol of the oligonucleotides S700 and S699 (see Table S2 in the supplemental material) were end labeled with [γ-^{32}P]ATP (50 μCi/6,000 Ci/mmol), using 20 units of T4 polynucleotide kinase (Fermentas). Unincorporated nucleotides were removed by passing the sample through a Sephadex-G50 nick column (GE-Healthcare). Five micrograms of total RNA was incubated with 4 μl of the ^{32}P-labeled oligonucleotide (approximately 50 fmol) in a total volume of 10 μl for 5 min at 65°C and cooled on ice. Then, samples of all deoxynucleoside triphosphates (dNTPs) (2 μl of a 10 mM dNTP mixture), 4 μl 5× cDNA buffer, 1 μl 0.1 M dithiothreitol (DTT), 2 μl of H$_2$O, and 1 μl (15 units) of Thermoscript reverse transcriptase (Invitrogen) were added, and the samples were incubated at 50°C for 45 min. The reaction was stopped by heating the solution to 85°C for 5 min, the sample was phenol and chloroform extracted, and then the DNA was ethanol precipitated and resuspended in 5 μl of H$_2$O. A stop solution (T7 sequencing kit; USB) was added, and the samples were separated next to a sequencing ladder on a denaturing sequencing gel (6% acrylamide/bisacrylamide, 19:1; 7 M urea, 0.9× TBE). The sequencing ladders were generated using the same labeled primers and the T7 sequencing kit (USB), as described above, with the following modifications. Briefly, 16 μl of the end-labeled primer with 2 μg of plasmid DNA were ethanol precipitated and resuspended in 12 μl of H$_2$O. Then, 2 μl of annealing buffer, 4

TABLE 1. Strains and plasmids

Strain	Relevant genotype or structure[a]	Reference or construction source[b]
EK25	MC4100 *leuO*::*cat* (stored as S2706)	26
MG1655	CGSC6300 *E. coli* K-12 wild type (stored as S527)	Coli Genetic Stock Center
S219	CSH50 Δ*bgl*-AC11 *stpA*::*tet*	15
S541	CSH50 Δ*bgl*-AC11 Δ*lacZ*	14
S3010	S541 Δ*hns*::*kan*$_{KD4}$	44
S3346	S541 Δ*hns*::FRT	44
S3108	S541 *attB*::[Spec^r *yjjP*(+182 to −450) *lacZ*]	S541 × pKES141
S3322	S541 *attB*::[Spec^r *yjjP*(+182 to −450) *lacZ*] Δ(*yjjP-bglJ*)::*cat*$_{KD3}$	S3108/pKD46 × pKD3 PCR S676/S783^c
S3375	S541 Δ(*yjjP-bglJ*)::*cat*$_{KD3}$	S541/pKD46 × pKD3 PCR S676/S783^c
S3469	S541 Δ(*yjjP-bglJ*)::FRT	S3375 × pCP20 *flp*
S3470	S541 *attB*::[Spec^r *yjjP*(+182 to −450) *lacZ*] Δ(*yjjP-bglJ*)::FRT	S3322 × pCP20 *flp*
S3472	S3469 *attB*::[Spec^r *yjjQ*(−347 to +166) *lacZ*]	S3469 × pKES111
S3474	S3469 *attB*::[Spec^r *yjjQ*(−147 to +166) *lacZ*]	S3469 × pKES119
S3476	S3469 *attB*::[Spec^r *yjjQ*(−347 to +26) *lacZ*]	S3469 × pKES157
S3478	S3469 *attB*::[Spec^r *yjjQ*(−147 to +26) *lacZ*]	S3469 × pKES158
S3480	S3469 *attB*::[Spec^r *yjjQ*(−87 to +26) *lacZ*]	S3469 × pKES159
S3482	S3469 *attB*::[Spec^r *yjjQ*(−47 to +26) *lacZ*]	S3469 × pKES160
S3484	S3469 *attB*::[Spec^r *yjjP*(+25 to −450) *lacZ*]	S3469 × pKES172
S3486	S3469 *attB*::[Spec^r *yjjP*(+25 to −250) *lacZ*]	S3469 × pKES173
S3488	S3469 *attB*::[Spec^r *yjjP*(+25 to −50) *lacZ*]	S3469 × pKES174
S3494	S3470 *leuO*::*cat*	S3470 × T4*GT7* (EK25)
S3496	S3470 Δ*hns*::*kan*$_{KD4}$	S3470 × T4*GT7* (S3010)
S3587	S3470 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3494 × T4*GT7* (S3010)
S3588	S3472 *leuO*::*cat*	S3472 × T4*GT7* (EK25)
S3590	S3472 Δ*hns*::*kan*$_{KD4}$	S3472 × T4*GT7* (S3010)
S3592	S3474 *leuO*::*cat*	S3474 × T4*GT7* (EK25)
S3594	S3474 Δ*hns*::*kan*$_{KD4}$	S3474 × T4*GT7* (S3010)
S3596	S3476 *leuO*::*cat*	S3476 × T4*GT7* (EK25)
S3598	S3476 Δ*hns*::*kan*$_{KD4}$	S3476 × T4*GT7* (S3010)
S3600	S3478 *leuO*::*cat*	S3478 × T4*GT7* (EK25)
S3602	S3478 Δ*hns*::*kan*$_{KD4}$	S3478 × T4*GT7* (S3010)
S3604	S3480 *leuO*::*cat*	S3480 × T4*GT7* (EK25)
S3606	S3480 Δ*hns*::*kan*$_{KD4}$	S3480 × T4*GT7* (S3010)
S3608	S3488 *leuO*::*cat*	S3488 × T4*GT7* (EK25)
S3610	S3482 Δ*hns*::*kan*$_{KD4}$	S3482 × T4*GT7* (S3010)
S3612	S3484 *leuO*::*cat*	S3484 × T4*GT7* (EK25)
S3614	S3484 Δ*hns*::*kan*$_{KD4}$	S3484 × T4*GT7* (S3010)
S3616	S3486 *leuO*::*cat*	S3486 × T4*GT7* (EK25)
S3618	S3486 Δ*hns*::*kan*$_{KD4}$	S3486 × T4*GT7* (S3010)
S3620	S3482 *leuO*::*cat*	S3488 × T4*GT7* (EK25)
S3622	S3488 Δ*hns*::*kan*$_{KD4}$	S3488 × T4*GT7* (S3010)
S3631	S3472 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3588 × T4*GT7* (S3010)
S3633	S3474 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3592 × T4*GT7* (S3010)
S3635	S3476 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3596 × T4*GT7* (S3010)
S3637	S3478 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3600 × T4*GT7* (S3010)
S3638	S3480 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3604 × T4*GT7* (S3010)
S3641	S3488 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3609 × T4*GT7* (S3010)
S3642	S3484 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3612 × T4*GT7* (S3010)
S3645	S3486 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3616 × T4*GT7* (S3010)
S3646	S3482 Δ*hns*::*kan*$_{KD4}$ *leuO*::*cat*	S3620 × T4*GT7* (S3010)
S3663	S3470 Δ*hns*::FRT *leuO*::*cat*	S3587 × pCP20 *flp*
S3665	S3472 Δ*hns*::FRT *leuO*::*cat*	S3631 × pCP20 *flp*
S3667	S3474 Δ*hns*::FRT *leuO*::*cat*	S3633 × pCP20 *flp*
S3669	S3476 Δ*hns*::FRT *leuO*::*cat*	S3635 × pCP20 *flp*
S3671	S3478 Δ*hns*::FRT *leuO*::*cat*	S3637 × pCP20 *flp*
S3673	S3488 Δ*hns*::FRT *leuO*::*cat*	S3641 × pCP20 *flp*
S3675	S3484 Δ*hns*::FRT *leuO*::*cat*	S3642 × pCP20 *flp*
S3677	S3486 Δ*hns*::FRT *leuO*::*cat*	S3645 × pCP20 *flp*
S3689	S3480 Δ*hns*::FRT *leuO*::*cat*	S3638 × pCP20 *flp*
S3691	S3482 Δ*hns*::FRT *leuO*::*cat*	S3646 × pCP20 *flp*

[a] All experiments were performed using isogenic *E. coli* K-12 CSH50 (40) derivatives. Integrations into *attB* carry the spectinomycin resistance (Spec^r) gene, fragments encompassing the *yjjQ* or *yjjP* promoter with positions given relative to that of the transcription start and the *lacZ* gene.

[b] The construction of strains by transduction with phage T4*GT7* by replacement of genes with resistance cassettes and by deletion of the resistance gene cassettes present in the Δ*hns* and Δ(*yjjP-bglJ*) mutants using plasmid pCP20 were performed as described previously (11, 14).

[c] Strain transformed with a PCR fragment generated with primers S676 and S783 using pKD3 as the template, as described elsewhere (11).
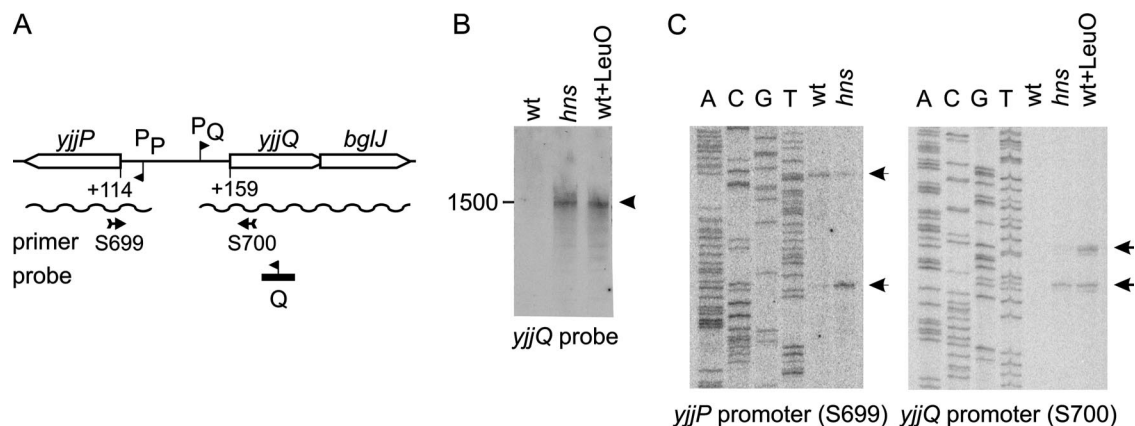
FIG. 2. Mapping of the transcription units and the *yjjP* and *yjjQ* promoters. (A) Schematic map of the *yjjP-yjjQ-bglJ* locus. Shown are positions of a riboprobe used in Northern blotting (B) and oligonucleotides S699 and S700 (see Table S2 in the supplemental material) used in the primer extension analyses (C) are indicated. (B) Northern blotting analysis of RNA isolated from the wild-type strain S541 (wt), the Δ*hns* mutant S3346 (*hns*), and the S541 strain transformed with plasmid pKEDR13 for the expression of LeuO (wt+LeuO) and separated with a denaturing gel, and the blot was hybridized to a riboprobe complementary to the *yjjQ* RNA. (C) Primer extension mapping of the *yjjP* promoter and the *yjjQ* promoter of RNA isolated from the wild-type, the *hns* mutant, and the wild type expressing LeuO (as in panel B) using the end-labeled oligonucleotides S699 and S700, respectively. The reaction products were separated next to a sequencing ladder generated with the same end-labeled primer. The 5′ end of the *yjjP* mRNA maps to 113 bases upstream of the *yjjP* translation start codon, with a second faint signal at 155 bases upstream of *yjjP*. The 5′ end of the *yjjQ* mRNA maps to 158 upstream of the *yjjQ* translation start codon, with a second signal mapping to 172 upstream of the *yjjQ* promoter codon AUG (Fig. 3).

μl labeling mixture (1.375 μM of all dNTPs, 333.5 mM NaCl), and 2 μl of diluted T7-DNA polymerase were added. After the solution was incubated for 5 min at room temperature, 4.5-μl aliquots were transferred to tubes containing 2.5 μl of the A, C, G, and T termination mixtures, respectively, and the reaction was stopped 5 min later by adding 5 μl of stop solution.

Purification of LeuO and H-NS. For the purification of C-terminally histidine-tagged LeuO (LeuO-His$_6$), strain S541 was transformed with the pKEAP21 plasmid (*lacI*^{q} *tac leuO-His$_6$*, Amp^r). One-liter cultures of LB medium plus ampicillin were inoculated to an OD$_{600}$ of 0.1, using a fresh overnight culture of S541/pKEAP21. The cultures were grown to an OD$_{600}$ of 0.3, at which point IPTG was added to a final concentration of 1 mM. Then, the cultures were grown for an additional 1 h and harvested on ice, having reached an OD$_{600}$ of 0.8. The cells were spun down and washed twice with Mg-saline (10 mM MgSO$_4$, 0.85% NaCl), and the pellets were stored in aliquots at −80°C. For lysate preparation, the pellets derived from 5 liter of culture were resuspended in 16 ml lysis buffer (20 mM Tris-HCl [pH 7.5], at 4°C, 100 mM KCl, 1 mM DTT, 1 mM phenyl-methylsulfonyl fluoride [PMSF], 5% glycerol, 50 mM imidazole), and the cells were lysed by sonication. The lysates were cleared by high-speed centrifugation and by filtration through a 0.2-μm filter unit. Then, 10 ml of the lysate was loaded onto a 1-ml HisTrap HP column equilibrated with the same buffer, using an Äkta fast-performance liquid chromatography system (GE-Healthcare). The column was washed with the same buffer containing 100 mM imidazole. LeuO-His$_6$ was eluted by increasing the imidazole concentration stepwise to 200 mM and 500 mM. A fraction of the eluate with 500 mM imidazole contained the highest LeuO-His$_6$ concentration of 120 μg/ml (or 3.4 μM) LeuO-His$_6$ (~37 kDa) and was stored in aliquots at −80°C.

H-NS was purified essentially as described previously (15). Transformants of strain S219 (*stpA::tet*) with plasmids pFDY400 (a high-copy pBR derivative plasmid carrying the *hns* gene under control of the *tac* promoter) and pFDX500 (a pACYC derivative carrying the *lacI*^{q} gene) were grown in LB medium with ampicillin and kanamycin to an OD$_{600}$ of 0.8. The expression of *hns* was induced by adding 1 mM IPTG, and cells were harvested after 90 min of further growth. The cell pellets were resuspended in 10 ml of H-NS lysis buffer (100 mM NH$_4$Cl, 30 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10% glycerol, 1 mM PMSF, 7 mM β-mercaptoethanol) per liter of culture, and the cells were lysed by sonication. The lysates were cleared by two subsequent high-speed centrifugations. In total, 30 ml of lysate was loaded onto a 50-ml phosphocellulose (P11; Whatman) column preequilibrated with the same buffer. Proteins were eluted with an NH$_4$Cl gradient, with H-NS eluting at approximately 300 mM NH$_4$Cl. The pooled fractions containing H-NS were diluted to a final buffer concentration of 100 mM NH$_4$Cl, 30 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10% glycerol, 1 mM PMSF, and 7 mM β-mercaptoethanol and loaded onto a heparin fast-flow col-

umn (GE-Healthcare). Again, an NH$_4$Cl gradient was used, with H-NS eluting at approximately 700 mM NH$_4$Cl. The pooled fractions were diluted to adjust the buffer to 100 mM NH$_4$Cl and then loaded onto a 1-ml Q-Sepharose column (GE-Healthcare) equilibrated with 100 mM KCl, 30 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10% glycerol, 1 mM PMSF, 7 mM β-mercaptoethanol. From this column, H-NS eluted at approximately 300 mM KCl. Fractions containing H-NS were pooled and rebuffered using a Centricon YM-3 centrifugal filter unit (Millipore). H-NS (~15.5 kDa) was stored at a concentration of 60 ng/μl (or 4 μM) in 75 mM KCl, 20 mM Tris-HCl (pH 7.5), 1.5 mM β-mercapto-ethanol, and 1 mM DTT.

Electrophoretic mobility shift assays (EMSA). DNA fragments were amplified by PCR (see Table S2 in the supplemental material), and their approximate concentrations were determined by the comparison of band intensities in agarose gels. Binding of H-NS and LeuO was carried out in 10-μl samples containing 20 to 25 ng of DNA per fragment in the case of the longer fragments or with 10 ng of DNA in the case of 75-bp fragments. The binding buffer was 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 2 mM DTT, and 10% glycerol, and the amounts of protein added are indicated in Fig. 3. The binding reaction was incubated at 30°C for 20 min, and then 6 μl of each sample was separated next to a GeneRuler size ladder (Fermentas) on 8% nondenaturing polyacrylamide gels (acrylamide/bisacrylamide, 29:1; 0.5× TBE) that were run under cold conditions (4°C). The gels were stained with ethidium bromide for visualization of the DNA.

RESULTS

H-NS represses and LeuO activates the *yjjQ-bglJ* operon. To determine whether the overlapping *yjjQ* and *bglJ* genes form an operon, Northern blotting was performed, using a riboprobe complementary to *yjjQ* (Fig. 2). With RNA isolated from the wild-type strain S541 carrying the native chromosomal *yjjP-yjjQ-bglJ* locus, no signal was detected (Fig. 2B). However, with RNA isolated from the isogenic Δ*hns* mutant, a weak signal was detected (Fig. 2B). The signal corresponded to an RNA of approximately 1,500 bases, which is the size expected for the transcription of *yjjQ* and *bglJ* as a bicistronic mRNA, suggesting that *yjjQ* and *bglJ* form an operon. The results show, furthermore, that H-NS represses the expression of this *yjjQ-bglJ* operon. In agreement with findings for repression by H-NS,

```
                        f1                 .          .          .          .          .          .     f1.2  f1.1
          .            .          .          .          .          .          .          .          .
TTTGCATAACTCGCCCTTTGTCATAGACGTGCTGCGTATTGTTCAGATAATGAGAATCCATTTGTATCATGAAGCACGGTTGTTTTGCGGTAGGTTAAGA
AAACGTATTGAGCGGGAAACAGTATCTGCACGACGCATAACAAGTCTATTACTCTTAGGTAAACATAGTACTTCGTGCCAACAAAACGCCATCCAATTCT
   .GlnMetValArgGlyLysAspTyrValHisGlnThrAsnAsnLeuTyrHisSerAspMetGlnIleMet
  ▷                                                                           yjjP
  +182

                    -347         .          .          .          .        f1.3   f1.2  .          .
          .          ▶           .          .          .          .          .          .          .
TCAAAATTGCGCTGATTAATCCTGGTTATCGGGAGAAGCTCGGTGATAAAAAGCGAATTATCTCTCGCCGCAAACAAACTATCACATGAATATTATCATC
AGTTTTAACGCGACTAATTAGGACCAATAGCCCTCTTCGAGCCACTATTTTTCGCTTAATAGAGAGCGGCGTTTGTTTGATAGTGTACTTATAATAGTAG
                                                       ▷                          +1       -10
                                                      +25                          P yjjP

            f1.4 f2 f1.3        .          .          .          .        f1  .          .          .
          .     [          .          .          .          .          .          .          .          .
ATAATGAATTTATTGTTTGGCCTTTACGAATCAGGATAATAGATAACCGGGCACGTTTTTTAATGTTAACAAGCTAAAACCATCAGATTTCATTGAATAA
TATTACTTAAATAACAAACCGGAAATGCTTAGTCCTATTATCTATTGGCCCGTGCAAAAAATTACAATTGTTCGATTTTGGTAGTCTAAAGTAACTTATT
          (+1)          ◁ (-10)
                        -50

  f1.4         .       -147        .          .        bend.       .       f3  .          .    -87    .          f2
          .          ▶           .          .          .          .          ▶           .          .
ATATTGGTTGTGTGATTTTGCATTCTGCGGGAAGGGATATCATTTAATTATAAATTAAATGAATGTGATTTCTGTTTTTCATTAAGTATTCCTAACAACT
TATAACCAACACACTAAAACGTAAGAGCGCCCTTCCCTATAGTAAATTAATATTTAATTTACTTACACTAAAGACAAAAAGTAATTCATAAGGATTGTTGA

    -47          .       .  -35       .    (-10)    .  (+1)  -10  .          +1   .          .    +26  .          f3
          .          ▶           .          .          .          .          .          .          ◁
TAATGTAGATATTTTAAATGTCTCCAGGCTATTTCACCAGGTTATCATTTGATGCTAACCTTCTGACACATGCAGTGGAGTTGTTGTGCAGCAGGAGTAT
ATTACATCTATAAAATTTACAGAGGTCCGATAAAGTGGTCCAATAGTAAACTACGATTGGAAGACTGTGTACGTCACCTCAACAACACGTCGTCCTCATA
                        -250                                              f4

          .          .          .          .          .          .          .          .          .
GCTGATATGAAAGTGAATGCTAAGGATAATTTATTCGCTTAATCTATTAATTTGCTGGAAACATTTAAGGAATGAATATCCTGAATAAACCATCATAATT
CGACTATACTTTCACTTACGATTCCTATTAAATAAGCGAATTAGATAATTAAACGACCTTTGTAAATTCCTTACTTATAGGACTTATTTGGTAGTATTAA

                             +166
     yjjQ            MetLeuProGlyCysCysLysAsnGlyIleValIleSerLysIleProValMetGlnAlaGlyLeuLysGluVal
TTTAGACAGTGGATGTGGAGGAAATATGTTGCCAGGATGCTGCAAAAATGGAATTGTTATCAGTAAAATACCTGTTATGCAAGCAGGGTTAAAAGAGGTC
AAATCTGTCACCTACACCTCCTTTATACAACGGTCCTACGACGTTTTTACCTTAACAATAGTCATTTTATGGCAATACGTTCGTCCCAATTTTCTCCAG
                    -450                                          f4
```
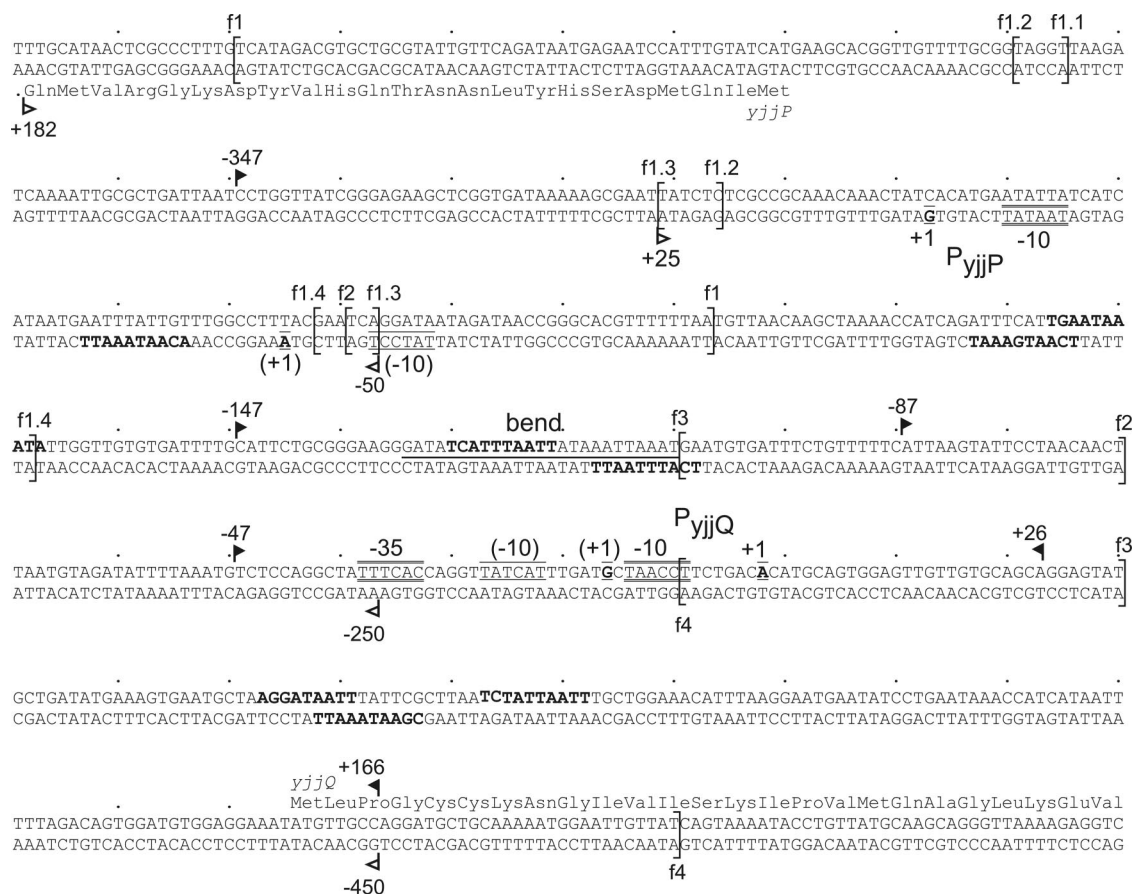
FIG. 3. Sequence of the intergenic region of the *yjjP* and *yjjQ* divergent genes. The putative transcription starts mapped by primer extension are indicated and the possible −10 and −35 boxes are marked. The endpoints of fragments used for the construction of the *yjjQ* and *yjjP* promoter-*lacZ* fusions are marked with arrows with closed and open arrowheads, respectively. The endpoints of fragments used in the gel shift assays are marked by brackets and are labeled with "f" and the number of the fragment. A predicted bend sequence is underlined. Putative H-NS nucleation sites with seven or more matches to the 10-bp H-NS consensus (28) are indicated in boldface letters.

the intergenic region between the *yjjQ* and the *yjjP* genes located upstream and in a divergent orientation shows features reminiscent of H-NS-repressed loci (16). The sequence is very AT rich (Fig. 3), and a strong bend at the center of this region is predicted by bend.it software (http://hydra.icgeb.trieste.it /~kristian/dna/bend_it.html) (43).

Furthermore, we determined whether the transcription factor LeuO regulates expression of the *yjjQ-bglJ* operon (Fig. 2, and see discussion below). The rationale for this experiment was that the constitutive expression of BglJ as well as that of LeuO relieves H-NS-mediated repression of the *bgl* operon (23, 39, 60). Since the *leuO* gene is also repressed by H-NS (7, 26), LeuO was provided in *trans* by the induction of plasmid-encoded *leuO* under the control of a *lacI^q tac* promoter cassette with IPTG (using the pKEDR13 plasmid) (39). Northern analysis of RNA isolated from the wild-type strain expressing LeuO revealed signals obtained with the *yjjQ* probe that were similar to the signals obtained with RNA isolated from the Δ*hns* mutant (Fig. 2B), which suggests that LeuO activates the *yjjQ-bglJ* operon.

Mapping of the *yjjQ* and *yjjP* transcription start sites. To map the transcription start sites of the *yjjQ-bglJ* operon and of the divergent *yjjP* gene, primer extension analyses were per-

formed using end-labeled oligonucleotides complementary to the 5′ end of *yjjP* and *yjjQ*, respectively (Fig. 2A and C). For the *yjjQ* promoter, no signals were obtained with RNA isolated from the wild-type strain. However, with RNA isolated from the Δ*hns* mutant, as well as with RNA isolated after the induction of LeuO, expression of the wild-type signals was obtained (Fig. 2C). One signal was mapped to the 5′ end of the *yjjQ-bglJ* operon-specific RNA to a position 158 bp upstream of the *yjjQ* coding region (Fig. 3). A second signal, which was more pronounced when LeuO was provided in *trans* than that of the Δ*hns* mutant, mapped to a position 172 bp upstream of the *yjjQ* coding region (Fig. 2C). For both of the putative transcription start sites, −10 boxes can be identified in the sequence, and a modest −35 may be present at the proper distance to the putative transcription start that was mapped to a position 158 bp upstream of the *yjjQ* coding region (Fig. 3). For the *yjjP* promoter, two signals were obtained. One of the signals was detected only for RNA isolated from the Δ*hns* mutant (Fig. 2C). The position of this signal suggests that the 5′ end of the *yjjP* gene-specific RNA maps to a position 113 bp upstream of the *yjjP* open reading frame (Fig. 2C). The second signal was mapped to a position 155 bp upstream of the *yjjP* gene coding region. Putative −10 boxes are located at the

proper distance to both, while no −35 boxes are evident in the sequence (Fig. 3). Taken together, the data confirm the regulation of the *yjjQ-bglJ* operon by H-NS and LeuO, and they demonstrate the repression of *yjjP* by H-NS. The primer extension analyses further reveal that both of the divergent transcription units, *yjjQ-bglJ* as well as *yjjP*, carry long untranslated leader sequences of at least 158 and 113 bases, respectively.

Binding of H-NS and LeuO to the intergenic region of the *yjjP-yjjQ-bglJ* locus. The expression directed by the divergent *yjjQ* and *yjjP* promoters is repressed by H-NS. In addition, LeuO activates the *yjjQ* promoter. To analyze the binding of H-NS and LeuO to the intergenic region, EMSAs were performed by using fragments ranging in size from 140 to 243 bp (Fig. 3 and Fig. 4 and see Table S2 in the supplemental material). In addition, a 360-bp fragment derived from the *lacZ* gene was used as a negative control. Mixtures of fragments 1 and 2 and of fragments 3 and 4 with the *lacZ* control fragment were incubated with increasing amounts of H-NS and then separated on native acrylamide gels (Fig. 4B). As shown in Fig. 4B, fragments 2 and 4 were shifted very efficiently by H-NS; fragment 2 encompasses the predicted bend region located between the divergent *yjjP* and *yjjQ* promoters, while fragment 4 was mapped to a position downstream of the *yjjQ* promoter (from positions −7 to +193 relative to that of the *yjjQ* transcription start site, which was mapped to 158 bp upstream of *yjjQ*); fragment 1, which encompasses the *yjjP* promoter and sequences located downstream to it (from position −80 to + 163 relative to that of the *yjjP* transcription start at 113 bp upstream of *yjjP*) was also shifted specifically (Fig. 4B); fragment 3, which encompasses the *yjjQ* promoter was not shifted compared to that of the *lacZ* control fragment, which suggests that in this region no sites or only weak H-NS sites are present. This lack of binding between H-NS and fragment 3 is not due to its small size (140 bp), since a 140-bp fragment mapped to within fragment 2 was efficiently shifted by H-NS (data not shown). Taken together, these results demonstrate that H-NS binds to the center region between the divergent promoters and to sites located distal to the two promoters (Fig. 4A). Furthermore, scanning of the DNA sequence for putative H-NS nucleation sites, using the H-NS consensus (28), revealed several putative matches which cluster in the predicted bend region and downstream of the *yjjQ* promoter (Fig. 3). The locations of these putative sites are in agreement with specific binding of H-NS to fragments 2 and 4, encompassing these two regions.

Binding of LeuO was likewise analyzed by EMSAs, using C-terminal LeuO-His$_6$ (Fig. 4C). These assays demonstrated binding of LeuO to fragments 1, 2, and 4 (Fig. 4B), while no retardation of fragment 3 encompassing the *yjjQ* promoter was detected (Fig. 4C). Binding of LeuO to fragment 1 resulted in a stable shift, while binding of LeuO to fragments 2 and 4 appeared as a smear, suggesting that the LeuO binding site with the highest affinity was mapped to fragment 1. The mapping of the LeuO binding sites is summarized in Fig. 4A. No LeuO consensus binding sequence is available to date.

LeuO counteracts the repression of *yjjQ* by H-NS. To further analyze the regulation of *yjjQ* by H-NS and LeuO, a series of fragments encompassing the putative *yjjQ* promoters was fused to a promoterless *lacZ* gene as the reporter for expression (Fig. 5A). The *yjjQ* promoter-*lacZ* fusions were integrated into the
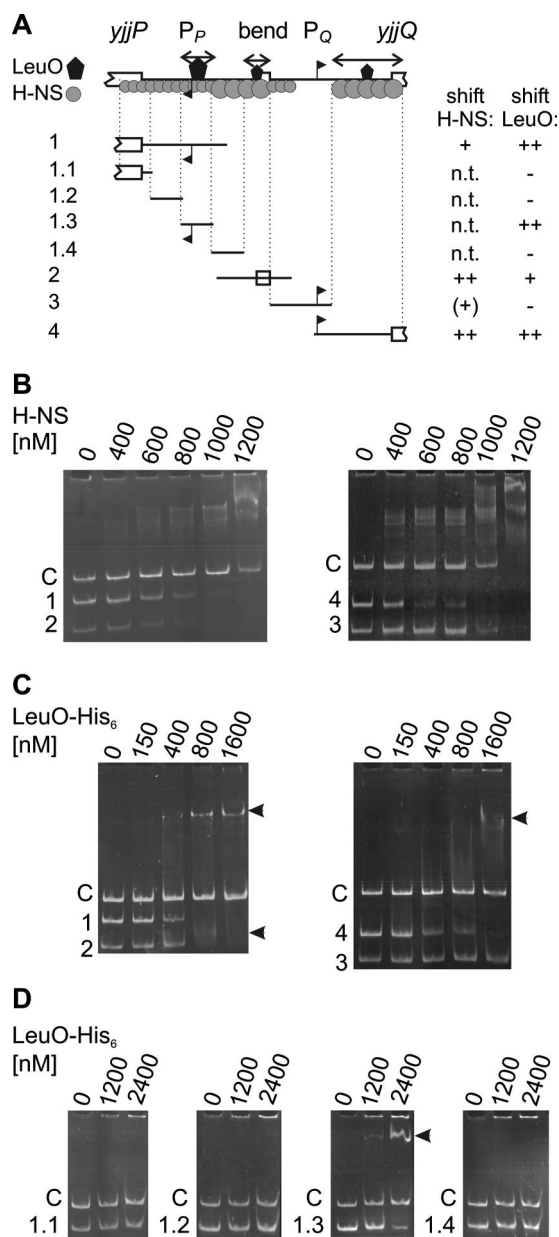

FIG. 4. Binding of H-NS and LeuO to the regulatory region of the *yjjP-yjjQ-bglJ* locus. (A) Schematic representation of the intergenic region. Regions bound by H-NS and LeuO, as deduced from the results shown in panels B to D, are indicated by a black polygon (LeuO) and gray circles forming an extended complex (H-NS). The positions of the fragments used in the EMSA are schematically indicated (with the specific positions given in Fig. 3). n.t., not tested. (B) Binding of H-NS. Fragments 1 (243 bp) and 2 (170 bp) and the 360-bp *lacZ* control fragment "C" as well as fragments 3 (140 bp) and 4 (200 bp) and the 360-bp *lacZ* control were mixed and incubated with the indicated amounts of H-NS. Subsequently, the samples were separated on native acrylamide gels stained with ethidium bromide. (C) Binding of LeuO-His$_6$ to fragments 1 to 4 in comparison to the binding of the 360-bp control fragment "C." (D) Binding of LeuO to fragments 1.1 to 1.4 (75 bp each) in comparison to that of the 140-bp *lacZ* control fragment "C."
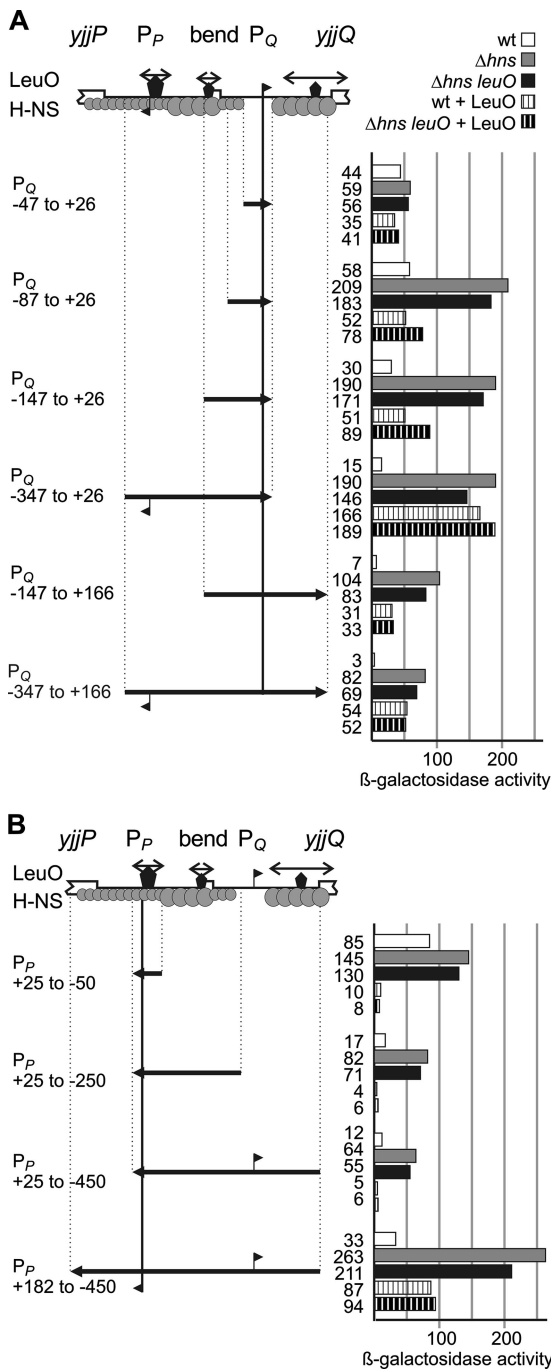
FIG. 5. Regulation of the *yjjQ* and the *yjjP* promoters by H-NS and LeuO. (A) The expression directed by the chromosomal *yjjQ* promoter-*lacZ* fusions was determined in the wild-type strain, the Δ*hns* mutant, and the Δ*hns leuO*::*cat* double mutant, as well as in transformants of the wild-type strain and the Δ*hns leuO*::*cat* double with plasmid pKEDR13 for the expression of LeuO. The expression of LeuO was induced with 1 mM IPTG in the overnight and exponential cultures. The positions of the fragments are given relative to that of the transcription start of the *yjjQ* promoter mapping to 158 bp upstream of the *yjjQ* coding region. Binding of H-NS and LeuO is schematically indicated as in Fig. 4. (B) Expression analyses using chromosomal *yjjP* promoter-*lacZ* fusions. Data are represented as in panel A, where positions are given relative to the *yjjP* promoter mapping to 113 bp upstream of the translation start.

chromosome at the *attB* site of strain S541 (Δ*lacZ*), and the expression directed by these fusions was analyzed in the wild-type strain, in the Δ*hns* mutant, and in the Δ*hns leuO*::*cat* double mutant. All strains carry a deletion of the chromosomal *yjjP-yjjQ-bglJ* locus. In addition, the role of LeuO in the regulation of the *yjjQ* promoter-*lacZ* fusions was analyzed with the wild-type strain as well as with the Δ*hns leuO* double mutant by using the pKEDR13 plasmid carrying *leuO* under the control of the *lacI*^q *tac* promoter cassette (Fig. 5A).

The *lacZ* fusion of the smallest *yjjQ* promoter fragment, which encompasses sequences from position −47 to +26 (relative to the transcription start site mapping 158 bp upstream of *yjjQ*), directed 44 units of β-galactosidase activity in the wild-type strain, 59 units in the Δ*hns* mutant, and 56 units in the Δ*hns leuO* double mutant (Fig. 5A, P_Q −47 to +26). When LeuO was provided in *trans* (by the induction of the plasmid-encoded *leuO* gene), the expression level decreased slightly to 35 units in the wild-type strain and to 41 units in the Δ*hns leuO* double mutant (Fig. 5A, P_Q −47 to +26). These data confirm the primer extension result that promoters map within the fragment. The results further show that the fragment lacks binding sites for regulation by H-NS and LeuO. When the promoter fragment was extended to include sequences from positions −87 to +26 and −147 to +26, the expression was repressed by H-NS, suggesting that H-NS binds in between −147 and −47 (Fig. 5A, P_Q −87 to +26 and P_Q −147 to +26). Furthermore, the unrepressed expression level directed by these fusions in the *hns* mutant was fourfold increased compared to that in the P_Q (−47 to +26) fusion, which may indicate that upstream DNA contacts by RNA polymerase are important for efficient transcription initiation. Providing LeuO in *trans* had no effect on the expression of these *yjjQ* promoter-*lacZ* fusions in the wild-type strain but caused repression in the Δ*hns leuO* double mutant (Fig. 5A, P_Q −87 to +26 and P_Q −147 to +26), suggesting the presence of a weak LeuO binding site between positions −147 and −47. In contrast, the expression of the *yjjQ* promoter-*lacZ* fusion encompassing position −347 to +26 was activated by LeuO and was repressed more efficiently by H-NS than the shorter constructs were (Fig. 5A, P_Q −347 to +26). This suggests the presence of a LeuO binding site in between positions −347 and −147 and also binding of H-NS within that region. The presence of regulatory sites for LeuO and H-NS between positions −347 and −147 is further supported by the result obtained with the *yjjQ* promoter −147-to-+166 and −347-to-+166 fusions. Again, LeuO had little effect on the expression of the −147-to-+166 promoter fusion but efficiently activated the −347-to-+166 promoter fusion in the wild-type strain. Furthermore, these two fusions, which include sequences downstream of the promoter (up to position +166) are more specifically repressed by H-NS (up to ~27-fold) than the corresponding fusions that lack the downstream region. The expression level directed by the −347-to-+166 fusion had a 27-fold increase (from 3 units in the wild-type strain to 82 units in the *hns* mutants), while the expression of the corresponding −347-to-+26 fusion had only a 13-fold increase, from 15 units in the wild-type strain to 190 units in the *hns* mutant (Fig. 5A). Similarly, the expression level directed by the −147-to-+166 fusion had a ~15-fold increase (compare the wild-type strain with the *hns* mutant), while the expression of the −147-to-+26 fusion had a ~6-fold

increase (Fig. 5A). These data suggest binding of H-NS to the leader DNA fragment. The finding that the absolute expression level was higher for the +26 than for the +166 fusions (Fig. 5A, compare, e.g., 190 units for the −347-to-+26 fusion in the *hns* mutant with 104 units for the −347-to-+166 fusion) may indicate that the leader contains signals that decrease expression, possibly at the posttranscriptional level. Taken together, the data are in agreement with the binding of H-NS over the complete intergenic region, with the exception of the *yjjQ* promoter, as determined by the EMSA (Fig. 4). H-NS sites appear to map to positions downstream of the *yjjQ* promoter, to the center region, and further upstream to between positions −47 and −347 (Fig. 5A). Furthermore, the data suggest that a LeuO binding site important for derepression of the *yjjQ* promoter maps to between positions −347 and −147.

Regulation of the *yjjP* promoter by H-NS and LeuO. Similar to the analysis performed for the *yjjQ* promoter region, four *yjjP* promoter-*lacZ* fusions were constructed and integrated into the chromosomal *attB* site (Fig. 5B). The *yjjP* promoter-*lacZ* fusion with the smallest fragment, encompassing sequences from position −50 to +25 (relative to that of the *yjjP* transcription start site that was mapped to a position 113 bp upstream of *yjjP*) directed the expression of 85 units of β-galactosidase activity in the wild-type strain, 145 units in the Δ*hns* mutant, and 130 units in the Δ*hns leuO* double mutant, confirming that a promoter maps on this fragment. Interestingly, when LeuO was provided in *trans*, expression dropped to 10 units in the wild-type strain and to 8 units in the Δ*hns leuO* double mutant (Fig. 5B), suggesting that LeuO binds next to this *yjjP* promoter and represses it. Extending the *yjjP* promoter fragment to include the central region (Fig. 5B, P$_P$ −250 to +25) or the complete intergenic region (Fig. 5B, P$_P$ −450 to +25) resulted in more effective repression by H-NS, in agreement with results described above that suggest that H-NS binds to the central region and to sequences distal to the divergent *yjjQ* promoter region (Fig. 5). These extended *yjjP* promoter-*lacZ* fusions were also repressed by LeuO (Fig. 5B, P$_P$ −250 to +25 and P$_P$ −450 to +25). Furthermore, the expression level directed by a *yjjP* promoter-*lacZ* fusion that encompasses the complete intergenic region (from position −450 to +182 relative to that of the transcription start site mapping to a position 113 bp upstream of *yjjP*) increased from 33 units in the wild-type strain to 263 units and 211 units in the Δ*hns* and the Δ*hns leuO* double mutants, respectively (Fig. 5B), demonstrating that in the complete context, H-NS represses the *yjjP* promoter ~eightfold and suggesting that H-NS also binds in between the *yjjP* promoter and the *yjjP* gene. Interestingly, this full construct was activated by LeuO in the wild-type strain and repressed only 2.2-fold by LeuO in the Δ*hns leuO* double mutant (Fig. 5B), indicative of a more complex pattern of regulation. Possibly, LeuO has a dual role in the regulation of the *yjjP* promoter; it may repress the promoter directly and also counteract its repression by H-NS when the binding site downstream of the *yjjP* promoter is present. The location of binding sites for LeuO and H-NS deduced from the *yjjP* promoter-*lacZ* fusions are summarized schematically in Fig. 5B.

To further address the regulation of the *yjjP* promoter by LeuO, gel retardation experiments were performed using a series of 75-bp fragments that cover the *yjjP* promoter and the leader region (Fig. 4D, fragments 1.1 to 1.4). The fragments were mixed with a 140-bp control fragment derived from *lacZ* to exclude unspecific binding. In this shift assay, LeuO bound only to the 75-bp fragment that encompassed the *yjjP* promoter but not to the other fragments. This fragment encompasses positions −50 to +25 relative to that of the transcription start and is, thus, identical to the fragment used in the *yjjP* promoter-*lacZ* fusion carrying the smallest *yjjP* promoter fragment (Fig. 5B). Binding of LeuO to this fragment is in agreement with repression of the *yjjP* promoter by LeuO.

DISCUSSION

We have shown that the *yjjP-yjjQ-bglJ* locus is regulated by H-NS and the LysR-type regulator LeuO. The *yjjQ* and *bglJ* genes encoding LuxR-type transcription factors form an operon whose repression by H-NS is counteracted by LeuO. The *yjjP* gene located upstream of the *yjjQ-bglJ* operon and in a divergent orientation is likewise repressed by H-NS but is not activated by LeuO. The H-NS binding sites are presumably spread throughout the extended 555-bp intergenic region, except for the *yjjQ* core promoter. For LeuO, three sites were mapped. One of these LeuO sites that overlapped the divergent *yjjP* promoter was required for the complete derepression of the promoter of the *yjjQ-bglJ* operon. The arrangement of binding sites suggests that H-NS forms an extended repressing nucleoprotein complex that prevents transcription initiation at both divergent promoters. Binding of LeuO and putative concomitant DNA looping could prevent spreading of the H-NS nucleoprotein complex into the *yjjQ* promoter and thus relieve its repression by H-NS. Repression of *yjjP*, *yjjQ*, and *bglJ* by H-NS has also been detected by genome scale analyses with *E. coli* and *S. enterica* (36, 46, 48).

For the activation of H-NS-repressed loci, several mechanisms have been reported (reviewed in references 16, 45, 51, and 55). For example, in the case of the *virF* promoter in *Shigella flexneri*, DNA bending is temperature dependent, and repression is relieved at 37°C (49). Repression of the *hdeAB* promoter in *E. coli* depends on the trapping of σ70-associated RNA polymerase, which induces a bend, while transcription initiation of σS-associated RNA polymerase is not repressed (57). Furthermore, binding of a transcription factor next to an essential H-NS nucleation site can prevent the binding of H-NS, as shown for the relief of the H-NS-mediated repression of the *icsB* promoter in *Shigella flexneri* by VirB (59). Alternatively, a bound transcription factor may form a barrier that prevents spreading of the H-NS nucleoprotein complex into the promoter, as proposed for autoregulation of the H-NS-repressed *leuO* gene and the divergent *leuABCD* operon encoding enzymes of the leucine synthesis pathway (7). In the case of the antagonistic regulation of the *yjjQ-bglJ* operon, analyzed here, the arrangement of the binding sites indicates that LeuO may form a DNA loop by binding to the *yjjP* promoter and to the center of the intergenic region. LeuO-mediated DNA looping may prevent spreading of the H-NS complex into the promoter of the *yjjQ-bglJ* operon. It is also possible that H-NS traps RNA polymerase at the *yjjQ* promoter by binding upstream and downstream and that LeuO prevents the formation of that repressing DNA loop. In a genome scale analysis, RNA polymerase and H-NS were both found to be associated with the *yjjQ* locus (48). An alternative

mechanism in which LeuO prevents the binding of H-NS to a nucleation site seems less likely. Such a mechanism was shown for LeuO-mediated antirepression of the H-NS and StpA-repressed *Salmonella enterica ompS1* gene (12). However, in the case of the *yjjP-yjjQ-bglJ* locus, H-NS binds to the center of the intergenic region with the highest affinity, while LeuO binds with highest affinity to the *yjjP* promoter fragment. DNA looping by LeuO is further supported by the result that the *yjjQ* promoter is moderately repressed by LeuO in the *hns* mutant when the distal LeuO site overlapping the *yjjP* promoter is missing (Fig. 4A).

The divergent arrangement of transcription units at the *yjjP-yjjQ-bglJ* locus raises the possibility that transcription-induced supercoiling (34) plays a role in its antagonistic regulation by H-NS and LeuO, which again would relate to autoregulation of the *leuO* gene (20, 21, 62). A similar divergent arrangement and topological coupling has also been shown to be important for the regulation of the *ilvYC* locus by the LysR-type transcription factor IlvY (47). In addition, divergent transcription units are a feature common to many loci that are regulated by LysR-type transcription factors (54).

Regulation of the *yjjQ-bglJ* operon by H-NS and LeuO adds an additional aspect to the role of LeuO in the emerging regulatory network important for in vivo induction of genes and for pathogenicity. LeuO is involved in the control of biofilm formation and pathogenicity in several bacteria (29, 42, 58). YjjQ encodes a putative pathogenicity determinant in APEC and in *S. enterica* (see the introduction) (29, 33). BglJ counteracts repression of the *bgl* operon by H-NS, as does LeuO (23, 39, 60). Although the importance of LeuO has been clearly defined in in vivo models for several bacterial pathogens, the regulatory effect of LeuO under in vitro growth conditions has so far been observed only when LeuO was constitutively overexpressed. In addition, no coinducer (or effector) of LeuO has been defined so far, although the activity of other transcription factors of the LysR-family is controlled by the binding of small molecules (see for example references 19, 27, 35, and 54). Furthermore, it is a common feature of LysR-type transcription factors that the effector is not required for the binding of the LysR-type regulator to DNA but alters its interaction with the DNA (27, 35). For example, the LysR-type transcription factor ArgP acts either as a repressor (when associated with lysine) or as an activator (when associated with arginine), stimulating promoter clearing at *argO* (27).

REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 2005. Current protocols in molecular biology. John Wiley and Sons, Inc., New York, NY.
2. **Badaut, C., R. Williams, V. Arluison, E. Bouffartigues, B. Robert, H. Buc, and S. Rimsky.** 2002. The degree of oligomerization of the H-NS nucleid structuring protein is related to specific binding to DNA. J. Biol. Chem. **277:**41657–41666.
3. **Bouffartigues, E., M. Buckle, C. Badaut, A. Travers, and S. Rimsky.** 2007. H-NS cooperative binding to high-affinity sites in a regulatory element results in transcriptional silencing. Nat. Struct. Mol. Biol. **14:**441–448.
4. **Chen, C. C., M. Y. Chou, C. H. Huang, A. Majumder, and H. Y. Wu.** 2005. A cis-spreading nucleoprotein filament is responsible for the gene silencing activity found in the promoter relay mechanism. J. Biol. Chem. **280:**5101–5112.
5. **Chen, C. C., M. Fang, A. Majumder, and H. Y. Wu.** 2001. A 72-base pair AT-rich DNA sequence element functions as a bacterial gene silencer. J. Biol. Chem. **276:**9478–9485.
6. **Chen, C. C., M. Ghole, A. Majumder, Z. Wang, S. Chandana, and H. Y. Wu.** 2003. LeuO-mediated transcriptional derepression. J. Biol. Chem. **278:**38094–38103.
7. **Chen, C. C., and H. Y. Wu.** 2005. LeuO protein delimits the transcriptionally active and repressive domains on the bacterial chromosome. J. Biol. Chem. **280:**15111–15121.
8. **Daley, D. O., M. Rapp, E. Granseth, K. Melen, D. Drew, and G. von Heijne.** 2005. Global topology analysis of the *Escherichia coli* inner membrane proteome. Science **308:**1321–1323.
9. **Dame, R. T., C. Wyman, R. Wurm, R. Wagner, and N. Goosen.** 2002. Structural basis for H-NS-mediated trapping of RNA polymerase in the open initiation complex at the *rrnB* P1. J. Biol. Chem. **277:**2146–2150.
10. **Dame, R. T., M. C. Noom, and G. J. L. Wuite.** 2006. Bacterial chromatin organization by H-NS protein unravelled using dual DNA manipulation. Nature **444:**387–390.
11. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
12. **De la Cruz, M. A., M. Fernandez-Mora, C. Guadarrama, M. A. Flores-Valdez, V. H. Bustamante, A. Vazquez, and E. Calva.** 2007. LeuO antagonizes H-NS and StpA-dependent repression in *Salmonella enterica ompS1*. Mol. Microbiol. **66:**727–743.
13. **Diederich, L., L. J. Rasmussen, and W. Messer.** 1992. New cloning vectors for integration into the lambda attachment site *attB* of the *Escherichia coli* chromosome. Plasmid **28:**14–24.
14. **Dole, S., S. Kühn, and K. Schnetz.** 2002. Post-transcriptional enhancement of *Escherichia coli bgl* operon silencing by limitation of BglG-mediated antitermination at low transcription rates. Mol. Microbiol. **43:**217–226.
15. **Dole, S., V. Nagarajavel, and K. Schnetz.** 2004. The histone-like nucleoid structuring protein H-NS represses the *Escherichia coli bgl* operon downstream of the promoter. Mol. Microbiol. **52:**589–600.
16. **Dorman, C. J.** 2004. H-NS: a universal regulator for a dynamic genome. Nat. Rev. Microbiol. **2:**391–400.
17. **Dorman, C. J.** 2007. Probing bacterial nucleoid structure with optical tweezers. BioEssays **29:**212–216.
18. **Dorman, C. J.** 2007. H-NS, the genome sentinel. Nat. Rev. Microbiol. **5:**157–161.
19. **Ezezika, O. C., S. Haddad, T. J. Clark, E. L. Neidle, and C. Momany.** 2007. Distinct effector-binding sites enable synergistic transcriptional activation by BenM, a LysR-type regulator. J. Mol. Biol. **367:**616–629.
20. **Fang, M., and H. Y. Wu.** 1998. Suppression of *leu*-500 mutation in *topA*⁺ *Salmonella typhimurium* strains. The promoter relay at work. J. Biol. Chem. **273:**29929–29934.
21. **Fang, M., and H.-Y. Wu.** 1998. A promoter relay mechanism for sequential gene activation. J. Bacteriol. **180:**626–633.
22. **Fernández-Mora, M., J. L. Puente, and E. Calva.** 2004. OmpR and LeuO positively regulate the *Salmonella enterica* serovar Typhi *ompS2* porin gene. J. Bacteriol. **186:**2909–2920.
23. **Giel, M., M. Desnoyer, and J. Lopilato.** 1996. A mutation in a new gene, bglJ, activates the *bgl* operon in *Escherichia coli* K-12. Genetics **143:**627–635.
24. **Grainger, D. C., D. Hurd, M. D. Goldberg, and S. J. Busby.** 2006. Association of nucleoid proteins with coding and non-coding segments of the *Escherichia coli* genome. Nucleic Acids Res. **34:**4642–4652.
25. **Hommais, F., E. Krin, C. Laurent-Winter, O. Soutourina, A. Malpertuy, J. P. Le Caer, A. Danchin, and P. Bertin.** 2001. Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. Mol. Microbiol. **40:**20–36.
26. **Klauck, E., J. Bohringer, and R. Hengge-Aronis.** 1997. The LysR-like regulator LeuO in *Escherichia coli* is involved in the translational regulation of *rpoS* by affecting the expression of the small regulatory DsrA-RNA. Mol. Microbiol. **25:**559–569.
27. **Laishram, R. S., and J. Gowrishankar.** 2007. Environmental regulation operating at the promoter clearance step of bacterial transcription. Genes Dev. **21:**1258–1272.
28. **Lang, B., N. Blot, E. Bouffartigues, M. Buckle, M. Geertz, C. O. Gualerzi, R. Mavathur, G. Muskhelishvili, C. L. Pon, S. Rimsky, S. Stella, M. M. Babu, and A. Travers.** 2007. High-affinity DNA binding sites for H-NS provide a molecular basis for selective silencing within proteobacterial genomes. Nucleic Acids Res. **35:**6330–6337.
29. **Lawley, T. D., K. Chan, L. J. Thompson, C. C. Kim, G. R. Govoni, and D. M. Monack.** 2006. Genome-wide screen for *Salmonella* genes required for long-term systemic infection of the mouse. PLoS Pathog. **2:**e11.
30. **Lawrenz, M. B., and V. L. Miller.** 2007. Comparative analysis of the regulation of *rovA* from the pathogenic *Yersiniae*. J. Bacteriol. **189:**5963–5975.
31. **Lease, R. A., M. E. Cusick, and M. Belfort.** 1998. Riboregulation in *Esche-*

richia coli: DsrA RNA acts by RNA:RNA interactions at multiple loci. Proc. Natl. Acad. Sci. USA **95:**12456–12461.

32. **Lease, R. A., and S. A. Woodson.** 2004. Cycling of the Sm-like protein Hfq on the DsrA small regulatory RNA. J. Mol. Biol. **344:**1211–1223.

33. **Li, G., C. Laturnus, C. Ewers, and L. H. Wieler.** 2005. Identification of genes required for avian *Escherichia coli* septicemia by signature-tagged mutagenesis. Infect. Immun. **73:**2818–2827.

34. **Liu, L. F., and J. C. Wang.** 1987. Supercoiling of the DNA template during transcription. Proc. Natl. Acad. Sci. USA **84:**7024–7027.

35. **Lorca, G. L., A. Ezersky, V. V. Lunin, J. R. Walker, S. Altamentova, E. Evdokimova, M. Vedadi, A. Bochkarev, and A. Savchenko.** 2007. Glyoxylate and pyruvate are antagonistic effectors of the *Escherichia coli* IclR transcriptional regulator. J. Biol. Chem. **282:**16476–16491.

36. **Lucchini, S., G. Rowley, M. D. Goldberg, D. Hurd, M. Harrison, and J. C. D. Hinton.** 2006. H-NS mediates the silencing of laterally acquired genes in bacteria. PLoS Pathog. **2:**e81.

37. **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.

38. **Luijsterburg, M. S., M. C. Noom, G. J. Wuite, and R. T. Dame.** 2006. The architectural role of nucleoid-associated proteins in the organization of bacterial chromatin: a molecular perspective. J. Struct. Biol. **156:**262–272.

39. **Madhusudan, S., A. Paukner, Y. Klingen, and K. Schnetz.** 2005. Independent regulation of H-NS mediated silencing of the *bgl* operon at two levels: upstream by BglJ and LeuO and downstream by DnaKJ. Microbiology **151:**3349–3359.

40. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

41. **Miller, J. H.** 1992. A short course in bacterial genetics. A laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

42. **Moorthy, S., and P. I. Watnick.** 2005. Identification of novel stage-specific genetic requirements through whole genome transcription profiling of *Vibrio cholerae* biofilm development. Mol. Microbiol. **57:**1623–1635.

43. **Munteanu, M. G., K. Vlahovicek, S. Parthasarathy, I. Simon, and S. Pongor.** 1998. Rod models of DNA: sequence-dependent anisotropic elastic modelling of local bending phenomena. Trends Biochem. Sci. **23:**341–347.

44. **Nagarajavel, V., S. Madhusudan, S. Dole, A. R. Rahmouni, and K. Schnetz.** 2007. Repression by binding of H-NS within the transcription unit. J. Biol. Chem. **282:**23622–23630.

45. **Navarre, W. W., M. McClelland, S. J. Libby, and F. C. Fang.** 2007. Silencing of xenogeneic DNA by H-NS-facilitation of lateral gene transfer in bacteria by a defense system that recognizes foreign DNA. Genes Dev. **21:**1456–1471.

46. **Navarre, W. W., S. Porwollik, Y. Wang, M. McClelland, H. Rosen, S. J. Libby, and F. C. Fang.** 2006. Selective silencing of foreign DNA with low GC content by the H-NS protein in *Salmonella*. Science **313:**236–238.

47. **Opel, M. L., S. M. Arfin, and G. W. Hatfield.** 2001. The effects of DNA supercoiling on the expression of operons of the *ilv* regulon of *Escherichia coli* suggest a physiological rationale for divergently transcribed operons. Mol. Microbiol. **39:**1109–1115.

48. **Oshima, T., S. Ishikawa, K. Kurokawa, H. Aiba, and N. Ogasawara.** 2006. *Escherichia coli* histone-like protein H-NS preferentially binds to horizontally acquired DNA in association with RNA Polymerase. DNA Res. **13:**141–153.

49. **Prosseda, G., M. Falconi, M. Giangrossi, C. O. Gualerzi, G. Micheli, and B. Colonna.** 2004. The *virF* promoter in *Shigella*: more than just a curved DNA stretch. Mol. Microbiol. **51:**523–537.

50. **Repoila, F., and S. Gottesman.** 2001. Signal transduction cascade for regulation of RpoS: temperature regulation of DsrA. J. Bacteriol. **183:**4012–4023.

51. **Rimsky, S.** 2004. Structure of the histone-like protein H-NS and its role in regulation and genome superstructure. Curr. Opin. Microbiol. **7:**109–114.

52. **Rodríguez-Morales, O., M. Fernández-Mora, I. Hernández-Lucas, A. Vázquez, J. L. Puente, and E. Calva.** 2006. *Salmonella enterica* serovar Typhimurium *ompS1* and *ompS2* mutants are attenuated for virulence in mice. Infect. Immun. **74:**1398–1402.

53. **Sambrook, J., and D. Russell.** 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

54. **Schell, M. A.** 1993. Molecular biology of the LysR family of transcriptional regulators. Annu. Rev. Microbiol. **47:**597–626.

55. **Schröder, O., and R. Wagner.** 2002. The bacterial regulatory protein H-NS—a versatile modulator of nucleic acid structures. Biol. Chem. **383:**945–960.

56. **Shi, X., and G. N. Bennett.** 1995. Effects of multicopy LeuO in the expression of the acid-inducible lysine decarboxylase gene in *Escherichia coli*. J. Bacteriol. **177:**810–814.

57. **Shin, M., M. Song, J. H. Rhee, Y. Hong, Y. J. Kim, Y. J. Seok, K. S. Ha, S. H. Jung, and H. E. Choy.** 2005. DNA looping-mediated repression by histone-like protein H-NS: specific requirement of Eσ70 as a cofactor for looping. Genes Dev. **19:**2388–2398.

58. **Tenor, J. L., B. A. McCormick, F. M. Ausubel, and A. Aballay.** 2004. *Caenorhabditis elegans*-based screen identifies *Salmonella* virulence factors required for conserved host-pathogen interactions. Curr. Biol. **14:**1018–1024.

59. **Turner, E. C., and C. J. Dorman.** 2007. H-NS antagonism in *Shigella flexneri* by VirB, a virulence gene transcription regulator that is closely related to plasmid partition factors. J. Bacteriol. **189:**3403–3413.

60. **Ueguchi, C., T. Ohta, C. Seto, T. Suzuki, and T. Mizuno.** 1998. The *leuO* gene-product has a latent ability to relieve the *bgl* silencing in *Escherichia coli*. J. Bacteriol. **180:**190–193.

61. **Wilson, G. G., K. Y. K. Young, G. J. Edlin, and W. Konigsberg.** 1979. High-frequency generalised transduction by bacteriophage T4. Nature **280:**80–82.

62. **Wu, H.-Y., J. Tan, and M. Fang.** 1995. Long-range interaction between two promoters: activation of the *leu-500* promoter by a distant upstream promoter. Cell **82:**445–451.