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The Small Nucleoid-Binding Proteins H-NS, HU, and Fis Affect *hilA* Expression in *Salmonella enterica* Serovar Typhimurium

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***hilA* encodes an activator of *Salmonella enterica* serovar Typhimurium virulence genes and is transcriptionally modulated by environmental conditions. We show that H-NS represses *hilA* under low-osmolarity conditions. H-NS, HU, and Fis also appear to affect the derepression of *hilA* by HilD. Modulation of *hilA* by counteracting repressing and derepressing mechanisms may allow *Salmonella* serovar Typhimurium to regulate its virulence genes in response to different situations in vivo.**

Genes on *Salmonella* pathogenicity island 1 (SPI1) are required for *Salmonella enterica* serovar Typhimurium to cause gastroenteritis (27). SPI1 encodes components of a type III secretion system that translocates bacterial effector proteins into the cytosol of mammalian cells and modifies host cell signaling pathways (18). A large number of environmental conditions and regulatory factors control the expression of the SPI1 secretion system by regulating *hilA* expression (18). *hilA* is located on SPI1 and encodes an OmpR/ToxR transcriptional regulator, which coordinately activates the genes that encode the SPI1 secretion system (18).

It has been proposed that counteracting repressing and derepressing mechanisms regulate *hilA* expression. The presence of an upstream DNA sequence represses a plasmid-borne *hilA* promoter (23). HilC and HilD, two SPI1-encoded AraC/XylS family members, have been called derepressors and are thought to counteract the repression of *hilA* by binding to sites within the upstream repressing sequence (URS) (24). However, recent results appear to contradict this model and support the idea that HilD interacts with the α subunit of RNA polymerase to activate *hilA* transcription (4). For example, replacement of the URS with 84 bp of unrelated sequence or a precise deletion of the URS in the *Salmonella* serovar Typhimurium chromosome was shown to reduce, rather than increase, *hilA* expression (4; unpublished results). Unfortunately, we could not rescue our deletion with the intact URS and wondered if a deleterious effect of the URS deletion selects for secondary genetic changes that both reduce *hilA-lacZ* expression and prevent replacement of the URS allele (unpublished results). Still, it is intriguing to consider that under certain conditions HilC and HilD may be able to activate *hilA* expression and that under other conditions they may be able to increase *hilA* expression by derepressing *hilA* transcription. Studies showing that HilC and HilD are not required for *hilA* expression from a plasmid in in vitro transcription assays or in the absence of the URS suggest that HilC and HilD can derepress *hilA* expression, possibly by antagonizing the repressing effects of proteins bound to the URS (21, 23).

We suspected that small nucleoid-binding proteins, such as H-NS, HU, and Fis, might control *hilA* expression by affecting its repression and/or derepression. Small nucleoid-binding proteins have been shown to regulate gene expression in response to specific environmental conditions that also control *hilA* expression (1, 18, 22). H-NS can silence promoters by interacting with distal sequences (5) and has been shown to be counteracted by AraC/XylS factors (8). HU may also antagonize the effects of H-NS on gene expression (6). The small nucleoid-binding protein Hha has been reported to bind the URS and repress *hilA* expression (4, 11). In contrast, Fis appears to have the opposite regulatory effect, since Wilson et al. have shown that a mutation in *fis* reduces *hilA* expression (28).

Effect of H-NS on *hilA* expression. To test whether H-NS regulates *hilA* expression in *Salmonella* serovar Typhimurium, we examined the effect of the *hns-1*::*kan* mutation on the expression of the chromosomal *hilA080*::Tn*5lacZY* (Tet^r) transcriptional fusion (2, 12). Although we routinely analyze *hilA* expression in *Salmonella* serovar Typhimurium SL1344, we found that *hns* mutations cause this strain to become mucoid and grow poorly. Therefore, we analyzed *hilA-lacZ* expression in *Salmonella* serovar Typhimurium LT2 (SGSC1412), which appears to better tolerate *hns* mutations. β-Galactosidase assays were performed on bacterial cultures grown under inducing or repressing conditions for *hilA* expression, as previously described (20, 23). Although *hilA* regulation in LT2 might not be identical to that in SL1344, we found that, as in SL1344, aerobic conditions and low-osmolarity conditions repress *hilA-lacZ* expression in LT2 (Fig. 1A). Interestingly, the *hns-1*::*kan* mutation increases *hilA-lacZ* expression under the low-osmolarity conditions but not under the aerobic growth conditions (Fig. 1A). Due to the counteracting nature of the repressing URS and derepressing HilC and HilD proteins that directly control *hilA* expression, the *hns-1*::*kan* mutation may increase *hilA* expression under the low-osmolarity conditions (i) by decreasing its repression and/or (ii) by increasing its derepression by HilC or HilD.

To test whether *hilC* and *hilD* are required for the *hns*

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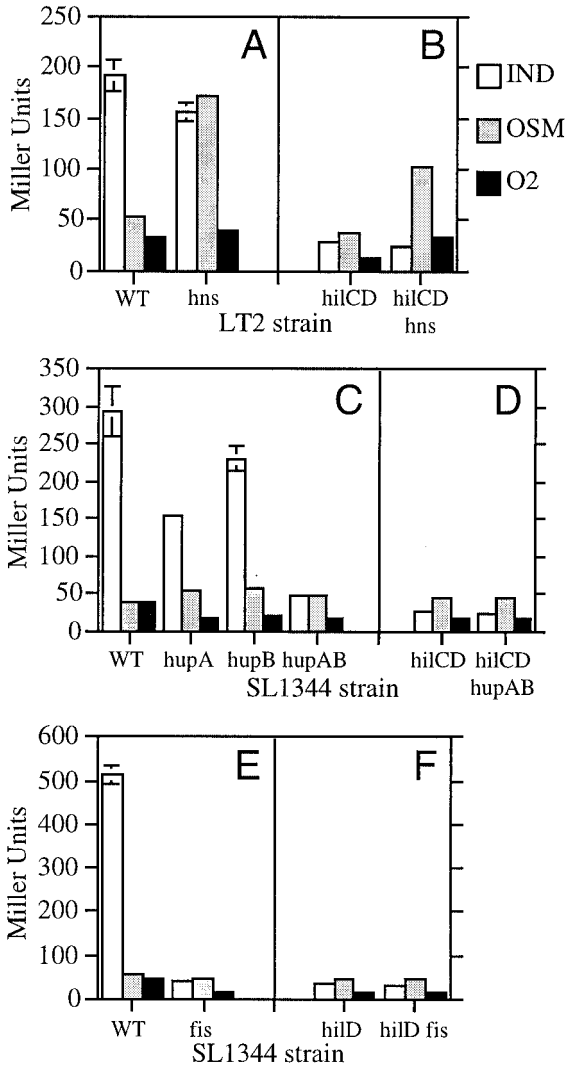

FIG. 1. Effects of small nucleoid-binding proteins on *hilA-lacZ* expression. *Salmonella* serovar Typhimurium LT2 and SL1344 strains containing the *hilA080*::Tn*5lacZY* chromosomal fusion were grown under inducing (IND; white bars), repressing low-osmolarity (OSM; gray bars), or repressing aerobic (O2; black bars) conditions. β-Galactosidase activities are reported in Miller units and are the averages (± standard deviations) of values obtained from the results of at least two independent cultures during one representative experiment. Similar results were obtained in repeated experiments. (A) Fusion strain (wild type [WT]) and its *hns-1*::*kan* (hns) derivative; (B) *hilC1*::*cam hilD1*::*kan* (hilCD) and *hilC1*::*cam hilD1*::*kan hns-1*::*kan* (hilCD hns) derivatives; (C) fusion strain (WT) and its *hupA*::*kan* (hupA), *hupB*::*kan* (hupB), and *hupA*::*kan hupB*::*kan* (hupAB) derivatives; (D) *hilC1*::*cam hilD1*::*kan* (hilCD) and *hilC1*::*cam hilD1*::*kan hupA*::*kan hupB*::*kan* (hilCD hupAB) derivatives; (E) fusion strain (WT) and its *fis3*::*cam* (fis) derivative; (F) *hilD1*::*kan* (hilD) and *hilD1*::*kan fis3*::*cam* (hilD fis) derivatives.

mutation to increase *hilA* expression in LT2, we introduced *hilC1*::*cam* and *hilD1*::*kan* mutations into the LT2 *hns-1*::*kan* strain. The *hilC1*::*cam* mutation was constructed by allele replacement with pLS112, a pLD55 derivative that contains a chloramphenicol resistance gene cassette inserted into the *Bcl*I site of *hilC* (20). The *hilD1*::*kan* mutation was constructed previously (24). The *hns-1*::*kan hilC1*::*cam hilD1*::*kan hilA080*::Tn*5lacZY* mutant was constructed by transducing LT2 *hns-1*::*kan* with a *hilC1*::*cam hilD1*::*kan hilA080*::Tn*5lacZY* P22 lysate (7, 23). Because *hilD1*::*kan* is located between *hilC1*::*cam* and *hilA080*::Tn*5lacZY*, chloramphenicol- and tetracycline-resistant transductants of LT2 *hns-1*::*kan* also contain the *hilD1*::*kan* mutation. The presence of the *hilD1*::*kan* mutation was confirmed by PCR.

Our analysis of *hilA-lacZ* expression in the *hilC hilD* mutant background shows that, even in the absence of *hilC* and *hilD*, the *hns-1*::*kan* mutation increases *hilA* expression under low-osmolarity conditions (Fig. 1B). These results are consistent with the idea that the *hns* mutation decreases the repression of *hilA* under these conditions. These results also provide further support for the idea that HilC and HilD function as derepressors, rather than activators, of *hilA* transcription. The *hns* mutation may also influence the derepression of *hilA* by HilC or HilD, since its ability to increase *hilA-lacZ* expression is partially attenuated in the *hilCD* mutant background (Fig. 1B).

Effect of HU and Fis on *hilA* expression. To see if HU regulates *hilA* expression in *Salmonella* serovar Typhimurium, we examined the effects of single *hupA* and *hupB* mutations on chromosomal *hilA-lacZ* expression in SL1344 (Fig. 1C) (16). Because *hupA* and *hupB* encode the α and β subunits of HU, which can form active homodimers, we also examined *hilA-lacZ* expression in a double *hupA hupB* mutant strain (Fig. 1C). The *Salmonella* serovar Typhimurium *hupA*::*kan hupB*::*kan* double mutant was constructed by transducing a *hupB*::*kan purD*::Tn*10* mutant with a *hupA*::*kan* P22 lysate and selecting for purine prototrophs. Because the *purD* gene is linked to *hupA*, *purD*$^+$ *hupA*::*kan hupB*::*kan* transductants were obtained and verified by genetic linkage. As shown in Fig. 1C, chromosomal *hilA-lacZ* expression is dramatically reduced in the *hupA*::*kan hupB*::*kan* mutant grown under conditions that normally induce *hilA* expression. Thus, in contrast to H-NS, HU plays a positive role in *hilA* expression. Fahlen et al. reported that a mutation in *hupB* increases *hilA* expression (10), which is contrary to our results. However, we used a *hupB*::*kan* mutation that disrupts the *hupB* open reading frame, while the *hupB1*::Tn*5* mutation used by Fahlen et al. is located 188 bp upstream of the *hupB* translation start site (10, 16). We speculate that the *hupB1*::Tn*5* mutation actually increases *hupB* expression and HU levels, which may stimulate *hilA* expression.

Wilson et al. have shown that a *fis*::*kan* mutation reduces *hilA* expression two- to threefold (28). We found that the *fis3*::*cam* mutation reduces chromosomal *hilA-lacZ* expression 13-fold under our conditions, which normally induce *hilA* (Fig. 1E). These results suggest that, like HU, Fis is required for full induction of *hilA* expression in *Salmonella* serovar Typhimurium. Previous studies have shown that HilD, not HilC, is primarily responsible for derepression of *hilA* expression when *Salmonella* serovar Typhimurium is grown under our inducing conditions (19, 23). Thus, the *hup* and *fis* mutations may decrease *hilA* expression (i) by decreasing its derepression by HilD and/or (ii) by increasing its repression. In the first case, we expect that mutations in *hupAB* or *fis* would not further reduce *hilA-lacZ* expression in strains lacking HilD. Consistent with the idea that HU and Fis affect the derepression of *hilA*, our results show that *hilA-lacZ* expression is not significantly reduced by the *hupAB* or *fis* mutations in a *hilCD* or *hilD* mutant strain background (Fig. 1DF). HU or Fis may bind to

the *hilA* promoter and enhance its ability to be derepressed by HilD. Alternatively, HU or Fis may influence *hilA* indirectly by increasing the levels and/or activity of HilD.

Effect of H-NS, HU, and Fis on *hilD* mRNA levels. To examine whether H-NS, HU, or Fis affects *hilD* mRNA levels, we first characterized the *hilD* transcript by identifying the *hilD* transcription start site. A primer extension analysis was performed on RNA from wild-type *Salmonella* serovar Typhimurium grown under inducing high-osmolarity, low-oxygen conditions. An overnight culture of SL1344 was subcultured at a dilution of 1:1,000 in 100 ml of high-salt (1% NaCl) Luria-Bertani medium in a 125-ml Erlenmeyer flask and grown with shaking at 150 rpm to a final optical density at 600 nm of 0.7 to 0.8. Ten micrograms of total RNA, isolated from these cells by using the RNAqueous kit (Ambion Inc.), was annealed to 100 fmol of LS49 (5′-GTCTGACTTTTAATTTGCTGC-3′), which was previously end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (NEN Life Science Products, Promega). The primer extension reaction was carried out by using the AMV reverse transcriptase system (Promega) and run on a 6% acrylamide–8 M urea gel; a dideoxy DNA sequencing reaction was carried out by using the fmol DNA sequencing system (Promega) and the end-labeled LS49 primer. Similar to the results of studies by Olekhnovich and Kadner (21), our results indicate that one or two *hilD* transcription start sites are located 34 or 35 bp upstream of the *hilD* translation start site and just downstream (6 or 7 bp) of a predicted σ^{70} binding site (TTTACA-16 bp-TAGGAT) (Fig. 2A and B).

Using this primer extension assay, we compared the *hilD* mRNA levels in our isogenic wild-type and mutant strains. We purified RNA from wild-type SL1344, the *hupA*::*kan hupB*::*kan* mutant, and the *fis3*::*cam* mutant grown under inducing conditions. In contrast, we grew the wild-type LT2 and the *hns-1*::*kan* mutant under low-osmolarity conditions, since our previous results showed that the *hns-1*::*kan* mutation specifically affects *hilA* expression in low-osmolarity media (Fig. 1A). Our results indicate that the level of *hilD* mRNA is reduced in the *hupA hupB* and *fis* mutants and increased in the *hns* mutant (Fig. 2C). Control primer extension assays were also conducted using primer ompA1 (5′-CGAAACCAGCCAGTGCCACTG-3′). *ompA* encodes a major outer membrane protein, which is important for structural integrity of the outer membrane. In *Escherichia coli*, *ompA* is highly expressed and its mRNA levels are only modestly affected by dramatic changes in growth conditions and growth rate (14, 15). As shown in Fig. 2C, each set of wild-type and mutant strains appears to contain similar levels of *ompA* mRNA. By quantitating the primer extension products obtained in two independent experiments with a phosphorimager, we normalized the *hilD* product to that of the *ompA* control and then calculated the level of *hilD* mRNA in each mutant strain relative to that in its isogenic wild-type strain. This analysis indicates that the *hupA*::*kan hupB*::*kan* mutations and *fis3*::*cam* mutation reduce *hilD* mRNA levels to 29 and 3% of that in wild-type SL1344, respectively. The dramatic reduction in *hilD* mRNA levels seen in the *fis* mutant suggests that Fis primarily influences the derepression of *hilA* by altering the production of HilD. The less dramatic change in *hilD* mRNA seen in the *hupAB* mutant indicates that HU may affect the derepression of *hilA* via more than one mechanism, such as by affecting regulatory pathways that modulate HilD posttranscriptionally (3). Our analysis indicated that the *hns-1*::*kan* mutation increases *hilD* mRNA levels sixfold over that in wild-type LT2. This result suggests that, in addition to decreasing the repression of *hilA* under low-osmolarity conditions (Fig. 1B), the *hns* mutation increases the derepression of *hilA* by HilD. Further studies are needed to understand how H-NS, HU, and Fis alter *hilD* mRNA levels and affect the derepression of *hilA*.

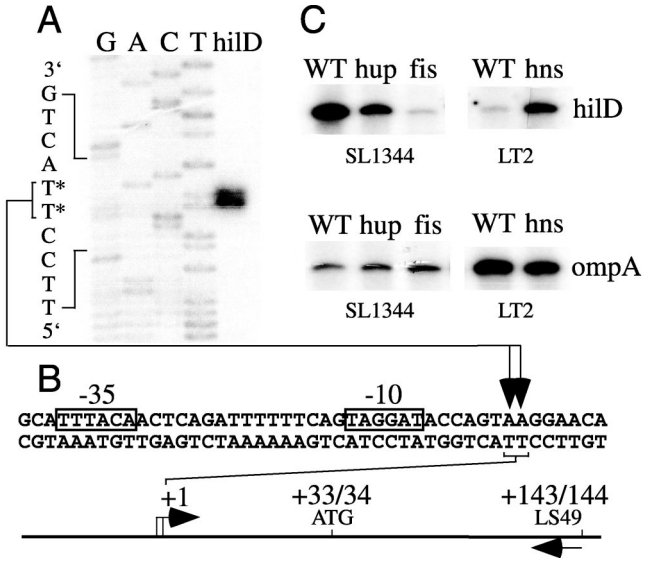

FIG. 2. Effect of small nucleoid-binding proteins on *hilD* mRNA levels. (A) The *hilD* transcription start site was determined by running primer extension products (hilD) from wild-type *Salmonella* serovar Typhimurium strain SL1344 RNA alongside dideoxy DNA sequencing reactions (GACT). Nucleotides marked with an asterisk represent two possible *hilD* transcription start sites on the template strand. (B) The positions of potential −10 and −35 σ^{70} binding sites, the *hilD* translation start site (ATG), and the primer used in the primer extension reactions (LS49) are shown below the autoradiograph of the gel in relation to the *hilD* transcription start sites (+1). (C) *hilD* and *ompA* primer extension products were generated by using total RNA from wild-type (WT), *hupA*::*kan hupB*::*kan* (hup), *fis3*::*cam* (fis), and *hns-1*::*kan* (hns) derivatives of SL1344 or LT2, grown under inducing or low-osmolarity conditions, respectively. The autoradiographs shown are from one representative experiment.

Repression of *hilA* expression. We previously proposed that a repressor protein binds to the URS and prevents the expression of *hilA* under repressing growth conditions (23). Our present results suggest that *hilA* expression may actually be repressed by different factors under different conditions. While H-NS may directly or indirectly repress the *hilA* promoter under low-osmolarity conditions, our finding that *hilA* remains repressed in a *hilCD hns* mutant grown under inducing or aerobic conditions suggests that other factors may repress *hilA* expression under these conditions (Fig. 1B). Additional repressors of *hilA* may include HilE, Pag, and Hha, as strains lacking these proteins exhibit increased *hilA* expression under inducing conditions (4, 10, 11). Hha may also repress *hilA* under low-osmolarity conditions (11). To determine whether HilE, Pag, or Hha affects repression, rather than derepression, of *hilA*, the effect that *hilE*, *pag*, and *hha* mutations have on *hilA* expression should be examined under repressing environmental conditions in strains lacking HilC and HilD. Since mutations in *hilE*,

pag, and *hha* do not abolish the repression of *hilA* expression in *Salmonella* serovar Typhimurium grown aerobically, other factors may repress *hilA* expression under aerobic conditions (10, 11).

There are several mechanisms by which H-NS might repress *hilA* under low-osmolarity conditions. H-NS might bind to the URS and directly occlude transcription initiation from the *hilA* promoter, as has been proposed for H-NS silencing of the *bgl* promoter in *E. coli* (5). Alternatively, H-NS, which bends DNA and affects global DNA supercoiling, may alter the DNA topology at the *hilA* promoter to repress *hilA* transcription (16, 17, 25, 26). Interestingly, expression of the SPI1 gene *invA* is sensitive to chemical inhibitors of DNA gyrase as well as topoisomerase mutations that change DNA supercoiling (13). Because HilA activates *invA* expression, alterations in DNA supercoiling may affect *invA* by influencing *hilA* expression (9).

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REFERENCES

1. **Atlung, T., and H. Ingmer.** 1997. H-NS: a modulator of environmentally regulated gene expression. Mol. Microbiol. **24:**7–17.
2. **Bajaj, V., R. L. Lucas, C. Hwang, and C. A. Lee.** 1996. Co-ordinate regulation of *Salmonella typhimurium* invasion genes by environmental and regulatory factors is mediated by control of *hilA* expression. Mol. Microbiol. **22:**703–714.
3. **Baxter, M. A., T. F. Fahlen, R. L. Wilson, and B. D. Jones.** 2003. HilE interacts with HilD and negatively regulates *hilA* transcription and expression of the *Salmonella enterica* serovar Typhimurium invasive phenotype. Infect. Immun. **71:**1295–1305.
4. **Boddicker, J. D., B. M. Knosp, and B. D. Jones.** 2003. Transcription of the *Salmonella* invasion gene activator, *hilA*, requires HilD activation in the absence of negative regulators. J. Bacteriol. **185:**525–533.
5. **Caramel, A., and K. Schnetz.** 1998. Lac and λ repressors relieve silencing of the *Escherichia coli bgl* promoter. Activation by alteration of a repressing nucleoprotein complex. J. Mol. Biol. **284:**875–883.
6. **Dame, R. T., and N. Goosen.** 2002. HU: promoting or counteracting DNA compaction? FEBS Lett. **529:**151–156.
7. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
8. **Egan, S. M.** 2002. Growing repertoire of AraC/XylS activators. J. Bacteriol. **184:**5529–5532.
9. **Eichelberg, K., and J. E. Galán.** 1999. Differential regulation of *Salmonella typhimurium* type III secreted proteins by pathogenicity island 1 (SPI-1)-encoded transcriptional activators InvF and HilA. Infect. Immun. **67:**4099–4105.
10. **Fahlen, T. F., N. Mathur, and B. D. Jones.** 2000. Identification and characterization of mutants with increased expression of *hilA*, the invasion gene transcriptional activator of *Salmonella typhimurium.* FEMS Immunol. Med. Microbiol. **28:**25–35.
11. **Fahlen, T. F., R. L. Wilson, J. D. Boddicker, and B. D. Jones.** 2001. Hha is a negative modulator of transcription of *hilA*, the *Salmonella enterica* serovar Typhimurium invasion gene transcriptional activator. J. Bacteriol. **183:**6620–6629.
12. **Falconi, M., V. McGovern, C. Gualerzi, D. Hillyard, and N. P. Higgins.** 1991. Mutations altering chromosomal protein H-NS induce mini-Mu transposition. New Biol. **3:**615–625.
13. **Galán, J. E., and R. Curtiss III.** 1990. Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling. Infect. Immun. **58:**1879–1885.
14. **Georgellis, D., S. Arvidson, and A. von Gabain.** 1992. Decay of *ompA* mRNA and processing of 9S RNA are immediately affected by shifts in growth rate, but in opposite manners. J. Bacteriol. **174:**5382–5390.
15. **Georgellis, D., T. Barlow, S. Arvidson, and A. von Gabain.** 1993. Retarded RNA turnover in *Escherichia coli*: a means of maintaining gene expression during anaerobiosis. Mol. Microbiol. **9:**375–381.
16. **Hillyard, D. R., M. Edlund, K. T. Hughes, M. Marsh, and N. P. Higgins.** 1990. Subunit-specific phenotypes of *Salmonella typhimurium* HU mutants. J. Bacteriol. **172:**5402–5407.
17. **Hinton, J. C. D., D. S. Santos, A. Seirafi, C. S. J. Hulton, G. D. Pavitt, and C. F. Higgins.** 1992. Expression and mutational analysis of the nucleoid-associated protein H-NS of *Salmonella typhimurium.* Mol. Microbiol. **6:**2327–2337.
18. **Lostroh, C. P., and C. A. Lee.** 2001. The *Salmonella* pathogenicity island-1 type III secretion system. Microbes Infect. **3:**1281–1291.
19. **Lucas, R. L., and C. A. Lee.** 2001. Roles of *hilC* and *hilD* in regulation of *hilA* expression in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **183:**2733–2745.
20. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Olekhnovich, I. N., and R. J. Kadner.** 2002. DNA-binding activities of the HilC and HilD virulence regulatory proteins of *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **184:**4148–4160.
22. **Pettijohn, D. E.** 1996. The nucleoid, p. 158–166. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*, 2nd ed., vol. 1. ASM Press, Washington, D.C.
23. **Schechter, L. M., S. M. Damrauer, and C. A. Lee.** 1999. Two AraC/XylS family members can independently counteract the effect of repressing sequences upstream of the *hilA* promoter. Mol. Microbiol. **32:**629–642.
24. **Schechter, L. M., and C. A. Lee.** 2001. AraC/XylS family members, HilC and HilD, directly bind and derepress the *S. typhimurium hilA* promoter. Mol. Microbiol. **40:**1289–1299.
25. **Spurio, R., M. Falconi, A. Brandi, C. L. Pon, and C. O. Gualerzi.** 1997. The oligomeric structure of nucleoid protein H-NS is necessary for recognition of intrinsically curved DNA and for DNA bending. EMBO J. **16:**1795–1805.
26. **Tupper, A. E., T. A. Owen-Hughes, D. W. Ussery, D. S. Santos, D. J. P. Ferguson, J. M. Sidebotham, J. C. D. Hinton, and C. F. Higgins.** 1994. The chromatin-associated protein H-NS alters DNA topology *in vitro.* EMBO J. **13:**258–268.
27. **Wallis, T. S., and E. E. Galyov.** 2000. Molecular basis of *Salmonella*-induced enteritis. Mol. Microbiol. **36:**997–1005.
28. **Wilson, R. L., S. J. Libby, A. M. Freet, J. D. Boddicker, T. F. Fahlen, and B. D. Jones.** 2001. Fis, a DNA nucleoid-associated protein, is involved in *Salmonella typhimurium* SPI-1 invasion gene expression. Mol. Microbiol. **39:**79–88.

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