Mechanism of *hilA* Repression by 1,2-Propanediol Consists of Two Distinct Pathways, One Dependent on and the Other Independent of Catabolic Production of Propionate, in *Salmonella enterica* Serovar Typhimurium

Shu-ichi Nakayama* and Haruo Watanabe

Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan

A glycerol dehydrogenase gene was selected as a multicopy suppressor rescuing the reduced *hilA* expression in the *Salmonella enterica* serovar Typhimurium *cpxA* mutant. A substrate of the enzyme, 1,2-propanediol, repressed *hilA* expression. The 1,2-propanediol-mediated repression at 150 mM, but not that at 300 mM, was abrogated by blocking the catabolism producing propionate from 1,2-propanediol.

Invasion into host epithelial cells in the intestine is the initial and essential step of pathogenesis by *Salmonella*. Most of the genes required for the invasion localize to a 40-kb region of the chromosome termed *Salmonella* pathogenicity island 1 (SPI-1) (9). Many genes within this region encode components of the Type III secretion machinery and effectors. These effectors are secreted through the functions of the type III secretion machinery. It has well been established that some of the SPI-1 region-encoded effectors, such as SipBCD, are essential mediators of invasion. The regulatory circuits of expression of the SPI-1 genes have been intensively analyzed (for reviews, see references 9, 16, and 26). One of the key steps of the circuits is regulation of the synthesis of the first global activator, HilA, since HilA is believed to be ultimately required for synthesis of both the effectors and the secretion machinery (11). Expression of the *hilA* gene and, consequently, the invasive phenotype is tightly regulated by a variety of environmental signals, such as oxygen concentration, osmolarity, and growth phase (13, 17, 25). Because of its important position in the circuits, many researchers have also investigated the regulatory mechanisms by which expression of the *hilA* gene is regulated. These works have identified many genetic factors controlling *hilA* expression. Briefly, a two-component system, BarA-SirA, activates *hilA* (3, 22). Fis, a DNA-nucleoid-associated protein (40); FadD, which is involved in uptake and degradation of fatty acids (28); and *fliZ*, a flagellar gene (21, 28), have been reported to be positive factors for *hilA* expression. Three AraC/XylS-type transcriptional factors, HilC, HilD, and RtsA, have been reported to be direct activation factors for *hilA* expression (12, 27, 36). The effect of CsrA-CsrB, a protein-RNA complex, on *hilA* expression seems be largely mediated through these three AraC/XylS-type factors (2, 3). Other two-component systems, i.e., PhoB-PhoR (28) and PhoP-PhoQ (33), have negative effects on *hilA* expression. Fahlen et al. screened genetic loci with negative effects on *hilA* and reported *ams*, *hupB*, *pag*, *hilE*, and *hha* as such loci (4, 14, 15).

Either, but not both, of the glycerol dehydrogenases is required for full activation of *hilA* expression. In a previous paper, we characterized the *cpxA* mutant of *Salmonella enterica* serovar Typhimurium (31). The *hilA* expression level was lower in the *cpxA* mutant than in the *cpxA*+ strain, and this effect was much greater when culture was at pH 6.0 than when it was at pH 8.0. At pH 6.0, the expression level in the *cpxA* mutant was only approximately 7% of that in the *cpxA*+ strain (31). Surprisingly, the putative cognate response regulator, CpxR, had no effect on *hilA* expression (31). In order to examine the mechanism of the CpxR-independent activation of *hilA* by CpxA, we screened multicopy suppressors that compensated for the phenotype of the *cpxA* mutant from a total *Salmonella* DNA library constructed with the pBR322 (7) vector. The *cpxA* mutant harboring pSN849 (Table 1) (31), which is white on pH 6.0-adjusted X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) agar (31), was transformed with the library. Dark blue colonies on the agar were screened, and the plasmids they contained were analyzed. We found that these suppressors fell into one of the following three genes: *hilC*, *rtsA*, or STM3529 (encoding a putative NAD-dependent glycerol dehydrogenase) (29). The effects of the *hilC* and *rtsA* genes on *hilA* expression have already been described in several reports (12, 27, 36). We were interested in the function of STM3529 (called *glhA* hereafter, for *g*lycerol dehydrogenase, *h*ypothetical or *a*lternative). We found that the multicopy effect of the cloned *glhA* on *hilA* expression was not necessarily complete but was partial. Indeed, at pH 6.0, introduction of the plasmid containing *glhA* into the *cpxA* mutant restored *hilA* expression to only 50% of the level in the *cpxA*+ strain (data not shown). This suggested that the phenotype of the *cpxA* mutant could not be explained by the involvement of *glhA* only, and we judged that *glhA* was a factor regulating *hilA* independently of the *cpxA* pathway. We further investigated the role of *glhA* in this regulation.

We constructed the *glhA*::*tetA* mutant SN3529 (Table 1) according to the method of allele exchange using the suicide vector pKH5002 (32) and monitored the *hilA* expression level in this mutant. However, the expression level was not significantly different from that in the wild-type (WT) parent, SL1344

* Corresponding author. Mailing address: Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan. Phone: 81-3-5285-1111, ext. 2226. Fax: 81-3-5285-1163. E-mail: shuichin@nih.go.jp.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype or description	Source (reference)
E. coli K-12		
JM109	*endA1 recA1 gyrA96 thi hsdR17*(r_K^- m_K^+) *relA1 supE44* Δ(*lac-proAB*) [F' *traD36 proAB+ lacI^{q}ZΔM15*], initial host for PCR-produced DNA cloning	C. Yanisch-Perron (41)
MS8	MC1061 *rnh::cat* Cm^r, host for manipulation of pKH5002 and its derivatives	H. Ohmori (32)
S. enterica serovar Typhimurium		
SL1344	Standard virulent strain, *hisG rpsL*	SGSC[a]
KK1501	LT2 *hsdSA29 hsdB121 hsdL6 metA22 metE551 trpC2 ilv-452 rpsL120 xyl-404 galE719* FelS2, initial host of DNAs prepared in *E. coli*	K. Kutsukake (23)
SN3529	SL1344 *glhA::tetA* Tet^r	This study
SN4108	SL1344 Δ*gldA::aphT* Km^r	This study
SN6000	SL1344 *glhA::tetA* Δ*gldA::aphT* Tet^r Km^r	This study
SN2036	SL1344 Δ*pocR::aphT* Km^r	This study
SN2040	SL1344 Δ*pduCDEGH::aphT* Km^r	This study
Plasmids		
pBR322	pMB1 replicon cloning vector, Amp^r Tet^r	F. Boliver (7)
pKH5002	Suicide vector for gene disruption, Amp^r	H. Ohmori (32)
pSN849	*hilA'-'lacZ* translational fusion gene cloned into pHSG595, Cm^r	S. Nakayama (31)
pGlhA	1,706-bp Sau3AI-NruI fragment consisting almost entirely of the *glhA* gene cloned into the BamHI-NruI sites of pBR322, Amp^r	This study
pPocR	PCR-generated fragment consisting of the *pocR* gene cloned into the SphI-SalI sites of pBR322, Amp^r	This study

[a] SGSC, *Salmonella* Genetic Stock Center, Calgary, Canada.

(Table 2), suggesting that GlhA was not necessarily required for activation of *hilA*. In the published genome sequence of *Salmonella enterica* serovar Typhimurium (29), there are two putative NAD-dependent glycerol dehydrogenase genes, *gldA* and *glhA*. We hypothesized that these two genes were redundant for activation of *hilA*. Hence, we constructed the Δ*gldA::aphT* mutant SN4108 (Table 1) and the *glhA::tetA* Δ*gldA::aphT* double mutant SN6000 (Table 1) according to the method reported by Datsenko and Wanner (10) and characterized them. *hilA* expression in the double mutant was decreased to approximately 60% of that in the parent, whereas in the Δ*gldA::aphT* single mutant, SN4108, the expression level was not significantly decreased (Table 2). These mutations, which are not linked on the chromosome, had a significant effect only when they were combined. We also confirmed that the *glhA*-expressing plasmid, pGlhA, which was selected as a multicopy suppressor (Table 1), fully complemented the *hilA* expression in the *glhA::tetA* Δ*gldA::aphT* double mutant (data not shown). On the other hand, attempts to clone the *gldA* gene on plasmid vectors resulted in instability of the resultant plasmids (data not shown). Overexpression of GldA, but not of

GlhA, thus might have some toxic effect on the bacterial cells under our culture conditions. Thus, we could not definitely demonstrate complementation of the double mutant by the cloned *gldA* gene. However, these results strongly suggest that either, but not both, of the two genes is required for full activation of *hilA* expression. We believe this means that the total finite activity of glycerol dehydrogenase is required for full activation, although demonstration of the glycerol dehydrogenase activities of the GlhA and GldA products is needed for this to be confirmed.

PDL represses *hilA* expression. The genetic data described above suggest that both *gldA* and *glhA* are involved in *hilA* activation. GldA and GlhA are predicted to have glycerol dehydrogenase activity (29). Therefore, we focused on how the glycerol dehydrogenases could be involved in gene regulation. We postulated that some organic compound, which could be a substrate of these enzymes, might influence *hilA* expression. A preliminary investigation indicated that glycerol, a representative substrate of the enzymes, had no effect on *hilA* expression when the concentration was not more than 3% (vol/vol) (about 326 mM) (data not shown). In *Escherichia coli*, 1,2-propanediol (PDL) is also established as a substrate of GldA, the *E. coli* glycerol dehydrogenase (1). Because the deduced amino acid sequences of *Salmonella* GldA and GlhA have significantly high similarity to that of *E. coli* GldA (1, 29), we examined the effect of PDL on *hilA* expression. We observed that addition of *R*-(−)-PDL into the medium at a concentration of 150 mM (at the initial point of growth and throughout the experiments described in this study) reduced *hilA* expression to 55.82% of that in the medium without PDL. In this initial experiment, we chose the *R*-(−) enantiomer of PDL because only this enantiomer is established as a substrate for the *E. coli* GldA (1).

This observation and the results described in the previous

TABLE 2. *hilA* expression levels reported by pSN849 in glycerol dehydrogenase mutants

Strain (relevant genotype)	β-Galactosidase activity[a]	% Expression compared to that in SL1344
SL1344 (*glhA+ gldA+*)	896 ± 119	100
SN3529 (*glhA::tetA gldA+*)	942 ± 63	110
SN4108 (*glhA+ ΔgldA::aphT*)	736 ± 25	88
SN6000 (*glhA::tetA ΔgldA::aphT*)	500 ± 8	60

[a] Activities are expressed as mean Miller units ± standard deviations. For culture conditions, see the legend to Fig. 1.

TABLE 3. Effect of R-(−)-1,2-propanediol on *hilA* expression
in strains SL1344 and SN6000

Strain (relevant genotype)	β-Galactosidase activity[a] at PDL concn (mM):		% Repression by 150 mM PDL[b]
	0	150	
SL1344 ($glhA^+$ $gldA^+$)	816 ± 78	455 ± 28	55.82
SN6000 ($glhA$::$tetA$ $\Delta gldA$::$aphT$)	491 ± 21	313 ± 17	63.74

[a] Activities are expressed as mean Miller units ± standard deviations. For culture conditions, see the legend to Fig. 1.
[b] Percent activity at 150 mM PDL compared to that at 0 mM PDL.

section raise two possibilities: first, that the glycerol dehydrogenases have a positive effect on *hilA* expression and R-(−)-PDL inhibits the effect through sequestration of the enzyme, and second, that R-(−)-PDL has a negative effect on *hilA* expression and the glycerol dehydrogenases suppress the effect through transformation of R-(−)-PDL into the dehydrogenated product, which rationally is hydroxyacetone. To distinguish these possibilities, we tested whether R-(−)-PDL further influenced *hilA* expression in the *glhA*::*tetA* $\Delta gldA$::*aphT* double mutant, SN6000. In this experiment, we found that R-(−)-PDL further repressed *hilA* expression in the mutant, although the degree of repression was slightly lower than that in the parent, SL1344 (Table 3). This result strongly supports the latter possibility described above, i.e., that R-(−)-PDL has a negative effect on *hilA* expression and the glycerol dehydrogenases partially suppress this effect, although this needs to be confirmed by direct demonstration that the GlhA and GldA products can catabolize PDL. However, the results of the preliminary experiment described in the fifth section of this report support the interpretation that GlhA and GldA catabolize PDL (see below).

Dose dependency of 1,2-propanediol-mediated repression. In the previous section, we described that addition of 150 mM R-(−)-PDL reduced *hilA* expression to 55.82% of that in medium without R-(−)-PDL. To better understand this repression, we investigated the dose dependency of this effect. At the same time, we examined whether the R-(−) and S-(+) enantiomers had different effects on *hilA* expression. The R-(−) and S-(+) enantiomers of PDL did not show a significant difference in their repressive effect at any concentration (data not shown). The reason why both enantiomers effectively repress *hilA* is unknown at present.

In the analysis of the dose dependency of the repressive effect, we found a striking characteristic. Namely, within the range of 50 to 150 mM, no significant decrease in *hilA* expression was observed with an increase in PDL concentration. This means that throughout this range of PDL concentrations, the level of *hilA* expression was maintained at around 55 to 60% of that in medium without PDL. However, when the concentration was further increased above this range, *hilA* expression again started to decrease. At 300 mM PDL, *hilA* expression was further reduced to 24% of that in medium without PDL. Such a unique, dual-phase nature of dose dependency implies that the PDL-mediated repression of *hilA* consisted of two distinct mechanisms, one functioning at lower concentrations and the other at higher concentrations. This was confirmed in the following analysis.

Involvement of *pocR* and the *pdu* operon in 1,2-propanediol-mediated *hilA* repression. Based on the above results, we attempted to analyze how PDL repressed *hilA* expression. In this background, the regulatory mechanism of the *pdu* operon provided some hints. The *pdu* operon, consisting of 21 genes (*pduABCDEGHJKLMNOPQSTUVWX*), encodes the enzymes that degrade PDL into propionate and propanol, although the functions of some of these genes are still unknown (6, 19, 20). Expression of the operon is activated by a transcriptional factor, PocR (5, 8, 34, 35). PocR belongs to the AraC family of regulators and requires PDL as a cofactor for activation of its own DNA-binding capacity (35). Thus, *pocR* is a genetic factor accomplishing PDL-dependent regulation of target genes. This prompted us to investigate whether PocR is also involved in the PDL-dependent repression of *hilA*. Hence, we constructed the $\Delta pocR$::*aphT* mutant SN2036 (Table 1) according to the method reported by Datsenko and Wanner (10) and compared its *hilA* expression with that of the parent WT strain, SL1344, in media containing 0 mM, 150 mM, and 300 mM PDL. As the two enantiomers of PDL had comparable effects on *hilA* expression (see above), we used a mixture of R-(−)- and S-(+)-PDL thereafter. In this analysis, we found that the $\Delta pocR$::*aphT* mutation almost completely abrogated the repression by 150 mM PDL (Fig. 1). Introduction of the plasmid pPocR (Table 1), which expresses PocR under control of the *tetA* promoter on pBR322, into the mutant restored the repression (Fig. 1). Thus, the simplest interpretation of the results was that PocR, through activation of its own DNA-binding capacity, directly repressed *hilA* expression in the presence of PDL, the cofactor of PocR. However, at the same time, we noted another possibility. PocR is an essential activator of the *pdu* operon, encod-
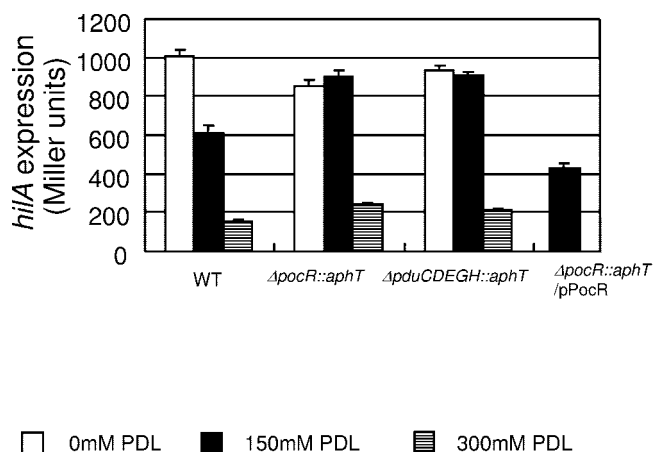

FIG. 1. Involvement of *pocR* and the *pdu* operon in *hilA* repression by 150 mM 1,2-propanediol. *hilA* expression levels reported by pSN849 in strains SL1344 (WT), SN2036 ($\Delta pocR$::*aphT*), SN2040 ($\Delta pduCDEGH$::*aphT*), and SN2036 harboring pPocR ($\Delta pocR$::*aphT*/pPocR) were monitored through β-galactosidase assay after cultivation in media supplemented with the indicated concentrations of a mixture of R-(−)- and S-(+)-1,2-propanediol. Throughout the experiments in this study, LB supplemented with 0.1 M NaCl and 0.1 M sodium phosphate buffer (pH 7.0) was employed as the basic medium. 1,2-Propanediol or sodium propionate was added at the indicated final concentrations when used. Bacterial cultures in the indicated media were incubated and kept static at 37°C until the optical density at 600 nm reached approximately 0.7. The cultured bacteria were harvested, suspended in Z buffer (30), and used for the β-galactosidase assay. Error bars indicate standard deviations.

ing enzymes responsible for propionate production from PDL (5, 8, 34, 35), and recently Lawhon et al. have reported that propionate represses *hilA* expression (24). Thus, it was plausible that the observed effect of the *pocR* mutation was the simple outcome of a defect in propionate production in the mutant. Hence, we further constructed the Δ*pduCDEGH*::*aphT* mutant SN2040 (Table 1) according to the method reported by Datsenko and Wanner (10). (Note that *pocR* remains intact in this mutant, because *pocR* is located upstream of the *pdu* operon and is transcribed divergently to it.) *pduCDE*, the three genes in the operon, encode the subunits of diol dehydratase, which catalyzes the conversion of PDL to propionaldehyde, the initial reaction to produce propionate from PDL, whereas *pduGH* encodes the subunits of diol dehydratase-reactivating factor (6, 19). Thus, it was expected that in this mutant, the catabolic pathway from PDL to propionate would be completely blocked but PocR would remain active. We investigated the response of this mutant to 150 mM PDL. This clearly showed that the pattern of *hilA* expression in the Δ*pduCDEGH*::*aphT* mutant was indistinguishable from that in the Δ*pocR*::*aphT* mutant (Fig. 1). Because cloning of the long region corresponding to the entire *pduCDEGH* was unsuccessful (data not shown), we could not definitely demonstrate the complementation of this Δ*pduCDEGH*::*aphT* mutant. However, these results strongly suggest that the repression by 150 mM PDL ultimately requires the production of propionate from PDL. Furthermore, we confirmed that in the Δ*pocR*::*aphT* mutant SN2036, in the Δ*pduCDEGH*::*aphT* mutant SN2040, and in strain SL1344, *hilA* was repressed by propionate (24). Indeed, in all three strains, the addition of 30 mM sodium propionate reduced *hilA* expression to about 32% of that in medium without sodium propionate. This observation is consistent with a previous report (24) and supports our interpretation that *hilA* repression by 150 mM PDL is exerted through propionate produced from PDL.

The effect of glycerol dehydrogenase activity on *hilA* expression is due to the catabolism of PDL. In the first section of this paper, we stated that the double mutations in glycerol dehydrogenases that are expected to catabolize PDL led to a reduction in *hilA* expression. Here, we found that the *hilA* repression by PDL at a lower concentration (150 mM) requires the function of the *pdu* operon. These data implied that the observed reduction of *hilA* expression in the *glhA*::*tetA* Δ*gldA*::*aphT* double mutant, SN6000, might also require the function of the *pdu* operon. Hence, we further inactivated *pduCDEGH* in SN6000 and monitored the *hilA* expression level in cells grown in medium without PDL or sodium propionate. This investigation showed that in this *glhA*::*tetA* Δ*gldA* Δ*pduCDEGH*::*aphT* mutant, the *hilA* expression level reported by pSN849 was 913 ± 25 Miller units, whereas that in SL1344 was 880 ± 21 Miller units. Thus, inactivation of *pduCDEGH* in SN6000 almost completely suppressed the phenotype of SN6000. This clearly indicates that the observed *hilA* repression in SN6000 requires the function of the *pdu* operon. This strongly suggests that when propionate production from the limited amount of endogenous PDL in the basic medium is blocked, PDL consumption by the glycerol dehydrogenases is no longer required for full activation of *hilA*. Thus, it can be interpreted that the ultimate role of the glycerol dehydrogenases in *hilA* regulation is to suppress the effect of propionate,

which is produced from PDL. Importantly, this result may also support our interpretation that the observed effect of the glycerol dehydrogenases is via PDL and that these glycerol dehydrogenases catabolize PDL (see previous sections).

***hilA* repression by a higher concentration of 1,2-propanediol does not require *pdu* operon.** In the third section, we stated the possibility that the mechanism of PDL-dependent repression at higher concentrations is different from that at lower concentrations. Accordingly, the levels of *hilA* expression in the WT strain SL1344, the Δ*pocR*::*aphT* mutant SN2036, and the Δ*pduCDEGH*::*aphT* mutant SN2040 at 300 mM PDL were also monitored. Rather surprisingly, all three strains showed almost the same level of *hilA* expression at this concentration of PDL (Fig. 1). The results of this analysis clearly indicate that repression by 300 mM PDL does not require *pocR* or *pduCDEGH* and, consequently, the production of propionate.

The simplest explanation for these results may be that PDL itself has no effect on *hilA* expression when the concentration is not more than 150 mM and that it has a repressive effect only at a concentration of 300 mM or higher. However, this simple interpretation is rather unlikely, as the repression by 300 mM PDL was as strong as fourfold (Fig. 1). Thus, it is rather hard to believe that PDL has no effect at all when the concentration is 150 mM. This issue should be clarified in the course of mechanistic studies of the pathways of repression by PDL in the future.

Conclusions and perspectives. PDL was shown to repress *hilA* expression. The mechanism of the PDL-dependent repression of *hilA* consists of two distinct pathways, one dependent on and the other independent of propionate production from PDL. These repression pathways could be weakened through consumption of PDL by the two NAD-dependent glycerol dehydrogenases of *Salmonella*. The specific mechanisms, as well as the biological significance, of the two repression pathways should be elucidated in the future.

PDL has been established as a safe compound (18, 38, 39) and is used as a solvent for many kinds of industrial products, including foods and cosmetics. Therefore, it can be anticipated that ingestion of PDL could prevent, at least partially, penetration of *Salmonella* into the intestinal epithelium through inhibition of *hilA* expression, without toxic effects in animals.

REFERENCES

1. **Altaras, N. E., and D. C. Cameron.** 1999. Metabolic engineering of a 1,2-propanediol pathway in *Escherichia coli*. Appl. Environ. Microbiol. **65:**1180–1185.
2. **Altier, C., M. Suyemoto, and S. D. Lowhon.** 2000. Regulation of *Salmonella enterica* serovar Typhimurium invasion genes by *csrA*. Infect. Immun. **68:**6790–6797.
3. **Altier, C., M. Suyemoto, A. I. Ruiz, K. D. Burnham, and R. Maurer.** 2000. Characterization of two novel regulatory genes affecting *Salmonella* invasion gene expression. Mol. Microbiol. **35:**635–646.
4. **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.
5. **Bobik, T. A., M. Ailion, and J. R. Roth.** 1992. A single regulatory gene integrates control of vitamin B$_{12}$ synthesis and propanediol degradation. J. Bacteriol. **174:**2253–2266.

6. **Bobik, T. A., G. D. Havemann, R. J. Busch, D. S. Williams, and H. C. Aldrich.** 1999. The propanediol utilization (*pdu*) operon of *Salmonella enterica* serovar Typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B_{12}-dependent 1,2-propanediol degradation. J. Bacteriol. **181:**5967–5975.

7. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Hyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–113.

8. **Chen, P., M. Ailion, T. A. Bobik, G. Stormo, and J. Roth.** 1995. Five promoters integrate control of the *cob/pdu* regulon in *Salmonella typhimurium*. J. Bacteriol. **177:**5401–5410.

9. **Darwin, K. H., and V. L. Miller.** 1999. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin. Microbiol. Rev. **12:**405–408.

10. **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.

11. **Eichelberg, K., and J. E. Galan.** 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.

12. **Ellermeier, C. D., and J. M. Slauch.** 2003. RtsA and RtsB coordinately regulate expression of the invasion and flagellar genes in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **185:**5096–5108.

13. **Ernst, R. K., D. M. Dombroski, and J. M. Merrick.** 1990. Anaerobiosis, type 1 fimbriae, and growth phase are factors that affect invasion of HEp-2 cells by *Salmonella typhimurium*. Infect. Immun. **58:**2014–2016.

14. **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.

15. **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.

16. **Galan, J. E.** 1996. Molecular genetic bases of *Salmonella* entry into host cells. Mol. Microbiol. **20:**263–271.

17. **Galan, 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.

18. **Gaunt, I. F., F. M. Carpanini, P. Grasso, and A. B. Lansdown.** 1972. Long-term toxicity of propylene glycol in rats. Food Cosmet. Toxicol. **10:**151–162.

19. **Havemann, G. D., and T. A. Bobik.** 2003. Protein content of polyhedral organelles involved in coenzyme B_{12}-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. **185:**5086–5095.

20. **Havemann, G. D., E. M. Sampson, and T. A. Bobik.** 2002. PduA is a shell protein of polyhedral organelles involved in coenzyme B_{12}-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. J. Bacteriol. **184:**1253–1261.

21. **Iyoda, S., T. Kamidoi, K. Hirose, K. Kutsukake, and H. Watanabe.** 2001. A flagellar gene *fliZ* regulates the expression of invasion genes and virulence phenotype in *Salmonella enterica* serovar Typhimurium. Microb. Pathog. **30:**81–90.

22. **Johnston, C., D. A. Pegues, C. J. Hueck, C. A. Lee, and S. I. Miller.** 1996. Transcriptional activation of *Salmonella typhimurium* invasion genes by a member of the phosphorylated response-regulator superfamily. Mol. Microbiol. **22:**715–727.

23. **Kutsukake, K., S. Iyoda, K. Ohnishi, and T. Iino.** 1994. Genetic and molecular analyses of the interaction between the flagellum-specific sigma and anti-sigma factors in *Salmonella typhimurium*. EMBO J. **13:**4568–4576.

24. **Lawhon, S. D., R. Maurer, M. Suyemoto, and C. Altier.** 2002. Intestinal short-chain fatty acids alter *Salmonella typhimurium* invasion gene expression and virulence through BarA/SirA. Mol. Microbiol. **46:**1451–1464.

25. **Lee, C. A., and S. Falkow.** 1990. The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. Proc. Natl. Acad. Sci. USA **87:**4304–4308.

26. **Lucas, R. L., and C. A. Lee.** 2000. Unravelling the mysteries of virulence gene regulation in *Salmonella typhimurium*. Mol. Microbiol. **36:**1024–1033.

27. **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.

28. **Lucas, R. L., C. P. Lostroh, C. C. DiRusso, M. P. Spector, B. L. Wanner, and C. A. Lee.** 2000. Multiple factors independently regulate *hilA* and invasion gene expression in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **182:**1872–1882.

29. **McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson.** 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:**852–856.

30. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

31. **Nakayama, S., A. Kushiro, T. Asahara, R. Tanaka, L. Hu, D. J. Kopecko, and H. Watanabe.** 2003. Activation of *hilA* expression at low pH requires the signal sensor CpxA, but not the cognate response regulator CpxR, in *Salmonella enterica* serovar Typhimurium. Microbiology **149:**2809–2817.

32. **Ohmori, H., M. Saitoh, T. Yasuda, T. Nagata, T. Fujii, M. Wachi, and K. Nagai.** 1995. The *pcsA* gene is identical to *dinD* in *Escherichia coli*. J. Bacteriol. **177:**156–165.

33. **Pegues, D. A., M. J. Hantman, I. Behlau, and S. I. Miller.** 1995. PhoP/PhoQ transcriptional repression of *Salmonella typhimurium* invasion genes: evidence for a role in protein secretion. Mol. Microbiol. **17:**169–181.

34. **Rondon, M. R., and J. C. Escalante-Semerena.** 1992. The *poc* locus is required for 1,2-propanediol-dependent transcription of the cobalamin biothynthetic (*cob*) and propanediol utilization (*pdu*) genes of *Salmonella typhimurium*. J. Bacteriol. **174:**2267–2272.

35. **Rondon, M. R., and J. C. Escalante-Semerena.** 1996. In vitro analysis of the interactions between the PocR regulatory protein and the promoter region of the cobalamin biosynthetic (*cob*) operon of *Salmonella typhimurium* LT2. J. Bacteriol. **178:**2196–2203.

36. **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.

37. **Schmieger, H.** 1972. Phage *P22*-mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.

38. **Trancik, R. J., and H. I. Maibach.** 1982. Propylene glycol: irritation or sensitization? Contact Dermatitis **8:**185–189.

39. **Weil, C. S., M. D. Woodside, H. F. Jr. Smyth, and C. P. Carpenter.** 1971. Results of feeding propylene glycol in the diet to dogs for two years. Food Cosmet. Toxicol. **9:**479–490.

40. **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.

41. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC vectors. Gene **33:**103–119.