JOURNAL OF BACTERIOLOGY, Sept. 1990, p. 5293–5298
0021-9193/90/095293-06$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 172, No. 9

Integration Host Factor Is a Negative Effector of In Vivo and In Vitro Expression of *ompC* in *Escherichia coli*

LIN HUANG, PING TSUI, AND MARTIN FREUNDLICH*

Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, New York 11794-5215

Received 24 April 1990/Accepted 15 June 1990

Integration host factor (IHF) of *Escherichia coli* is a DNA-binding protein involved in gene expression and other cellular functions in *E. coli* and some of its bacteriophages and plasmids. We report here that IHF is a direct negative effector of the *ompC* operon of *E. coli*. IHF binds to *ompC* DNA and protects a region of 35 base pairs located upstream from the *ompC* promoters. The addition of IHF to a purified in vitro transcription system inhibited transcription from two of the three *ompC* promoters. In vivo experiments suggest that the in vitro results are physiologically relevant. IHF mutants show increased expression of OmpC. In addition, the OmpC$^-$ phenotype of certain strains is completely suppressed by a mutation in IHF.

Integration host factor (IHF) of *Escherichia coli* is a multifunctional DNA-binding protein (3) that is a direct participant in certain site-specific recombination events and other processes in *E. coli* and some of its bacteriophages and plasmids (reviewed in references 5 and 11). This histonelike protein (5) has also been implicated in the expression of some *E. coli* (4, 9, 12, 27, 44) and phage (15, 22, 23, 26) genes. IHF alters gene expression in a number of ways, but its exact mechanism of action in these events is unknown (11). IHF binding can change DNA structure (reviewed in reference 11), and this property has been suggested to be central to its pleiotropic activities (5, 35, 40).

We have recently shown that IHF binds to multiple sites in the *ompB* promoter and blocks transcription (P. Tsui, L. Huang, R. Brissette, K. Tsung, M. Inouye, and M. Freundlich, submitted for publication). The protein products of the *ompB* operon, OmpR and EnvZ, are required for the expression and osmoregulation of the major outer membrane proteins OmpF and OmpC (16, 17). OmpR and EnvZ are members of a family of bacterial signal transduction proteins (36). OmpR is a DNA-binding protein that regulates *ompF* and *ompC* transcription (31, 32, 45). EnvZ is thought to act as an osmosensor and to affect *ompF* and *ompC* by phosphorylating (1, 6, 19) and dephosphorylating (20) OmpR. Two observations suggest that the effects of IHF on *ompB* are physiologically significant. One, IHF mutants have increased expression of *ompB* (Tsui et al., submitted), and two, IHF mutants have altered osmoregulation and increased expression of OmpF (44).

In the present study, we continued our investigation of the role of IHF in the *omp* system by examining the effects of this protein on *ompC* expression. We found that IHF is a negative effector of *ompC*. An IHF mutation completely suppresses the OmpC$^-$ phenotype of certain *ompR* and *envZ* mutants. In addition, in vitro experiments show that IHF strongly binds upstream from the *ompC* promoter region and inhibits transcription from two of the three *ompC* promoters. These data suggest that IHF exerts negative control of *ompC* expression in two ways—directly, by binding upstream from the *ompC* promoter region, and indirectly, by influencing the expression of OmpR and EnvZ.

* Corresponding author.

MATERIALS AND METHODS

Bacteria and plasmids. The *E. coli* strains and the plasmid used in this work are described in Table 1.

Growth of bacteria and enzyme assays. The bacteria were grown as described previously (44) in 0.8% nutrient broth (Difco Laboratories, Detroit, Mich.) supplemented with sucrose as noted in the text. β-Galactosidase activity was determined as described by Miller (28) on cells removed during mid-exponential growth.

Preparation of outer membrane proteins. Cells in mid-exponential growth were centrifuged at 10,000 × *g* and suspended in 4 ml of sodium phosphate (10 mM, pH 7.0). The outer membranes were prepared as described by Ramakrishnan et al. (34) and analyzed by 8 M urea–sodium dodecyl sulfate–polyacrylamide gel electrophoresis (30).

Gel retardation assays. Gel retardation assays were done essentially as described previously (43). Approximately 0.1 pmol of an end-labeled *Hpa*I–*Bam*HI (−406 to +108) fragment was incubated with IHF at 22°C for 30 min in the following buffer: 50 mM Tris hydrochloride (pH 6.5)–70 mM KCl–1 mM EDTA–7 mM $MgCl_2$–2 mM $CaCl_2$–5% (vol/vol) glycerol–1 mM β-mercaptoethanol. Poly(dI · dC) (Promega Biotec, Madison, Wis.) at 9 μg/ml was added to inhibit nonspecific binding of IHF to the DNA. The mixture was loaded onto a 6.8% polyacrylamide gel prepared in a buffer containing 6.7 mM Tris hydrochloride (pH 8.0), 3.3 mM sodium acetate, and 1 mM EDTA.

DNase I footprinting. Approximately 0.1 pmol of an *Hpa*I-*Bam*HI (−406 to +108) fragment was incubated with IHF at 22°C in the same buffer used in the gel retardation experiments. After 30 min, 2.5 ng of DNase I per ml was added and the incubation continued for 2 min. The reaction was terminated by adding 150 μl of stop solution (1.5 ammonium acetate, 37.5 mM EDTA, 50 μg of yeast tRNA per ml). The reaction products were isolated and analyzed by polyacrylamide gel electrophoresis as described previously (8).

In vitro transcription. The reaction mixture (25 μl) contained 80 mM Tris acetate (pH 7.9), 400 mM KCl, 0.4 mM EDTA, 0.4 mM dithiothreitol, 250 μM each ATP, GTP, and CTP, 25 μM UTP, [α-^{32}P]UTP (3,000 Ci/mmol), 0.1 pmol of an *Hpa*I-*Bam*HI (−406 to +108) fragment, and 1 U of RNA polymerase. After preincubation for 10 min at 37°C, the reaction was started by adding 4 mM magnesium acetate and rifampin (10 μg/ml). The reaction was terminated by adding proteinase K (20 μg/ml) and heating at 65°C for 20 min.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype	Source
MC4100	Wild type	T. Silhavy
MF2763	MC4100 Δ*himA82*	This study
MH760	MC4100 *ompR472*	T. Silhavy
MF2761	MH760 Δ*himA82*	This study
MH1160	MC4100 *ompR101*	T. Silhavy
MF2765	MH1160 Δ*himA82*	This study
CS12	MC4100 Φ(*ompC-lacZ*$^+$) *envZ*	M. Inouye
MF2767	CS12 Δ*himA82*	This study
JA221	*recA1*	M. Inouye
MF5001	JA221(pKI0041-C)	This study
pKI0041-C	pBR322 containing an *ompC* *Hpa*I-*Bam*HI fragment	M. Inouye

When used, IHF was preincubated in the reaction mixture for 20 min before RNA polymerase was added. The terminated reaction mixtures were loaded directly onto 8% polyacrylamide gels containing 7 M urea and fractionated by electrophoresis.

Materials. Restriction enzymes and DNA polymerase I (Klenow fragment) were obtained from New England BioLabs, Inc. (Beverly, Mass.). DNase I and urea were purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). RNA polymerase was obtained from U.S. Biochemical Corp. (Cleveland, Ohio). Proteinase K was purchased from 5 Prime-3 Prime Inc. Radioactive chemicals were purchased from ICN Radiochemicals. Most other chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

IHF mutation suppresses the OmpC$^-$ phenotype in an *ompR* and in an *envZ* mutant. We have found that *ompB* is negatively regulated by IHF (Tsui et al., submitted) and that IHF mutants have increased levels and altered osmoregulation of OmpF (44). However, only small changes in OmpC expression were found in IHF mutants (44). This was surprising since the levels of OmpF and OmpC are usually controlled in tandem by the products of the *ompB* operon (16, 17) and by numerous other regulatory factors (21, 37). To investigate this in more detail, we examined the effect of an IHF mutation on OmpC expression in a number of mutants in the *ompB* operon that are phenotypically OmpF$^+$ OmpC$^-$. The strains used in these studies are described in Table 1. MH760 contains an *ompR2* mutation which results in the complete loss of OmpC expression without a concomitant reduction in the levels of OmpF (16). We transduced an IHF mutation into this strain, and outer membranes were prepared and analyzed after growth of the cells in nutrient broth with or without 20% sucrose. The data in Fig. 1 show that the introduction of the IHF mutation into MH760 strongly suppressed the OmpC$^-$ phenotype of this strain. The levels of OmpC detected in the IHF mutant were comparable to those found in the wild type (Fig. 1). In addition, these levels increased when the cells were grown with added 10% sucrose, suggesting that the IHF mutation also allows for normal osmoregulation of OmpC. It is also apparent that osmoregulation of OmpF, which is lacking in MF760 (Fig. 1, lanes 5 and 6), is at least partially restored by the IHF mutation (Fig. 1, lanes 7 and 8).

The involvement of IHF in OmpC expression was further examined by using a mutant that has a cold-sensitive defect

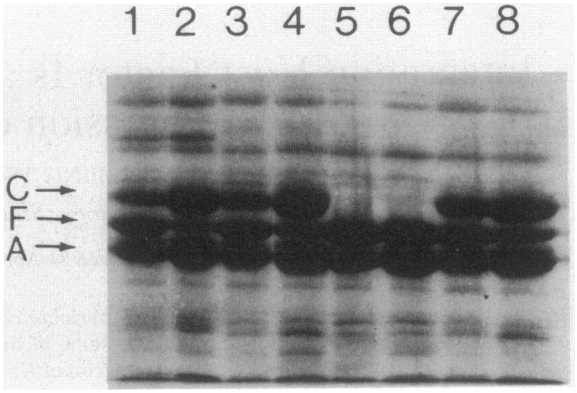


FIG. 1. Effect of an IHF mutation on OmpC expression in the *ompR2* mutant MH760. Strains M4100 (lanes 1 and 2), MF2763 (lanes 3 and 4), MH760 (lanes 5 and 6), and MF2761 (lanes 7 and 8) were grown in nutrient broth (lanes 1, 3, 5, and 7) or nutrient broth supplemented with 10% sucrose (lanes 2, 4, 6, and 8). C, F, A, Positions of OmpC, -F, and -A, respectively.

in EnvZ. This strain does not produce OmpC when it is grown in nutrient broth at 23°C. However, OmpC expression is close to normal if the mutant is grown at 37 or 23°C in media of high osmolarity (7). In our studies, we used strain CS12, which has the cold-sensitive defect in EnvZ and a chromosomal fusion between *lacZ* and the *ompC* promoter. We transduced an IHF mutation into this strain, and examined *ompC* expression by measuring β-galactosidase activity in cells grown under various conditions. The data in Table 2 show a complete absence of β-galactosidase activity in CS12 grown in nutrient broth at 23°C. In contrast, the introduction of an IHF mutation into this strain allowed considerable β-galactosidase activity under these conditions. In addition, β-galactosidase activity under all conditions was significantly higher in the IHF mutant. This ranged from 5.5-fold in cells grown in nutrient broth at 37°C to 3.1- to 4.8-fold when the cultures were grown at either temperature in nutrient broth supplemented with 10% sucrose.

IHF binds to *ompC* promoter region. The results presented above suggest that IHF negatively affects the expression of OmpC. The data, however, do not distinguish between a direct or indirect effect of IHF on this gene. To examine whether IHF directly participates in *ompC* expression, we investigated possible in vitro interactions of IHF with the *ompC* promoter region by the gel retardation technique (10, 13). The results in Fig. 2 show strong binding of IHF to an

TABLE 2. Effect of IHF mutation on *ompC* expression in CS12[a]

Strain	Growth temp (°C)	Sucrose (%)	β-Galactosidase activity[b]
CS12	37	0	1,219
	37	10	1,707
	23	0	0
	23	10	1,113
MF2767	37	0	6,784
	37	10	8,141
	23	0	586
	23	10	3,420

[a] Cells were grown in nutrient broth with or without 10% sucrose. The data are the average of three separate experiments.
[b] β-Galactosidase activity is shown in Miller units (28).

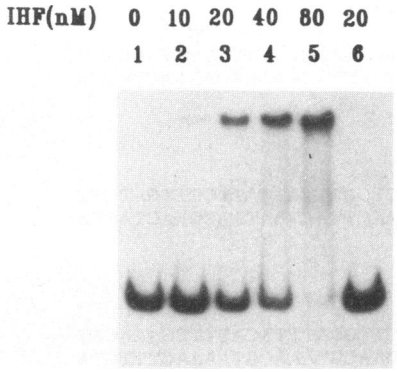


FIG. 2. Analysis of IHF binding by gel retardation. Approximately 0.1 pmol of an end-labeled *Hpa*I-*Bam*HI fragment (see Fig. 4) was incubated with IHF and the DNA and DNA-protein complexes were separated on a polyacrylamide gel as described in Materials and Methods. In lane 6, unlabeled fragment was added to the binding assay as competitor.

ompC promoter fragment. A single retarded DNA-protein complex was progressively formed as the concentration of IHF was increased from 10 to 80 nM.

Localization of IHF binding in *ompC* promoter region. The DNA fragment used for gel retardation was end labeled and used in DNase I footprinting experiments to further analyze the binding of IHF to the *ompC* promoter region. The data in Fig. 3 show a strongly protected area from −158 to −193 which is upstream from the *ompC* promoter region. This site contains a sequence (Fig. 4) identical to the 13-base-pair (bp) IHF DNA-binding consensus sequence found in known IHF-binding sites (3, 24). Approximately 6 nM IHF was required for 50% protection against DNase I digestion.

IHF inhibits transcription from two *ompC* promoters. The in vitro binding data, taken together with the results showing increased OmpC expression in IHF mutants, suggest that IHF directly blocks *ompC* transcription. We examined this possibility by adding IHF to an in vitro transcription reaction with a DNA fragment containing the *ompC* promoter region. The transcription pattern from this fragment is shown in Fig. 5. Estimation of the size of these RNAs suggests they are the transcripts previously reported to be produced in vitro by the three *ompC* promoters (32). The addition of IHF to the reaction mixture inhibited two of these promoters, P1 and P3. Transcription from the third promoter, P2, was slightly increased by IHF (Fig. 5). The amount of IHF required for 50% inhibition of transcription from P1 (80 nM) and P3 (40 nM) was higher than that needed for 50% binding (6 nM). This difference has been observed previously in other systems (22, 23) and may be due to differences in the assay conditions used in transcription compared with those used in the binding studies. Alternatively, high concentrations of IHF may be necessary for saturation of a low-affinity IHF-binding site that is required for inhibition. However, we have failed to identify any additional IHF-binding sites using concentrations of IHF as high as 160 nM (L. Huang and M. Freundlich, unpublished data).

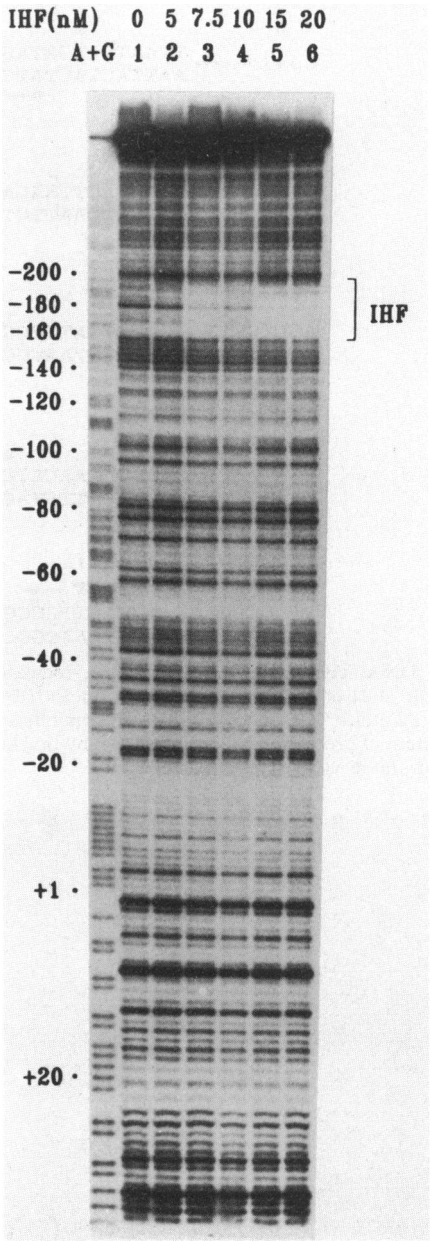


FIG. 3. DNase I footprinting of IHF in the *ompC* promoter-regulatory region. The sequence is numbered as shown in Fig. 4. The extent of protection by IHF is indicated by the brackets. The DNA fragment used was the same as in Fig. 2. Lane A+G shows the products of an A+G sequencing reaction of the fragment.

DISCUSSION

The data in this report clearly show that IHF is a negative effector of *ompC* expression. We have reported previously that IHF negatively regulates *ompF* (44) and *ompB* (Tsui et al., submitted), two additional operons involved in the expression of the *E. coli* major outer membrane proteins (16, 17). The products of the *ompB* operon, OmpR and EnvZ, are necessary for the osmoregulation and expression of *ompF* and *ompC* (16, 17). Therefore, the negative effects of IHF on *ompF* and *ompC* could be due to the inhibitory action of IHF on *ompB*. This could explain the suppression of the OmpC$^-$ phenotype in CS12 by the IHF mutation since it is known that the OmpC defect in *envZ* mutants can be suppressed by increased OmpR expression (14, 38). However, it is unlikely that suppression of the OmpC$^-$ phenotype in MH760 can be explained by this mechanism. This strain produces an altered OmpR protein that is unable to bind to the *ompC*

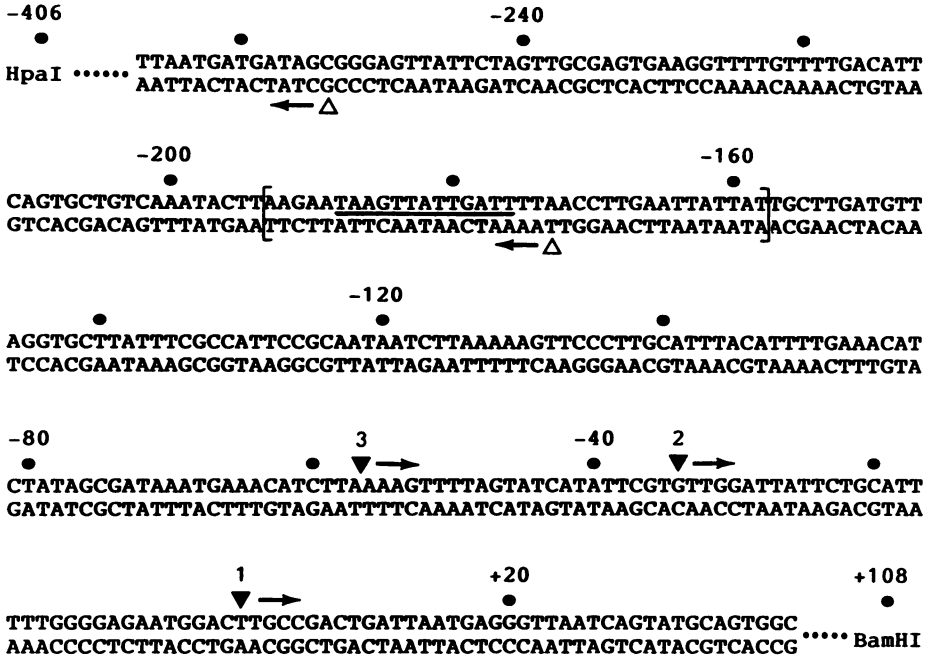
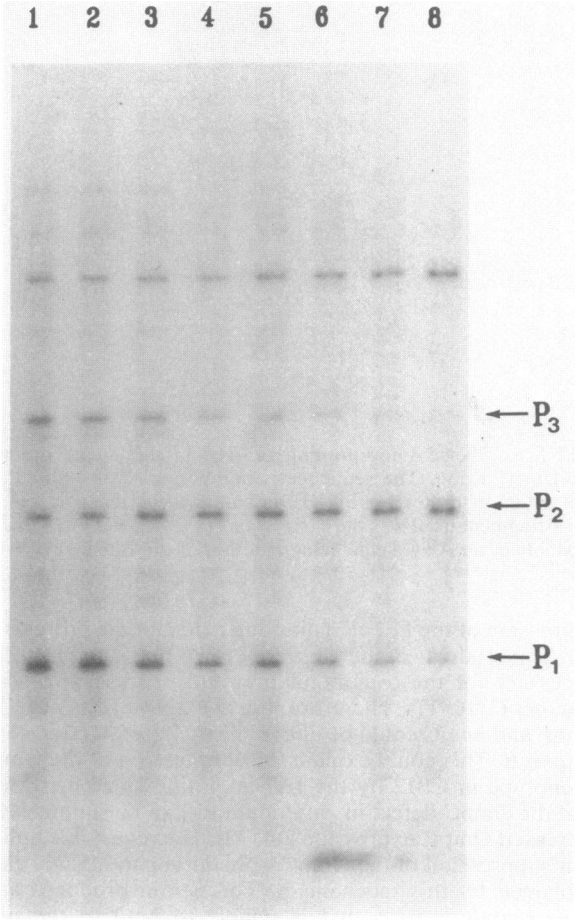


FIG. 4. DNA sequence of *ompC* promoter-regulatory region. The sequence is taken from Norioka et al. (32). The solid triangle at +1 indicates the start of transcription from promoter P1 as determined in vivo (32). Solid triangles 2 and 3 are the estimated start sites from promoters P2 and P3, respectively (32). The open triangles represent the start sites for the divergently transcribed 6S and 4.5S *micF* RNAs (2). The extent of protection by IHF is shown by brackets, and the IHF consensus sequence is underlined. The 514-bp *Hpa*I-*Bam*HI fragment was used in the in vitro experiments.



promoter in vitro or in vivo (7, 31, 45). In addition, it has been shown that MH760 remains OmpC$^-$ even in the presence of increased amounts of the altered OmpR protein (25, 33). Perhaps high expression of *ompB* in IHF mutants leads to changed levels of EnvZ in relation to OmpR. EnvZ can phosphorylate (1, 6, 19) and dephosphorylate (20) OmpR, which alters the DNA-binding properties of OmpR (19, 20). It has been suggested that the correct ratio of these two proteins is important for the normal activity of OmpR (14), and it has recently been found that quantitative and qualitative differences in EnvZ can suppress the OmpC$^-$ phenotype in MH760 (39). In addition, in vivo footprinting experiments suggest that the introduction of an IHF mutation into MH760 allows binding of the altered OmpR protein to the OmpR sites in the *ompC* promoter region (K. Tsung and M. Inouye, personal communication). Thus, different levels of EnvZ in IHF mutants could lead to changes in the binding properties of OmpR in MH760. Alternatively, these results may reflect a direct interaction between IHF and *ompC*. Our results show strong binding of IHF to a site centered approximately 80 bp upstream from the first of the three *ompC* promoters. In addition, transcription from two of these promoters is inhibited by IHF. These results are consistent with the

FIG. 5. Effect of IHF on in vitro transcription. Transcription was done with the *Hpa*I-*Bam*HI fragment (Fig. 4) as a template. There was little or no difference in the pattern of inhibition if IHF was added before or after the addition of RNA polymerase (L. Huang and M. Freundlich, unpublished data). Arrows P1, P2, and P3 indicate the transcripts from promoters P1, P2, and P3, respectively. Lanes: 1, 0 nM IHF; 2, 20 nM IHF; 3, 40 nM IHF; 4, 60 nM IHF; 5, 80 nM IHF; 6, 100 nM IHF; 7, 120 nM IHF; 8, 140 nM IHF. We do not know the origin of the high-molecular-weight band seen near the top of the gel.

positive effects on *ompC* expression found in IHF mutants. IHF may also influence the properties of other DNA-binding proteins, such as OmpR, that alter *ompC* expression. In this regard, there are four OmpR-binding sites that overlap the three *ompC* promoters (45). Thus, IHF may interact at many levels to negatively regulate the expression of *ompC*. Multilevel control by IHF of λ (26) and Mu (22) development has been previously reported.

IHF has been shown to have a direct positive or negative role in the expression of a number of genes in *E. coli*, λ, and Mu (reviewed in references 5 and 11). In some of the cases in which IHF inhibits gene expression, IHF binds to a site in the promoter that overlaps the −10 and/or −35 region (15, 23, 42; Tsui et al., submitted). Thus, in these systems, IHF may influence transcription directly by contacting RNA polymerase or altering promoter DNA conformation. However, in *ompC* the IHF-binding site is 50 to 120 bp upstream from the promoters (P1 and P3) that are inhibited by this protein. It is therefore not apparent how IHF alters promoter function in this system. IHF inhibits the PcM promoter in Mu when it is bound to a site 80 bp downstream from the start of transcription (22). Action at a distance by IHF has also been suggested for the positive role of this protein in *ilvBN* transcription in *E. coli* (P. Tsui and M. Freundlich, unpublished data), DNA replication in pSC101 (40), and Mu transposition (41). How IHF acts to influence these events is not known, but it has been suggested that the ability of IHF to bend the DNA to which it binds is essential to its mode of action in these systems (18, 35, 40, 41). Perhaps a change in DNA conformation when IHF binds in *ompC* results in alterations in DNA structure that lead to a reduction in P1 and P3 promoter function.

Finally, the IHF-binding site described in this report is not only close to the *ompC* promoter region but is also in close proximity to the *micF* promoter region (2). *micF* RNA is involved in the process of osmoregulation (29), and the *micF* gene is known to be coordinately regulated with *ompC* (32). Therefore, this "*ompC*" IHF site may participate in the tandem control of these two genes.

ACKNOWLEDGMENTS

We thank T. Silhavy for strains MH760 and MH1160 and M. Inouye for strain CS12. We are grateful to H. Nash for his gift of purified IHF protein.

This research was supported by Public Health Service grant GM17152 from the National Institutes of Health.

LITERATURE CITED

1. **Aiba, H., T. Mizuno, and S. Mizushima.** 1989. Transfer of phosphoryl group between two regulatory proteins involved in osmoregulatory expression of the *ompF* and *ompC* genes in *Escherichia coli*. J. Biol. Chem. **264**:8563–8567.
2. **Anderson, J., N. Delihas, K. Ikenaka, P. J. Green, O. Pines, O. Iceril, and M. Inouye.** 1987. The isolation and characterization of RNA coded by the *micF* gene in *Escherichia coli*. Nucleic Acids Res. **15**:2089–2101.
3. **Craig, N., and H. A. Nash.** 1984. *E. coli* integration host factor binds to specific sites in DNA. Cell **39**:707–716.
4. **Dorman, C. J., and C. F. Higgins.** 1987. Fimbrial phase variation in *Escherichia coli*: dependence on integration host factor and homologies with other site-specific recombinases. J. Bacteriol. **169**:3840–3843.
5. **Drlica, K., and J. Rouviere-Yaniv.** 1987. Histone-like proteins of bacteria. Microbiol. Rev. **51**:301–319.
6. **Forst, S., J. Delgado, and M. Inouye.** 1989. Phosphorylation of OmpR by the osmosensor EnvZ modulates expression of the *ompF* and *ompC* genes in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86**:6052–6056.
7. **Forst, S., and M. Inouye.** 1988. Environmentally regulated gene expression for membrane proteins in *Escherichia coli*. Annu. Rev. Cell Biol. **4**:21–42.
8. **Friden, P., P. Tsui, K. Okamoto, and M. Freundlich.** 1984. Interaction of cyclic AMP receptor protein with the *ilvB* biosynthetic operon in *E. coli*. Nucleic Acids Res. **12**:8145–8160.
9. **Friden, P., K. Voelkel, R., Sternglanz, and M. Freundlich.** 1984. Reduced expression of the isoleucine and valine enzymes in integration host factor mutants of *Escherichia coli*. J. Mol. Biol. **172**:573–579.
10. **Fried, M., and D. Crothers.** 1981. Equilibria and kinetics of Lac repressor operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6526.
11. **Friedman, D. I.** 1988. Integration host factor: a protein for all reasons. Cell **55**:545–554.
12. **Friedman, D. I., E. J. Olson, D. Carver, and M. Gellert.** 1984. Synergistic effect of *himA* and *gyrB* mutations: evidence that Him functions control expression of *ilv* and *xyl* genes. J. Bacteriol. **157**:484–489.
13. **Garner, M. M., and A. Revzin.** 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: applications to components of the *E. coli* lactose operon regulatory system. Nucleic Acids Res. **9**:3047–3060.
14. **Gibson, M. M., E. M. Ellis, K. A. Graeme-Cook, and C. F. Higgins.** 1987. OmpR and EnvZ are pleiotropic regulatory proteins: positive regulation of the tripeptide permease (*tppB*) of *Salmonella typhimurium*. Mol. Gen. Genet. **207**:120–129.
15. **Griffo, G., A. B. Oppenheim, and M. E. Gottesman.** 1989. Repression of the p*cin* promoter by integrative host factor. J. Mol. Biol. **209**:55–64.
16. **Hall, M. N., and T. J. Silhavy.** 1981. The *ompB* locus and the regulation of the major outer membrane porin proteins of *Escherichia coli* K-12. J. Mol. Biol. **146**:23–43.
17. **Hall, M. N., and T. J. Silhavy.** 1981. Genetic analysis of the *ompB* locus in *Escherichia coli* K-12. J. Mol. Biol. **151**:1–15.
18. **Higgins, N. P., D. A. Collier, M. W. Kilpatrick, and H. M. Krause.** 1989. Supercoiling and integration host factor change the DNA conformation and alter the flow of convergent transcription in phage Mu. J. Biol. Chem. **264**:3035–3042.
19. **Igo, M. M., A. J. Ninfa, and T. J. Silhavy.** 1989. A bacterial environmental sensor that functions as a protein kinase and stimulates transcriptional activation. Genes Dev. **3**:598–605.
20. **Igo, M. M., A. J. Ninfa, J. B. Stock, and T. J. Silhavy.** 1989. Phosphorylation and dephosphorylation of a bacterial transcriptional activator by a transmembrane receptor. Genes Dev. **3**:1725–1734.
21. **Kawaji, H., T. Mizuno, and S. Mizushima.** 1979. Influence of molecular size and osmolarity of sugars and dextrans on the synthesis of outer membrane proteins O-8 and O-9. J. Bacteriol. **140**:843–847.
22. **Krause, H. M., and N. P. Higgins.** 1986. Positive and negative regulation of the Mu operator by Mu repressor and *Escherichia coli* integration host factor. J. Biol. Chem. **261**:3744–3752.
23. **Kur, J., N. Hasam, and W. Szybalski.** 1989. Physical and biological consequences of interactions between integration host factor (IHF) and coliphage lambda late p'_R promoter and its mutants. Gene **81**:1–15.
24. **Leong, J. M., S. Nunes-Duby, C. F. Lesser, P. Youderian, M. M. Susskind, and A. Landy.** 1985. The φ80 and P22 attachment sites: primary structure and interaction with *Escherichia coli* integration host factor. J. Biol. Chem. **260**:4468–4477.
25. **Liljestrom, P., J. Luokkamaki, and E. Tapio Palva.** 1987. Isolation and characterization of a substitution mutation in the *ompR* gene of *Salmonella typhimurium* LT2. J. Bacteriol. **169**:438–441.
26. **Miller, H. I.** 1981. Multilevel regulation of bacteriophage λ lysogeny by the *E. coli himA* gene. Cell **5**:269–276.
27. **Miller, H. I., M. Kirk, and H. Echols.** 1981. SOS induction and autoregulation of the *himA* gene for site-specific recombination in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:6754–6758.
28. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1984. A unique

mechanism regulating expression: translational inhibition by a complementary RNA transcript (micRNA). Proc. Natl. Acad. Sci. USA **81**:1966–1970.

30. **Mizuno, T., and M. Kageyama.** 1978. Separation and characterization of the outer membrane of *Pseudomonas aeruginosa*. J. Biochem. **84**:179–191.
31. **Mizuno, T., K. Masashi, Y. Jo, and S. Mizushima.** 1988. Interaction of OmpR, a positive regulator, with the osmoregulated *ompC* and *ompF* genes in *Escherichia coli*. J. Biol. Chem. **263**:1008–1012.
32. **Norioka, S., G. Ramakrishnan, K., Ikenaka, and M. Inouye.** 1986. Interaction of a transcriptional activator, OmpR, with reciprocally osmoregulated genes, *ompF* and *ompC*, of *Escherichia coli*. J. Biol. Chem. **261**:17113–17119.
33. **Pirhonen, M., H. T. Saarilahti, S. Kurkela, and E. T. Palva.** 1986. *In vivo* cloning and characterization of mutations of the regulatory locus *ompR* of *Escherichia coli* K12. Mol. Gen. Genet. **203**:520–523.
34. **Ramakrishnan, G., K. Ikenaka, and M. Inouye.** 1985. Uncoupling of osmoregulation of the *Escherichia coli* K-12 *ompF* gene from *ompB*-dependent transcription. J. Bacteriol. **163**:82–87.
35. **Robertson, C. A., and H. A. Nash.** 1988. Bending of the bacteriophage λ attachment site by *Escherichia coli* integration host factor. J. Biol. Chem. **263**:3544–3557.
36. **Ronson, C. W., B. T. Nixon, and F. M. Ausubel.** 1987. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell **49**:579–581.
37. **Scott, N. W., and C. R. Harwood.** 1980. Studies on the influence of the cyclic AMP system on major outer membrane proteins of *Escherichia coli* K12. FEMS Microbiol. Lett. **9**:95–98.
38. **Slauch, J. M., S. Garrett, D. E. Jackson, and T. J. Silhavy.** 1988. EnvZ functions through OmpR to control porin gene expression in *Escherichia coli* K-12. J. Bacteriol. **170**:439–441.
39. **Slauch, J. M., and T. J. Silhavy.** 1989. Genetic analysis of the switch that controls porin gene expression in *Escherichia coli* K-12. J. Mol. Biol. **210**:281–292.
40. **Stenzel, T. T., P. Patel, and D. Bastia.** 1987. The integration host factor of *Escherichia coli* binds to bent DNA at the origin of replication of the plasmid pSC101. Cell **49**:709–717.
41. **Surette, M. G., B. D. Lavoie, and G. Chaconas.** 1989. Action at a distance in Mu DNA transposition: an enhancer-like element is the site of action of supercoiling relief activity by integration host factor (IHF). EMBO J. **8**:3483–3489.
42. **Thompson, R. J., and G. Mosig.** 1988. Integration host factor (IHF) represses a *Chlamydomonas* chloroplast promoter in *E. coli*. Nucleic Acids Res. **16**:3313–3326.
43. **Tsui, P., and M. Freundlich.** 1988. Integration host factor binds specifically to sites in the *ilvGMEDA* operon in *Escherichia coli*. J. Mol. Biol. **203**:817–820.
44. **Tsui, P., V. Helu, and M. Freundlich.** 1988. Altered osmoregulation of *ompF* in integration host factor mutants of *Escherichia coli*. J. Bacteriol. **170**:4950–4953.
45. **Tsung, K., R. E. Brissette, and M. Inouye.** 1989. Identification of the DNA-binding domain of the OmpR protein required for transcriptional activation of the *ompF* and *ompC* genes of *Escherichia coli* by *in vivo* DNA footprinting. J. Biol. Chem. **264**:10104–10109.