JOURNAL OF BACTERIOLOGY, Sept. 1994, p. 5537–5540
0021-9193/94/$04.00+0
Copyright © 1994, American Society for Microbiology
Vol. 176, No. 17

The H-NS Protein Is Involved in the Biogenesis of Flagella in *Escherichia coli*

PHILIPPE BERTIN,[1]* ERIKO TERAO,[2] EUN HEE LEE,[3] PHILIPPE LEJEUNE,[1]†
CHARLES COLSON,[2] ANTOINE DANCHIN,[1] AND EKKEHARD COLLATZ[3]

Unité de Régulation de l'Expression Génétique, Institut Pasteur, F-75724 Paris Cedex 15,[1] and LRMA, Université Paris VI, F-75270 Paris Cedex 06,[3] France, and Département de Biologie, Université de Louvain, B-1348 Louvain-la-Neuve, Belgium[2]

Received 1 December 1993/Accepted 11 June 1994

The function of the flagellum-chemotaxis regulon requires the expression of many genes and is positively regulated by the cyclic AMP-catabolite activator protein (cAMP-CAP) complex. In this paper, we show that motile behavior was affected in *Escherichia coli hns* mutants. The loss of motility resulted from a complete lack of flagella. A decrease in the level of transcription of the *flhD* and *fliA* genes, which are both required for the synthesis of flagella, was observed in the presence of an *hns* mutation. Furthermore, the Fla$^-$ phenotype was not reversed to the wild type in the presence of a *cfs* mutation which renders the flagellum synthesis independent of the cAMP-CAP complex. These results suggest that the H-NS protein acts as a positive regulator of genes involved in the biogenesis of flagella by a mechanism independent of the cAMP-CAP pathway.

In order to survive and develop in highly varied environments, microorganisms have to constantly monitor external conditions and respond to changes in pH, osmolarity, temperature, or chemicals. One of these adaptative behaviors causes motile bacteria to swim toward attractants (e.g., amino acids or carbohydrates) and away from repellents (e.g., alcohols and other toxic substances) in response to environmental cues.

Bacterial motility is dependent on the presence of flagella. In *Escherichia coli*, the biosynthesis of these multicomponent structures requires the expression of about 40 genes clustered at several regions on the chromosome. The transcription of the flagellar operons forms an ordered cascade in which the expression of genes located at a given level requires the transcription of another gene at an upper level. At the top of the hierarchy, the *flhC* and *flhD* genes constitute the master operon which controls the expression of all other flagellar genes (15, 23).

The expression of the master operon is sensitive to catabolite repression and is positively regulated by the cyclic AMP-catabolite activator protein (cAMP-CAP) complex (2, 4). Furthermore, the transcription of flagellar genes requires the DnaK, DnaJ, and GrpE heat shock proteins (29). The organization of the bacterial membrane is also known to affect swarming properties of *E. coli*. For example, flagellum formation is impaired in lipopolysaccharide (LPS)-deficient strains (17, 26). Similarly, the *pss* mutation results in a drastic reduction in the membrane of phosphatidylethanolamine and in loss of flagellation (27). Finally, the swarming properties can be altered by adverse conditions (e.g., high temperature and high salt concentration) which result in transcriptional repression of the *flhD* operon (28).

Recently, it has been shown that mutations in the *hns* gene alter the motility of *Salmonella typhimurium* (11). This gene was identified as the structural gene for the DNA-binding protein H-NS (9, 13, 24), isolated from *E. coli* in 1971 as a transcription factor (14). This protein was later shown to be involved in the organization of the bacterial chromosome by affecting the level of DNA condensation (30). Moreover, its synthesis is increased during the cold shock response (20) and is under negative autoregulation in the exponential growth phase (7, 8, 30). In *E. coli*, mutations at the *hns* locus are highly pleiotropic and are known to affect the expression of several apparently unrelated genes (6, 10, 33).

We have previously described the isolation of pleiotropic *hns* mutants of *E. coli*. These strains showed an increased resistance to kanamycin in the presence of plasmid pGR71 (5, 21). In contrast, an increased susceptibility to chloramphenicol was observed in the *hns* mutants, in spite of similar chloramphenicol acetyltransferase activities in wild-type and mutant strains (21). This phenotype could be due to an alteration of membrane permeability and prompted us to investigate the possible role of the H-NS protein in cell envelope and/or flagellum formation. In the present paper, we show that H-NS affects the biogenesis of flagella by controlling the expression of the flagellum-chemotaxis master regulon.

Bacterial strains and growth conditions. The bacterial strains used in this study are listed in Table 1. Standard media were LB broth and M63 medium (25). For examination of flagella by electron microscopy, cells were grown in tryptone medium (25). For testing the effect of catabolite repression on flagellar gene expression, Vogel-Bonner minimal medium (31) was used with 100 mM D-glucose as a carbon source. When needed, thiamine and amino acids were added at 1 and 40 μg/ml, respectively. The following antibiotics were used at the indicated final concentrations (in micrograms per milliliter): ampicillin, 25; kanamycin, 50; and tetracycline, 15.

Mutation in *hns* causes a loss of motility. To determine whether the *hns* gene was involved in the assembly and/or function of *E. coli* flagella, we tested the motility of wild-type and mutant strains. Tryptone swarm plates containing 1% Bacto-Tryptone, 0.5% NaCl, and 0.3% Bacto-Agar were used

* Corresponding author. Mailing address: Unité de Régulation de l'Expression Génétique, Institut Pasteur, 28 rue du Dr. Roux, F-75724 Paris Cedex 15, France. Fax: (33) 45 68 89 48. Electronic mail address: phbertin@pasteur.fr.

† Present address: Laboratoire de Génétique Moléculaire des Microorganismes, INSA de Lyon, F-69621 Villeurbanne Cedex, France.

TABLE 1. Bacterial strains

E. coli strain	Relevant genotype	Reference or source
TP500	*zch-506*::Tn*10*	22
TP504	*zch-506*::Tn*10* Δ(*hns tdk adhE oppABCD*) *118*	22
PHL403	Δ*lacZ169 zch-506*::Tn*10*	P. Lejeune
PS2651	Δ*lacZ169 zch-506*::Tn*10 hns-1000*::Tn*seq1*	19
PS2652	Δ*lacZ169 zch-506*::Tn*10 hns-1001*::Tn*seq1*	J. Landgraf
YK4331	*nalA flhD*::Mu d(Ap^r *lac*)	16
YK4337	*nalA fliA*::Mu d(Ap^r *lac*)	16
BE1201	*nalA flhD*::Mu d(Ap^r *lac*) *zch-506*::Tn*10* Δ(*hns tdk adhE oppABCD*)*118*	This study
BE1202	*nalA fliA*::Mu d(Ap^r *lac*) *zch-506*::Tn*10* Δ(*hns tdk adhE oppABCD*)*118*	This study
BE1405	*nalA flhD*::Mu d(Ap^r *lac*) *hns-1001*::Tn*seq1*	This study
BE1406	*nalA fliA*::Mu d(Ap^r *lac*) *hns-1001*::Tn*seq1*	This study
JLV15-4	Δ*cya-854 cfs*	32
JLV45-3	Δ*cya-854* Δ*crp-45 cfs*	32
JLV70-2	Δ*crp-45 cfs*	32
BE1407	Δ*cya-854 cfs hns-1001*::Tn*seq1*	This study
BE1408	Δ*cya-854* Δ*crp-45 cfs hns-1001*::Tn*seq1*	This study
BE1409	Δ*crp-45 cfs hns-1001*::Tn*seq1*	This study

to assess the motility of bacteria (1). Swarm plates were inoculated with a stab of approximately 10^6 cells and incubated at 30°C for 15 h. Wild-type strain TP500 swarmed on semisolid medium. In contrast, the *hns-118* mutant TP504 was nonmotile (Fig. 1).

The TP504 mutant strain was isolated on the basis of its increased resistance to kanamycin in the presence of plasmid pGR71 (5, 22). This mutant carries a deletion including *hns*, *tdk*, *adhE*, and *oppABCD* but leaving *galU* intact (data not shown). To determine whether the *hns* mutation by itself was responsible for the loss of motility, we tested the swarming behavior of *hns* strains PS2651 and PS2652 in comparison with their wild-type parent PHL403. These strains were isolated as suppressors of a *glyA* mutation by transposon mutagenesis as described previously (19). The insertion is located in the promoter region of the *hns* gene in PS2651 (19) and in the 17th codon of the *hns* coding region in PS2652 (18). Nonmotile behavior similar to that observed in the presence of the *hns-118* mutation was shown by both strains (Table 2). We also tested the motility of strain PS2652(pDIA510). The plasmid was constructed by cloning a 900-bp *Eco*RI-*Sna*BI fragment

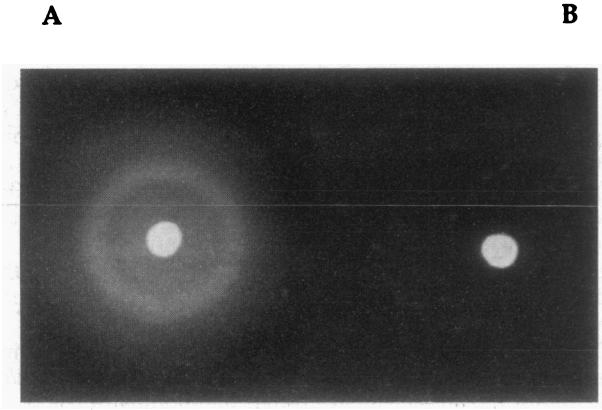

FIG. 1. Results of motility assay on semisolid medium. (A) Wild-type strain TP500; (B) *hns-118* mutant TP504.

TABLE 2. Motility of *E. coli* strains on semisolid agar plates

Strain	Relevant genotype	Motility[a]
PHL403	Wild type	31 ± 3
PS2651	*hns-1000*	3 ± 1
PS2652	*hns-1001*	3 ± 1
	hns-1001/pDIA510	24 ± 3
JLV15-4	Δ*cya cfs*	21 ± 2
BE1407	Δ*cya cfs hns-1001*	3 ± 1
JLV45-3	Δ*cya* Δ*crp cfs*	16 ± 1
BE1408	Δ*cya* Δ*crp cfs hns-1001*	3 ± 1
JLV70-2	Δ*crp cfs*	32 ± 3
BE1409	Δ*crp cfs hns-1001*	3 ± 1

[a] Expressed as the diameter of the swarming ring (in millimeters) after 15 h at 30°C. Data are the mean values ± standard deviations of six independent experiments.

carrying the *hns* gene into pBR322 and was introduced into *hns* derivatives as previously described (5). These strains were cultivated in the presence of 25 μg of ampicillin per ml. We had observed that use of higher antibiotic concentrations resulted in the selection of plasmid mutations with altered levels of *hns* expression (data not shown). In the presence of plasmid pDIA510, the altered motility resulting from the *hns-1001* mutation was greatly, but not fully, reversed to the wild-type level (Table 2). This partial reversion could be due to an *hns* gene dosage effect. A critical parameter might be the copy number of *hns*, as has been suggested from complementation experiments with *hns* mutants in which OmpC and OmpF synthesis and *proU* expression were measured (3, 24). Taken together, these results suggest that *hns* mutation is itself responsible for the loss of motility.

The nonmotile phenotype results from the lack of flagella. The swarming behavior can be affected either by an alteration of chemotaxis or by a defect in the assembly of flagella (15, 23). Bacterial motility was examined by phase-contrast microscopy. While the wild-type strain TP500 was fully motile, TP504 cells were completely nonmotile under the same conditions. In order to determine whether the failure of TP504 to swarm was due to nonfunctional flagella or to an impairment of flagellum formation, we examined wild-type and mutant strains by both transmission and scanning electron microscopy. Wild-type and mutant strains were grown without shaking at 28°C and fixed with 2.5% glutaraldehyde. To visualize bacteria by transmission electron microscopy, cells in 1 drop of the suspension were allowed to sediment for 5 min onto 300-mesh grids (Graticules, Tonbridge, United Kingdom) coated with Formvar (1% in chloroform). The grids were gently washed in water and dried on Whatman 3MM paper. Unstained bacteria were examined at 80 keV with a Jeol 100 SX electron microscope. For observation by scanning electron microscopy, the samples were filtered onto a polyester membrane with a 0.2-μm pore size (Cyclopore, Louvain-la-Neuve, Belgium). Membranes were washed in 0.1 M phosphate buffer (pH 7.4) and postfixed for 1 h in osmium tetroxide (2% in phosphate buffer). After a washing in the same buffer, samples were dehydrated through graded concentrations of acetone and finally critical-point dried with liquid CO_2 (critical-point dryer apparatus from Balzers Union, Balzers, Liechtenstein). The dried specimens were fixed on copper stubs, coated with gold in a sputtering apparatus (Balzers Union), and examined at 25 keV with a Jeol 840 scanning electron microscope. The presence of flagella on the wild-type strain TP500 was observed (Fig. 2A and C). In contrast, the *hns-118* strain TP504 showed no flagella (Fig. 2B and D).

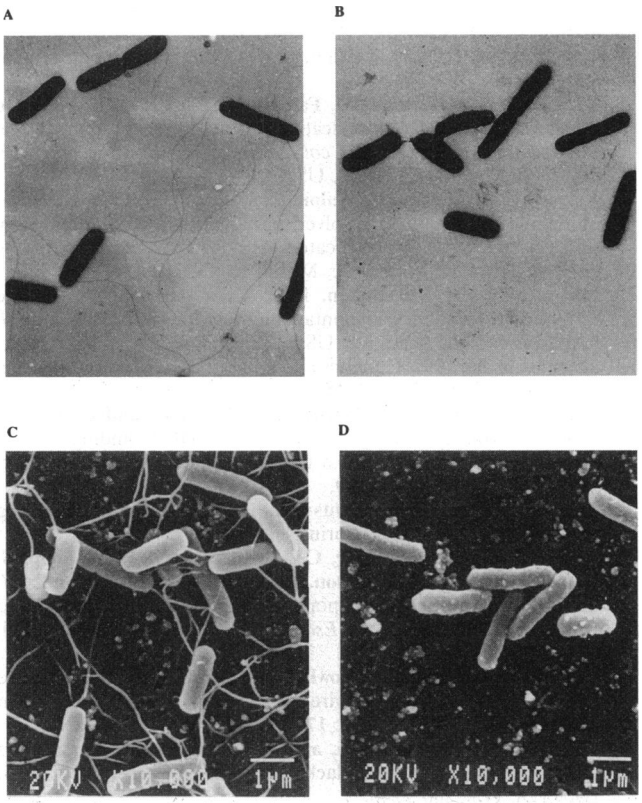

FIG. 2. Transmission and scanning electron microscopy of wild-type strain TP500 (A and C, respectively) and *hns-118* mutant TP504 (B and D, respectively).

H-NS affects the transcription of the flagellum biosynthesis genes. The so-called deep-rough mutants of *E. coli* are known to produce outer membranes with incomplete LPS and are nonmotile. For example, the *galU* and *rfaG* mutants are nonmotile because of an impairment in flagellum formation (17, 26). To investigate the possible influence of the H-NS protein on the organization of this outer membrane component, we have analyzed the LPS banding patterns of strain TP504 and its wild-type parent after polyacrylamide gel electrophoresis by the method of Hitchcock and Brown (12). No difference in the electrophoretic profiles of purified LPS between wild-type and *hns* strains was observed (data not shown). This observation suggests that the loss of motility of the *hns* strains does not result from sugar side chain alterations of the LPS.

Alternatively, the lack of flagella could result from a decrease in transcription of genes involved in the biogenesis of flagella. In order to explore this possibility, we introduced the *hns-118* and *hns-1001* mutations into strains YK4331 and YK4337, using phage P1 *vir* as previously described (5). These strains carry transcriptional *flhD-lacZ* and *fliA-lacZ* fusions, respectively (16). The *flhD* operon encodes FlhD and FlhC, which are believed to act as alternative sigma factors required for expression of all genes in the flagellum regulon, while the *fliA* gene encodes the flagellar sigma factor σ^F (15, 23). The β-galactosidase activity in these pairs of isogenic strains was measured by the method of Miller (25). The results are presented in Table 3. In the presence of both *hns* mutations, a threefold reduction and a fourfold reduction in the activity of the *flhD-lacZ* and *fliA-lacZ* transcriptional fusions were observed, respectively. It has been shown previously that cells grown in the presence of 100 mM D-glucose are nonmotile and lack flagella (28, 29). Growth under such conditions resulted in a less than twofold reduction of β-galactosidase activity from the *flhD-lacZ* fusion and in a threefold reduction of activity from the *fliA-lacZ* fusion (28, 29) (Table 2). Therefore, the reduced activity of the two transcriptional fusions observed in *hns* mutants seems to be sufficient to explain the lack of flagella and the concomitant loss of motility.

TABLE 3. Effect of *hns* mutation on the activity of *flhD-lacZ* and *fliA-lacZ* transcriptional fusions

Strain (relevant genotype) and medium[a]	β-Galactosidase sp act[b]
YK4331 (wild type)	371 ± 6
100 mM D-Glucose	261 ± 4
BE1201 (*hns-118*)	130 ± 3
BE1405 (*hns-1001*)	134 ± 2
YK4337 (wild type)	298 ± 5
100 mM D-Glucose	125 ± 2
BE1202 (*hns-118*)	81 ± 3
BE1406 (*hns-1001*)	75 ± 2

[a] Cells were grown in LB medium at 37°C except for the study of catabolite repression that utilized Vogel-Bonner medium supplemented with D-glucose.
[b] Expressed as defined by Miller (25). Data are the mean values of duplicate samples ± standard deviations from three independent experiments.

The flagellum-chemotaxis master operon is positively regulated by cAMP-CAP. Consequently, flagellar synthesis and motility are abolished in *cya* and *crp* mutants and under conditions of catabolite repression (15, 23). Suppressor mutations of this nonmotile phenotype in the *flhD* operon have been selected. In the presence of such a constitutive flagellar synthesis (*cfs*) mutation, synthesis of flagella is independent of cAMP-CAP (32). We wondered whether the lack of motility in *hns* strains was related to this regulatory pathway. Therefore, we introduced the *hns-1001* mutation by P1 transduction into *cfs* strains JLV15-4, JLV45-3, and JLV70-2, giving rise to strains BE1407, BE1408, and BE1409, respectively. The motile behavior of each *cfs* strain was suppressed by the presence of the *hns* mutation (Table 2). This suggests that the nonflagellate phenotype caused by the *hns* mutation is independent of the cAMP-CAP regulatory pathway.

The results presented here show that *hns* mutations affect the function of the flagellum-chemotaxis regulon by a mechanism independent of the cAMP-CAP pathway. The expression of several genes involved in flagellum biosynthesis was decreased in the *hns* mutant strains, which indicates that their transcription requires the H-NS protein. Such a positive effect of this transcriptional regulator on gene expression is the first described so far. Analysis of the molecular mechanism by which the H-NS protein affects the biogenesis of flagella is in progress.

We thank J. Adler, C. A. Gross, and J. Landgraf for gifts of strains. We are grateful to A. Baulard for photographic assistance and to E. Pradel for critical reading of the manuscript.

This study was supported in part by a grant (CJF-8901) from the Institut National de la Santé et de la Recherche Médicale, Paris.

REFERENCES

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153**:708–716.
2. **Adler, J., and B. Templeton.** 1967. The effect of environmental conditions on the motility of *Escherichia coli*. J. Bacteriol. **46**:175–184.
3. **Barr, G. C., N. Ní Bhriain, and C. J. Dorman.** 1992. Identification of two new genetically active regions associated with the *osmZ*

locus of *Escherichia coli*: role in regulation of *proU* expression and mutagenic effect at *cya*, the structural gene for adenylate cyclase. J. Bacteriol. **174:**998–1006.
4. **Bartlett, D. H., B. B. Frantz, and P. Matsumura.** 1988. Flagellar transcriptional activators FlbB and FlaI: gene sequences and 5′ consensus sequences of operons under FlbB and FlaI control. J. Bacteriol. **170:**1575–1581.
5. **Bertin, P., P. Lejeune, C. Colson, and A. Danchin.** 1992. Mutations in *bglY*, the structural gene for the DNA-binding protein H1 of *Escherichia coli*, increase the expression of the kanamycin resistance gene carried by plasmid pGR71. Mol. Gen. Genet. **233:**184–192.
6. **Bertin, P., P. Lejeune, C. Laurent-Winter, and A. Danchin.** 1990. Mutations in *bglY*, the structural gene for the DNA-binding protein H1, affect expression of several *Escherichia coli* genes. Biochimie **72:**889–891.
7. **Dersch, P., K. Schmidt, and E. Bremer.** 1993. Synthesis of the *Escherichia coli* K-12 nucleoid-associated DNA-binding protein H-NS is subjected to growth-phase control and autoregulation. Mol. Microbiol. **8:**875–889.
8. **Falconi, M., N. P. Higgins, R. Spurio, C. L. Pon, and C. O. Gualerzi.** 1993. Expression of the gene encoding the major bacterial nucleoid protein H-NS is subject to transcriptional autorepression. J. Bacteriol. **10:**273–282.
9. **Göransson, M., B. Sondén, P. Nilsson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature (London) **344:**682–685.
10. **Higgins, C. F., J. C. D. Hinton, C. J. S. Hulton, T. Owen-Hughes, G. D. Pavitt, and A. Seirafi.** 1990. Protein H1: a role for chromatin structure in the regulation of bacterial gene expression and virulence? Mol. Microbiol. **4:**2007–2012.
11. **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 H-NS of *Salmonella typhimurium*. Mol. Microbiol. **6:**2327–2337.
12. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154:**269–277.
13. **Hulton, C. S. J., A. Seirafi, J. C. D. Hinton, J. M. Sidebotham, L. Waddell, G. D. Pavitt, T. Owen-Hughes, A. Spassky, H. Buc, and C. F. Higgins.** 1990. Histone-like protein H1 (H-NS), DNA supercoiling and gene expression in bacteria. Cell **63:**631–642.
14. **Jacquet, M., R. Cukier-Kahn, J. Pla, and F. Gros.** 1971. A thermostable protein factor acting on in vitro DNA transcription. Biochem. Biophys. Res. Commun. **45:**1597–1607.
15. **Jones, C. J., and S. Aizawa.** 1991. The bacterial flagellum and flagellar motor: structure, assembly and function. Adv. Microb. Physiol. **32:**109–172.
16. **Komeda, Y.** 1982. Transcriptional control of flagellar genes in *Escherichia coli* K-12. J. Bacteriol. **168:**1315–1318.
17. **Komeda, Y., T. Icho, and T. Iino.** 1977. Effects of *galU* mutation on flagellar formation in *Escherichia coli*. J. Bacteriol. **129:**908–915.
18. **Landgraf, J. R.** Personal communication.
19. **Landgraf, J. R., M. Levinthal, and A. Danchin.** Submitted for publication.
20. **La Teana, A., A. Brandi, M. Falconi, R. Spurio, C. L. Pon, and C. O. Gualerzi.** 1991. Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS. Proc. Natl. Acad. Sci. USA **88:**10907–10911.
21. **Lejeune, P., P. Bertin, C. Walon, K. Willemot, C. Colson, and A. Danchin.** 1989. A locus involved in kanamycin, chloramphenicol and L-serine resistance is located in the *bglY-galU* region of the *Escherichia coli* chromosome. Mol. Gen. Genet. **218:**361–363.
22. **Lejeune, P., and A. Danchin.** 1990. Mutations in the *bglY* gene increase the frequency of spontaneous deletions in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **87:**360–363.
23. **Macnab, R. M.** 1992. Genetics and biogenesis of bacterial flagella. Annu. Rev. Genet. **26:**131–158.
24. **May, G., P. Dersch, M. Haardt, A. Middendorf, and E. Bremer.** 1990. The *osmZ* (*bglY*) gene encodes the DNA-binding protein H-NS (H1a), a component of the *Escherichia coli* K12 nucleoid. Mol. Gen. Genet. **224:**81–90.
25. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Parker, C. T., A. W. Kloser, C. A. Schnaitman, M. A. Stein, S. Gottesman, and B. W. Gibson.** 1992. Role of the *rfaG* and *rfaP* genes in determining the lipopolysaccharide core structure and cell surface properties of *Escherichia coli* K-12. J. Bacteriol. **174:**2525–2538.
27. **Shi, W., M. Bogdanov, W. Dowhan, and D. R. Zusman.** 1993. The *pss* and *psd* genes are required for motility and chemotaxis in *Escherichia coli*. J. Bacteriol. **175:**7711–7714.
28. **Shi, W., C. Li, C. J. Louise, and J. Adler.** 1993. Mechanism of adverse conditions causing lack of flagella in *Escherichia coli*. J. Bacteriol. **175:**2236–2240.
29. **Shi, W., Y. Zhou, J. Wild, J. Adler, and C. A. Gross.** 1992. DnaK, DnaJ, and GrpE are required for flagellum synthesis in *Escherichia coli*. J. Bacteriol. **174:**6256–6263.
30. **Ueguchi, C., M. Kakeda, and T. Mizuno.** 1993. Autoregulatory expression of the *Escherichia coli hns* gene encoding a nucleoid protein: H-NS functions as a repressor of its own transcription. Mol. Gen. Genet. **236:**171–178.
31. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
32. **Vogler, A. P., and J. W. Lengeler.** 1987. Indirect role of adenylate cyclase and cyclic AMP in chemotaxis to phosphotransferase system carbohydrates in *Escherichia coli* K-12. J. Bacteriol. **169:**593–599.
33. **Yamada, H., T. Yoshida, K. Tanaka, C. Sasakawa, and T. Mizuno.** 1991. Molecular analysis of the *Escherichia coli hns* gene encoding a DNA-binding protein, which preferentially recognizes curved DNA sequences. Mol. Gen. Genet. **230:**332–336.