JOURNAL OF BACTERIOLOGY, June 1997, p. 3818–3821 Vol. 179, No. 11
0021-9193/97/$04.00+0

The *Escherichia coli* Flagellar Transcriptional Activator *flhD* Regulates Cell Division through Induction of the Acid Response Gene *cadA*

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FlhD is a positive regulator of *cadA*. A mutant with a transposon-mediated *lacZ* fusion to *cadA* exhibited a cell division phenotype similar to that of the *flhD* mutant and had FlhD-dependent β-galactosidase activity. Under different growth conditions, the cell division rate correlated with the level of expression of *cadA*.

In a previous study (13), we demonstrated that *Escherichia coli flhD* is involved in a process other than flagellar expression, namely, reducing the cell division rate as cells enter stationary phase. It was demonstrated that *flhD* mutant cells grown in tryptone broth (TB) (1% tryptone, 1% NaCl) continued to divide at a rate that is typical for mid-exponential growth at a time when wild-type cells started to reduce their cell division rate. *flhD* mutant cells were smaller than wild-type cells as they entered stationary phase, and it was demonstrated that this characteristic correlated with their inability to sense the depletion of serine from the medium, which signals wild-type cells to reduce their cell division rate. It was discussed that the signal cascade probably included phosphorylation of OmpR (15) by acetyl phosphate (14). It was unclear how the expression of *flhD* led to the reduction of the cell division rate.

We now demonstrate that the effect of FlhD upon the cell division rate is mediated through induction of *cadA*. The *cad* operon consists of *cadB* and *cadA*, encoding the lysine/cadaverine antiporter CadB and the enzyme lysine decarboxylase CadA. Expression of this operon is dependent on the regulator CadC, whose gene is located upstream of *cadA*. The expression from the p_{cad} promoter is induced by low pH, low oxygen, rich medium, and excess lysine. All of these signals activate *cadBA* expression via a *cadC*-dependent pathway (10–12, 18).

Construction of the *cadA* transposon mutant. All strains, plasmids, and phage used in this study are listed in Table 1. Plasmid pPB10 was constructed by cloning the *flhD* gene, obtained from plasmid pPM61 (1) by PCR, into the arabinose-inducible plasmid pKB130 (17), kindly provided by L. Katz (Abbott Laboratories, Chicago, Ill.). *pcnB1 zad-981*::mini-Kn from strain RP7947 (8), kindly provided by J. S. Parkinson (University of Utah, Salt Lake City), was used to reduce the plasmid copy number and the background expression. The resulting strain BP78 exhibited complementation of the *flhD* mutation in the presence of arabinose. The *cadA*::Tn*10-lacZ* fusion was obtained by transposon mutagenesis. Cells of strain BP78 were infected with phage lysate λ Tn*phoA′-2* (19), kindly provided by B. Wanner (Purdue University, West Lafayette, Ind.). Cells were plated on tetracycline to identify chromosomal insertions. One tetracycline-resistant colony was blue in the presence of arabinose and white in its absence. This strain was designated BP87 and is characterized in this report. *cadA*::Tn*10-lacZ* was transduced by P1 transduction into the wild-type strain YK410 and the *flhD* mutant strain YK4131. In Luria-Bertani medium (LB) (TB plus 0.5% yeast extract) and late-exponential phase, the β-galactosidase activity of wild-type cells (strain BP101) was 210 ± 37.5 Miller units. The β-galactosidase activity of the *flhD* mutant (strain BP102) was 13.7 ± 3.1 Miller units. By endonuclease digestion with *Sac*I, the chromosomal junction was cloned into pUC18. The sequence revealed identity with *cadA*. Consistent with this observation, the lysine decarboxylase activity (5) of the mutant was reduced to 5%. The remaining activity is probably due to the constitutive lysine decarboxylase encoded by *ldcC* (20). The expression of *lacZ* from *cadA*::Tn*10-lacZ* was acid inducible by a factor of 14 (3,000 Miller units) at pH 5.8.

Figure 1 demonstrates the phenotype of the *cadA* mutant. The presence of Tn*10-lacZ* in *cadA* caused the same cell division phenotype as described previously (13) for the *flhD* mutant. Wild-type (strain YK410) and *flhD* (strain YK4131) and *cadA* (strain BP101) mutant cells and *cadA* mutant cells containing plasmid pKB130 (BP186) or the *cadA*-expressing plasmid pBP15 (strain BP211) were grown in TB. pBP15 was constructed in a manner similar to that of pBP10 and contains *cadA* under the control of the arabinose promoter. The optical density at 600 nm was monitored, and the number of cells was determined by microscopy as described previously (13). Neither the mutations in *flhD* or *cadA* nor the presence of a plasmid exhibited a significant effect upon the optical density of the cultures (Fig. 1A). All cultures entered stationary phase after approximately 2 h at an optical density of 0.3 to 0.5. Wild-type cells reduced their cell division rate from 1.6 to 0.85 generation (gen)/h after 1.5 h of growth and reached a cell density of 6×10^7 cells per ml after 3.5 h (Fig. 1B). As demonstrated previously (13), the *flhD* mutant kept dividing at a rapid rate of 2.1 gen/h, reaching a cell density of 10^9 cells per ml. The *cadA* mutant exhibited a behavior identical to that of the *flhD* mutant, reaching cell densities of 10^9 cells per ml after 3.5 h. At this time (3.5 h), the average length of a *cadA* mutant cell was 0.98 ± 0.05 μm in comparison to the average lengths of 2.27 ± 0.1 μm for wild-type cells and 0.85 ± 0.05 μm for *flhD* mutant cells, determined by electron microscopy as described previously (13). In early exponential phase, cells of all strains had the same size. The control plasmid, pKB130, did not exhibit any effect upon the cell division rate. The expression of *cadA* from plasmid pBP15 restored the lysine decarboxylase activity and complemented the cell division phenotype. Cells divided at a steady rate of 1 gen/h from the time of inoculation. This is approximately the division rate of wild-type cells in stationary phase. Mutant cells did not exhibit the biphasic cell division behavior wild-type cells did, presumably

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TABLE 1. Strains, phage, and plasmids used in this study

Strain, phage, or plasmid	Genotype or description	Plasmid	Source, construction, or reference
E. coli			
BP78	MC4100 ara+ *pcnB*	pBP10	This study
BP87	BP78 *cadA*::Tn*10-lacZ*	pBP10	This study
BP101	YK410 *cadA*::Tn*10-lacZ*		BP87 × YK410
BP102	YK4131 *cadA*::Tn*10-lacZ*		BP87 × YK4131
BP168	YK410 *cadC*::Tn*10*		EP247 × YK410
BP186	BP101	pKB130	This study
BP204	BP168	pXL27	This study
BP205	BP168	pXL25	This study
BP206	BP168	pXL26	This study
BP207	BP168	pT7-7	This study
BP211	BP101	pBP15	This study
DM1	MC4100 ara+ *pcnB*		RP7947 × MC4100
EP247	W3110 *cadC*::Tn*10*		E. Olson
MC4100 ara+	F⁻ *lacU169 rpsL relA thi flhD*		Malcolm Casadaban
RP7947	RP437 *pcnB1 zad-981*:: mini-Kn		8
YK410	F⁻ *lacU169 thi araD139 strA pyrC46 nalA thyA his*		6
YK4131	YK410 *flhD* point mutation		7
Phage			
λ Tn*phoA′-2*	*phoA′ lacZ tetAR tnp*		19
Plasmids			
pKB130	pUC18 p_{BAD} *lacZ*		17
pBP10	pKB130 *flhD*		This study
pBP15	pKB130 *cadA*		This study
pCX38	pFZY1 *ftsQAZ*::*lacZ*		Larry Rothfield
pPM61	pMK2004 *flhDC*		1
pT7-7	Overexpression vector		16
pXL25	pT7-7 *flhD*		9
pXL26	pT7-7 *flhC*		9
pXL27	pT7-7 *flhDC*		9

because *cadA* was expressed constitutively. The complementation of the *cadA* phenotype by providing *cadA* in *trans* indicates that the effect of the transposon upon the cell division rate was due to the mutation in *cadA* and not to a polar effect upon downstream genes.

The cell division rate correlates with the expression level of *cadA*. Above a certain threshold level of *cadA* expression, cells are able to reduce their cell division rate. Wild-type cells (strain BP101) and *flhD* mutant cells (strain BP102), each containing *cadA*::Tn*10-lacZ*, were grown in different media, and the β-galactosidase activity was determined (Fig. 2). In addition, the cell division rate was determined for wild-type cells (strain YK410) and *flhD* mutant cells (strain YK4131). Overall, the expression patterns of wild-type cells and the mutant cells were the same. However, in all media tested, the expression of *cadA* was higher in wild-type cells than in *flhD* mutant cells, clearly indicating that expression of *cadA* can be induced by FlhD. Expression increased with the richness of the medium from minimal M9 (1.3% $Na_2HPO_4 \cdot 7H_2O$, 0.3% KH_2PO_4, 5% NaCl, 0.1% NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 1 mM $FeCl_2$, 0.2% glycerol) to TB and LB. L-Lysine (20 mM) or morpholineethanesulfonic acid (MES) (100 mM) (pH 5.8) was added to the M9 cultures (M9 5.8) after 3 h of growth to induce *cadA* expression (11, 18). Expression was highest at low pH. The area between the dashed lines in Fig. 2 documents a threshold expression of *cadA*. At levels above 60 Miller units, cell division rates were reduced during the postexponential phase, and below 17 Miller units, cell division rates were not reduced. Wild-type cells achieved this threshold level of *cadA* expression in TB, LB 7.6, LB, M9 with lysine, M9 5.8, and LB 5.8. *flhD* mutant cells need acidic pH to induce enough *cadA* to be able to reduce the cell division rate. Lysine is not able to adequately increase the expression of *cadA* in an *flhD* mutant to cause a reduction of the cell division rate. The different effects which lead to an expression of *cadA* seem to be additive, with acid having the most significant effect. The cell division rate of *cadA* mutants was not affected by acid or lysine (data not shown). Therefore, it was concluded that the effect of acid or lysine addition upon the cell division rate was directly caused by the induction of *cadA* and not by other acid- or lysine-inducible genes.

FlhD regulates *cadA* expression in a CadC-independent manner. The overexpression of FlhD itself was sufficient to reverse the cell division phenotype in a *cadC* mutant. Wild-type cells (strain YK410) and *flhD* (strain YK4131), *cadA* (strain BP101), and *cadC* (strain BP168) mutant cells and *cadC* mutant cells containing plasmid pT7-7, pXL25, pXL26, or

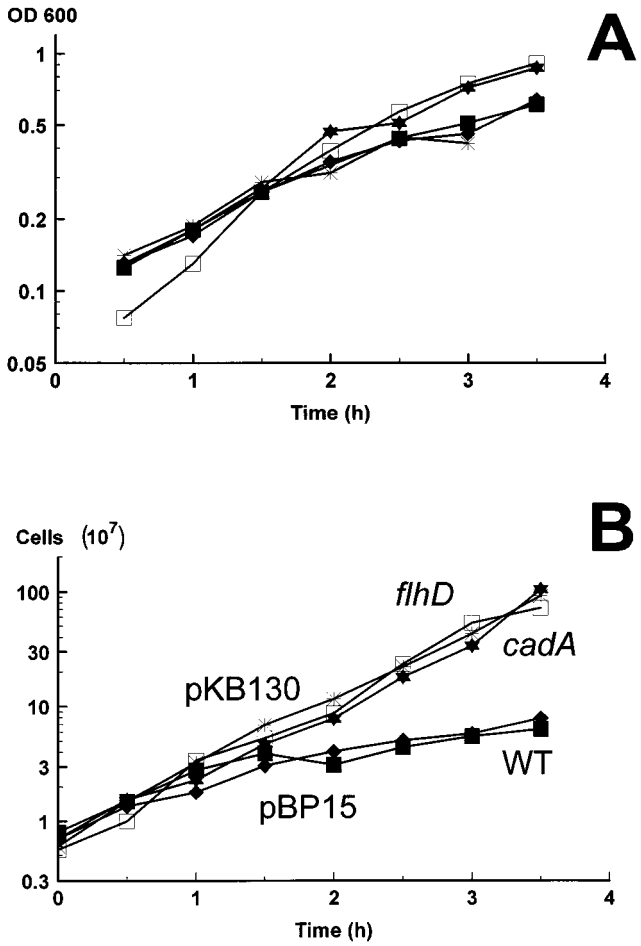

FIG. 1. Growth of wild-type and *flhD* and *cadA* mutants. The cells used were the wild-type (WT) (strain YK410; solid squares) and *flhD* mutant (strain YK4131; open squares) and *cadA* mutant (strain BP101; stars) cells and *cadA* mutant cells bearing plasmid pKB130 (strain BP186; asterisks) or pBP15 (strain BP211; diamonds). Cells were grown in TB over a period of 3.5 h. The growth rate was measured as optical density at 600 nm (OD 600) (A), and the number of cells was determined by microscopy (B). The experiment was done two to four times, and the means of the populations were determined. Standard errors were less than 20%.

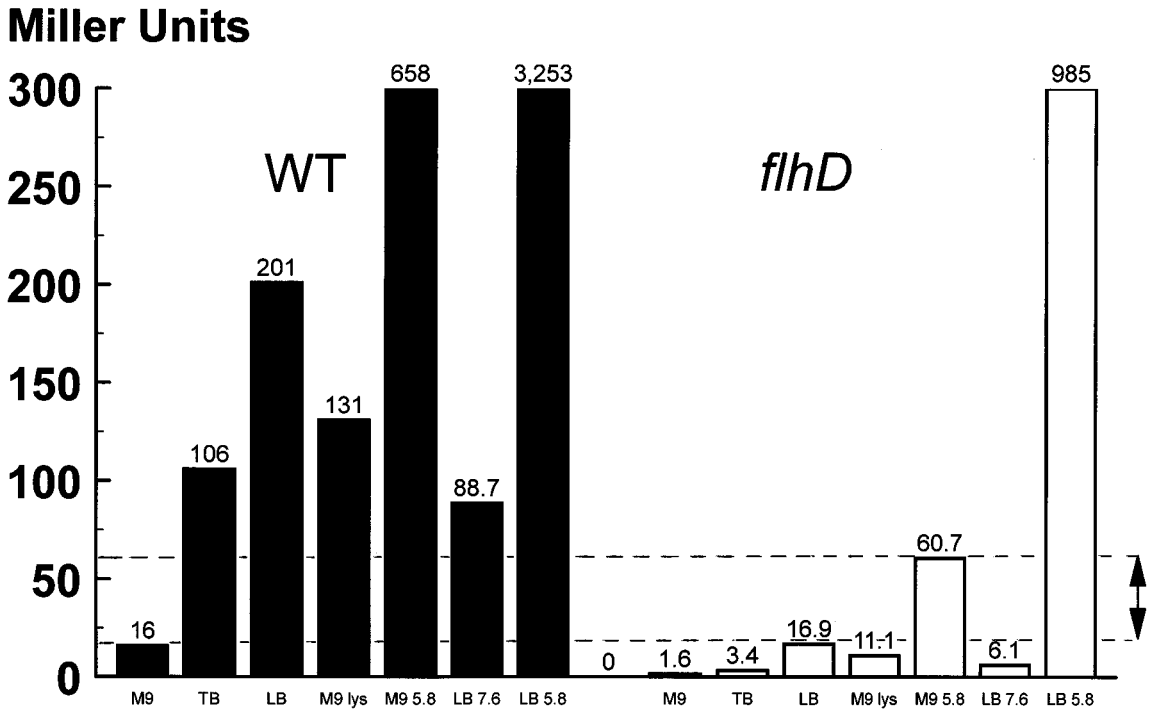

FIG. 2. Expression of *cadA* in wild-type (WT) (strain BP101) and *flhD* mutant (strain BP102) cells under different growth conditions. Cells were grown in minimal M9, TB, LB, M9 with lysine (M9 lys), M9 5.8, LB 7.6, and LB 5.8. The activity of β-galactosidase was determined during growth. Activities are expressed in terms of Miller units at an optical densities of 0.15 for TB cultures, 0.5 for LB cultures, and 0.2 for M9 cultures. In M9 lys and M9 5.8 cultures, β-galactosidase activity was determined 30 min after induction. The experiment was done twice, and the means of the populations were determined. Standard errors were between 10 and 30%.

pXL27 (9) were grown in LB, and the number of cells was determined. The cell division rates during the first 2 h of growth are given in Table 2. *cadC* mutants exhibited a behavior identical to that of *flhD* and *cadA* mutants. The presence of the control plasmid pT7-7 exhibited no significant effect upon the optical density and number of cells. Expression of *flhC* from plasmid pXL26 had no effect upon the cell division rate. The expression of *flhDC* or *flhD* led to a reduction of the cell division rate from 1.7 to 1 gen/h. It was concluded that signalling FlhD can occur in a CadC-independent manner. These data also confirm that the phenotype is specific for *flhD* and that the other flagellar regulator, *flhC*, is not involved in regulating the cell division rate. The multiple signals that lead to a regulation of the cell division rate by either affecting *flhD* or *cadA* expression are summarized in Fig. 3. One of the signals that regulate the expression of *flhD* is the growth phase-dependent depletion of serine (13) which is believed to be me-

TABLE 2. Involvement of CadC in the effect of FlhD on *cadA* expression

Strain	Relevant genotype	Plasmid	Cell division rates[a]
YK410	Wild-type	None	1.8/1.0
YK4131	*flhD*	None	1.9/1.9
BP101	*cadA*	None	1.9/1.9
BP168	*cadC*[b]	None	1.8/1.8
BP207	*cadC*	pT7-7	1.7/1.8
BP206	*cadC*	pT7-7 *flhC*	2.0/1.7
BP205	*cadC*	pT7-7 *flhDC*	1.1/1.1
BP204	*cadC*	pT7-7 *flhD*	1.1/1.0

[a] The cell division rate during the first 2 h in LB was determined. The first value is the cell division rate between 0.5 h and 1.5 h of growth, and the second value is the cell division rate between 1.5 h and 2.5 h of growth. These two values are before and after the reduction of the cell division rate that occurs in wild-type cells, respectively.

[b] *cadC*::Tn*10* was kindly provided by E. Olson (Parke-Davis Research Division, Ann Arbor, Mich.) and transduced into YK410.

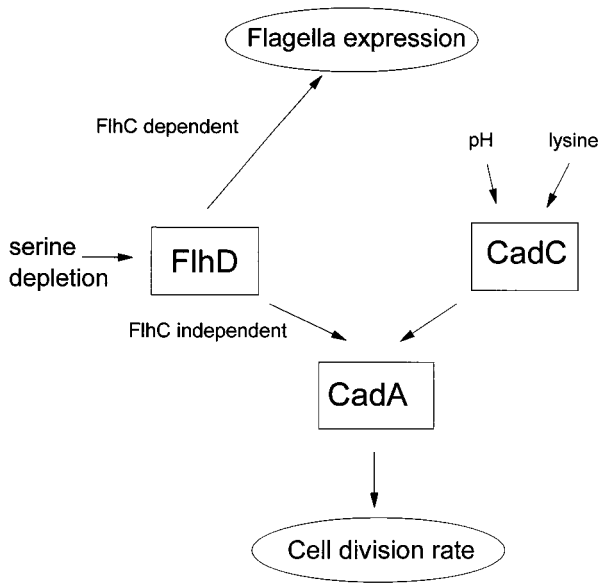

FIG. 3. Model showing the multiple signals that lead to a regulation of the cell division rate through *cadA*. The central regulator is FlhD, simultaneously regulating the expression of flagella and the cell division rate in response to the nutritional situation of the cell. Independent of FlhD are the responses to other signals, such as pH and the concentration of lysine, which are mediated by CadC.

diated by acetyl phosphate (14) and phosphorylation of OmpR (15).

The finding of *cadA* as a target of *flhD* that is involved in the regulation of the cell division rate raises two major questions. First, how does FlhD regulate the expression of *cadA*? Second, how does CadA affect cell division? This study provides evidence that FlhD can act upon the *cad* promoter in a CadC-independent manner. Whether FlhD binds directly to the *cad* promoter or requires some other intermediate is currently under investigation.

To address the question of how CadA might regulate cell division, a number of experiments have been performed. For *E. coli* cells, growth to a critical mass is believed to lead to initiation of chromosome replication and subsequent cell division. A protein that is also involved in regulating the rate of cell division is FtsZ (2). Small increases in the level of FtsZ lead to a hyperdivision phenotype (4), whereas lowering the level blocks division (3). Therefore, regulation of FtsZ by FlhD was considered to explain the regulation of the cell division rate. The expression of *ftsQAZ* was measured from plasmid pCX38, kindly provided by L. Rothfield (University of Connecticut, Farmington). This plasmid contains the entire *ftsQAZ* operon, with all of the known promoters, fused to *lacZ*. The expression from this plasmid in TB was not significantly different in *flhD* mutant cells than in wild-type cells. Expression of *ftsQAZ* in both strains was approximately 200 Miller units at the time when the phenotype is expressed (after 1.5 h of growth). After 4 h, the expression was about 20% higher in *flhD* mutant cells than in wild-type cells. It was concluded that the effects of FlhD and CadA upon the cell division rate were not mediated by regulation of *ftsQAZ*.

We thank M. Casadaban (University of Chicago, Chicago, Ill.), L. Katz (Abbott Laboratories, Chicago, Ill.), E. Olson (Parke-Davis Research Division), J. S. Parkinson (University of Utah), L. Rothfield (University of Connecticut), and B. Wanner (Purdue University) for providing plasmids, strains, and phage, and we thank H. Ohmori and Y. Yamamoto for providing unpublished data. We also thank J. Gibbons (Department of Biology, University of Illinois at Chicago, Ill.) for the electron microscopy and P. Goodlove (University of Illinois at Urbana) for sequencing; V. Carr-Brendel, S. Chang, and A. Mandić for constructing plasmids; and P. O'Neill for critical reading of the manuscript.

This work was supported by grant AI 18985 from the National Institutes of Health.

REFERENCES

1. **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.
2. **Bi, E., and J. Lutkenhaus.** 1990. FtsZ regulates frequency of cell division in *Escherichia coli*. J. Bacteriol. **172:**2765–2768.
3. **Dai, K., and J. Lutkenhaus.** 1992. The proper ratio of FtsZ to FtsA is required for cell division to occur in *Escherichia coli*. J. Bacteriol. **174:**6145–6151.
4. **Doublet, P., J. van Heijenoort, J.-B. Bohin, and D. Mengin-Lecreux.** 1992. Identification of the *Escherichia coli mur* gene, which is required for the biosynthesis of D-glutamic acid, a specific component of bacterial peptidoglycan. J. Bacteriol. **174:**5772–5779.
5. **Falkow, S.** 1958. Activity of lysine decarboxylase as an aid in the identification of *Salmonellae* and *Shigellae*. Am. J. Clin. Pathol. **29:**598–600.
6. **Komeda, Y., and T. Iino.** 1979. Regulation of expression of the flagellin gene (*hag*) in *Escherichia coli* K-12: analysis of *hag-lac* fusions. J. Bacteriol. **139:** 721–729.
7. **Komeda, Y., K. Katsukake, and T. Iino.** 1980. Definition of additional flagellar genes in *Escherichia coli* K-12. Genetics **94:**277–290.
8. **Liu, J., and J. S. Parkinson.** 1989. Genetics and sequence analysis of the *pcnB* locus, an *Escherichia coli* gene involved in plasmid copy number control. J. Bacteriol. **171:**1254–1261.
9. **Liu, X., and P. Matsumura.** 1994. The FlhD/FlhC complex, a transcriptional activator of the *Escherichia coli* flagellar class II operons. J. Bacteriol. **176:** 7345–7351.
10. **Meng, S. Y., and G. N. Bennett.** 1992. Nucleotide sequence of the *Escherichia coli cad* operon: a system for neutralization of low extracellular pH. J. Bacteriol. **174:**2659–2669.
11. **Meng, S. Y., and G. N. Bennett.** 1992. Regulation of the *Escherichia coli cad* operon: location of a site required for acid induction. J. Bacteriol. **174:**2670–2678.
12. **Neely, M. N., and E. R. Olson.** 1996. Kinetics of expression of the *Escherichia coli cad* operon as a function of pH and lysine. J. Bacteriol. **178:**5522–5528.
13. **Prüß, B. M., and P. Matsumura.** 1996. A regulator of the flagellar regulon of *Escherichia coli*, *flhD*, also affects cell division. J. Bacteriol. **178:**668–674.
14. **Prüß, B. M., and A. J. Wolfe.** 1994. Regulation of acetyl phosphate synthesis and degradation, and the control of flagellar expression in *Escherichia coli*. Mol. Microbiol. **12:**973–984.
15. **Shin, S., and C. Park.** 1995. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. J. Bacteriol. **177:** 4696–4702.
16. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
17. **Taylor, A., D. P. Brown, S. Kadam, M. Maus, W. E. Kohlbrenner, D. Weigl, M. C. Turon, and L. Katz.** 1992. High-level expression and purification of mature HIV-1 protease in *Escherichia coli* under control of the *ara*BAD promoter. Appl. Microbiol. Biotechnol. **37:**205–210.
18. **Watson, N., D. S. Dunyak, E. L. Rosey, J. L. Slonczewski, and E. R. Olson.** 1992. Identification of elements involved in transcriptional regulation of the *Escherichia coli cad* operon by external pH. J. Bacteriol. **174:**530–540.
19. **Wilmes-Riesenberg, M. R., and B. L. Wanner.** 1992. Tn*phoA* and Tn*phoA′* elements for making and switching fusions for study of transcription, translation, and cell surface localization. J. Bacteriol. **174:**4558–4575.
20. **Yamamoto, Y., and H. Ohmori.** Personal communication.