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The Two-Component Response Regulator RcsB Regulates Type 1 Piliation in *Escherichia coli*[▽]

William R. Schwan,[1] Satoshi Shibata,[2,3] Shin-Ichi Aizawa,[2,3] and Alan J. Wolfe[4]*

Department of Microbiology, University of Wisconsin—La Crosse, La Crosse, Wisconsin 54601[1]; Department of Life Sciences, Prefectural University of Hiroshima, 562 Nanatsuka, Shobara, Hiroshima 727-0023, Japan[2]; CREST Soft Nano-Machine Project, Innovation Plaza Hiroshima, 3-10-23 Kagamiyama, Higashi-Hiroshima 739-0046, Japan[3]; and Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, Illinois 60153[4]

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The ability of *Escherichia coli* cells to produce type 1 pili depends upon the orientation of the *fimA* promoter. The orientation depends upon the ratios of the FimB and FimE recombinases. Here, we report that the two-component response regulator RcsB influences the piliation state by controlling *fimB* and *fimE* transcription.

Type 1 pili are filamentous proteinaceous appendages produced by many members of the family *Enterobacteriaceae* (4) that play a major role in biofilm development and pathogenesis during the course of human infections (26). *Escherichia coli* cells can switch from a completely piliated state to a completely nonpiliated state (7). This ability depends on a process called phase variation (Fig. 1), in which a 314-bp invertible DNA element switches between two interconvertible orientations (1). When this element is in the "phase-on" orientation, the *fimA* promoter (*fimAp*) faces the *fim* operon and thus can drive its transcription. Since the *fim* operon includes genes for structural components and the machinery required for pilus assembly, cells in the "phase-on" orientation elaborate numerous type 1 pili. When the element is oriented in the "phase-off" state, the promoter faces the opposite direction, the promoter cannot drive *fimA* transcription, and the cells lack type 1 pili altogether. The invertible nature of the *fimAp* element depends upon two site-specific recombinases, FimB and FimE. Whereas FimE recombinase activity favors switching from "phase-on" to "phase-off," FimB facilitates switching in both directions (16, 22, 23).

We previously reported that the *fim* operon is regulated by acetyl phosphate (35), a central metabolite that functions as a global signal (12, 34) by donating its phosphoryl group to a subset of response regulators (RRs) of the family of two-component signal transduction (2CST) pathways (8, 15a, 19). The most fundamental of 2CST pathways consists of an RR and a sensor kinase (SK). The SK autophosphorylates a conserved histidinyl residue, using ATP as its phosphoryl donor. The phospho-SK then serves as the phosphoryl donor to the RR, which autophosphorylates a conserved aspartyl residue (6, 29, 33). For a subset of RRs, the central metabolite acetyl phosphate can serve as an alternative phosphoryl donor (reviewed in reference 34). As its name implies, the RR is typically associated with a response domain, often one that permits binding to DNA. Thus, many RRs function as transcription factors (6, 29, 33).

The phosphorelay, a more complex version of the 2CST pathway, contains two additional domains. As in the fundamental 2CST pathway, ATP donates a phosphoryl group to the SK, which then donates it to an RR. In the phosphorelay, a histidine phosphotransferase transfers the phosphoryl group from the first RR to a second one (reviewed in references 2, 14, and 24). The core of the Rcs phosphorelay is composed of three proteins: RcsC (a hybrid SK-RR), RcsD (a histidine phosphotransferase also known as YojN), and RcsB (the terminal RR) (reviewed in references 10 and 20). RcsB can bind DNA either as a homodimer (3) or as a heterodimer in association with the accessory protein RcsA (31, 32). The stability of RcsA is controlled by the proteases Lon (30) and ClpYQ (18). Another accessory protein, the outer membrane lipoprotein RcsF, serves to activate the kinase activity of RcsC (20).

The Rcs phosphorelay is estimated to regulate some 5% of the *Escherichia coli* genome (reviewed in references 21 and 25). Most of these genes encode functions associated with the cell envelope. For instance, the Rcs phosphorelay activates the genes required for the biosynthesis of colanic acid, an extracellular polysaccharide required for biofilm development (13). It also activates the expression of several multiple-stress effectors that localize to the periplasm (3, 5), while repressing genes required for the biogenesis of flagella (11).

While studying the impact exerted by acetyl phosphate upon the network of 2CST pathways (12), we obtained electron microscopic evidence that led us to hypothesize that RcsB functions as a positive regulator of type 1 pili. Here, we report attempts to test that hypothesis.

We grew cells at 37°C in tryptone broth (1% [wt/vol] tryptone, 0.5% [wt/vol] NaCl), harvested them during mid-exponential growth and shortly after entry into stationary phase, and monitored piliation by transmission electron microscopy as described previously (12). At both stages of growth, about 60% of wild-type (WT) cells (strain AJW678) (17) elaborated

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL 60153. Phone: (708) 216-5814. Fax: (708) 216-9574. E-mail: awolfe@lumc.edu.

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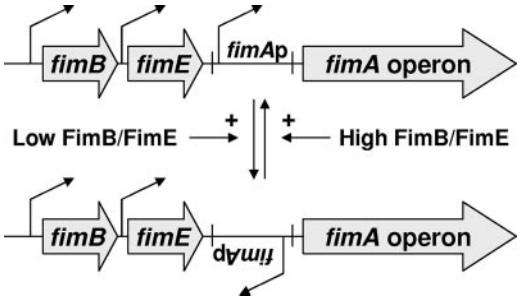

FIG. 1. In *E. coli*, the abilities of cells to assemble type 1 pili depend upon the orientation of the *fimA* promoter. The orientation depends primarily on the ratio of the activities of FimB and FimE. A high activity favors the "phase-on" orientation (top); a low activity favors the "phase-off" orientation (bottom).

pili (Fig. 2A and C); isogenic *rcsC* mutant cells (strain AJW2144) (12) displayed similar behavior (Fig. 2C). In contrast, most isogenic *rcsB* mutant cells (strain AJW2143) (12) were nonpiliated (Fig. 2B). About 40% of the *rcsB* mutant cells elaborated pili after entry into stationary phase, while only 20% displayed pili during exponential growth (Fig. 2C).

The observation that the percentage of piliated cells was affected rather than the number of type 1 pili per cell or their length led us to hypothesize that RcsB influenced the orientation of the 314-bp *fimAp* invertible element. To test this hypothesis, we initially performed multiplex PCR amplifications on chromosomal DNA extracted from the *rcsB* mutant, the *rcsC* mutant, or their WT parent, using oligonucleotide primers specific for the "phase-on" and "phase-off" orientations of the invertible element (28) or, as a control, the *E. coli ftsZ* gene (27).

We previously reported that pH values and salt relevant to murine urine exerted substantial effects on phase variation (27). Therefore, we grew cells at 37°C to mid-exponential phase in LB (1% [wt/vol] peptone 140, 0.5% [wt/vol] yeast extracts, 1% [wt/vol] glycerol, 0.1 M sodium phosphate, 0.5% [wt/vol] NaCl) at either neutral pH (7.0) or acidic pH (5.5) and in either the presence of additional NaCl (final concentration, 490 mM [referred to as high salt]) or its absence (final concentration, 90 mM [referred to as low salt]).

Overall, the PCR results followed those of our previous study (27): more WT cells grown at neutral pH (Fig. 3A, lanes 1 and 4, and B) than cells grown at acidic pH (Fig. 3A, lanes 7 and 10, and B) positioned their invertible *fimAp* element in the "phase-on" orientation. This analysis also showed that *rcsB* mutant cells grown under neutral-pH, low-salt conditions (Fig. 3A, lane 2) positioned their invertible element more in the "phase-off" orientation than did cells of either their WT parent (lane 1) or the isogenic *rcsC* mutant (lane 3) grown under the same conditions. Under neutral pH, high-salt conditions, the *rcsC* mutant (lane 6) appeared to position the *fimAp* promoter element less in the "phase-on" orientation than did either its WT parent (lane 4) or the *rcsB* mutant (lane 5). At acidic pH, regardless of salt, all strains produced similar "phase-off" and "phase-on" distributions (lanes 7 to 12).

To confirm the *rcsB* finding, we complemented the *rcsB* mutant with a plasmid containing a His-tagged WT *rcsB* gene (pHRcsB) (5), harvested cells during mid-exponential growth,

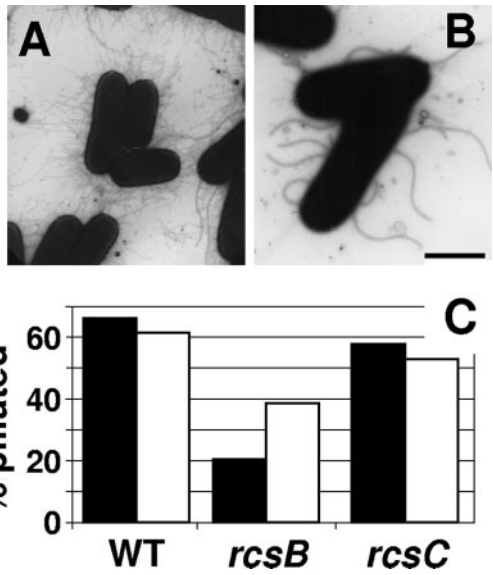

FIG. 2. RcsB enhances piliation. Cells were grown aerobically with 250-rpm agitation at 37°C in tryptone broth. Samples were negatively stained with 2% phosphotungstic acid (pH 7.0) and observed with a JEM-1200EXII electron microscope (JEOL, Tokyo, Japan). Micrographs were taken at an accelerating voltage of 80 kV. Transmission electron micrographs of WT cells (strain AJW678) (A) and *rcsB* mutant cells (strain AJW2143) (B) harvested at an optical density at 600 nm of 0.5 are shown . The thin and thick appendages are type 1 pili and flagella, respectively. The bar represents 2 μm. (C) Histogram showing percentages of piliated cells harvested at either 0.5 (solid bar) or 1.0 (open bar) optical density units at 600 nm. The sample sizes ranged from 83 to 178 cells.

and compared the complemented strain to the WT strain and the *rcsB* mutant parent (Fig. 3C and D). At the neutral-pH, low-salt condition, the complemented *rcsB* mutant (lane 3) oriented its invertible element more like the WT strain (lane 1) than the *rcsB* mutant (lane 2). Similar results were obtained when cells were harvested following entry into stationary phase (data not shown).

Taken together, these results support the hypothesis that RcsB regulates piliation under neutral-pH, low-salt conditions by influencing the orientation of the *fimAp* invertible element. These results also suggest that RcsC may influence the positioning of the invertible element under high-salt growth conditions. Finally, they show that neither RcsB nor RcsC exerts much influence at acidic pH and that some other factor must be involved.

To determine how RcsB may regulate the orientation of the invertible element, we asked whether RcsB and/or RcsC influences the transcription of *fimB*, *fimE*, or both recombinase genes. The *rcsB* mutant, the *rcsC* mutant, and their WT parent were transformed with single-copy plasmids pJB5A and pJLE4-3, which express the transcriptional fusions *fimB-lacZYA* and *fimE-lacZYA*, respectively (27). Because pH and salt have been shown to influence the transcriptional states of both *fimB* and *fimE* (27), we performed these reporter studies under the growth conditions used for the PCR analyses.

When cells were grown at acidic pH in the presence of either the low or high salt, transcription of either *fimB* or *fimE* was largely unaffected by the status of the Rcs phosphorelay (Table

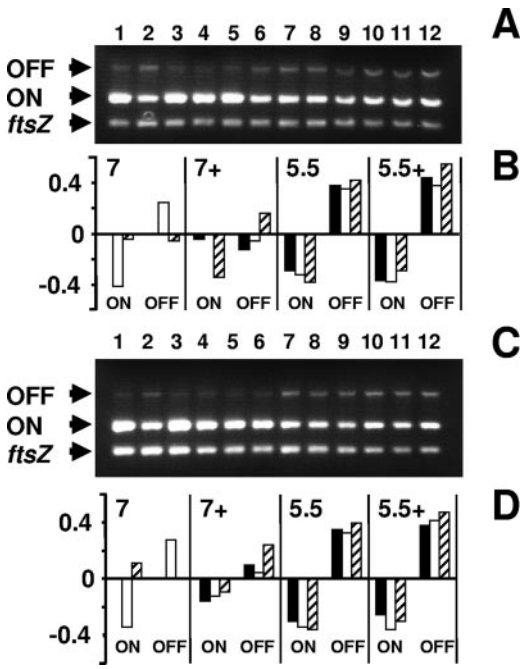

FIG. 3. Determination of the invertible element orientation by PCR. (A) Analysis was performed on chromosomal DNA isolated from WT cells (strain AJW678), an *rcsB* mutant (strain AJW2143), and an *rcsC* mutant (strain AJW2144). Cells were harvested during mid-exponential phase following aerobic growth with 250-rpm agitation at 37°C in pH 7.0 LB medium with either no added NaCl (low salt) or 400 mM added NaCl (+; high salt) or in pH 5.5 LB medium with either no added NaCl (low salt) or 400 mM NaCl (high salt). Multiplex PCRs were set up with INV and FIMA primers to amplify "phase-on"-oriented DNA (ON; 450-bp product) (28), FIME and INV primers to amplify "phase-off"-oriented DNA (OFF, 750-bp product) (28), and EcFtsZ 1 and 2 primers to amplify the *ftsZ* gene (302-bp product) (27). Each multiplex was run at least three separate times. The lanes were loaded as follows: lane 1, AJW678 (pH 7.0, low salt); lane 2, AJW2143 (pH 7.0, low salt); lane 3, AJW2144 (pH 7.0, low salt); lane 4, AJW678 (pH 7.0, high salt); lane 5, AJW2143 (pH 7.0, high salt); lane 6, AJW2144 (pH 7.0, high salt); lane 7, AJW678 (pH 5.5, low salt); lane 8, AJW2143 (pH 5.5, low salt); lane 9, AJW2144 (pH 5.5, low salt); lane 10, AJW678 (pH 5.5, high salt); lane 11, AJW2143 (pH 5.5, high salt); and lane 12, AJW2144 (pH 5.5, high salt). (B) Quantification of the data from panel A. Using ImageQuant software (Molecular Dynamics), the number of pixels for each band was quantified. For each lane, the intensities of the OFF and ON states were corrected to the intensity of the *ftsZ* band. The corrected values for both states were standardized to the respective WT band (lane 1). Since the resultant values were plotted as $\log_{10}$ numbers, the WT strain for both the OFF and ON states had a value of zero, while increased and decreased PCR products resulted in positive and negative values, respectively. Solid bars, strain AJW678 (WT); open bars, strain AJW2143 (*rcsB* mutant); hatched bars, strain AJW2144 (*rcsC* mutant). (C) Analysis was performed on chromosomal DNA isolated from WT cells (strain AJW678), an *rcsB* mutant (strain AJW2143), and an *rcsB* mutant complemented with the pHRcsB plasmid (strain AJW2307) without induction. Multiplex PCR amplifications were performed with the same primer pairs, using DNAs isolated from the strains grown as described for panel A. Each multiplex was run at least three separate times. Lane 1, AJW678 (pH 7.0, low salt); lane 2, AJW2143 (pH 7.0, low salt); lane 3, AJW2307 (pH 7.0, low salt); lane 4, AJW678 (pH 7.0, high salt); lane 5, AJW2143 (pH 7.0, high salt); lane 6, AJW2307 (pH 7.0, high salt); lane 7, AJW678 (pH 5.5, low salt); lane 8, AJW2143 (pH 5.5, low salt); lane 9, AJW2307 (pH 5.5, low salt); lane 10, AJW678 (pH 5.5, high salt); lane 11, AJW2143 (pH 5.5, high salt); and lane 12, AJW 2307 (pH 5.5, high salt). All PCR products were subjected to electrophoresis on 1.5% agarose gels. (D) Quantification of the data from panel C as described for panel B. Solid bars, strain AJW678 (WT); open bars, strain AJW2143 (*rcsB* mutant); hatched bars, strain AJW2307 (complemented *rcsB* mutant).

TABLE 1. Effects of pH and salt on *fimB-lacZ* and *fimE-lacZ* fusions in WT *E. coli* compared to effects on isogenic *rcsB* and *rcsC* mutants

Fusion and strain	Expression level (Miller units) under indicated growth condition[a]			
	pH 7.0	pH 7.0 (+)	pH 5.5	pH 5.5 (+)
fimB-lacZ fusion				
WT	345 ± 30	259 ± 36	271 ± 20	219 ± 23
rcsB	**201 ± 19**[b]	229 ± 27	274 ± 62	182 ± 48
rcsB/pRcsB[c]	329 ± 21	315 ± 13	297 ± 11	231 ± 11
rcsC	305 ± 34	235 ± 36	284 ± 51	238 ± 57
fimE-lacZ fusion				
WT	335 ± 26	318 ± 54	252 ± 35	237 ± 26
rcsB	332 ± 34	**463 ± 68**	260 ± 54	221 ± 60
rcsB/pRcsB	339 ± 32	377 ± 70	231 ± 55	245 ± 13
rcsC	365 ± 21	**437 ± 53**	260 ± 32	253 ± 48

[a] Values indicate *fimB* and *fimE* promoter expression in terms of β-galactosidase activity and are means ± standard deviations from at least three independent experiments. Cells were grown in LB at pH 7.0 or pH 5.5 with the low or high (+) salt and harvested during mid-exponential phase.

[b] Bold denotes that the differential expression is significant ($P < 0.05$) as determined by Student's *t* test.

[c] *rcsB*/pRcsB is the *rcsB* mutant transformed with pHRcsB, which encodes a His-tagged WT *rcsB* allele under the control of the *lac* promoter. Induction, however, was unnecessary.

1). This is consistent with the lack of any significant effect upon the populations of invertible elements (Fig. 3). In contrast, when cells were grown at neutral pH, the states of certain Rcs phosphorelay components influenced transcription. Furthermore, the critical Rcs component and the promoter affected depended upon the salt. For example, at the low salt, *rcsB* mutant cells transcribed *fimB* at significantly reduced levels relative to their WT parent and the *rcsC* mutant (Table 1). This effect appears to be specific because expression of the His-tagged WT *rcsB* allele from a compatible plasmid (pHRcsB) restored transcription to WT levels. In contrast, growth in high-salt medium resulted in a distinctly different pattern. Under this condition, relative to WT cells, both the *rcsB* and rcsC mutants exhibited elevated *fimE* transcription. Here, both the RcsB and RcsC effects appeared to be specific because expression from a compatible plasmid either with the His-tagged WT *rcsB* allele in the *rcsB* mutant (Table 1) or with the WT *rcsC* allele (pSG980) (9) in the *rcsC* mutant restored *fimE* transcription to WT levels (data not shown). In contrast, the vector controls had no effect (data not shown).

The PCR analysis (Fig. 3) and the *fim-lacZ* fusion data (Table 1) supported the hypothesis that RcsB can influence the orientation of the *fimAp* invertible element by controlling the transcription of the recombinase genes *fimB* and *fimE*. This RcsB-dependent behavior occurred only at neutral pH and depended upon RcsC only in the presence of the high salt. On the basis of these observations, we predicted that type 1 pilus expression would be affected in a condition-dependent manner by mutations in *rcsB* and *rcsC*. To test this prediction, enzyme immunoassays were performed according to the procedure of Hultgren et al. (15). As predicted, at neutral pH with the low salt, the *rcsB* mutant strain displayed significantly reduced type 1 pilus expression relative either to its WT parent or to the *rcsC* mutant (Fig. 4). At neutral pH with the high salt, however, both the *rcsB* and *rcsC* mutants displayed lower levels of type

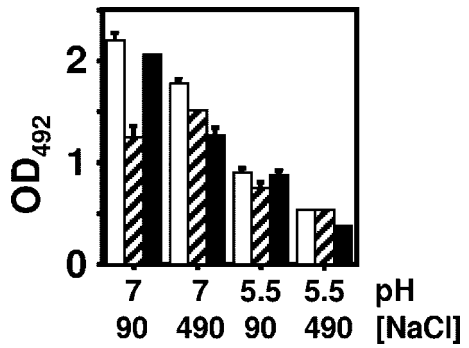

FIG. 4. Enzyme immunoassay of strains. WT (AJW678; open columns), *rcsB* mutant (AJW2143; striped columns), and *rcsC* mutant (AJW2144; filled columns) cells were harvested in stationary phase after growth in pH 7.0 and 5.5 LB media with either 90 mM or 490 mM NaCl. Optical densities at 492 nm (OD_{492}) were determined. The mean values ± standard deviations for two separate runs are indicated.

1 pili than did their WT parent. In contrast, at acidic pH (regardless of salt), the status of *rcsB* or *rcsC* had, at most, a minor effect.

At neutral pH, therefore, it appears that RcsB helps mediate the inversion of the *fimAp* element either by increasing *fimB* transcription (low salt) or by decreasing *fimE* transcription (high salt). These results are consistent with our observation that significantly fewer *rcsB* mutant cells than cells of their WT parent expressed pili. Whether RcsB acts directly upon *fim* transcription awaits further experimentation; however, inspection of the sequence upstream of the *fimB* and *fimE* open reading frames reveals several sequences with some similarity to RcsB and RcsAB boxes (31, 32). Although RcsC appeared to have no discernible effect under neutral-pH, low-salt growth conditions, it appeared to influence the orientation of the invertible element under neutral-pH, high-salt conditions, presumably by decreasing *fimE* transcription. These results argue for the hypothesis that RcsB receives its phosphoryl groups from RcsC (its cognate SK) under neutral-pH, high-salt conditions but from an alternative donor (e.g., acetyl phosphate or a noncognate SK) under neutral-pH, low-salt conditions.

Our previous work showed that acidic growth conditions reduce type 1 pilus expression, while implicating the EnvZ/OmpR 2CST pathway as a *fim* inhibitor at least in the presence of the high salt (27). The current study supports the former conclusion and shows that it occurs in an RcsB-independent manner. Whether *fim* regulation under acidic growth conditions involves the EnvZ/OmpR pathway or some other acid tolerance gene product is currently under examination.

In summary, we propose that the *fim* locus is part of the RcsB regulon and that this global regulator inversely affects the transcription of *fimB* and *fimE*. Furthermore, this inverse regulation increases the probability of the "phase-on" orientation of the *fimAp* element and consequently the elaboration of type 1 pili. On the basis of the current study and our previous report (12), we can conclude that RcsB enhances the production of type 1 pili as well as the synthesis of the capsule, while inhibiting the biogenesis of flagella. Since these surface organelles play critical and/or essential roles in biofilm development and urinary tract infections, RcsB should now be considered a coordinator of these processes.

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REFERENCES

1. **Abraham, S. N., J. P. Babu, C. S. Giampapa, D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1985. Protection against *Escherichia coli*-induced urinary tract infections with hybridoma antibodies directed against type 1 fimbriae or complementary D-mannose receptors. Infect. Immun. **48:**625–628.
2. **Appleby, J. L., J. S. Parkinson, and R. B. Bourret.** 1996. Signal transduction via the multi-step phosphorelay: not necessarily a road less traveled. Cell **86:**845–848.
3. **Carballes, F., C. Bertrand, J. P. Bouche, and K. Cam.** 1999. Regulation of *Escherichia coli* cell division genes *ftsA* and *ftsZ* by the two-component system *rcsC-rcsB*. Mol. Microbiol. **34:**442–450.
4. **Clegg, S., and G. F. Gerlach.** 1987. Enterobacterial fimbriae. J. Bacteriol. **169:**934–938.
5. **Davalos-Garcia, M., A. Conter, I. Toesca, C. Gutierrez, and K. Cam.** 2001. Regulation of *osmC* gene expression by the two-component system *rcsB-rcsC* in *Escherichia coli*. J. Bacteriol. **183:**5870–5876.
6. **Dutta, R., L. Qin, and M. Inouye.** 1999. Histidine kinases: diversity of domain organization. Mol. Microbiol. **34:**633–640.
7. **Eisenstein, B. I.** 1981. Phase variation of type 1 fimbriae in *Escherichia coli* is under transcriptional control. Science **214:**337–339.
8. **Feng, J., M. R. Atkinson, W. McCleary, J. B. Stock, B. L. Wanner, and A. J. Ninfa.** 1992. Role of phosphorylated metabolic intermediates in the regulation of glutamine synthetase synthesis in *Escherichia coli*. J. Bacteriol. **174:** 6061–6070.
9. **Ferrieres, L., and D. J. Clarke.** 2003. The RcsC sensor kinase is required for normal biofilm formation in *Escherichia coli* K-12 and controls the expression of a regulon in response to growth on a solid surface. Mol. Microbiol. **50:**1665–1682.
10. **Francez-Charlot, A., J. Filee, M. P. Castanie-Cornet, and K. Cam.** 2005. Regulation of *flhDC* by the His-Asp phosphorelay RcsCDB, p. 93–106. *In* B. M. Pruss (ed.), Global regulatory networks in enteric bacteria. Research Signpost, Kerala, India.
11. **Francez-Charlot, A., B. Laugel, A. Van Gemert, N. Dubarry, F. Wiorowski, M.-P. Castanie-Cornet, C. Gutierrez, and K. Cam.** 2003. RcsCDB His-Asp phosphorelay system negatively regulates the *flhDC* operon in *Escherichia coli*. Mol. Microbiol. **49:**823–832.
12. **Fredericks, C. E., S. Shibata, S.-I. Aizawa, S. A. Reimann, and A. J. Wolfe.** 2006. Acetyl phosphate-sensitive regulation of flagellar biogenesis and capsular biosynthesis depends on the Rcs phosphorelay. Mol. Microbiol. **61:** 734–747.
13. **Gottesman, S., P. Trisler, and A. Torres-Cabassa.** 1985. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. J. Bacteriol. **162:**1111–1119.
14. **Hoch, J. A., and T. J. Silhavy (ed.).** 1995. Two-component signal transduction. American Society for Microbiology, Washington, DC.
15. **Hultgren, S. J., J. L. Duncan, A. J. Schaeffer, and S. K. Amundsen.** 1990. Mannose-sensitive haemagglutination in the absence of piliation in *Escherichia coli*. Mol. Microbiol. **4:**1311–1318.

15a. **Klein, A. H., A. Shulla, S. A. Reimann, D. H. Keating, and A. D. Wolfe.** 2007. The intracellular concentration of acetyl phosphate in *Escherichia coli* is sufficient for direct phosphorylation of two-component response regulators. J. Bacteriol. **189:**5574–5581.

16. **Klemm, P.** 1986. Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type 1 fimbriae in *Escherichia coli*. EMBO J. **5:**1389–1393.
17. **Kumari, S., C. M. Beatty, D. F. Browning, S. J. Busby, E. J. Simel, G. Hovel-Miner, and A. J. Wolfe.** 2000. Regulation of acetyl coenzyme A synthetase in *Escherichia coli*. J. Bacteriol. **182:**4173–4179.
18. **Kuo, M. S., K. P. Chen, and W. F. Wu.** 2004. Regulation of RcsA by the ClpYQ (HslUV) protease in *Escherichia coli*. Microbiology **150:**437–446.
19. **Lukat, G. S., W. R. McCleary, A. M. Stock, and J. B. Stock.** 1992. Phosphorylation of bacterial response regulator proteins by low molecular weight phospho-donors. Proc. Natl. Acad. Sci. USA **89:**718–722.
20. **Majdalani, N., and S. Gottesman.** 2005. The Rcs phosphorelay: a complex signal transduction system. Annu. Rev. Microbiol. **59:**379–405.
21. **Majdalani, N., M. Heck, V. Stout, and S. Gottesman.** 2005. Role of RcsF in signaling to the Rcs phosphorelay pathway in *Escherichia coli*. J. Bacteriol. **187:**6770–6778.
22. **McClain, M. S., I. C. Blomfield, K. J. Eberhardt, and B. I. Eisenstein.** 1993. Inversion-independent phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **175:**4335–4344.
23. **McClain, M. S., I. C. Blomfield, and B. I. Eisenstein.** 1991. Roles of *fimB* and *fimE* in site-specific DNA inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **173:**5308–5314.
24. **Mizuno, T.** 1998. His-Asp phosphotransfer signal transduction. J. Biochem. (Tokyo) **123:**555–563.
25. **Prüss, B. M., C. Besemann, A. Denton, and A. J. Wolfe.** 2006. A complex

transcription network controls the early stages of biofilm development by *Escherichia coli*. J. Bacteriol. **188:**3731–3739.
26. **Schilling, J. D., M. A. Mulvey, and S. J. Hultgren.** 2001. Structure and function of *Escherichia coli* type 1 pili: new insight into the pathogenesis of urinary tract infections. J. Infect. Dis. **183:**S36–S40.
27. **Schwan, W. R., J. L. Lee, F. A. Lenard, B. T. Matthews, and M. T. Beck.** 2002. Osmolarity and pH growth conditions regulate *fim* gene transcription and type 1 pilus expression in uropathogenic *Escherichia coli*. Infect. Immun. **70:**1391–1402.
28. **Schwan, W. R., H. S. Seifert, and J. L. Duncan.** 1992. Growth conditions mediate differential transcription of *fim* genes involved in phase variation of type 1 pili. J. Bacteriol. **174:**2367–2375.
29. **Stock, A. M., V. L. Robinson, and P. N. Goudreau.** 2000. Two-component signal transduction. Annu. Rev. Biochem. **69:**183–215.
30. **Stout, V., A. Torres-Cabassa, M. R. Maurizi, D. Gutnick, and S. Gottesman.** 1991. RcsA, an unstable positive regulator of capsular polysaccharide synthesis. J. Bacteriol. **173:**1738–1747.
31. **Wehland, M., and F. Bernhard.** 2000. The RcsAB box. Characterization of a new operator essential for the regulation of exopolysaccharide biosynthesis in enteric bacteria. J. Biol. Chem. **275:**7013–7020.
32. **Wehland, M., C. Kiecker, D. L. Coplin, O. Kelm, W. Saenger, and F. Bernhard.** 1999. Identification of an RcsA/RcsB recognition motif in the promoters of exopolysaccharide biosynthetic operons from *Erwinia amylovora* and *Pantoea stewartii* subspecies *stewartii*. J. Biol. Chem. **274:**3300–3307.
33. **West, A. H., and A. M. Stock.** 2001. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem. Sci. **26:**369–376.
34. **Wolfe, A. J.** 2005. The acetate switch. Microbiol. Mol. Biol. Rev. **69:**12–50.
35. **Wolfe, A. J., D.-E. Chang, J. D. Walker, J. E. Seitz-Partridge, M. D. Vidaurri, C. F. Lange, B. M. Pruess, M. C. Henk, J. C. Larkin, and T. Conway.** 2003. Evidence that acetyl phosphate functions as a global signal during biofilm development. Mol. Microbiol. **48:**977–988.