JOURNAL OF BACTERIOLOGY, Aug. 2002, p. 4334–4342 Vol. 184, No. 16
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.16.4334–4342.2002

The *N*-Acetyltransferase RimJ Responds to Environmental Stimuli To Repress *pap* Fimbrial Transcription in *Escherichia coli*

Christine A. White-Ziegler,* Alia M. Black, Stacie H. Eliades, Sarah Young, and Kimberly Porter

Department of Biological Sciences, Smith College, Northampton, Massachusetts

Received 22 January 2002/Accepted 17 May 2002

In uropathogenic *Escherichia coli*, P pili (Pap) facilitate binding to host epithelial cells and subsequent colonization. Whereas P pili can be produced at 37°C, the expression of these fimbriae is suppressed at 23°C. Previously, insertion mutations in *rimJ*, a gene encoding the N-terminal acetyltransferase of ribosomal protein S5, were shown to disrupt this thermoregulatory response, allowing *papBA* transcription at low temperature. In this study, we created an in-frame deletion of *rimJ*. This deletion relieved the repressive effects not only of low temperature but also of rich (Luria-Bertani [LB]) medium and glucose on *papBA* transcription, indicating that RimJ modulates *papBA* transcription in response to multiple environmental stimuli. *papI* transcription was also shown to be regulated by RimJ. *papBA* transcription is also controlled by a phase variation mechanism. We demonstrated that the regulators necessary to establish a phase ON state—PapI, PapB, Dam, Lrp, and cyclic AMP-CAP–are still required for *papBA* transcription in a *rimJ* mutant strain. *rimJ* mutations increase the rate at which bacteria transition into the phase ON state, indicating that RimJ inhibits the phase OFF→ON transition. A Δ*rimJ hns651* mutant is viable on LB medium but not on minimal medium. This synthetic lethality, along with transcriptional analyses, indicates that RimJ and H-NS work through separate pathways to control *papBA* transcription. Mutations in *rimJ* do not greatly influence the transcription of the *fan*, *daa*, or *fim* operon, suggesting that RimJ may be a *pap*-specific regulator. Overexpression of *rimJ* under conditions repressive for *papBA* transcription complements the Δ*rimJ* mutation but has little effect on transcription under activating conditions, indicating that the ability of RimJ to regulate transcription is environmentally controlled.

A variety of environmental signals, including temperature, growth medium, carbon source, osmolarity, pH, oxygen level, and various ions, are known to regulate virulence gene expression in pathogenic bacteria. The expression of several genes is often coordinately regulated by one or more environmental cues (reviewed in references 15 and 36). Presumably, the bacterium uses these stimuli to determine whether it is within a host or, more specifically, to identify a particular environmental niche within the host. This regulation allows for a more efficient utilization of the bacterium's resources and may be necessary for productive colonization of the host.

Pyelonephritis-associated pilus (Pap) expression is regulated by both phase variation and environmental regulatory mechanisms. In many strains of uropathogenic *Escherichia coli*, Pap expression allows the attachment of bacteria to uroepithelial cells, facilitating colonization of the upper urinary tract (41, 42). Phase variation enables individual bacteria within a given population to alternate between two states of expression: phase ON, in which they are expressing fimbriae, and phase OFF, in which they are not expressing fimbriae (33). Phase variation is controlled at the transcriptional level by the formation of specific DNA methylation patterns of two GATC sites, $GATC^{prox}$ and $GATC^{dist}$, within the *pap* regulatory region (6, 9, 52). Formation of these patterns relies upon the global regulators deoxyadenosine methylase (Dam), leucine-responsive regulatory protein (Lrp), and the cyclic AMP (cAMP) receptor protein CAP, as well as the operon-specific proteins PapI and PapB (reviewed in references 30 and 52).

We previously demonstrated that four environmental cues—low temperature, rich (Luria-Bertani [LB]) medium, glucose as a carbon source, and high osmolarity—decrease *papBA* transcription (6, 57, 60). These environmental cues control several *E. coli* fimbrial operons, confirming their importance in regulating virulence gene expression (18, 20, 21, 25, 29, 37, 39, 45, 46, 60). For the *papBA* operon, low temperature causes all cells to transition to a phase OFF state, both phenotypically and at the level of DNA methylation (7, 57). Glucose and high osmolarity decrease the rate at which cells transition into a phase ON state (7, 60).

Two proteins are known to be important in the regulation of *papBA* transcription in response to environmental conditions, H-NS and RimJ. H-NS is a histone-like nucleoid structuring protein that binds to A-T-rich bent regions of DNA and regulates the expression of a number of environmentally controlled virulence genes (1, 51, 61). Under all growth conditions, *papBA* transcription is decreased, relative to a wild-type strain, in an *hns651* mutant, indicating that H-NS plays a positive role in *papBA* transcription (54, 57, 60). However, the repression caused by environmental signals is either fully or partially relieved by an *hns651* mutation such that transcription approximates levels measured for the mutant under activating conditions (57, 60). Under environmentally repressive conditions, H-NS inhibits the phase OFF→ON transition and can prevent methylation of the *pap* $GATC^{prox}$ and $GATC^{dist}$ sites at 23°C, but not 37°C (57).

RimJ was initially identified in a thermoregulatory mutant screen in which random chromosomal mini-Tn*10* (mTn*10*) in-

* Corresponding author. Mailing address: Department of Biological Sciences, Smith College, Northampton, MA 01063. Phone: (413) 585-3815. Fax: (413) 585-3786. E-mail: cwhitezi@science.smith.edu.

TABLE 1. Bacterial strains, bacteriophages, and plasmids used in this study

Strain, plasmid, or bacteriophage	Description	Reference or source
E. coli strains		
MC4100	F⁻ *araD139* Δ(*lacIPOZYA-argF*)*U169 rpsL thi-1*	11
NH757	B178 *hns651 tyrT*β::Tn*10*	22
DL479	MC4100 λ246 lysogen (*papBA-lacZYA*) *rimJ-2*::mTn*10*	58
DL812	MC4100 λMW01 lysogen (*fanABC'-lacZYA*)	53
DL1504	MC4100 λ354 lysogen (*papBA-lacZYA*)	9
DL1509	DL1504 *rimJ-2*::mTn*10*	This work
DL1530	MC4100 λ366 lysogen (*daa-lacZYA*)	55
DL1910	DL1504 Δ*lrp*	54
DL1947	DL1504 *hns651*	54
DL2208	MC4100 λ354-15 lysogen (*papBA-lacZYA* lysogen with ATG start codon of *papB* changed to CTG)	57
DL2838	MC4100 λ491 lysogen (*papI-lacZYA*)	57
DL3052	MC4100 λ354-73 lysogen (*papBA-lacZYA* lysogen containing *papI* frameshift mutation)	57
DL2873	DL1910 *rimJ-2*::mTn*10*	This work
DL3089	DL1504 Δ*crp-45 zhd-3083*::Tn*10*	D. A. Low
AAEC198A	MG1655 Δ*lacZYA fimA-lacZYA*	5
CWZ381	DL812 *rimJ-2*::mTn*10*	This work
CWZ382	DL1530 *rimJ-2*::mTn*10*	This work
CWZ387	MC4100 Δ*rimJ*	This work
CWZ388	DL1504 Δ*rimJ*	This work
CWZ395	CWZ388 containing pCWZ101 and pMV101	This work
CWZ400	AAEC198A *rimJ-2*::mTn*10*	This work
CWZ403	CWZ388 *hns651*	This work
CWZ405	CWZ388 Δ*crp-45 zhd-3083*::Tn*10*	This work
CWZ406	CWZ387 *hns651*	This work
CWZ410	DL1504 *dam-13*::Tn*9*	This work
CWZ411	DL1509 *dam-13*::Tn*9*	This work
CWZ412	DL2838 *rimJ-2*::mTn*10*	This work
CWZ418	CWZ387 λ354-73 lysogen	This work
CWZ419	CWZ387 λ354-15 lysogen	This work
Bacteriophages		
P1L4	Virulent phage P1	D. A. Low
λ491	*papI-lacZYA* fusion phage	57
λ354	*papBA-lacZYA* fusion phage	9
λ354-15	*papBA-lacZYA* lysogen with ATG start codon of *papB* changed to CTG	57
λ354-73	*papBA-lacZYA* lysogen containing *papI* frameshift mutation	57
λ366	*daa-lacZYA* fusion phage	55
λMW01	*fanABC'-lacZYA* fusion phage	10, 53
Plasmids		
pUHS*2 Pzl-2	ColE1 replicon containing the $P_{lac/ara-1}$ promoter	35
pMV101	pMC9 derivative containing $lacI^q$ that is Ap^s Tc^r	14
pMV106	pUHS*2 Pzl-2 with replacement of Kn^r with Ap^r	14
pKO3	pSC101 replicon containing *repA* (Ts) replication origin, *sacB*, and Cm^r	32
pCWZ100	pKO3 containing Δ*rimJ* deletion	This work
pCWZ101	pMV106 containing *rimJ* under $P_{lac/ara-1}$ promoter	This work

sertions isolated within *rimJ* allowed *papBA* transcription at a low temperature (23°C) (58, 59). RimJ is the N-terminal acetyltransferase that modifies the ribosomal protein S5 (16). RimJ, unlike H-NS, is exclusively a negative regulator of *papBA* transcription: transcriptional levels in *rimJ* mutants are similar to levels measured in the wild-type strain grown under transcriptionally activating conditions (reference 59 and this study). The mechanism by which RimJ represses *papBA* transcription and how the modification of a ribosomal protein might be involved in this process are unknown.

In this study, we provide evidence that RimJ controls *papBA* and *papI* transcription in response to multiple environmental cues and inhibits the phase OFF→ON transition. In the absence of RimJ, *papBA* transcription still relies upon the regulators necessary to establish a phase ON state (Lrp, cAMP-CAP, Dam, PapI, and PapB), while our analyses indicate that RimJ and H-NS work in separate pathways to control *papBA* transcription. RimJ appears to be a *pap*-specific regulator that does not control other fimbrial operons in response to environmental conditions. Additionally, our experiments indicate that the ability of RimJ to control transcription is environmentally regulated.

MATERIALS AND METHODS

Strains and media. The strains, plasmids, and bacteriophages used in this study are shown in Table 1. Media and antibiotics were prepared as described previously (38, 47, 60).

Construction of mutant strains by P1 transduction. The preparation of P1 lysates and P1 transductions were carried out as described previously (47, 60). *rimJ-2*::mTn*10*, *dam-13*::Tn*9*, Δ*crp-45*, and *hns651* mutant strains were created by P1 transduction of the individual mutations into the appropriate recipient strain (Table 1).

UV induction and lysogenization of UV-induced phage. UV induction and lysogenization were performed as described previously (47, 58). UV induction was performed on DL3052 and DL2208, with the resulting phage lysates used to lysogenize CWZ387, creating CWZ418 and CWZ419, respectively (Table 1).

Construction of Δ*rimJ* strain. Crossover PCR was used to create an internal, in-frame deletion within *rimJ* by the method of Link et al. (32). Primers *rimJ*(A), 5′-CGCGGATCCGGCGATACCCATTGTGGC-3′, and *rimJ*(B), 5′-CCCATC CACTAAACTTAAACAACTGCGATAGCCAAACAT-3′, were used to generate a 573-bp upstream fragment, and primers *rimJ*(C), 5′-TGTTTAAGTTTAG TGGATGGGGCATTAACTACCCCAGAC-3′, and *rimJ*(D), 5′-CGCGGATC CCGCGTTTACCCGGTTCGC-3, were used to generate a downstream 556-bp fragment. The two PCR products were combined in a secondary PCR using primers *rimJ*(A) and *rimJ*(D) for amplification. The *Bam*HI-*Sal*I-digested PCR product was cloned into *Bam*HI-*Sal*I-digested pKO3 to create pCWZ100 (Table 1).

pCWZ100 was transformed (12) into DL1504, and the selection for integration of the Δ*rimJ* deletion onto the chromosome was performed as described previously, with the exception that the incubation on sucrose was completed at 23 rather than 30°C (32). Colony PCR was used to detect clones in which the amplification of the *rimJ* region showed the expected decrease in size. In the resulting Δ*rimJ* strain, CWZ388 (Table 1), the region overlapping the deletion was sequenced to confirm the correct replacement. Steps identical to those described above were followed to construct CWZ387 (Table 1).

Construction of pCWZ101 for overexpression of RimJ. *rimJ* was amplified from wild-type DL1504 chromosomal DNA using primers 5′-CGGAATTCGC GTATTAAAGACGTTAC-3′ and 5′-GCTCTAGACAAGGGCAGTAAGTTG AT-3′. The amplified fragment and pMV106 were each digested with *Eco*RI and *Bam*HI and subsequently ligated to create pCWZ101 (Table 1). pCWZ101 and pMV101, containing the *lacI*^{q} gene, were cotransformed (12) into CWZ388 to yield strain CWZ395 (Table 1).

Growth conditions. Media (M9 glyc, M9 gluc, M9 NaCl, and LB) were prepared as described previously (60). For growth conditions that are activating for *papBA* transcription, the bacteria were cultured in 10 ml of M9 glyc at 37°C. Low temperature was tested by growing the bacteria at 23°C in M9 glyc, whereas rich medium was tested by growth of bacteria in LB broth at 37°C. Cultures grown at 37°C in M9 gluc or M9 NaCl medium were used to measure the effect of a change in carbon source and osmolarity, respectively. Glucose was substituted for glycerol in the M9 minimal medium (M9 gluc). The sodium chloride concentration was increased by 300 mM (M9 NaCl) compared to 8.5 mM sodium chloride in the M9 glyc medium to test osmolarity.

Culture inoculation and measurement of β-galactosidase activity. For the assays determining the effects of environmental stimuli on fimbrial transcription, each bacterial culture was inoculated as described previously (60). To assess the effect of *rimJ* overexpression in CWZ395, two phase ON (Lac$^+$) colonies were excised from M9 glyc agar at 37°C and resuspended in 2 ml of M9 salts. Flasks containing 10 ml of the prewarmed medium (M9 glyc or LB) with the appropriate concentration of isopropyl-β-D-thiogalactopyranoside (IPTG) were inoculated with 140 μl of the colony suspension. These inoculation methods ensured that all the bacterial cultures had grown for approximately 9 to 11 generations under the new conditions prior to the measurement of β-galactosidase activity. The bacterial cultures were grown to exponential phase (optical density at 600 nm, 0.25 to 0.9), and β-galactosidase activities were measured as described previously (38). All the values for the β-galactosidase activities represent averages from two or more independent cultures grown under identical conditions.

Calculation of switch frequencies. Phase transition rates were calculated as described previously (7, 60). Weighted averages were calculated from at least two independent analyses and are given as the number of events per cell per generation.

RESULTS

An in-frame deletion of *rimJ* causes a loss of *papBA* thermoregulation. An in-frame deletion of *rimJ* was created to analyze the effect of a total loss of the RimJ protein on *papBA* transcription. The two originally characterized *rimJ*::mTn*10* mutations, previously designated *tcp*, for thermoregulatory control of *pap*, are insertions within the 3′ end of *rimJ*, and minicell analysis demonstrated that for both insertions a fusion protein was expressed (59). Thus, the effect of the *rimJ*::mTn*10* mutations on *papBA* thermoregulation could be due to either a total loss of RimJ activity in these mutants or an alteration in the levels or specificity of RimJ activity. In addition, because all of the previously mapped insertions in *rimJ* from our laboratory and others were in the C terminus, the possibility was raised that *rimJ* might be an essential gene (59, 63).

A deletion of *rimJ* was created by crossover PCR, cloned into the allelic exchange vector pKO3, and recombined onto the chromosome of DL1504, producing the Δ*rimJ* strain CWZ388 (Table 1). In the Δ*rimJ* mutation, the DNA sequences for the first 5 and the last 11 amino acids of RimJ are retained while the internal 179 amino acids of RimJ are replaced by an insertion that encodes 8 amino acids. The insertion is in frame, preventing any polar effects on two downstream genes of unknown function that appear to be in the same operon as *rimJ*.

The Δ*rimJ* deletion strain CWZ388 showed a phase variation phenotype at both 37 and 23°C on M9 glyc, demonstrating that the Δ*rimJ* mutation disrupts thermoregulation, similar to the *rimJ*::mTn*10* insertions characterized previously (58) (data not shown). Our results also demonstrate that *rimJ* is not essential. Acetylation of S5 is not required for cell growth, as the Δ*rimJ* strain displays a growth rate similar to that of the wild-type strain DL1504 under all of the environmental conditions tested in this study (data not shown).

RimJ represses *papBA* transcription in response to multiple environmental cues. To determine if RimJ controlled *papBA* transcription in response to environmental cues other than temperature, β-galactosidase activity was measured in the wild-type strain DL1504 and the *rimJ* mutant strains, CWZ388 (Δ*rimJ*) and DL1509 (*rimJ-2*::mTn*10*), under differing environmental conditions. A phase ON (Lac$^+$) colony was used to inoculate each culture, ensuring that transcriptionally active cells were used to initiate the culture. Within a Lac$^+$ colony, 20 to 50% of cells are in a phase ON state (data not shown).

In the wild-type strain, *papBA* transcription is decreased by low temperature, LB medium, glucose as a carbon source, and high osmolarity compared to the activating conditions of M9 glyc at 37°C (Fig. 1) (60). The Δ*rimJ* mutation and the *rimJ-2*::mTn*10* mutations relieve the repression due to low temperature and LB medium such that *papBA* transcriptional levels under these normally repressive conditions are similar to levels observed at 37°C in M9 glyc (Fig. 1). While we previously reported a greater reduction due to glucose (60), more recent experiments indicate that *papBA* transcription is decreased approximately 1.8-fold, similar to the 3.4-fold reduction measured by Båga et al. (2). Both *rimJ* mutations increased transcription in glucose to levels greater than that seen in M9 glyc at 37°C (Fig. 1). These results extend the function of RimJ beyond that of a thermoregulator, as RimJ responds to multiple environmental cues to control *papBA* transcription.

We note that RimJ is not a major regulator in response to osmolarity. While the levels of *papBA* transcription are slightly elevated in the Δ*rimJ* and the *rimJ-2*::mTn*10* mutant strains grown in M9 NaCl compared to the wild-type strain, high osmolarity still has a repressive effect on *papBA* transcription in the mutant strains (Fig. 1).

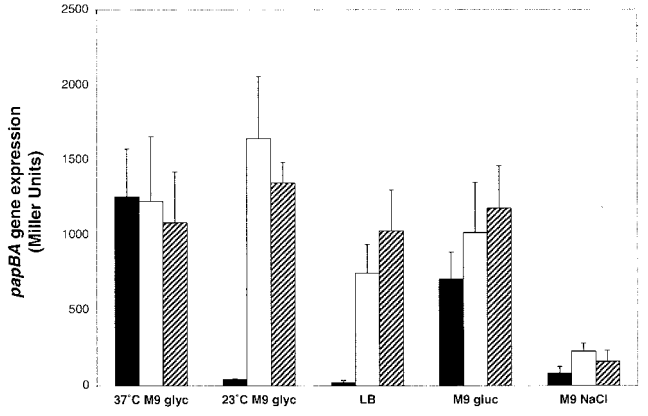

FIG. 1. Effects of environmental stimuli on *pap* fimbrial transcription in wild-type and *rimJ* mutant strains. The bars indicate β-galactosidase activities measured in the wild-type strain DL1504 (solid bars), in strain CWZ388 containing the Δ*rimJ* mutation (open bars), and in strain DL1509 containing the *rimJ-2*::mTn*10* mutation (hatched bars). β-Galactosidase activity was measured as described in Materials and Methods. Error is expressed as 1 standard deviation from the mean.

RimJ controls *papI* transcription. The PapI regulatory protein is necessary to establish the phase ON state and activate *papBA* transcription (8). *papI* is transcribed on a monocistronic operon, divergent from *papBA* (26, 57). To determine if RimJ also controls *papI* transcription, the *rimJ-2*::mTn*10* mutation was transduced into DL2838, which contains a *papI-lacZYA* fusion, creating strain CWZ412 (Table 1). Overall levels of *papI* transcription were decreased in the wild-type strain DL2838 at 23 (11 ± 4 Miller units [MU] [38]) compared to 37°C (203 ± 35 MU), whereas in CWZ412, *papI* transcription levels were similar at 37 (157 ± 9 MU) and 23°C (120 ± 14 MU). Similar to *papBA* transcription, LB medium decreased *papI* transcription in the wild-type strain (3 ± 0 MU). The *rimJ-2*::mTn*10* mutation increased *papI* transcription (28 ± 5 MU) but did not restore it to the levels seen in M9 glyc. Glucose did not greatly alter *papI* transcription in the wild-type (218 ± 10 MU) or the *rimJ-2*::mTn*10* mutant (113 ± 19 MU) strain.

Maximal *papBA* transcription in a *rimJ* mutant strain requires PapI, PapB, Lrp, Dam, and cAMP-CAP. In order to establish a phase ON state for *papBA* transcription, PapI, Lrp, cAMP-CAP, and Dam are required, while PapB plays primarily an indirect role in phase variation, that of activating *papI* transcription (reviewed in reference 30). To determine if these same regulators are still required for *papBA* transcription in the absence of RimJ, a *rimJ* mutation was tested for its effect on transcription in the absence of each individual regulator. Regardless of whether RimJ was present or absent, no phase variation was seen in strains lacking PapI, Lrp, or Dam, and transcription measured at 37 or 23°C was low (Table 2), indicating that these regulators are still required to initiate transcription in the absence of RimJ. In CWZ419 lacking PapB and RimJ, a phase variation phenotype was observed at 37°C in which Lac^+ colonies displayed a pale-blue phenotype and only Lac^- colonies were observed at 23°C. The phase ON cells observed in the *papB* Δ*rimJ* mutant might result from increased *papI* transcription due to the *rimJ* mutation. However, the level of *papI* transcription in the absence of PapB activation must not be equivalent to that in the Δ*rimJ* strain CWZ388, as overall *papBA* transcription levels are minimal (Table 2).

TABLE 2. Effects of a *rimJ* mutation on *papBA* transcription in various mutant strains

Strain	Relevant genotype	β-Galactosidase activity[a]	
		37°C M9 glyc	23°C M9 glyc
DL3052[b]	*papI* frameshift mutation	9 ± 0	6 ± 0
CWZ418	*papI* frameshift Δ*rimJ*	23 ± 1	11 ± 1
DL2208[b]	*papB* CTG start codon mutation	4 ± 0	2 ± 0
CWZ419	*papB* CTG start codon Δ*rimJ*	35 ± 4	5 ± 0
DL1910[b]	Δ*lrp*	4 ± 2	3 ± 2
DL2873	Δ*lrp rimJ-2*::mTn*10*	3 ± 1	3 ± 0
CWZ410	*dam-13*::Tn*9*	73 ± 2	11 ± 0
CWZ411	*dam-13*::Tn*9 rimJ-2*::mTn*10*	68 ± 5	12 ± 1

[a] β-Galactosidase activity is expressed as Miller units (38) and was measured as described in Materials and Methods. Error is expressed as ±1 standard deviation from the mean.
[b] β-Galactosidase activities were previously published (57).

A Δ*crp-45* Δ*rimJ* strain was viable on LB medium but was unable to grow on M9 glyc or M9 gluc agar, indicating that the absence of both proteins was deleterious for growth on minimal medium. Measurement of transcription in the wild-type strain DL1504 in LB medium yielded 8 ± 0 MU due to the repression of LB medium on *papBA* transcription, while transcription was elevated to 71 ± 5 MU in the Δ*rimJ* strain CWZ388. The double-mutant strain CWZ403 (Δ*rimJ* Δ*crp-45*) displayed a level of *papBA* transcription identical to that of the Δ*crp-45* strain DL3089 (5 ± 0 MU), demonstrating that *papBA* transcription remains cAMP-CAP dependent in the absence of RimJ. Taken together, these data indicate that all of the regulators necessary to attain a phase ON state are still required in the absence of RimJ.

RimJ inhibits the transitioning of cells to the phase ON state. Phase transition rates were calculated to determine if the loss of repression due to the *rimJ* mutations could be attributed to alterations in switch frequencies. While LB medium results in repression of *papBA* transcription, all colonies display a uniform colony phenotype on LB medium, and phase transition rates could not be calculated.

For the wild-type and *rimJ* mutant strains, the phase ON→OFF rates on M9 gluc and M9 NaCl are similar to the rates calculated on M9 glyc at 37°C, indicating that the carbon source, high osmolarity, and the *rimJ* mutations do not greatly influence the rate at which cells transition to the phase OFF state (Table 3) (60). At a low temperature for the wild-type strain, all colonies display a phase OFF colony phenotype, correlating with a phase OFF DNA methylation state (57). The rate at which cells transition from the phase ON to a phase OFF state at 23°C in the *rimJ* mutants is similar to the rate on M9 glyc at 37°C. These results indicate that the absence of RimJ removes the temperature repression of phase variation, but once it is removed, cells transition to a phase OFF state at a rate similar to that under the other conditions tested.

In contrast, the phase OFF→ON transition rates for the *papBA* operon are influenced by the *rimJ* mutations and environmental conditions (Table 3). At 37°C on M9 glyc, the phase

TABLE 3. Effects of Δ*rimJ* and *rimJ-2*::mTn*10* mutations on phase transition rates for *papBA* operon under different environmental conditions

Phase	Environmental conditions[a]	Phase transition rate[b]		
		Wild type[c]	Δ*rimJ*	*rimJ-2*::mTn*10*
ON→OFF	37°C M9 glyc	3.37×10^{-2}	2.60×10^{-2}	2.20×10^{-2}
	23°C M9 glyc	NA	3.97×10^{-2}	3.68×10^{-2}
	M9 gluc	4.40×10^{-2}	1.95×10^{-2}	4.04×10^{-2}
	M9 NaCl	4.23×10^{-2}	3.99×10^{-2}	3.89×10^{-2}
OFF→ON	37°C M9 glyc	3.50×10^{-4}	1.43×10^{-3}	8.40×10^{-4}
	23°C M9 glyc	NA	2.55×10^{-5}	6.14×10^{-5}
	M9 gluc	ND	8.26×10^{-5}	1.11×10^{-4}
	M9 NaCl	1.59×10^{-4}	7.28×10^{-5}	4.97×10^{-5}

[a] The same growth medium was used for the isolation of the initial colony (Lac^+ or Lac^-) and for the subsequent quantitation of phase transition rates from the initial colony.

[b] Phase transition rates were measured in the wild-type strain DL1504, Δ*rimJ* mutant strain CWZ388, and *rimJ-2*::mTn*10* mutant strain DL1509. The weighted averages were calculated from at least two independent analyses as described by Blyn et al (7). The frequencies are given as the number of events per cell per generation. The phase transition rates for DL1504 were previously published (60).

[c] NA, not applicable; wild-type strain DL1504 does not undergo phase variation at low temperature. ND, not determined; a weighted average could not be calculated for DL1504, as no Lac^+ colonies were observed in a screening of approximately 37,000 colonies from four independent analyses. An earlier study using a similar, but not identical, *papBA-lacZYA* transcriptional fusion yielded a phase transition frequency of 4.51×10^{-6}/cell/generation (7).

OFF→ON transition rates are increased in the Δ*rimJ* and *rimJ-2*::mTn*10* mutant strains compared to the wild-type strain, demonstrating that the *rimJ* mutations increase the phase OFF→ON rate in the absence of an environmental change. The Δ*rimJ* and *rimJ-2*::mTn*10* mutations allow cells to transition to the phase ON state at a low temperature and increase the phase OFF→ON transition rates on glucose compared to the wild-type strain (Table 3). While the phase OFF→ON rates are significantly increased over the wild-type rates under these conditions, they are reduced compared to the rates observed at 37°C on M9 glyc, indicating that the stimuli of low temperature and carbon source still retain a partial repressive effect on the phase OFF→ON transition rate in the absence of RimJ. This partial repression may be mediated by H-NS, which also inhibits the phase OFF→ON transition rate (60). High osmolarity also inhibits the rate at which cells transition to a phase ON state in the wild-type strain DL1504 (Table 2). Unlike the other conditions tested, the phase OFF→ON transition rates are further decreased in the *rimJ* mutant strains when they are grown on M9 NaCl (Table 3), in agreement with the transcriptional analyses, in which the *rimJ* mutations do not relieve the repression due to high osmolarity.

RimJ and H-NS control *papBA* transcription through separate pathways. While the *rimJ* and *hns651* mutant strains differ in many ways, both H-NS and RimJ have been shown to control *papBA* transcription in response to multiple environmental signals and share the common function of inhibiting the phase OFF→ON transition rate (57, 60), raising the question of whether RimJ and H-NS work through the same or separate regulatory pathways to control *papBA* transcription.

A Δ*rimJ hns651* double mutant strain, CWZ403, was constructed that was viable on LB medium but was unable to grow on M9 glyc or M9 gluc agar, indicating that the absence of both proteins was deleterious for growth on minimal medium. This phenotype is independent of *papBA* gene expression, as the same lethality was seen in the Δ*rimJ hns651* mutant strain CWZ387, which does not contain the *papBA-lacZYA* transcriptional fusion (data not shown). On M9 glyc, the *hns651* mutant strain DL1947 has a significantly decreased growth rate and displays a mucoid phenotype, whereas the Δ*rimJ* mutant strain CWZ388 is indistinguishable from the wild-type strain DL1504 in growth rate and colony morphology. On LB medium, the Δ*rimJ hns651* double-mutant strain grows more slowly than the wild-type or Δ*rimJ* strain, similar to the *hns651* mutant strain DL1947. The lethality of the double-mutant strain on minimal medium indicates that RimJ and H-NS work through parallel pathways, since an additional change in phenotype in the Δ*rimJ hns651* mutant strain, relative to the single mutants, would not be expected if both regulators were in the same pathway.

Due to the loss of viability of the double-mutant strain on M9 glyc, *papBA* transcription was measured after growth in LB medium at 37°C. In both of the single-mutant strains, CWZ388 (Δ*rimJ*) and DL1947 (*hns651*), the repression due to LB medium was relieved by the individual mutations compared to the wild-type strain DL1504 (Fig. 2). Overall transcription in the Δ*rimJ* strain was not as high as previously measured in cultures initiated from a Lac^+ colony (Fig. 1). Because the strains in this experiment were initially streaked on LB medium, it could not be determined if cultures were started with a phase ON (Lac^+) or phase OFF (Lac^-) colony, possibly accounting for the lower level of *papBA* transcription. Plating of LB medium-grown colonies for CWZ388 onto M9 glyc showed an average of 5% of cells in the phase ON state, correlating with the low transcription measured in these cultures. In the *hns651* strain, only a very low percentage of phase ON cells (2%) were observed even under transcriptionally activating conditions (54). In the Δ*rimJ hns651* double mutant, *papBA* transcription was elevated to levels slightly higher than those with either mutation alone (Fig. 2). Using a nested analysis of variation, the differences in β-galactosidase activity due to the genotype of the strain were found to be statistically significant, supporting the conclusion that RimJ and H-NS work through different pathways.

RimJ does not control transcription of the *fan*, *daa*, or *fim* operon in response to environmental conditions. The *fan*, *daa*, and *fim* fimbrial operons share common regulators and regulatory mechanisms with *pap*, leading us to hypothesize that RimJ might control their transcription. Transcription of all three fimbriae is regulated by Lrp and H-NS (5, 10, 25, 60). Transcription of the *daa* operon that encodes F1845 fimbriae

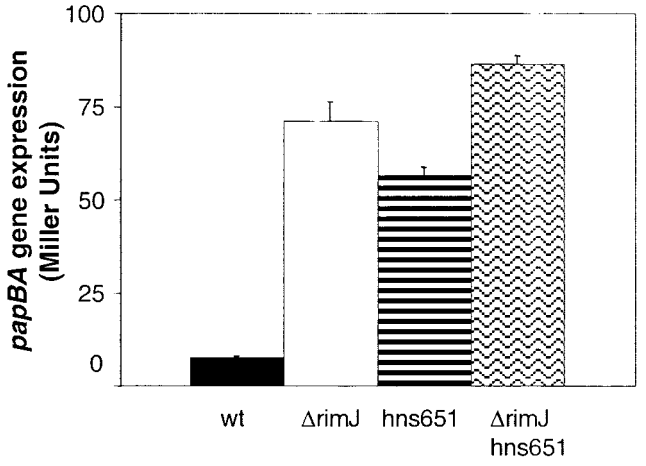

FIG. 2. Effects of the Δ*rimJ* and *hns651* mutations on *papBA* transcription. The bars indicate β-galactosidase activities measured in the wild-type (wt) strain DL1504, in the Δ*rimJ* mutant strain CWZ388, in the *hns651* mutant strain DL1947, and in the Δ*rimJ hns651* double-mutant strain CWZ403. β-Galactosidase activity was measured as described in Materials and Methods. Error is expressed as 1 standard deviation from the mean.

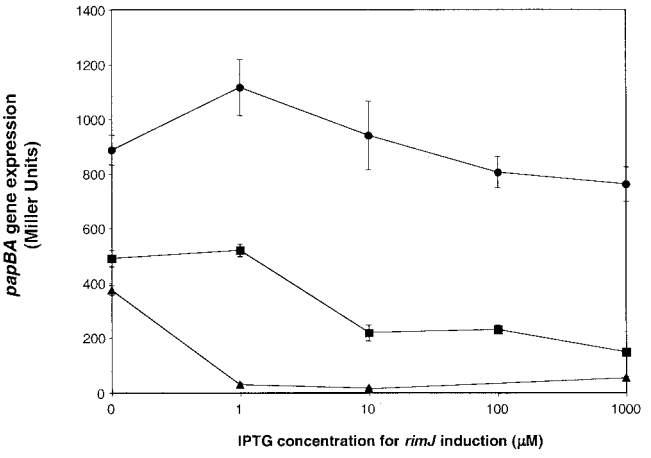

FIG. 3. Effect of increasing levels of *rimJ* on *papBA* transcription. The strain CWZ395 containing pCWZ101 (*rimJ* under the control of the $P_{lac/ara-1}$ promoter) and pMV101 ($lacI^q$) was used in this experiment. IPTG was added at concentrations ranging from 0 to 1,000 μM as indicated to induce expression of *rimJ*. The data points indicate β-galactosidase activities measured after growth in M9 glyc at 37°C (circles), LB at 37°C (squares), and M9 glyc at 23°C (triangles). β-Galactosidase activity was measured as described in Materials and Methods. Error is expressed as ±1 standard deviation from the mean.

(3) is controlled by a methylation-dependent phase variation mechanism similar to *pap* (55), whereas transcription of the *fim* operon encoding type I fimbriae (4, 13, 28) relies upon an invertible promoter phase variation mechanism (18, 43, 44). Transcription of the *fan* operon encoding K99 fimbriae (24) is not known to be subject to phase variation.

Previously, we showed that *fan* and *daa* transcription is repressed by the same environmental cues as that of *pap*—low temperature, LB medium, glucose as a carbon source, and high osmolarity (Table 4) (60). In this study, we demonstrate that *fim* transcription is also reduced by growth at a low temperature and in LB medium, in agreement with other studies showing that temperature and medium influence the rate at which cells transition to a phase OFF state (25) (Table 3).

For all three operons, the *rimJ-2*::mTn*10* mutation did not relieve repression due to low temperature or growth in LB medium. The level of *fan* transcription in the *rimJ-2*::mTn*10* mutant strain CWZ381 was similar to that of the wild-type strain DL812 under these conditions (Table 4). Similarly, the transcription of the *daa* operon in CWZ382 was not altered by introduction of the *rimJ-2*::mTn*10* mutation, nor was the transcription of *fim* in CWZ400 (Table 4). Taken together, these data suggest that RimJ may be a *pap*-specific regulator in response to environmental conditions.

Overexpression of *rimJ* complements the Δ*rimJ* mutation at 23°C or in LB medium but does not repress *papBA* transcription at 37°C in M9 glyc. To analyze the effect of *rimJ* overexpression on *papBA* transcription, *rimJ* was cloned under the control of the $P_{lac/ara-1}$ promoter in pCWZ101 (Table 1). At 23°C without the addition of IPTG, *papBA* transcription was reduced 2.4-fold compared to the level seen at 37°, indicating that some transcription of *rimJ* occurs in the absence of IPTG induction and that this low level partially complements the Δ*rimJ* mutation on the chromosome of CWZ395 (Fig. 3). At IPTG levels of 10 to 1,000 μM, *papBA* transcription was reduced to levels similar to those of the wild-type strain DL1504 at low temperature, demonstrating that pCWZ101 is able to fully complement the Δ*rimJ* mutation on the chromosome in M9 glyc at 23°C. At 37°C, *papBA* transcriptional levels were similar to the initial measurement made at 37°C in the absence of IPTG (Fig. 3). Thus, the overexpression of *rimJ* does not

TABLE 4. Effects of the *rimJ-2*::mTn*10* mutation on fimbrial transcription of the *fan, daa*, and *fim* operons

Operon fusion	Relevant genotype	β-Galactosidase activity[a]		
		37°C M9 glyc	23°C M9 glyc	LB
fanABC'-lacZYA	Wild type	8,163 ± 1,526	95 ± 6	1,538 ± 188
	rimJ-2::mTn*10*	4724 ± 440	92 ± 39	1,977 ± 706
daa-lacZYA	Wild type	72 ± 14	45 ± 4	7 ± 1
	rimJ-2::mTn*10*	36 ± 13	38 ± 6	10 ± 6
fimA-lacZYA[b]	Wild type	2,271 ± 273	716 ± 195	69 ± 10
	rimJ-2::mTn*10*	2,036 ± 239	847 ± 194	85 ± 7

[a] β-Galactosidase activity is expressed as Miller units (38) and was measured as described in Materials and Methods. Error is expressed as ±1 standard deviation from the mean.

[b] Strains DL812 (wild type) and CWZ381 (*rimJ-2*::mTn*10*) were used to analyze *fan* transcription, DL1530 (wild type) and CWZ382 (*rimJ-2*::mTn*10*) were used for *daa* transcription, and AAEC198A (wild type) and CWZ400 (*rimJ-2*::mTn*10*) were used for *fim* transcription.

repress *papBA* transcription at 37°C in M9 glyc. Similar to the results seen at low temperature in M9 glyc, *papBA* transcription in LB medium at 37°C was reduced 1.8-fold in the absence of IPTG induction (Fig. 3). At increasing concentrations of IPTG (1 to 1,000 μM), the overexpression of *rimJ* in LB medium repressed *papBA* transcription. The complementation in this case was not as complete as that observed at 23°C in M9 glyc, as *papBA* transcription did not decrease to the levels measured for the wild-type strain in LB medium (Fig. 1).

DISCUSSION

In this study, we provide evidence that the function of RimJ extends beyond that of a thermoregulator. RimJ is involved in cellular response to other environmental cues, including growth (LB) medium and glucose as a carbon source. We can envision two different, and not necessarily mutually exclusive, models for the role of RimJ in decreasing *papBA* transcription in response to these signals. In one model, RimJ alters phase variation frequencies by decreasing the rate at which cells transition to a phase ON state and/or increasing the rate at which cells transition to a phase OFF state. Alternatively, RimJ may act to inhibit *papBA* transcription by a mechanism independent of phase variation.

In accordance with the first model, our results demonstrate that RimJ inhibits the transition of cells into the phase ON state. This effect on the transition rate may be the only mechanism required to account for the effect of RimJ on *papBA* transcription when glucose is provided as the sole carbon source. Because glucose does not alter the ON→ OFF rate (Table 3) (60), we postulate that the only effect of glucose is to prevent cells that are phase OFF from transitioning into the phase ON state due to limiting cAMP-CAP. Phase ON cells used to initiate the culture maintain a transcriptionally active state after transfer into M9 gluc, but in the absence of a mechanism to recruit new cells into the phase ON state, *papBA* transcription gradually decreases (Fig. 1). Growth of cultures for longer periods in M9 gluc show further reduction in overall *papBA* transcription and the percentage of cells in the phase ON state, consistent with this model (data not shown). The increase in *papBA* transcription in the *rimJ* mutants, relative to the wild-type strain, results from an increased frequency of cells switching to the phase ON state. It is a paradox how phase OFF cells in a *rimJ* mutant strain attain a phase ON state in glucose, since cAMP-CAP should be limiting. We note that the phase transition rates measured in glucose for the *rimJ* mutants are greater than those measured in the wild type in M9 gluc but are significantly decreased compared to the wild type and *rimJ* mutants in glycerol.

In contrast to glucose, low temperature and LB medium cause a more dramatic reduction in *papBA* transcription, suggesting that RimJ may play an additional role unrelated to inhibiting the phase OFF→ON transition. At low temperature, all of the cells transition to a phase OFF state in the wild-type strain. If temperature regulation were dependent only upon phase variation, it would suggest that at low temperature RimJ both increases the phase ON→OFF rate and decreases the phase OFF→ON rate. Yet in the *rimJ* mutant strains, the ON→OFF rates at 23°C on M9 glyc are basically unchanged relative to the wild-type strain at 37°C, arguing that RimJ does not function by simply altering this transition rate. Previous temperature downshift experiments show that *papBA* transcription is rapidly repressed within 1 generation of growth at 23°C while approximately 20% of the cells are still in the phase ON state based on analysis of the DNA methylation states (57). Thus, RimJ may have an additional role in the rapid repression of *papBA* transcription prior to transition to the phase OFF methylation state. Additional experiments are being pursued to understand the interrelationship between environmental regulation and phase variation, particularly in response to LB medium.

It is not known whether the acetyltransferase activity of RimJ is necessary for the regulation of *papBA* transcription, although the evidence presented here is suggestive. The two sequenced *rimJ*::mTn*10* insertions are inserted between motifs A and B (59), motifs conserved in the *N*-acetyltransferase superfamily and that encompass the acetyl-coenzyme A binding site (19, 34, 40, 49). Minicell analysis demonstrated that fusion proteins of RimJ with the mTn*10* elements are expressed (59), suggesting that it may be the disruption of the acetyl-coenzyme A site and not loss of the entire protein that leads to the loss of *papBA* repression. This conclusion is further supported by the observation that the *rimJ-2*::mTn*10* and Δ*rimJ* mutations have similar effects on *papBA* transcription and phase variation. A search using only the N-terminal portion of RimJ did not detect homology to any known conserved domain: no other known function can, at present, be attributed to RimJ.

RimJ may be acting indirectly by altering the quantity of a regulatory protein or directly by modifying a protein involved in *papBA* transcription and influencing its activity. While it has been shown that RimJ is highly specific for its ribosomal substrate, S5, RimJ may have additional nonribosomal substrates (27, 63). Given that H-NS controls transcription of the *papBA* operon and that studies have indicated that H-NS is posttranslationally modified (17, 51), one possible model argues that RimJ acetylates H-NS, modulating its activity under varying environmental conditions. Our results are not consistent with this conclusion but rather indicate that RimJ and H-NS function in separate pathways. In addition, RimJ does not alter H-NS levels, as these levels remain unchanged at 23 and 37°C (57). While alternative substrates for RimJ must be considered, it is possible that the acetylation of S5 determines whether full-length *papBA* transcription is completed only under the activating, but not the repressive, conditions. In addition to their well-known structural roles, the ribosomal proteins S4, S10, and L4 also play roles as transcriptional antiterminators (23, 31, 48, 50, 56, 62). S5 may play a similar dual role. Studies in our laboratory are aimed at determining RimJ substrate specificity and the importance of the acetylase activity for the repression of *papBA* transcription.

With these ideas in mind, it is intriguing to consider how RimJ responds to environmental conditions to repress *papBA* transcription. Transcription of *rimJ* may itself be modulated by environmental signals, but this simple mechanism is undercut by the *rimJ* overexpression results. An inducible promoter is used in this experiment, making *rimJ* transcription unresponsive to environmental conditions. Nevertheless, increasing *rimJ* mRNA levels under activating conditions is insufficient to repress *papBA* transcription, indicating that functionally active

RimJ protein, capable of decreasing *papBA* transcription, is not being produced in M9 glyc at 37°C. Thus, alternative hypotheses must be proposed for how the function of RimJ is sensitive to environmental conditions. It is possible that *rimJ* mRNA stability, RimJ protein stability, or RimJ translation is environmentally controlled, thus ensuring production of RimJ only under repressive conditions. Alternatively, it may be that RimJ protein is equally expressed under all growth conditions but that it is only active under repressing environmental conditions. Lastly, it may be that the substrate of RimJ is itself regulated by environmental conditions. Our preliminary experiments rule out the environmental modulation of at least one important RimJ substrate: S5 is present under all of the conditions tested, and S5 expression levels are not altered by the Δ*rimJ* mutation (data not shown).

From our overexpression data, we note that the function of RimJ can be modulated by multiple environmental cues and that the presence of a single repressive cue will determine the activity of RimJ. When grown in M9 glyc, the ability of RimJ to repress *papBA* transcription is temperature dependent. However, when the medium is changed to LB, RimJ is able to repress *papBA* transcription even at a higher growth temperature. Thus, while one stimulus is activating (temperature), the other stimulus is repressive (growth medium), and the repressive stimulus dictates the activity of RimJ. This represents an efficient mechanism for regulation that may be important in vivo, where it may be necessary to control virulence gene expression based on multiple environmental cues.

Taken together, our investigations of RimJ demonstrate its importance for regulating the expression of *papBA* expression in response to multiple environmental cues. Environmental cues play an integral role in regulating virulence gene expression that may impact a pathogen's ability to colonize a host and its survival in external environments. Consequently, RimJ may play a significant role in the adaptation of uropathogenic *E. coli* to changing environments.

ACKNOWLEDGMENTS

We thank Marjan van der Woude, David Low, George Church, and Ian Blomfield for generous gifts of bacterial strains and plasmids. In addition, we thank Marjan van der Woude and Rob Dorit for critical reading of the manuscript. We also thank the Smith College students who have provided technical assistance on this project, including Olivia Carrick, Jennifer Hoot, Stephanie Jakus, Michelle Ploutz, and Angela Rasmussen.

This work was supported by National Institutes of Health grant GM62792 to Christine White-Ziegler, by the Albert F. Blakeslee Trust, and by Smith College.

REFERENCES

1. **Atlung, T., and H. Ingmer.** 1997. H-NS: a modulator of environmentally regulated gene expression. Mol. Microbiol **24:**7–17.
2. **Båga, M., M. Göransson, S. Normark, and B. E. Uhlin.** 1985. Transcriptional activation of a *pap* pilus virulence operon from uropathogenic *Escherichia coli*. EMBO J. **4:**3887–3893.
3. **Bilge, S. S., C. R. Clausen, W. Lau, and S. L. Moseley.** 1989. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J. Bacteriol. **171:**4281–4289.
4. **Bloch, C. A., B. A. Stocker, and P. E. Orndorff.** 1992. A key role for type 1 pili in enterobacterial communicability. Mol. Microbiol. **6:**697–701.
5. **Blomfield, I. C., P. J. Calie, K. J. Eberhardt, M. S. McClain, and B. I. Eisenstein.** 1993. Lrp stimulates phase variation of type 1 fimbriation in *Escherichia coli* K-12. J. Bacteriol. **175:**27–36.
6. **Blyn, L. B., B. A. Braaten, and D. A. Low.** 1990. Regulation of *pap* pilin phase variation by a mechanism involving differential Dam methylation states. EMBO J. **9:**4045–4054.
7. **Blyn, L. B., B. A. Braaten, C. A. White-Ziegler, D. H. Rolfson, and D. A. Low.** 1989. Phase-variation of pyelonephritis-associated pili in *Escherichia coli*: evidence for transcriptional regulation. EMBO J. **8:**613–620.
8. **Braaten, B. A., L. B. Blyn, B. S. Skinner, and D. A. Low.** 1991. Evidence for a methylation-blocking factor (*mbf*) locus involved in *pap* pilus expression and phase variation in *Escherichia coli*. J. Bacteriol. **173:**1789–1800.
9. **Braaten, B. A., X. Nou, L. S. Kaltenbach, and D. A. Low.** 1994. Methylation patterns in *pap* regulatory DNA control pyelonephritis-associated pili phase variation in *E. coli*. Cell **76:**577–588.
10. **Braaten, B. A., J. V. Platko, M. W. van der Woude, B. H. Simons, F. K. DeGraaf, J. M. Calvo, and D. A. Low.** 1992. Leucine-responsive regulatory protein controls the expression of both the *pap* and *fan* pili operons in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **89:**4250–4254.
11. **Casadaban, M.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *E. coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104:**541–555.
12. **Chung, C. T., S. L. Niemela, and R. H. Miller.** 1989. One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. USA **86:**2172–2175.
13. **Connell, H., L. K. Poulsen, and P. Klemm.** 2000. Expression of type 1 and P fimbriae *in situ* and localisation of a uropathogenic *Escherichia coli* strain in the murine bladder and kidney. Int. J. Med Microbiol **290:**587–597.
14. **Correnti, J., V. Munster, T. Chan, and M. van der Woude.** 2002. Dam-dependent phase variation of Ag43 in *E. coli* is altered in a *seqA* mutant. Mol. Microbiol. **44:**521–532.
15. **Cotter, P. A., and J. F. Miller.** 1998. *In vivo* and *ex vivo* regulation of bacterial virulence gene expression. Curr. Opin. Microbiol. **1:**17–26.
16. **Cumberlidge, A. G., and K. Isono.** 1979. Ribosomal protein modification in *Escherichia coli*. I. A mutant lacking the N-terminal acetylation of protein S5 exhibits thermosensitivity. J. Mol. Biol. **131:**169–189.
17. **Donato, G. M., and T. H. Kawula.** 1999. Phenotypic analysis of random *hns* mutations differentiate DNA-binding activity from properties of *fimA* promoter inversion modulation and bacterial motility. J. Bacteriol. **181:**941–948.
18. **Dorman, C. J., and N. N. Bhriain.** 1992. Thermal regulation of *fimA*, the *Escherichia coli* gene coding for the type 1 fimbrial subunit protein. FEMS Microbiol. Lett. **78:**125–130.
19. **Dutnall, R. N., S. T. Tafrov, R. Sternglanz, and V. Ramakrishnan.** 1998. Structure of the histone acetyltransferase Hat1: a paradigm for the GCN5-related N-acetyltransferase superfamily. Cell **94:**427–438.
20. **Edwards, R. A., L. H. Keller, and D. M. Schifferli.** 1998. Improved allelic exchange vectors and their use to analyze 987P fimbria gene expression. Gene **207:**149–157.
21. **Edwards, R. A., and D. M. Schifferli.** 1997. Differential regulation of *fasA* and *fasH* expression of *Escherichia coli* 987P fimbriae by environmental cues. Mol. Microbiol. **25:**797–809.
22. **Falconi, M., V. McGovern, C. Gualerzi, D. Hillyard, and N. P. Higgins.** 1991. Mutations altering chromosomal protein H-NS induce mini-Mu transposition. New Biol. **3:**615–625.
23. **Friedman, D. I., A. T. Schauer, M. R. Baumann, L. S. Baron, and S. L. Adhya.** 1981. Evidence that ribosomal protein S10 participates in control of transcription termination. Proc. Natl. Acad. Sci. USA **78:**1115–1118.
24. **Gaastra, W., and F. K. deGraaf.** 1982. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. **46:**129–161.
25. **Gally, D. L., J. A. Bogan, B. I. Eisenstein, and I. C. Blomfield.** 1993. Environmental regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media. J. Bacteriol. **175:**6186–6193.
26. **Göransson, M., K. Forsman, and B. E. Uhlin.** 1989. Regulatory genes in the thermoregulation of *Escherichia coli* pili gene transcription. Genes Dev. **3:**123–130.
27. **Isono, K., and S. Isono.** 1980. Ribosomal protein modification in *Escherichia coli*. II. Studies of a mutant lacking the N-terminal acetylation of protein S18. Mol. Gen. Genet. **177:**645–651.
28. **Johnson, J. R.** 1991. Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. **4:**80–128.
29. **Jordi, B. J., B. Dagberg, L. A. de Haan, A. M. Hamers, B. A. van der Zeijst, W. Gaastra, and B. E. Uhlin.** 1992. The positive regulator CfaD overcomes the repression mediated by histone-like protein H-NS (H1) in the CFA/I fimbrial operon of *Escherichia coli*. EMBO J. **11:**2627–2632.
30. **Krabbe, M., N. Weyand, and D. Low.** 2000. Environmental control of pilus gene expression, p. 305–322. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, D.C.
31. **Li, X., L. Lindahl, and J. M. Zengel.** 1996. Ribosomal protein L4 from *Escherichia coli* utilizes nonidentical determinants for its structural and regulatory functions. RNA **2:**24–37.
32. **Link, A. J., D. Phillips, and G. M. Church.** 1997. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: application to open reading frame characterization. J. Bacteriol. **179:**6228–6237.

33. **Low, D. A., E. N. Robinson, Jr., Z. A. McGee, and S. Falkow.** 1987. The frequency of expression of pyelonephritis-associated pili is under regulatory control. Mol. Microbiol. **1:**335–346.
34. **Lu, L., K. A. Berkey, and R. A. Casero, Jr.** 1996. RGFGIGS is an amino acid sequence required for acetyl coenzyme A binding and activity of human spermidine/spermine N1 acetyltransferase. J. Biol. Chem. **271:**18920–18924.
35. **Lutz, R., and H. Bujard.** 1997. Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O and TetR/O and AraCI1-I2 regulatory elements. Nucleic Acids Res. **25:**1203–1210.
36. **Mahan, M. J., J. M. Slauch, and J. J. Mekalanos.** 1996. Environmental regulation of virulence gene expression in *Escherichia*, *Salmonella*, and *Shigella* spp., p. 2803–2816. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
37. **Martinez-Laguna, Y., E. Calva, and J. L. Puente.** 1999. Autoactivation and environmental regulation of *bfpT* expression, the gene coding for the transcriptional activator of *bfpA* in enteropathogenic *Escherichia coli*. Mol. Microbiol. **33:**153–166.
38. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
39. **Nagy, L. K., T. Mackenzie, D. J. Pickard, and G. Dougan.** 1986. Effects of immune colostrum on the expression of a K88 plasmid encoded determinant: role of plasmid stability and influence of phenotypic expression of K88 fimbriae. J. Gen. Microbiol. **132:**2497–2503.
40. **Neuwald, A. F., and D. Landsman.** 1997. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem. Sci. **22:**154–155.
41. **Normark, S., D. Lark, R. Hull, M. Norgren, M. Båga, P. O'Hanley, G. Schoolnik, and S. Falkow.** 1983. Genetics of digalactoside-binding adhesion from a uropathogenic *Escherichia coli*. Infect. Immun. **41:**942–949.
42. **O'Hanley, P., D. A. Low, I. Romero, D. Lark, K. Vosti, S. Falkow, and G. Schoolnik.** 1985. Gal-Gal binding and hemolysin phenotypes and genotypes associated with uropathogenic *Escherichia coli*. N. Engl. J. Med. **313:**414–420.
43. **Olsen, P. B., and P. Klemm.** 1994. Localization of promoters in the *fim* gene cluster and the effect of H-NS on the transcription of *fimB* and *fimE*. FEMS Microbiol. Lett. **116:**95–100.
44. **Olsen, P. B., M. A. Schembri, D. L. Gally, and P. Klemm.** 1998. Differential temperature modulation by H-NS of the *fimB* and *fimE* recombinase genes which control the orientation of the type 1 fimbrial phase switch. FEMS Microbiol. Lett. **162:**17–23.
45. **Puente, J. L., D. Bieber, S. W. Ramer, W. Murray, and G. K. Schoolnik.** 1996. The bundle-forming pili of enteropathogenic *Escherichia coli*: transcriptional regulation by environmental signals. Mol. Microbiol. **20:**87–100.
46. **Schmoll, T., M. Ott, B. Oudega, and J. Hacker.** 1990. Use of a wild-type gene fusion to determine the influence of environmental conditions on expression of the S fimbrial adhesin in an *Escherichia coli* pathogen. J. Bacteriol. **172:**5103–5111.
47. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
48. **Squires, C. L., and D. Zaporojets.** 2000. Proteins shared by the transcription and translation machines. Annu. Rev. Microbiol. **54:**775–798.
49. **Tercero, J. C., L. E. Riles, and R. B. Wickner.** 1992. Localized mutagenesis and evidence for post-transcriptional regulation of MAK3. A putative N-acetyltransferase required for double-stranded RNA virus propagation in *Saccharomyces cerevisiae*. J. Biol. Chem. **267:**20270–20276.
50. **Torres, M., C. Condon, J. M. Balada, C. Squires, and C. L. Squires.** 2001. Ribosomal protein S4 is a transcription factor with properties remarkably similar to NusA, a protein involved in both non-ribosomal and ribosomal RNA antitermination. EMBO J. **20:**3811–3820.
51. **Ussery, D. W., J. C. D. Hinton, B. J. A. M. Jordi, P. E. Granum, A. Seirafi, R. J. Stephen, A. E. Tupper, G. Berridge, J. M. Sidebotham, and C. F. Higgins.** 1994. The chromatin-associated protein H-NS. Biochimie **76:**968–980.
52. **van der Woude, M., B. Braaten, and D. Low.** 1996. Epigenetic phase variation of the *pap* operon in *Escherichia coli*. Trends Microbiol. **4:**5–9.
53. **van der Woude, M. W.** 1990. Regulation of the production of fimbriae by enterotoxigenic *Escherichia coli*. Ph.D. thesis. Free University of Amsterdam, Amsterdam, The Netherlands.
54. **van der Woude, M. W., L. S. Kaltenbach, and D. A. Low.** 1995. Leucine-responsive regulatory protein plays dual roles as both an activator and a repressor of the *Escherichia coli pap* fimbrial operon. Mol. Microbiol. **17:**303–312.
55. **van der Woude, M. W., and D. A. Low.** 1994. Leucine-responsive regulatory protein and deoxyadenosine methylase control phase variation and expression of the *sfa* and the *daa* pili operons in *Escherichia coli*. Mol. Microbiol **11:**605–618.
56. **Warren, F., and A. Das.** 1984. Formation of termination-resistant transcription complex at phage lambda nut locus: effects of altered translation and a ribosomal mutation. Proc. Natl. Acad. Sci. USA **81:**3612–3616.
57. **White-Ziegler, C. A., M. L. Angus Hill, B. A. Braaten, M. W. van der Woude, and D. A. Low.** 1998. Thermoregulation of *Escherichia coli pap* transcription: H-NS is a temperature-dependent DNA methylation blocking factor. Mol. Microbiol. **28:**1121–1137.
58. **White-Ziegler, C. A., L. B. Blyn, B. A. Braaten, and D. A. Low.** 1990. Identification of a genetic locus in *Escherichia coli* involved in the thermoregulation of the *pap* operon. J. Bacteriol. **172:**1775–1782.
59. **White-Ziegler, C. A., and D. A. Low.** 1992. Thermoregulation of the *pap* operon: evidence for the involvement of RimJ, the N-terminal acetylase of ribosomal protein S5. J. Bacteriol. **174:**7003–7012.
60. **White-Ziegler, C. A., A. Villapakkam, K. Ronaszeki, and S. D. Young.** 2000. H-NS controls *pap* and *daa* fimbrial transcription in *Escherichia coli* in response to multiple environmental cues. J. Bacteriol. **182:**6391–6400.
61. **Williams, R. M., and S. Rimsky.** 1997. Molecular aspects of the *E. coli* nucleoid protein, H-NS: a central controller of gene regulatory networks. FEMS Microbiol. Lett. **156:**175–185.
62. **Worbs, M., R. Huber, and M. C. Wahl.** 2000. Crystal structure of ribosomal protein L4 shows RNA-binding sites for ribosome incorporation and feedback control of the S10 operon. EMBO J. **19:**807–818.
63. **Yoshikawa, A., S. Isono, A. Sheback, and K. Isono.** 1987. Cloning and nucleotide sequencing of the genes *rimI* and *rimJ* which encode enzymes acetylating ribosomal proteins S18 and S5 of *Escherichia coli* K12. Mol. Gen. Genet. **209:**481–488.