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Christine A. White-Ziegler  
*Smith College*, cwhitezi@smith.edu

Alia M. Black  
*Smith College*

Stacie H. Eliades  
*Smith College*

Sarah Young  
*Smith College*

Kimberly Porter  
*Smith College*

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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

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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 ΔrimJ 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 ΔrimJ 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\textsuperscript{prox} and GATC\textsuperscript{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\textsuperscript{prox} and GATC\textsuperscript{dist} sites at 23°C, but not 37°C (57).

RimJ was initially identified in a thermoregulatory mutant screen in which random chromosomal mini-Tn10 (mTn10) in-
RimJ and environmental cues regulate pap transcription

In this study, we provide evidence that RimJ controls papBA 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).

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

<table>
<thead>
<tr>
<th>Strain, plasmid, or bacteriophage</th>
<th>Description</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli strains</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| MC4100                            | F−  
  Δ(lacIPOZYA-argF)U169  
  ρsl thi-1 | 11                   |
| NH757                             | B178 hns651 tyrT::Tn10 | 22                   |
| DL479                             | MC4100 λ246 lysogen (papBA-lacZYA) rimJ-2::mTn10 | 58                   |
| DL612                             | MC4100 AMW01 lysogen (fapABC- lacZYA) | 53                   |
| DL1504                            | MC4100 λ354 lysogen (papBA-lacZYA) | 9                    |
| DL1509                            | DL1504 rimJ-2::mTn10 | This work |
| DL1530                            | MC4100 λ366 lysogen (daa-lacZYA) | 55                   |
| DL1910                            | DL1504 Δlp | 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::mTn10 | This work |
| DL3089                            | DL1504 Δcp-45  
  zhd-3083::Tn10 | D. A. Low |
| AAEC198A                          | MG1655 ΔlacZYA fimA-lacZYA | 5                    |
| CWZ381                            | DL812 rimJ-2::mTn10 | This work |
| CWZ382                            | DL1530 rimJ-2::mTn10 | This work |
| CWZ387                            | MC4100 ΔrimJ | This work |
| CWZ388                            | DL1504 ΔrimJ | This work |
| CWZ395                            | CWZ388 containing pCWZ101 and pMV101 | This work |
| CWZ400                            | AAEC198A rimJ-2::mTn10 | This work |
| CWZ403                            | CWZ388 hns651 | This work |
| CWZ405                            | CWZ388 Δcp-45  
  zhd-3083::Tn10 | This work |
| CWZ406                            | CWZ387 hns651 | This work |
| CWZ410                            | DL1504 dam-13::Tn9 | This work |
| CWZ411                            | DL1509 dam-13::Tn9 | This work |
| CWZ412                            | DL2838 rimJ-2::mTn10 | 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 Pz+2                       | ColE1 replicon containing the P<sub>lac</sub>-ara-1 promoter | 35                   |
| pMV101                            | PMC9 derivative containing lacI<sup>+</sup> that is Ap<sup>+</sup> Tc<sup>+</sup> | 14                   |
| pMV106                            | pUHS*2 Pz+2 with replacement of Kan<sup>R</sup> with Ap<sup>+</sup> | 14                   |
| pKO3                              | pSC101 replicon containing renA (Ts) replication origin, sacB, and Cm<sup>R</sup> | 32                   |
| pCWZ100                           | pKO3 containing ΔrimJ deletion | This work |
| pCWZ101                           | pMV106 containing rimJ under P<sub>lac</sub>-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 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.
Construction of mutant strains by PI transduction. The preparation of PI lysates and PI transductions were carried out as described previously (60). rimJ::mTn10, dam-13::Tn9, Δrep-45, and hns651 mutant strains were created by PI 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 CWZ357, creating CWZ318 and CWZ349, respectively (Table 1).

Construction of ΔrimJ 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′-CGCGGATCCTCGGCCATACCATGTTGCGC-3′, and rimJ(B), 5′-CCCCATCCTAAACAAAAGCCGTATAGCCAAAACAT-3′, were used to generate a 573-bp upstream fragment, and primers rimJ(C), 5′-TGTTTAAAGTTAG TGGATGGGCCATATTAACTACCCCGACG-3′, and rimJ(D), 5′-CCGCGGTTC CGGGTTACCCTCGTCGC-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 BamHI-SalI-digested PCR product was cloned into BamHI-SalI-digested pKO3 to create pCWZ100 (Table 1).

pCWZ100 was transformed (12) into DL1504, and the selection for integration of the ΔrimJ deletion onto the chromosome was performed as described previously, with the exception that the induction 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 ΔrimJ strain, CWZ388 (Table 1), the region overlapping the deletion was sequenced to confirm the correct replacement. Sequences 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′-CGCGGATCCTCGGCCATACCATGTTGCGC-3′ and 5′-CCTCGTACTAGAAGGGCAGTAAGTTG AT-3′. The amplified fragment and pMV106 were each digested with EcoRI and BamHI and subsequently ligated to create pCWZ101 (Table 1). pCWZ101 and pMV101, containing the lecF gene, were cotransformed (12) into CWZ388 to yield strain CWZ389 (Table 1).

Growth conditions. Media (M9 glyc, M9 gluc, M9 NaCl, and LB) were prepared as described previously (60). For 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 glyc or M9 NaCl medium were used to measure the effect of a change in carbon source and osmolarity, respectively. Glucose was substituted for glyc- erol 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+D-thiogalactopyranoside (IPTG) were inoculated as described previously (12) into CWZ388 to measure β-galactosidase activities represent average levels observed at 37°C. β-galactosidase activity was measured in the two originally characterized ΔrimJ::mTn10 mutant strains, CWZ388 and CWZ357, under differing 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 (ΔrimJ) and DL1509 (rimJ-2::mTn10), 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 ΔrimJ mutation and the rimJ-2::mTn10 mutations relieve the repression due to low temperature and LB medium such that papBA transcription 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 Bāga 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 ΔrimJ and the rimJ-2::mTn10 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).
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::mTn10 mutation was transduced into DL2838, which contains a papI-lacZYA fusion, creating strain CWZ412 (Table 1). Overall levels of \(\text{papI}^{\text{ON}}\) transcription were decreased in the wild-type strain DL2838 at 23 °C (11 ± 4 Miller units [MU]) compared to 37 °C (203 ± 35 MU), whereas in CWZ412, papI transcription levels were similar at 37 °C (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::mTn10 mutation increased papI transcription (28 ± 5 MU) but did not increase the levels seen in M9 glyc. Glucose did not greatly alter papI transcription in the wild-type (218 ± 10 MU) or the rimJ-2::mTn10 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-10 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 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 rimJ strain CWZ388, as overall papBA transcription levels are minimal (Table 2).

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 glyc 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

\[\text{beta-Galactosidase activity}^a \]

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>(37^\circ)C M9 glyc</th>
<th>(23^\circ)C M9 glyc</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL3052</td>
<td>papI frameshift mutation</td>
<td>9 ± 0</td>
<td>6 ± 0</td>
</tr>
<tr>
<td>CWZ418</td>
<td>papI frameshift (\Delta\text{rimJ})</td>
<td>23 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>DL2208</td>
<td>papB CTG start codon mutation</td>
<td>4 ± 2</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>CWZ419</td>
<td>papB CTG start codon (\Delta\text{rimJ})</td>
<td>35 ± 4</td>
<td>5 ± 0</td>
</tr>
<tr>
<td>DL1910</td>
<td>(\Delta\text{lp})</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>DL2873</td>
<td>(\Delta\text{lp rimJ-2::mTn10})</td>
<td>3 ± 1</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>CWZ410 dam-13::Tn9</td>
<td></td>
<td>75 ± 2</td>
<td>11 ± 0</td>
</tr>
<tr>
<td>CWZ411 dam-13::Tn9 rimJ-2::xTn10</td>
<td></td>
<td>68 ± 5</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>

\(^a\)beta-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.

\(^b\)beta-Galactosidase activities were previously published (57).
OFF → ON transition rates are increased in the ∆rimJ and rimJ-2::mTn10 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 ∆rimJ and rimJ-2::mTn10 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 gly, 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 ∆rimJ hns651 double mutant strain, CWZ403, was constructed that was viable on LB medium but was unable to grow on M9 gly 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 ∆rimJ hns651 mutant strain CWZ387, which does not contain the papBA-lacZ/YA transcriptional fusion (data not shown). On M9 gly, the hns651 mutant strain DL1947 has a significantly decreased growth rate and displays a mucoid phenotype, whereas the ∆rimJ mutant strain CWZ388 is indistinguishable from the wild-type strain DL1504 in growth rate and colony morphology. On LB medium, the ∆rimJ hns651 double-mutant strain grows more slowly than the wild-type or ∆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 ∆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 gly, papBA transcription was measured after growth in LB medium at 37°C. In both of the single-mutant strains, CWZ388 (∆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 ∆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 gly 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 ∆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
Transcription of the \(\text{fanABC}\) operon is similar to \(\text{pap}\) (55), whereas transcription of the \(\text{fim}\) operon encoding type I fimbriae (4, 13, 28) relies upon an invertible promoter phase variation mechanism (18, 43, 44). Transcription of the \(\text{fan}\) operon encoding K99 fimbriae (24) is not known to be subject to phase variation.

Previously, we showed that \(\text{fan}\) and \(\text{daa}\) transcription is repressed by the same environmental cues as that of \(\text{pap}\) — low temperature, LB medium, glucose as a carbon source, and high osmolarity (Table 4) (60). In this study, we demonstrate that \(\text{fim}\) 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 \(\text{rimJ-2::mTn10}\) mutation did not relieve repression due to low temperature or growth in LB medium. The level of \(\text{fan}\) transcription in the \(\text{rimJ-2::mTn10}\) mutant strain CWZ381 was similar to that of the wild-type strain DL812 under these conditions (Table 4). Similarly, the transcription of the \(\text{daa}\) operon in CWZ382 was not altered by introduction of the \(\text{rimJ-2::mTn10}\) mutation, nor was the transcription of \(\text{fim}\) in CWZ400 (Table 4). Taken together, these data suggest that RimJ may be a \(\text{pap}\)-specific regulator in response to environmental conditions.

**Overexpression of \(\text{rimJ}\) complements the \(\Delta\text{rimJ}\) mutation at \(37^\circ\text{C}\) or in LB medium but does not repress \(\text{papBA}\) transcription at \(37^\circ\text{C}\) in M9 gly.

To analyze the effect of \(\text{rimJ}\) overexpression on \(\text{papBA}\) transcription, \(\text{rimJ}\) was cloned under the control of the \(\text{P}_{\text{lac/ara-1}}\) promoter in pCWZ101 (Table 1). At \(23^\circ\text{C}\) without the addition of IPTG, \(\text{papBA}\) transcription was reduced 2.4-fold compared to the level seen at \(37^\circ\text{C}\), indicating that some transcription of \(\text{rimJ}\) occurs in the absence of IPTG induction and that this low level partially complements the \(\Delta\text{rimJ}\) mutation on the chromosome of CWZ395 (Fig. 3). At IPTG levels of 10 to 1,000 \(\mu\text{M}\), \(\text{papBA}\) 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 \(\Delta\text{rimJ}\) mutation on the chromosome in M9 gly at \(23^\circ\text{C}\). At \(37^\circ\text{C}\), \(\text{papBA}\) transcriptional levels were similar to the initial measurement made at \(37^\circ\text{C}\) in the absence of IPTG (Fig. 3). Thus, the overexpression of \(\text{rimJ}\) does not

![FIG. 2. Effects of the \(\Delta\text{rimJ}\) and \(\text{hns651}\) mutations on \(\text{pap}\) transcription. The bars indicate \(\beta\)-galactosidase activities measured in the wild-type (wt) strain DL1504, in the \(\Delta\text{rimJ}\) mutant strain CWZ388, in the \(\text{hns651}\) mutant strain DL1947, and in the \(\Delta\text{rimJ} \text{hns651}\) double-mutant strain CWZ403. \(\beta\)-Galactosidase activity was measured as described in Materials and Methods. Error is expressed as 1 standard deviation from the mean.](https://journals.asm.org/journal/jb on 19 September 2022 by 24.62.206.239.

![FIG. 3. Effect of increasing levels of \(\text{rimJ}\) on \(\text{pap}\) transcription. The strain CWZ395 containing pCWZ101 (\(\text{rimJ}\) under the control of the \(\text{P}_{\text{lac/ara-1}}\) promoter) and pMV101 (\(\text{lacP}\)) was used in this experiment. IPTG was added at concentrations ranging from 0 to 1,000 \(\mu\text{M}\) as indicated to induce expression of \(\text{rimJ}\). The data points indicate \(\beta\)-galactosidase activities measured after growth in M9 gly at \(37^\circ\text{C}\) (circles), LB at \(37^\circ\text{C}\) (squares), and M9 gly at \(23^\circ\text{C}\) (triangles). \(\beta\)-Galactosidase activity was measured as described in Materials and Methods. Error is expressed as 1 standard deviation from the mean.](https://journals.asm.org/journal/jb on 19 September 2022 by 24.62.206.239.

### TABLE 4. Effects of the \(\text{rimJ-2::mTn10}\) mutation on fimbrial transcription of the \(\text{fan}\), \(\text{daa}\), and \(\text{fim}\) operons

<table>
<thead>
<tr>
<th>Operon fusion</th>
<th>Relevant genotype</th>
<th>(\text{37^\circ\text{C}}) M9 gly</th>
<th>(\text{23^\circ\text{C}}) M9 gly</th>
<th>LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{fanABC-lacZYA})</td>
<td>Wild type</td>
<td>8,163 ± 1,526</td>
<td>95 ± 6</td>
<td>1,538 ± 188</td>
</tr>
<tr>
<td>(\text{rimJ-2::mTn10})</td>
<td>4724 ± 440</td>
<td>92 ± 39</td>
<td>1,977 ± 706</td>
<td></td>
</tr>
<tr>
<td>(\text{daa-lacZYA})</td>
<td>Wild type</td>
<td>72 ± 14</td>
<td>45 ± 4</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>(\text{rimJ-2::mTn10})</td>
<td>36 ± 13</td>
<td>38 ± 6</td>
<td>10 ± 6</td>
<td></td>
</tr>
<tr>
<td>(\text{fimA-lacZYA})</td>
<td>Wild type</td>
<td>2,271 ± 273</td>
<td>716 ± 195</td>
<td>69 ± 10</td>
</tr>
<tr>
<td>(\text{rimJ-2::mTn10})</td>
<td>2,036 ± 239</td>
<td>847 ± 194</td>
<td>85 ± 7</td>
<td></td>
</tr>
</tbody>
</table>

\(\beta\)-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.

\(\text{Strains DL812 (wild type) and CWZ381 (rimJ-2::mTn10) were used to analyze \(\text{fan}\) transcription, DL1530 (wild type) and CWZ382 (rimJ-2::mTn10) were used for \(\text{daa}\) transcription, and AAEC198A (wild type) and CWZ400 (rimJ-2::mTn10) were used for \(\text{fim}\) 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 to the phase ON state due to limiting cAMP-CAP. Phase ON cells used to initiate the culture maintain a transcriptionally active phase ON state due to limiting cAMP-CAP. Phase ON cells switching to the phase OFF state. It is a paradox how phase OFF cells in a phase OFF state in the wild-type strain, results from an increased frequency of cells switching to the 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 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*:mTn10 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 mTn10 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::mTn10 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 substrates, 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 ΔrimJ 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.

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