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Thermoregulation of the *pap* Operon: Evidence for the Involvement of RimJ, the N-Terminal Acetylase of Ribosomal Protein S5

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Our previous work showed that *pap* pilin gene transcription is subject to a thermoregulatory control mechanism under which *pap* pilin is not transcribed at a low temperature (23°C) (L. B. Blyn, B. A. Braaten, C. A. White-Ziegler, D. H. Rolfson, and D. A. Low, EMBO J. 8:613–620, 1989). In order to isolate genes involved in this temperature regulation of gene expression, chromosomal mini-Tn*10* (mTn*10*) mutations that allowed transcription of the *pap* pilin gene at 23°C were identified, and the locus was designated *tcp*, for "thermoregulatory control of *pap*" (C. A. White-Ziegler, L. B. Blyn, B. A. Braaten, and D. A. Low, J. Bacteriol. 172:1775–1782, 1990). In the present study, quantitative analysis showed that the *tcp* mutations restore *pap* pilin transcription at 23°C to levels similar to those measured at 37°C. By in vivo recombination, the *tcp* mutations were mapped to phage E4H10S of the Kohara library of the *Escherichia coli* chromosome (Y. Kohara, K. Akiyama, and K. Isono, Cell 50:495–508, 1987). The *tcp* locus was cloned by complementation, in which a 1.3-kb DNA fragment, derived from the Kohara phage, was shown to restore thermoregulation to the mTn*10* mutants. DNA sequencing revealed two open reading frames (ORFs) encoding proteins with calculated molecular masses of 22.7 and 20.3 kDa. The sequence of the 22.7-kDa ORF was identical to that of *rimJ*, the N-terminal acetylase of the ribosomal protein S5. The gene encoding the 20.3-kDa ORF, designated *g20.3* here, did not display significant homology to any known DNA or protein sequence. On the basis of Northern (RNA) blot data, *rimJ* and *g20.3* are located within the same operon. Two of the mTn*10* transposons in the thermoregulatory mutants were inserted within the coding region of *rimJ*, indicating that the RimJ protein plays an important role in the temperature regulation of *pap* pilin gene transcription. However, *rimJ* itself is not thermoregulated, since *rimJ* transcripts were detected at both 23 and 37°C. Disruption of the *g20.3* gene by insertion and deletion mutagenesis did not affect thermoregulation of the *pap* operon, suggesting that, although *g20.3* lies within the same operon as *rimJ*, it does not play a role in thermoregulation.

Many uropathogenic strains of *Escherichia coli* uniquely express pyelonephritis-associated pili (Pap) at their cell surfaces (24, 26). The expression of Pap pili facilitates bacterial attachment to epithelial cells and colonization of the urinary tract (12, 20, 25, 31).

The expression of cell surface molecules is subject to different regulatory control mechanisms, many of which are responsive to environmental signals. Possibly, the ability of bacteria to regulate the expression of certain genes in response to environmental signals increases the efficiency of resource utilization, either within or outside of a host. The expression of Pap pili is subject to two distinct control mechanisms. Using a *papBAp-lac* operon fusion in which the β-galactosidase gene is under the control of the *pap* pilin promoter (the *papBAp* promoter), we showed that *pap* pilin transcription was subject to both phase variation and thermoregulatory control mechanisms (3). Under the phase variation mechanism at 37°C, both Lac^+ and Lac^- colony phenotypes were displayed. Lac^+ bacteria correspond to phase-on cells that transcribe pilin, while Lac^- bacteria represent phase-off cells that do not transcribe pilin. In contrast, under the thermoregulatory control mechanism at 23°C, only a Lac^- colony phenotype was observed, suggesting a loss of *pap* pilin gene transcription at a low temperature.

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To identify the genes involved in the temperature regulation of gene expression, mini-Tn*10* (mTn*10*) mutagenesis was performed on a strain containing the *papBAp-lac* fusion (DL357) (Table 1) with selection at 23°C. Mutants defective in thermoregulation of the *pap* operon were isolated (41). These mutants were able to transcribe pilin at the nonpermissive temperature, as evidenced by both Lac^+ and Lac^- colony phenotypes which were similar to the phenotype seen at 37°C. The mTn*10* insertions mapped to 23.4 min on the *E. coli* chromosome, and the locus was designated *tcp*, for "thermoregulatory control of *pap*."

Thermoregulation of the *pap* operon has been studied by other researchers as well. Using a system in which a *pap-lac* fusion is carried on a multicopy plasmid, Goransson and Uhlin demonstrated that *pap* pilin gene transcription was under thermoregulatory control (11). Thermoregulation in this system was shown to be derepressed by spontaneous mutations in *drdX*, which appears to encode the histone-like protein H-NS (10).

In this study, we have cloned a gene at the *tcp* locus necessary for complementation of the mTn*10* mutations in the thermoregulatory mutants. Placement of this gene in *trans* allows the restoration of thermoregulatory control at 23°C. The sequence of this gene is identical to that of *rimJ*, a gene that encodes the N-terminal acetylase that modifies the ribosomal protein S5 in *E. coli* (43). The function that the acetylation of the N-terminal alanine of the S5 protein serves within the bacterium is unknown. Another open reading

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

Strain, plasmid, or phage	Description[a]	Reference or source
E. coli		
MC4100	F⁻ *araD139* Δ(*lacIPOZYA-argF*)*U169 rpsL thi-1*	4
MC4100 *recA*1	F⁻ *araD139* Δ(*lacIPOZYA-argF*)*U169 rpsL thi-1 recA1*	R. Isberg
DL357	MC4100 *recA1* λ246 lysogen	41
DL379	MC4100 λ246 lysogen	3
DL478	DL357 *tcp-1*::mTn*10*	41
DL479	DL357 *tcp-2*::mTn*10*	41
DL582	DL379 *tcp-1*::mTn*10*	This study
DL583	DL379 *tcp-2*::mTn*10*	This study
DL632	MC4100 *tcp-2*::mTn*10*	41
DL652	MC4100 *tcp-1*::mTn*10*	41
SKB178 λ lysogen	*galE su*	Margaret Karow; 9
JC7623	AB1157 *recB21 rec-22 sbcB15 sbcC201*	27
DL1288	JC7623 *g20.3*::Ω*spc*	This study
DL1423	DL379 *g20.3*::Ω*spc*	This study
ORN103	*thr-1 leu-6 thi-1* Δ(*argF-lac*)*U169 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA2 minA minB recA13* Δ(*pilABCDFE hyp*)	28
Bacteriophages		
λ246	*papBAp-lac* operon fusion phage	3
P1L4	Virulent phage P1	L. Caro
Plasmids		
pREG153	*amp* R388 replicon containing *cos*	18
pDAL294B	pREG153 containing 6.5-kb *Eco*RI fragment from E4H10S	This study
pDAL295B	pREG153 containing 14-kb *Eco*RI fragment from E4H10S	This study
pDAL296B	pREG153 containing 2.3-kb *Bam*HI fragment from E4H10S	This study
pSPT18	*amp* cloning vector	Boehringer Mannheim
pDAL299B	pSPT18 containing 1.3-kb *rimJ*-partial *g20.3* DNA sequence	This study
pDAL302B	Deletion clone of 299B	This study
pDAL303B	Deletion clone of 299B	This study
pDAL305B	Deletion clone of 299B	This study
pDAL308B	Deletion clone of 299B	This study
pDAL312B	Deletion clone of 299B	This study
pDAL318B	pSPT18 containing 7.0-kb *Eco*RV *rimJ* and *g20.3* DNA sequence	This study
pDAL319B	Deletion subclone of pDAL318B	This study
pDAL320B	Deletion subclone of pDAL318B	This study
pDAL321B	Deletion subclone of pDAL318B	This study
pDAL322B	Deletion subclone of pDAL318B	This study
pGB2	*spc* low-copy-number cloning vector	6
pDAL326B	pGB2 containing 5.6-kb *rimJ-g20.3* DNA sequence	This study
pDAL327B	*tcp-1*::mTn*10* recombined onto pDAL326B	This study
pDAL328B	*tcp-2*::mTn*10* recombined onto pDAL326B	This study
pDAL332B	pSPT18 containing 1.8-kb *Hpa*I-*Eco*RI *rimJ*-partial *g20.3*	This study
pDAL335B	pDAL332B *g20.3*::Ω*spc*	This study

[a] Resistance determinants: *amp*, ampicillin; *spc*, spectinomycin.

frame (ORF) downstream from the *rimJ* coding sequences, designated *g20.3*, is also reported in this study. Although this ORF appears to be present on the same mRNA as *rimJ*, a chromosomal insertion within *g20.3* does not appear to disrupt thermoregulation of the *pap* operon. Its function within the bacterium remains unknown.

MATERIALS AND METHODS

Strains and media. Bacterial strains and bacteriophages used in this study are listed in Table 1. Luria-Bertani (LB) broth, LB agar, tryptone (TB) broth, TB agar, TB top agar, M9 minimal broth, and M9 minimal agar were prepared as previously described (21, 33). When used, supplements were at the following final concentrations: lactose, 0.2% (wt/vol); maltose, 0.2% (wt/vol); glycerol, 0.2% (vol/vol); ampicillin, 50 or 100 μg/ml; kanamycin, 25 μg/ml; tetracycline, 15 μg/ml; streptomycin, 20 μg/ml; spectinomycin, 25 or 40 μg/ml; and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), 40 μg/ml. All M9 solid media contained X-Gal.

Measurement of β-galactosidase activity. Each *E. coli* strain was inoculated onto an M9 minimal agar plate containing glycerol and incubated at 37°C. A single Lac⁺ colony (a Lac⁻ colony from those strains that were Δ*lac*) was isolated and resuspended in 1 ml of M9 minimal salts. An inoculum of 400 μl was added to 5 ml of M9 minimal broth containing glycerol at both 37 and 23°C. These starter cultures were used in log or early stationary phase to inoculate 30 ml of M9 minimal broth containing glycerol at the appropriate temperature. The cultures were grown to log phase (optical density at 600 nm [OD_{600}] = 0.2 to 0.9), and β-galactosidase activities were determined for each culture as described previously (22). Measurements of β-galactosidase activities are based on at least three independent measurements from a single culture.

Propagation of Kohara λ phage. To create a bacterial lawn for spot lysis, a 5-ml overnight culture of the appropriate strain was grown in TB broth. The culture was diluted in TB containing 0.2% maltose and grown to an OD_{590} of 0.5. A 0.5-ml aliquot of this culture was mixed with 3 ml of TB top agar and poured over a TB agar plate. Approximately 5 μl of stock lysate of the appropriate Kohara phage was spotted on this bacterial lawn and allowed to incubate at 37°C overnight.

For the preparation of a high-titer lysate of a given Kohara phage, a culture of the appropriate bacterial strain was grown to an OD_{590} of 1.0 in TB broth containing 0.2% maltose. The culture was diluted with TB broth to an OD_{590} of 0.05. Two phage plugs obtained from the lysis spot (prepared as described above) were then added to 4 ml of this diluted culture and allowed to incubate at 37°C on a tube roller. Lysis generally occurred in 2 to 3 h. Those cultures that did not lyse were diluted 10-fold with TB broth and incubated for an additional 30 to 60 min. To harvest lysates, 0.2 ml of chloroform was added and vortexed, and the lysate was centrifuged at 3,000 × *g* for 15 min. The supernatant was moved to an amber vial, and the following reagents were added to the lysate: gelatin, 0.1%; glycerol, 1%; and $MgSO_4$, 10 mM (final concentrations).

In vivo recombination of tetracycline resistance to Kohara λ phage. Kohara lysates were produced by growing Kohara phages 230 to 235 (from the miniset collection) on strain DL652, which contains the *tcp-1*::mTn*10* insertion (Table 1). At a low frequency, the phage(s) which contained sequences homologous to those of the region carrying the *tcp-1*::mTn*10* insertion in DL652 should recombine with the chromosome and acquire the mTn*10* DNA sequences for tetracycline resistance. As a host for testing successful recombination to the phage, a 5-ml culture of the bacterial strain SKB178 λ lysogen was grown to an approximate OD_{590} of 0.6 in TB broth containing 0.2% maltose. The culture was centrifuged and resuspended in 2.5 ml of TB broth. To test each phage for the ability to recombine the mTn*10* insertion back onto the chromosome of the SKB178 λ lysogen, 0.5 ml of a given phage lysate (approximate titer, 10^9 PFU/ml) was added to 0.5 ml of the bacterial culture, and the phage was allowed to adsorb for 15 min at room temperature. The bacterium-phage mixture was allowed to grow at 37°C for 90 min on a tube roller. The mixture was then centrifuged, and the cells were plated on LB agar containing tetracycline.

DNase I deletion subclones. DNase I deletion clones were made as previously described (3). DNase I was titered to give approximately 1 double-stranded cut per molecule. To obtain deletions from the *Eco*RI end of the insert, the DNase I-digested plasmid pDAL299B (Fig. 1B; Table 1) was subsequently cut at the *Eco*RI site. To create deletions from the *Cla*I end of the clone, the *Hin*dIII site was utilized. The *Hin*dIII site is located in the polylinker immediately 5′ to the *Acc*I site into which the *Cla*I end of the complementing fragment was cloned. The DNA ends were end filled with the Klenow fragment of DNA polymerase, and the blunt ends were ligated intramolecularly. A complete series of deletion clones was made for use in both complementation analysis and sequencing (Fig. 1B; data not shown).

Sequencing of plasmid DNA. CsCl-purified plasmid DNA was used for all DNA sequencing experiments. Sequencing reactions were run with the TaqTrack kit (Promega, Madison, Wis.) or T7 Sequencing kit (Pharmacia LKB Biotechnology, Piscataway, N.J.). The plasmid deletion clones of pDAL299B were sequenced with the SP6 and T7 primers purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). To sequence beyond the *Eco*RI site of the complementing clone pDAL299B, a larger plasmid clone, pDAL318B (Table 1), was created by cloning the 7.0-kb *Eco*RV fragment of the Kohara phage E4H10S into the *Sma*I-digested vector pSPT18. Deletion subclones of plasmid pDAL318B, including pDAL319B, pDAL320B, pDAL321B, and pDAL322B (Table 1), were used to complete sequencing of the *g20.3* ORF. Additionally, the primers 5′-CGATCTGAAACTGAGCGCAG-3′ and 5′-TTCGCGGG CCAGACGCAC-3′ were used to anneal to opposite strands within the region adjacent to the *Eco*RI site, in order to confirm the sequence that spanned this restriction site.

A primer with the sequence 5′-GGTCACCAACGCTTT TCCCG-3′ was used to determine the insertion sites of mTn*10* in *rimJ*. This primer anneals to a unique DNA sequence found at one end of the mTn*10* element (38).

DNA sequence analysis was completed with the Genetics Computer Group sequence analysis software package programs and the GENINFO BLAST Network Service. The nucleotide sequences for *rimJ* and *g20.3* reported in this study were compared with the nucleotide sequences in the GenEMBL data base on 10 August 1992. The peptide sequence deduced from the nucleotide sequence of the *g20.3* gene was compared with the protein sequences in the SwissProt data base on 12 August 1992.

Insertion mutation in *g20.3*. To create an insertion mutation in *g20.3*, the plasmid pDAL332B, which contains the DNA sequences for *rimJ* and the DNA sequences extending to the *Eco*RI site of *g20.3* (Table 1), was digested with the restriction endonuclease *Bst*EII, removing a 171-bp DNA fragment from *g20.3*. This deletion results in the loss of codons 23 to 77 of the protein. The DNA ends were end filled with the Klenow fragment of DNA polymerase and ligated to the *Sma*I-digested omega (Ω*spc*) fragment of pHP45 (29). The Ω*spc* fragment was inserted into the beginning of the *g20.3* coding sequences to act as both a transcriptional and a translational terminator. The resulting plasmid was transformed into the bacterial strain JC7623 (27) (Table 1). This strain is *recBC sbcBC*, and it displays a high cure rate for plasmids. Selection for recombination of *g20.3*::Ω*spc* onto the chromosome was carried out on LB agar plates containing spectinomycin at 30°C. Individual colonies were passaged several times at 30°C on LB agar containing spectinomycin. The same colonies were screened for ampicillin sensitivity to identify bacteria in which the *g20.3*::Ω*spc* insertion had recombined onto the chromosome, and the remaining plasmid sequences were lost from the cell. The resulting strain was designated DL1288 (Table 1).

The spectinomycin resistance inserted in the chromosomal copy of *g20.3* was transferred from DL1288 to strain DL379 by P1 transduction (22) to obtain strain DL1423 (Table 1). Southern blot analysis was used to confirm the insertion of the Ω*spc* fragment into *g20.3* (data not shown).

Nucleic acid isolation and manipulation. The isolation of λ DNA was performed as described previously (21). Bacterial plasmid (21) and chromosomal (1) DNAs were isolated as described previously. For Southern blot analysis, chromosomal DNA was digested with restriction endonucleases as described previously (21) and concentrated by ammonium acetate-ethanol precipitation at 4°C. Chromosomal DNA was size fractionated on a 0.7% agarose gel, and electrophoresis was carried out for 3.5 h at 90 V. The DNA was transferred to Biotrace RP nylon membranes (Gelman Sciences, Inc., Ann Arbor, Mich.) in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) by using the Posiblot system (Stratagene, La Jolla, Calif.) and fixed to the nylon

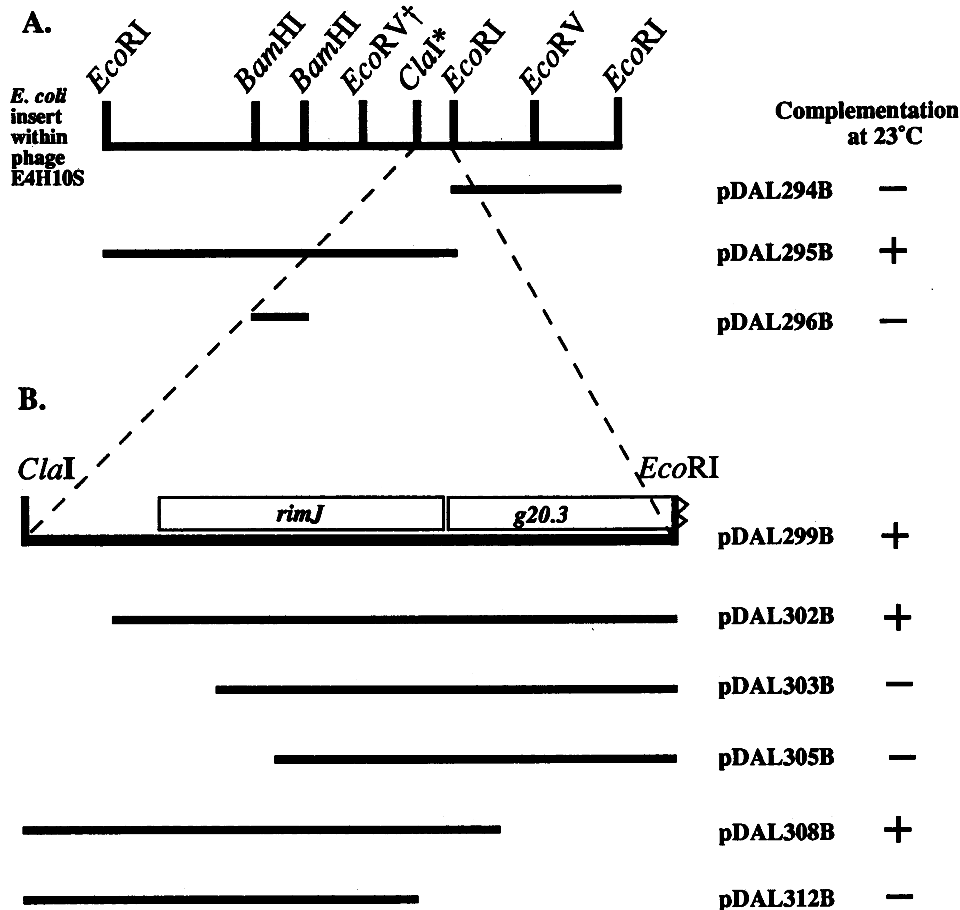

FIG. 1. Complementation of the *tcp* mutant thermoregulatory phenotype by plasmid constructs. +, complementation, as evidenced by a Lac^- colony phenotype at 23°C; −, noncomplementation, as evidenced by a phase variation phenotype at 23°C with both Lac^+ and Lac^- colony phenotypes present. (A) Representation of the *E. coli* chromosomal DNA insert present in phage E4H10S (top drawing) and restriction fragments derived from the chromosomal insert and cloned into plasmid pREG153 (18) (subsequent drawings). *Eco*RV† indicates that additional *Eco*RV sites are found within the *E. coli* insert of the phage E4H10S. However, only the two *Eco*RV sites closest to the 1.3-kb *Cla*I-*Eco*RI fragment are presented here in order to demonstrate the sites used to clone the 7.0-kb *Eco*RV fragment of plasmid pDAL318B. This plasmid was used for completing sequencing of the *g20.3* reading frame. *Cla*I* indicates that three additional *Cla*I sites were found within the *E. coli* chromosomal insert of phage E4H10S, but their positions were not unambiguously mapped. Therefore, these sites are not presented here. (B) Complementing plasmid pDAL299B, containing the 1.3-kb *Cla*I-*Eco*RI fragment derived from phage E4H10S cloned in the vector pSPT18 (Boehringer Mannheim), and deletion subclones of this plasmid. The jagged edge at the end of the *g20.3* gene indicates that this gene is truncated by cloning at the *Eco*RI site.

membrane by UV cross-linking with a Stratalinker UV cross-linker (Stratagene). Hybridization was performed in 50% formamide at 42°C for 16 to 20 h with wash procedures as detailed by Amersham (Arlington Heights, Ill.).

For Northern (RNA) blot analysis, total bacterial RNA was isolated as described previously (13). RNA was isolated from bacteria cultured at either 37 or 23°C in M9 minimal broth containing glycerol. The RNA was size fractionated on formaldehyde-agarose gels as described previously (32), except that electrophoresis was performed for 3 to 4 h at approximately 80 V. The RNA was transferred and fixed to the nylon membrane as described above. Hybridization was performed in an $NaPO_4$-sodium dodecyl sulfate (SDS) buffer at 65°C, and the washes were completed at 65°C as described previously (8).

DNA probe isolation. To obtain probes for Southern and Northern analyses, the desired DNA fragments were purified from agarose gels with Prep-A-Gene (Bio-Rad, Richmond, Calif.). The probe used to specifically hybridize to *rimJ* was a 462-bp *Dpn*I fragment internal to *rimJ*. One of two probes was used to hybridize to *g20.3*: a 325-bp *Bst*EII-*Eco*RI fragment obtained by partial digestion or a mixture of the 171-bp *Bst*EII fragment with the 144-bp *Bst*EII-*Eco*RI fragment; this mixture encompasses the DNA sequences within the 325-bp *Bst*EII-*Eco*RI probe derived by partial cutting. All *g20.3* probes are internal to the coding region of *g20.3*. Hybridization with the Ω*spc* fragment used for insertional mutagenesis was carried out with the 1.9-kb *Sma*I fragment of plasmid pHP45 (29). The *Sma*I DNA fragment was nick translated with [α-^{32}P]dCTP as described by Bethesda Research Laboratories (Gaithersburg, Md.). All other DNA probes were labeled with [α-^{32}P]dCTP by the random-priming method (Stratagene or Boehringer Mannheim Biochemicals).

Minicell analysis. The isolation of minicells was performed as described previously (3). For samples isolated at 23°C, the

periods for preincubation, labeling, and chase were doubled compared with the periods used at 37°C. Protein samples were separated on an SDS-polyacrylamide gel electrophoresis (PAGE) system with a 12% acrylamide gel (19). Gels were treated with En^{3}Hance (New England Nuclear, Boston, Mass.) to increase signal intensity prior to being dried.

Nucleotide sequence accession number. The nucleotide sequence presented in this paper was submitted to GenBank. Its accession number is M99278.

RESULTS

Previously, we described a locus, designated *tcp*, involved in the thermoregulation of the *pap* operon (41). mTn*10* mutations at this locus were shown to allow transcription of pilin at the nonpermissive temperature of 23°C. The mTn*10* mutations were mapped to 23.4 min on the *E. coli* chromosome. The goals of the current study were to quantitate the effects of the mTn*10* insertions on *pap* thermoregulation and to isolate and sequence the gene(s) disrupted by these mTn*10* insertions.

The effects of the *tcp*::mTn*10* insertions on *pap* thermoregulation were quantitated by measuring β-galactosidase activities at both 37 and 23°C with *E. coli*-phage λ246 lysogens containing a single copy of the *papBAp-lac* transcriptional fusion (Table 1). This most closely represents the wild-type situation, in which the *pap* operon is found to be single copy on the chromosome (26). Phage λ246 contains the *papBAp-lac* fusion, which places *lacZYA* under the control of the *papBAp* pilin promoter. In this fusion, the *papA* pilin gene is replaced by the *lacZYA* genes (3). In wild-type strains, transcripts initiated at the *papBAp* promoter extend through the *papB* regulatory gene as well as the *papA* pilin gene on a polycistronic mRNA (2). However, in the *papBAp-lac* fusion, transcripts initiated at the *papBA* promoter extend through the *papB* regulatory gene and *lacZYA*. Therefore, in phage λ246 lysogens, β-galactosidase activity should provide an accurate measure of *pap* pilin transcription.

Analysis of the wild-type *E. coli* strain DL357 (Table 1) containing the *papBAp-lac* fusion indicated that there was almost a 100-fold reduction in *pap* pilin transcription at 23°C compared with that at 37°C, on the basis of β-galactosidase activity (Table 2). These results confirm and extend our previous results, which showed that strain DL357 displayed a phase variation phenotype (Lac$^+$ and Lac$^-$ colonies) at 37°C, whereas the colonies are all Lac$^-$ at 23°C (41). In contrast, both *tcp*::mTn*10* mutants (strains DL478 and DL479) (Table 1) displayed similar β-galactosidase levels at 37 and 23°C (Table 2). These results showed that both *tcp* mutations restore *pap* pilin transcription at 23°C to levels near those measured at 37°C. These results are in agreement with those of our previous work, which demonstrated that *tcp*::mTn*10*-containing strains DL478 and DL479 display a phase variation phenotype at 23 as well as at 37°C.

To identify the gene(s) disrupted by the mTn*10* insertions at the *tcp* locus, in vivo recombination was used to transfer the mTn*10* mutation in the *tcp-1*::mTn*10* mutant to the homologous phage of the Kohara library of the *E. coli* chromosome (17). The phages in the region of 23.4 min on the *E. coli* chromosome were chosen for testing. These included Kohara phages 9G7, 1C7, 1H7, E4H10S, E3G11, and 14C1 (phages 230 to 235 from the miniset). Bacterial strain DL652, which contains the *tcp-1*::mTn*10* mutation (Table 1), was used as the host for each Kohara phage to allow recombination of the mTn*10* mutation from the chromosome onto the phage containing homologous DNA sequences. These phage lysates were then used to infect the bacterial strain SKB178 λ lysogen (Table 1), and selection for tetracycline-resistant colonies was performed. Infection of the λ lysogen allowed recombination of the tetracycline resistance of the mTn*10* element to the chromosome on the basis of either homology in the region of the *tcp* mutation or homology to λ, while the immunity of the λ lysogen prevented the lytic Kohara phage from lysing the cell. Only phage E4H10S was able to transfer the mTn*10* mutation from strain DL652 to the strain SKB178 λ lysogen, suggesting that phage E4H10S contained the DNA sequences corresponding to the region interrupted by the *tcp-1*::mTn*10* mutation.

TABLE 2. Quantitation of the effects of *rimJ* and *g20.3* mutations on *pap* thermoregulation

Strain	β-galactosidase activity[a] at: 37°C	β-galactosidase activity[a] at: 23°C	β-galactosidase 37°C/23°C ratio
MC4100 *recA1* (Δ*lac* control strain)	<1[b]	<1	NA[c]
DL357 (MC4100 *recA1* λ246 lysogen)	781 ± 7	8 ± 4	98
DL478 (DL357 *tcp-1*::mTn*10*)	1,272 ± 51	1,122 ± 212	1
DL479 (DL357 *tcp-2*::mTn*10*)	1,210 ± 193	1,031 ± 38	1
MC4100 (Δ*lac* control strain)	<1	<1	NA
DL379 (MC4100 λ246 lysogen)	617 ± 46	37 ± 1	17
DL1423 (DL379 *g20.3*::Ω*spc*)	1,062 ± 116	58 ± 4	18

[a] β-galactosidase activity is expressed as Miller units (22) and was measured as described in Materials and Methods. Errors are expressed as ±1 standard deviation.

[b] β-galactosidase activity was measured at less than 1 Miller unit.

[c] NA, not applicable.

To determine which region of the Kohara phage contained the DNA sequences corresponding to the site of the *tcp-1*::mTn*10* insertion, specific restriction fragments of the Kohara phage were subcloned into the low-copy-number cosmid pREG153 (18). These cosmids were tested for their abilities to complement the phenotype of the *E. coli* strains containing the *tcp-1*::mTn*10* and *tcp-2*::mTn*10* mutations (Fig. 1A). Complementation was assessed on the basis of the ability of the plasmid clone to restore the Lac$^-$ colony phenotype at 23°C to the thermoregulatory mutants DL478 (*tcp-1*::mTn*10*) and DL479 (*tcp-2*::mTn*10*) (Table 1). Neither plasmid pDAL294B, containing the 6.5-kb *Eco*RI fragment, nor pDAL296B, containing the 2.3-kb *Bam*HI fragment, restored the normal thermoregulatory phenotype (Fig. 1A). In contrast, the introduction of plasmid pDAL295B, which contains the 14-kb *Eco*RI DNA fragment, into the *tcp* mutants restored thermoregulation, as evidenced by a Lac$^-$ colony phenotype at 23°C (Fig. 1A). These results suggested that the DNA sequence required for thermoregulation was located on the 14-kb *Eco*RI fragment. Subcloning of plasmid pDAL295B showed that a 1.3-kb *Cla*I-*Eco*RI fragment (pDAL299B) (Fig. 1B) complemented both *tcp* mutants.

Further analysis of the *tcp* locus was carried out by deletion subcloning of the complementing plasmid pDAL299B (Fig. 1B). DNase I deletions were made from either the *Eco*RI or the *Cla*I end of pDAL299B (see Mate-

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   1 GTCGATGAGCAGGAAATATTTACCCAGGTTCCTCGCCTGCGACACGCGGGACATTTCCCTCCCGGGAAATAAAAGATGAGCACTTTCTATTCTGCATTGC

 101 CGCTAAGACTTTTCCCATCACTTCGGGGACAATATTTTTTTATCAAAACGTCGCTTTGATAGAGAGTTTTTATCAAATATGTGAATAATGCAGAAAATGG
                -35            -10                                                       M  F  G  Y
 201 CATTTTGGACTTTTCACAGGGTCTGGTTGCGCAGGTATAGTAATACTTACAGCGTATTAAAGACGTTACGGGAAGGAGTAGGTATAGAATGTTTGGCTAT
                                                                                    rimJ ------->
     R  S  N  V  P  K  V  R  L  T  T  D  R  L  V  V  R  L  V  H  D  R  D  A  W  R  L  A  D  Y  Y  A  E  N
 301 CGCAGTAACGTGCCAAAAGTGCGCTTAACCACAGACCGACTGGTCGTGCGTCTGGTGCATGATCGTGATGCCTGGCGTCTTGCGGATTATTACGCAGAGA

      R  H  F  L  K  P  W  E  P  V  R  D  E  S  H  C  Y  P  S  G  W  Q  A  R  L  G  M  I  N  E  F  H  K
 401 ATCGCCATTTCCTCAAGCCCTGGGAGCCAGTGCGCGACGAAAGCCACTGTTATCCATCAGGCTGGCAGGCCAGGCTGGGGATGATTAACGAATTTCATAA

     Q  G  S  A  F  Y  F  G  L  F  D  P  D  E  K  E  I  I  G  V  A  N  F  S  N  V  V  R  G  S  F  H  A
 501 ACAAGGTTCAGCTTTCTACTTTGGCTTATTCGACCCGGACGAAAAAGAGATTATTGGCGTTGCCAATTTTTCCAATGTTGTTCGTGGCTCTTTTCATGCC

     C  Y  L  G  Y  S  I  G  Q  K  W  Q  G  K  G  L  M  F  E  A  L  T  A  A  I  R  Y  M  Q  R  T  Q  H  I
 601 TGCTATCTCGGTTATTCGATTGGGCAAAAATGGCAGGGCAAAGGACTCATGTTTGAAGCCCTGACCGCAGCCATTCGTTATATGCAGCGCACCCAACATA
                                                                                             tcp-2 ▲
      H  R  I  M  A  N  Y  M  P  H  N  K  R  S  G  D  L  L  A  R  L  G  F  E  K  E  G  Y  A  K  D  Y  L
 701 TTCATCGCATTATGGCTAATTATATGCCGCACAATAAACGCAGCGGTGATTTACTGGCGCGACTGGGTTTTGAAAAAGAAGGCTATGCGAAAGACTATCT
                                                                                  tcp-1 ▲
     L  I  D  G  Q  W  R  D  H  V  L  T  A  L  T  T  P  D  W  T  P  G  R  *              M  K  Y  Q  L  T
 801 GTTGATTGATGGACAATGGCGCGATCACGTACTGACGGCATTAACTACCCCAGACTGGACGCCCGGCCGCTAAGGAGAATCTCATGAAATATCAACTTAC
                                                                                 g20.3 ------->
     A  L  E  A  R  V  I  G  C  L  L  E  K  Q  V  T  T  P  E  Q  Y  P  L  S  V  N  G  V  V  T  A  C  N
 901 TGCCCTTGAAGCTCGCGTGATTGGTTGCTTGCTGGAAAAGCAGGTGACCACGCCGGAGCAATACCCGCTCTCAGTCAATGGTGTAGTCACGGCCTGTAAT

     Q  K  T  N  R  E  P  V  M  N  L  S  E  S  E  V  Q  E  Q  L  D  N  L  V  K  R  H  Y  L  R  T  V  S  G
1001 CAGAAAACGAACCGTGAACCGGTCATGAATCTGAGTGAATCCGAAGTGCAGGAACAGCTGGATAATCTGGTCAAACGTCATTATCTACGCACAGTGAGCG

      F  G  N  R  V  T  K  Y  E  Q  R  F  C  N  S  E  F  G  D  L  K  L  S  A  A  E  V  A  L  I  T  T  L
1101 GTTTTGGTAATCGGGTCACCAAATATGAGCAACGTTTTTGTAATTCAGAATTTGGCGATCTGAAACTGAGCGCAGCGGAAGTGGCGTTAATCACCACGTT

      L  L  R  V  P  R  R  Q  V  N  C  A  A  A  P  R  E  C  M  N  S  A  I  W  R  K  W  S  R  R  W  N  N
1201 GTTATTGCGTGTGCCCAGACGCCAGGTGAACTGCGCAGCCGCGCCGCGCGAATGTATGAATTCAGCGATATGGCGGAAGTGGAGTCGACGCTGGAACAAC

     W  Q  I  A  K  M  V  L  L  W  C  V  W  P  A  N  R  V  N  A  K  T  A  T  C  I  F  S  V  V  R  L  K  I
1301 TGGCAAATCGCGAAGATGGTCCTTTTGTGGTGCGTCTGGCCCGCGAACCGGGTAAACGCGAAAACCGCTACATGCATCTTTTCAGTGGTGAGGTTGAAGA

      S  R  R  *
1401 TCAGCCGGCGGTGACGGATATGTCGAACGCGGTTGACGGTGATTTACAGGCCCGCGTCGAAGGCCTGGAAATCGAAGTGGCAGAACTGAAACAGCGTCTT

1501 GATTCGTTGCTGGCCATCTGGGAGATTAAAGTGAAAAAATTACGTATCGGCGTAGTGGATTAGGTGCATTCGCAAAAGCGTGTTACGTCTGCGCAGCTCT

1601 GACTGACGT
```

FIG. 2. Nucleotide sequences of the *rimJ* and *g20.3* genes and flanking DNA sequences. The sequence of the coding region for *rimJ* is the same as that in Yoshikawa et al. (43). The beginning of each protein translation for *rimJ* and *g20.3* is indicated by an arrow. Protein translation of the genes is shown by amino acid designations above each codon. A potential RNA polymerase binding site for *rimJ* is underlined, and potential ribosome binding sites for both genes are double-underlined. Arrowheads designate the sites of insertion of the *tcp-1*::mTn*10* and *tcp-2*::mTn*10* transposon mutations.

rials and Methods). These deletion subclones were tested for their abilities to complement both the *tcp-1*::mTn*10* and *tcp-2*::mTn*10* mutations, as evidenced by restoration of a Lac$^-$ colony phenotype at 23°C to strains DL478 and DL479, respectively. This analysis demonstrated that a deletion of approximately 200 bp from the *Cla*I end of the plasmid pDAL299B did not disrupt complementation of the *tcp-1*::mTn*10* and *tcp-2*::mTn*10* mutations (plasmid pDAL302B). However, further deletions from the *Cla*I end resulted in plasmids that could no longer complement the mutant phenotype (e.g., plasmids pDAL303B and pDAL 305B). Plasmid pDAL308B, which contains a deletion from the *Eco*RI end, complemented both *tcp* mutants. However, plasmids containing larger deletions from the *Eco*RI end of the clone were unable to restore thermoregulation to the *tcp* mutants (e.g., plasmid pDAL312B). These results suggested that the overlapping regions of plasmids pDAL302B and pDAL308B were necessary and sufficient for complementation of the *tcp* mutants.

The entire 1.3-kb *Cla*I-*Eco*RI DNA fragment in pDAL 299B was sequenced (Fig. 2). Two ORFs were found within the DNA insert. The upstream ORF had a calculated molecular mass of 22.7 kDa, while the downstream ORF had a calculated molecular mass of 15.1 kDa. The downstream ORF, however, lacked a stop codon, suggesting that cloning at the *Eco*RI site disrupted this ORF. To obtain a plasmid that contained the entire coding region for the two ORFs, a 7-kb *Eco*RV DNA fragment from the Kohara phage E4H10S was cloned into pSPT18 (Boehringer Mannheim Biochemicals). Deletion subclones of this plasmid were used to continue DNA sequencing beyond the *Eco*RI site, providing the remainder of the sequence for the downstream ORF. The complete sequence of the downstream ORF yielded a protein with a calculated molecular mass of 20.3 kDa (Fig. 2).

The DNA sequence of the 22.7-kDa ORF was found to be identical to the DNA sequence of *rimJ* (43). RimJ is an acetylase that modifies the N-terminal alanine of the ribosomal protein S5 (7). The downstream ORF, designated *g20.3*, displayed no significant homology to any known DNA or protein sequences. Possible RNA polymerase and ribosome binding sites are present upstream of the ATG start codon of *rimJ* (Fig. 2). Within the 10 bp that separate the end of *rimJ* and the beginning of *g20.3*, a potential ribosome binding site is found 6 bp upstream of the ATG codon of *g20.3* (Fig. 2). However, a good consensus promoter sequence was not found for RNA polymerase binding 5′ of the *g20.3* gene, suggesting the possibility that these genes are within the same operon.

Sequencing of the *tcp-1*::mTn*10* and *tcp-2*::mTn*10* mutations demonstrated that they were inserted within the coding sequences of *rimJ* (Fig. 2). The mTn*10* elements in *rimJ* were both C terminal. The *tcp-1*::mTn*10* insertion was located at bp 491 of *rimJ*, whereas the *tcp-2*::mTn*10* mutation was present at bp 403 of *rimJ*. Three additional thermoregulatory mutants also contained mTn*10* insertions within *rimJ*, on the basis of complementation and Southern blot analyses (data not shown). The finding that these mTn*10* insertions were all within *rimJ* suggests that the RimJ acetylase plays an important role in the thermoregulation of the *pap* operon. In addition, the subclone pDAL308B (Fig. 1B), which contains only the coding sequence for RimJ, complemented the mutant thermoregulatory phenotypes. These complementa-

tion data demonstrate that *rimJ* alone is sufficient for restoring the thermoregulatory phenotype and confirm the importance of RimJ in this system.

The results presented above indicate that *rimJ* plays an important role in *pap* thermoregulation. To determine whether *g20.3* might also be involved in thermoregulation, a chromosomal insertion in the *g20.3* gene was made. This was accomplished by inserting a spectinomycin-resistant omega (Ω*spc*) fragment (29) into the chromosomal copy of the *g20.3* gene (see Materials and Methods). The Ω*spc* fragment was inserted into the beginning of the *g20.3* coding sequences to act as both a transcriptional and a translational terminator. On the basis of the construction of the mutation, the insertion of the Ω*spc* fragment also entailed the loss of codons 23 to 77 of the g20.3 protein. Phenotypic analysis of strain DL1423 (Table 1), which contains the chromosomal insertion in *g20.3*, demonstrated that the *g20.3*::Ω*spc* mutation did not disrupt thermoregulation, as the colonies were all uniformly Lac$^-$ at 23°C. This phenotype is similar to that of the nonmutant strain DL379 (Table 1) at this temperature. Quantitation of β-galactosidase levels expressed by the wild-type strain DL379 and strain DL1423, which contains the *g20.3*::Ω*spc* mutation, indicated that disruption of the *g20.3* gene did not affect *pap* thermoregulation (Table 2). Both strains showed similar reductions (17-fold in DL379 and 18-fold in DL1423) in β-galactosidase levels at 23°C compared with those at 37°C.

E. coli minicell analysis was performed to determine whether proteins were expressed from the ORFs *rimJ* and *g20.3*. Two proteins in addition to those produced by minicells containing plasmid vector pSPT18 were produced by *E. coli* minicells containing plasmid pDAL302B (Fig. 3). As shown in Fig. 1B, plasmid pDAL302B complemented the *tcp-1*::mTn*10* (DL478) and *tcp-2*::mTn*10* (DL479) thermoregulatory mutants. Plasmid pDAL302B contains the entire coding sequence for *rimJ*, but the *g20.3* gene is truncated at the *Eco*RI site. The apparent molecular masses of the two visualized proteins were approximately 22 and 13 kDa. These molecular masses corresponded well to the calculated molecular mass of RimJ (22.7 kDa) and the calculated molecular mass of the truncated g20.3 protein (15.1 kDa). *E. coli* minicells containing plasmid pDAL308B, which contains only the coding sequences for RimJ, expressed a 22-kDa protein, confirming both the location and the expression of the *rimJ* gene. Plasmid pDAL305B, which contains coding sequences for the truncated g20.3 protein but not the complete sequence for RimJ, expressed only the 13-kDa protein. Plasmid pDAL312B expressed a truncated RimJ protein, since the deletion in this clone removes the C-terminal end of the *rimJ* gene as well as the coding sequence for the *g20.3* gene. Minicells containing pDAL303B produced the 13-kDa truncated form of the g20.3 protein as well as a truncated form of the RimJ protein. In this plasmid construct, the N terminus of the *rimJ* gene appears to be removed by the deletion.

To examine the effect of the *tcp* mutations on RimJ expression, minicell analysis was carried out with *E. coli* in which the *tcp-1*::mTn*10* and *tcp-2*::mTn*10* mutations were each separately recombined onto plasmid pDAL326B (Table 1). The resulting plasmids, pDAL327B (*tcp-1*::mTn*10*) and pDAL328B (*tcp-2*::mTn*10*), along with the nonmutant plasmid pDAL326B, were used in minicell analysis (data not shown). The nonmutant plasmid pDAL326B expressed a 22-kDa RimJ protein. A RimJ-mTn*10* fusion protein of approximately 26 kDa resulted from the insertion of *tcp-1*::mTn*10* into *rimJ*, whereas the insertion of *tcp-2*::mTn*10*

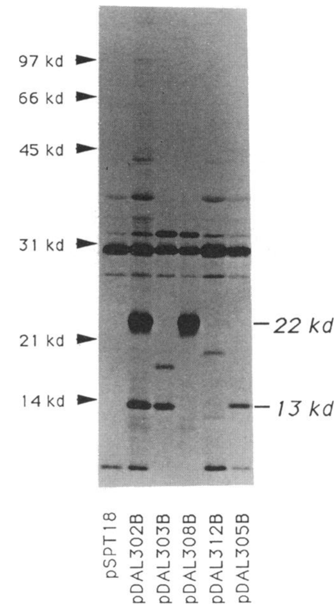

FIG. 3. Minicell analysis of plasmid-encoded proteins of plasmid pDAL299B deletion clones. Minicell analysis was carried out as described in Materials and Methods. Briefly, minicells were isolated by sucrose gradient centrifugation and incubated with a mixture of [^{35}S]methionine and [^{35}S]cysteine (Trans-^{35}S label; ICN, Costa Mesa, Calif.). Labeled proteins were separated on an SDS–12% PAGE gel (19) and visualized by fluorography. Each lane is labeled with the name of the plasmid construct tested for protein expression. See Fig. 1B for a representation of the coding regions covered by each clone. The numbers to the left of the figure are molecular mass markers, while the numbers to the right of the figure designate the positions and the apparent molecular masses of the RimJ and truncated g20.3 proteins (values are in kilodaltons).

resulted in the expression of a 17-kDa fusion protein. The different sites of insertion of the mTn*10* transposons as well as the opposite orientation of insertion of the mTn*10* elements in the two *tcp* mutants resulted in RimJ-mTn*10* fusion proteins with significantly different sizes.

Minicell analysis was also performed with *E. coli* containing the same plasmids (pDAL326B, pDAL327B, and pDAL328B) in which the minicells were isolated after growth at 37°C but were subsequently labeled at 23°C to examine RimJ protein expression at this low temperature. The results obtained at 23°C were identical to those obtained at 37°C, suggesting that the expression of the RimJ and the RimJ-mTn*10* fusion proteins is not regulated by temperature (data not shown).

To determine whether transcription of *rimJ* is thermoregulated, Northern analysis was performed with RNA isolated at 37 and 23°C from the nonmutant strain DL379 and the two thermoregulatory mutants DL582, containing *tcp-1*::mTn*10*, and DL583, containing *tcp-2*::mTn*10* (Fig. 4; Table 1). A ^{32}P-labeled, 462-bp *Dpn*I DNA fragment, internal to *rimJ*, was used in Northern blot analysis to visualize the transcript of this gene. In the nonmutant strain DL379, a transcript of about 2.4 kb was identified by the *rimJ* probe at both 37 and 23°C (Fig. 4, lanes 1 and 2), demonstrating that the transcription of *rimJ* is not thermoregulated.

On the basis of Northern analysis and minicell protein analysis, it was found that transcription of *rimJ* in the thermoregulatory mutants DL582 and DL583 also occurred

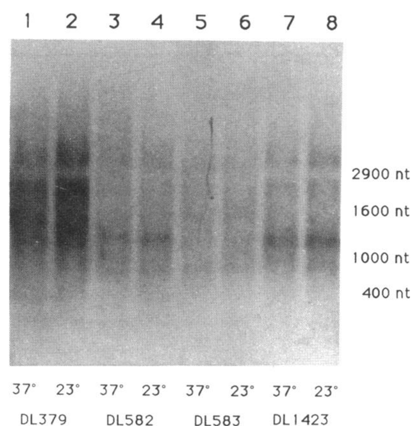

FIG. 4. RNA analysis of *rimJ* transcription. RNA was isolated from cells grown at either 37 or 23°C. RNA isolation and Northern analysis were performed as described in Materials and Methods. The blot was hybridized with a ^{32}P-labeled 462-bp *DpnI* fragment that is internal to *rimJ* coding sequences. The strains and temperatures used for Northern analysis are as indicated.

at both 37 and 23°C. In the bacterial strain DL582, the *rimJ* transcript was present in bacteria grown at both 37 and 23°C (Fig. 4, lanes 3 and 4). The transcript length was shortened by approximately 1.1 kb compared with that of the wild type by the insertion of the mTn*10* sequence. Transcription of *rimJ* in the bacterial strain DL583 appeared to be greatly decreased (Fig. 4, lanes 5 and 6). Small amounts of transcript have been visualized in other Northern blots, and in these studies, the length of the transcript is shortened from the wild-type length by the insertion of the mTn*10* sequence to a length of about 1.1 kb (data not shown). Identification of a 17-kDa *tcp-2*::mTn*10* fusion protein expressed in minicell analysis provides supportive evidence that at least a small amount of *rimJ* transcription must be initiated in the thermoregulatory mutant DL583. The shorter length of the *rimJ* transcript in DL583 corresponded well to the sequence data demonstrating that the *tcp-2*::mTn*10* mutation in DL583 is located closer to the N terminus than the *tcp-1*::mTn*10* mutation in DL582 (Fig. 2). In the bacterial strain DL1423 (Fig. 4, lanes 7 and 8), the chromosomal insertion within *g20.3* resulted in a similar shortening of the *rimJ* transcript, indicating that these two genes are both within the same operon.

RNA samples from the same bacterial strains analyzed above were subsequently probed with a 325-bp *Bst*EII-*Eco*RI DNA probe internal to *g20.3* coding sequences (data not shown). In the nonmutant strain DL379, the *g20.3* probe identified a transcript with the same size (2.4 kb) as the transcript identified by the *rimJ* probe, supporting the hypothesis that *rimJ* and *g20.3* are within the same operon. Moreover, the *rimJ*::mTn*10* insertions in the thermoregulatory mutants as well as the *g20.3*::Ω*spc* mutations in strain DL1423 caused the loss of transcripts identified by the *g20.3* probe. These results, taken together with the results presented above, strongly indicate that *rimJ* and *g20.3* are both within the same operon.

DISCUSSION

Our results indicate that RimJ plays a role in the thermoregulation of the *pap* operon. Previous work showed that RimJ acetylates the N-terminal alanine residue of the ribosomal protein S5 (7, 43). While it has been demonstrated previously that a mutation for temperature sensitivity in *rimJ* results in a loss of acetylation of S5 at the nonpermissive temperature (42°C) (7), it is not known what function this acetylation plays in either the assembly or the function of the ribosome.

Other ribosomal proteins in *E. coli* are known to be acetylated. The S18 protein is acetylated at its N-terminal alanine residue (15, 42), whereas the ribosomal L12 protein is acetylated at its N-terminal serine (16, 36), converting it to the L7 protein. Both the L12 protein and its acetylated form, L7, are present in the ribosome (14, 34). The acetylation of these proteins is carried out by other acetylases distinct from RimJ. The RimI protein acetylates S18 (43), whereas the RimL protein acetylates the L12 protein at its N-terminal serine residue to convert it to the L7 protein (35). As is the case with the modification of S5, the functions of the acetylations of S18 and L12 are unknown.

Modification of some ribosomal proteins has been shown to be responsive to environmental conditions. Studies detailing the relative content of the L12 and L7 proteins in the ribosome in response to growth phase have been performed. The relative content of the nonacetylated form of the L12 protein increased in the early logarithmic to mid-logarithmic stages (30). Thereafter, the relative contents of these two proteins shifted toward the acetylated form, L7, through the late log and stationary growth phases. Ramagopal and Subramanian suggest that the increase in the acetylated form, L7, may be an adaptation to stationary phase (30). Another modification of the ribosomal proteins has been found to be responsive to the environment. The temperature response of methylation of the L7-L12 ribosomal proteins has been characterized previously (5). Methylation of both the L7 and L12 proteins increases in response to decreasing temperatures. One hypothesis that Chang proposes is that the increased methylation may allow ribosomes to function at low temperatures (5). However, the actual function of this modification is unknown.

These studies on modification of ribosomal proteins in response to growth phase and temperature may support the model that the ribosome is an environmental sensor. Indeed, VanBogelen and Neidhardt (37) have suggested that the ribosome may be a temperature sensor, on the basis of studies in which the exposure of nonresistant bacteria to antibiotics that affect the ribosome induced the expression of either heat shock or cold shock proteins. The acetylation of the ribosomal proteins in response to growth temperature has not been studied. However, it is possible that the acetylation of S5 is part of another mechanism by which the ribosome responds to a change in its environment.

Efforts in our laboratory to produce a chromosomal insertion in *rimJ* by methods used to construct the *g20.3*::Ω*spc* knockout were unsuccessful, suggesting that *rimJ* may be an essential gene. An insertion mutation in *rimJ* was achieved in a diploid strain, supporting the hypothesis that the gene may be essential, as we were only able to retrieve this mutation in a diploid background. Additionally, all five of the naturally occurring mTn*10* insertions we isolated are located in the C-terminal region of the protein, suggesting that an N-terminal insertion of the mTn*10* element may be lethal to the bacterium (Fig. 2; also data not shown). Further studies need to be pursued to determine whether *rimJ* is an essential gene.

The *tcp-1*::mTn*10* and *tcp-2*::mTn*10* mutations in the thermoregulatory mutants do not appear to abrogate the expression of the RimJ acetylase, since fusion proteins are

present in both cases (data not shown). Whether these fusion proteins maintain acetylase activity is unknown. It is possible that insertion of the mTn*10* elements has either destroyed or modified the activity of RimJ. The hypothesis that the activity of RimJ might in some way be modified is suggested by the fact that the two mTn*10* insertions in the thermoregulatory mutants DL478 and DL479 have differential effects on the phase transition rates in these strains (41). The Lac^- → Lac^+ phase transition rate is increased 31-fold in the *tcp-1*::mTn*10* mutant DL478 at 37°C compared with that in the wild-type strain, DL357, whereas the *tcp-2*::mTn*10* mutant DL479 shows a 17-fold increase in its Lac^- → Lac^+ phase transition rate at 37°C when compared with that of the wild-type strain. However, at 23°C, the Lac^- → Lac^+ phase transition rate in mutant DL478 is similar to the phase transition rate seen at 37°C in the wild-type strain, while the Lac^- → Lac^+ phase transition rate in the mutant DL479 remains elevated at 23°C compared with that in the wild-type strain at 37°C. While such changes in the phase transition rates do not prove that the mTn*10* insertions modify the activity of RimJ, the two unique insertions do have a differential effect within the two mutants which would not be expected if the RimJ activity had been abolished. These results raise the possibility that *tcp*::mTn*10* fusion proteins expressed by the mutants may have partial activities that are responsible for the altered thermoregulatory phenotype. Further work needs to be completed to determine the effect of the mTn*10* insertions on RimJ activity.

The role of the downstream *g20.3* gene is unknown. Its position within the same operon as *rimJ* suggests that it might be involved in the temperature regulation of *pap* pilin transcription. However, a chromosomal insertion in this gene does not disrupt thermoregulation, on the basis of the observation that the colony phenotype of the *papBAp-lac* fusion strain DL1423 remains Lac^- at 23°C. Additionally, the expression of β-galactosidase activity is similar to that by the nonmutant strain DL379 at both temperatures. The sequence data of this ORF do not offer any clues as to the function of this protein, since there are no significant matches of this sequence with any known protein or DNA sequence.

It is unknown whether a change in acetylation of the ribosomal protein S5 is responsible for the disruption of thermoregulation of the *pap* operon or whether the acetylation of some other protein by RimJ is involved in temperature regulation. The RimJ and RimI acetylases, which both add an acetyl group to alanine, appear to be specific for their ribosomal protein substrates, since the S5 protein remains acetylated in the *rimI* mutant and the S18 protein remains acetylated in the *rimJ* mutant (43). Additionally, the acetylation of L12 still occurs in the *rimI* and *rimJ* mutants, suggesting that these acetylases are not responsible for the acetylation of the serine residue of the L12 protein (15). While these studies demonstrated that the RimJ acetylase has only a single ribosomal substrate, no studies have been performed to determine whether the RimJ acetylase has any other substrate outside the ribosome.

The possibility that the RimJ acetylase may have a substrate besides the ribosomal protein S5 is suggested by studies of the yeast *Saccharomyces cerevisiae* (23). Mullen et al. (23) have identified a mutant, *nat-1*, that contains a mutation in an N-terminal acetylase. Another yeast mutation, designated *ard-1* (39, 40), demonstrates phenotypic effects identical to those of *nat-1*. Mullen et al. (23) suggest that both mutations affect the N-terminal acetylase and that *NAT1* and *ARD1* encode different subunits of the acetylase. When this acetylase is mutated, there are many effects on the cell, including the derepression of the silent mating locus *HML* (23). Similarly, mutation of the *rimJ* acetylase releases the thermoregulatory repression of *pap* pilin transcription. Two-dimensional protein analysis of *nat-1* mutants indicates that at least 20 proteins are acetylated by *NAT1* (23). This result raises the possibility that the RimJ acetylase may have a number of substrates in addition to the ribosomal protein S5. Further studies need to be performed to determine whether RimJ acetylates many substrates or is specific for acetylation of the ribosomal protein S5 alone.

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REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1987. Current protocols in molecular biology, vol. 1. Wiley Interscience, New York.
2. **Baga, M., M. Goransson, S. Normark, and B. E. Uhlin.** 1988. Processed mRNA with differential stability in the regulation of *E. coli* pilin gene expression. Cell **52:**197–206.
3. **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.
4. **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.
5. **Chang, F. N.** 1978. Temperature-dependent variation in the extent of methylation of ribosomal proteins L7 and L12 in *Escherichia coli*. J. Bacteriol. **135:**1165–1166.
6. **Churchward, G., D. Belin, and Y. Nagamine.** 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene **31:**165–171.
7. **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.
8. **Dohrmann, P. R., G. Butler, K. Tamai, S. Dorland, J. R. Greene, D. J. Thiele, and D. J. Stillman.** 1991. Parallel pathways of gene regulation: homologous regulators SW15 and ACE2 differentially control transcription of HO and chitinase. Genes Dev. **6:**93–104.
9. **Georgopoulos, C.** 1971. Bacterial mutants in which the gene N of bacteriophage lambda is blocked have an altered RNA polymerase. Proc. Natl. Acad. Sci. USA **68:**2977–2981.
10. **Goransson, M., B. Sonden, P. Nilsson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature (London) **344:**682–685.
11. **Goransson, M., and B. E. Uhlin.** 1984. Environmental temperature regulates transcription of a virulence pili operon in *E. coli*. EMBO J. **3:**2885–2888.
12. **Hagberg, L., R. Hull, S. Hull, S. Falkow, R. Freter, and C. Svanborg-Eden.** 1983. Contribution of adhesion and bacterial persistence in the mouse urinary tract. Infect. Immun. **40:**265–272.
13. **Hagen, F. S., and E. T. Young.** 1978. Effect of RNaseIII on efficiency of translation of bacteriophage T7 lysozyme mRNA. Virology **26:**793–804.
14. **Hardy, S. J. S.** 1975. The stoichiometry of the ribosomal proteins of *Escherichia coli*. Mol. Gen. Genet. **140:**253–274.

15. **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.
16. **Isono, S., and K. Isono.** 1981. Ribosomal protein modification in *Escherichia coli*. III. Studies of mutants lacking an acetylase activity specific for protein L12. Mol. Gen. Genet. **183:**473–477.
17. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
18. **Koomey, J. M., R. E. Gill, and S. Falkow.** 1982. Genetic and biochemical analysis of gonococcal IgA1 protease: cloning in *Escherichia coli* and construction of mutants of gonococci that fail to produce the activity. Proc. Natl. Acad. Sci. USA **79:**7881–7885.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
20. **Lindberg, F. P., B. Lund, and S. Normark.** 1984. Genes of pyelonephritogenic *E. coli* required for digalactosidase-specific agglutination of human cells. EMBO J. **3:**1167–1173.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Mullen, J. R., P. S. Kayne, R. P. Moerschell, S. Tsunasawa, M. Gribskov, M. Colavito-Shepanski, M. Grunstein, F. Sherman, and R. Sternglanz.** 1989. Identification and characterization of genes and mutants for an N-terminal acetyltransferase from yeast. EMBO J. **8:**2067–2075.
24. **Normark, S., D. Lark, R. Hull, M. Norgren, M. Baga, P. O'Hanley, G. Schoolnik, and S. Falkow.** 1983. Genetics of digalactoside-binding adhesion from a uropathogenic *Escherichia coli*. Infect. Immun. **41:**942–949.
25. **O'Hanley, P., D. Lark, S. Falkow, and G. Schoolnik.** 1985. Molecular basis of *Escherichia coli* colonization of the upper urinary tract in BALB/c mice: Gal-Gal pili immunization prevents *Escherichia coli* pyelonephritis in the BALB/c mouse model of human pyelonephritis. J. Clin. Invest. **75:**347–360.
26. **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.
27. **Oden, K. L., L. C. DeVeaux, C. R. T. Vibat, J. E. Cronan, Jr., and R. B. Gennis.** 1990. Genomic replacement in *Escherichia coli* K-12 using covalently closed circular plasmid DNA. Gene **96:**29–36.
28. **Orndorff, P. E., P. A. Spears, D. Schauer, and S. Falkow.** 1985. Two modes of control of *pilA*, the gene encoding type 1 pilin in *Escherichia coli*. J. Bacteriol. **164:**321–330.
29. **Prentki, P., and H. M. Krisch.** 1984. In vitro insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
30. **Ramagopal, S., and A. Subramanian.** 1974. Alteration in the acetylation level of ribosomal protein L12 during growth of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **71:**2136–2140.
31. **Roberts, J. A., M. B. Kaack, and E. N. Fussell.** 1989. Bacterial adherence in urinary tract infections: preliminary studies in a primate model. Infection **17:**401–404.
32. **Rosen, K. M., E. D. Lamperti, and L. Villa-Komaroff.** 1990. Optimizing the Northern blot procedure. BioTechniques **8:**398–403.
33. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Subramanian, A. R.** 1975. Copies of proteins L7 and L12 and heterogeneity of the large subunit of *Escherichia coli* ribosome. J. Mol. Biol. **95:**1–8.
35. **Tanaka, S., Y. Matsushita, and K. Isono.** 1989. Cloning and molecular characterization of the gene *rimL* which encodes an enzyme acetylating protein L12 of *Escherichia coli* K12. Mol. Gen. Genet. **217:**289–293.
36. **Terhorst, C., W. Moller, R. Laursen, and B. Wittmann-Liebold.** 1973. The primary structure of an acidic protein from 50S ribosomes of *Escherichia coli* which is involved in GTP hydrolysis dependent on elongation factors G and T. Eur. J. Biochem. **34:**138–152.
37. **VanBogelen, R. A., and F. C. Neidhardt.** 1990. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5589–5593.
38. **Way, J. C., M. A. Davis, D. Morisanto, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.
39. **Whiteway, M., R. Freedman, S. Van Arsdell, J. W. Szostak, and J. Thorner.** 1987. The yeast *ARD1* gene product is required for repression of cryptic mating-type information at the *HML* locus. Mol. Cell. Biol. **7:**3713–3722.
40. **Whiteway, M., and J. W. Szostak.** 1985. The ARD1 gene of yeast functions in the switch between the mitotic cell cycle and alternative developmental pathways. Cell **43:**483–492.
41. **White-Ziegler, C. A., L. B. Blyn, B. A. Braaten, and D. A. Low.** 1990. Identification of an *Escherichia coli* genetic locus involved in thermoregulation of the *pap* operon. J. Bacteriol. **172:**1775–1782.
42. **Yaguchi, M.** 1975. Primary structure of protein S18 from the small *Escherichia coli* ribosomal subunit. FEBS Lett. **59:**217–220.
43. **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.