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Identification of an *Escherichia coli* Genetic Locus Involved in Thermoregulation of the *pap* Operon

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We previously showed, using a single-copy *papBAp-lac* fusion (previously designated *pap$_{BA}$-lac*), that pyelonephritis-associated pili (*pap*) pilin gene transcription is subject to both phase variation and thermoregulatory control mechanisms (L. B. Blyn, B. A. Braaten, C. A. White-Ziegler, D. H. Rolfson, and D. A. Low, EMBO J. 8:613–620, 1989). At 37°C, *Escherichia coli* strains carrying the *papBAp-lac* fusion displayed both Lac$^+$ and Lac$^-$ colony phenotypes. In contrast, at 23°C, colonies displayed a uniform Lac$^-$ phenotype, suggesting that pilin was not transcribed at this temperature. In this study, a strain carrying the *papBAp-lac* fusion was subjected to mini-Tn*10* (mTn*10*) mutagenesis to isolate mutants that could initiate transcription of pilin at the nonpermissive temperature. Two classes of thermoregulatory mutants were identified in which the mTn*10* mutation was linked to the mutant phenotype. Class I mutants displayed a phase variation phenotype at both 37°C and 23°C, whereas class II mutants displayed a uniform Lac$^+$ colony phenotype at both temperatures. Preliminary analysis of these mutants showed that the mTn*10* insertions in the class I mutants were chromosomally located, whereas the mTn*10* insertions in the class II mutants were located within the *papBAp-lac* fusion phage. Southern blot analysis of the class I mutants demonstrated that mTn*10* was present in the same 5.9-kilobase *SalI* DNA fragment in each mutant. Two of the class I mTn*10* mutations were mapped to approximately 23.4 min on the *E. coli* K-12 chromosome. The locus defined by the class I mTn*10* mutations was designated *tcp*, for thermoregulatory control of *pap*. Analysis of phase transition rates of the class I mutants showed that the phase-off (Lac$^-$) → phase-on (Lac$^+$) transition rates were higher than those observed with the nonmutant *E. coli* strain.

Most *Escherichia coli* strains that cause upper urinary tract infections express a pili-adhesin complex encoded by the pyelonephritis-associated pili (*pap*) operon (24). The *pap* operon encodes at least 11 proteins (22, 23), including the pilin monomer PapA, which is the main structural subunit of the pilus, and PapB and PapI, which are involved in the regulation of pilus expression. The *papI* and *papB* genes are transcribed from divergent promoters located in an intercistronic regulatory region between these two genes (3). The PapB and PapA genes are coded on a polycistronic message that initiates at the *papBAp* promoter (previously designated *pap$_{BA}$*) which is located upstream of *papB*.

Previously, we found that *pap* pili expression is subject to a phase variation control mechanism in which cells alternate between two pili expression states; i.e., phase-off (pili$^-$) and phase-on (pili$^+$) (15). 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 is also subject to phase variation (4). This phase variation is heritable and is responsive to at least two environmental signals: temperature and carbon source. Incubation of *E. coli* K-12 strains containing the *papBAp-lac* fusion at a low temperature (23°C) results in the loss of *pap* pilin transcription, as evidenced by a uniform Lac$^-$ colony phenotype. It seems likely that at least one function of the low-temperature response is to turn off the production of Pap pili when *E. coli* are not residing in a host.

Thermoregulation of the expression of cell surface molecules, similar to that which occurs with Pap pili, has been described for other bacterial pathogens. The K1 capsular antigen, which is associated with strains of *E. coli* that cause

neonatal meningitis, is not detected when the cells are incubated at 22°C (5). Both K88 and K99 pili, which are expressed by enterotoxigenic *E. coli* strains, are absent at the bacterial surface when bacteria are incubated at 18°C (11, 21, 26). Also, colonization factor antigen, which is produced by human-specific enterotoxigenic *E. coli* strains, is not detectable on cells grown at 18°C (9).

Virulence factors found on the surface of non-*E. coli* bacterial pathogens are also subject to repression by low growth temperatures. In *Yersinia* species, a subset of outer membrane proteins associated with virulence are expressed at 37°C but not at 28°C (25). A *Yersinia enterocolitica* gene, *virF*, has been shown to be involved in the thermoregulation of expression of these proteins (8). *virF* appears to encode a transcriptional activator related to *araC* that is transcribed at 37°C but not at 25°C. Another bacterial pathogen, *Shigella* species, is virulent and invasive at 37°C but becomes noninvasive when it is cultured at 30°C (17). A *Shigella flexneri* chromosomal gene, *virR*, that controls the expression of the *vir* genes has been identified, and a mutation in the gene results in the expression of an invasive phenotype at 30°C as well as 37°C (18). *Bordetella pertussis* also has virulence genes whose expression is regulated by temperature (13). These genes are expressed at 37°C, but not at 30°C. The *bvg* (*vir*) operon in *B. pertussis* appears to play a role in the transcriptional activation of these genes at 37°C (19, 27). Sequencing of the genes in the *bvg* operon has shown that two of the predicted proteins, BvgA and BvgC, are homologous to other proteins of two-component regulatory systems that are responsive to environmental stimuli (1).

In this study, we used the *papBAp-lac* fusion to isolate mutants that were defective in thermoregulation of *pap* pilin gene transcription. Two classes of mutants were isolated

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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	6
MC4100 recA1	F⁻ araD139 Δ(lacIPOZYA-argF) U169 rpsL thi-1 recA1	R. Isberg
MC1061	araD139 Δ(ara-leu)7697 ΔlacX74 galU galK hsr strA	7
DL357	MC4100 recA1 λ246 lysogen	This study
DL379	MC4100 λ246 lysogen	4
DL478	DL357 tcp-1::mTn10 (class I mutant)	This study
DL479	DL357 tcp-2::mTn10 (class I mutant)	This study
DL480	DL357 tcp-3::mTn10 (class I mutant)	This study
DL481	DL357 tcp-4::mTn10 (class I mutant)	This study
DL482	DL357::mTn10 (class II mutant)	This study
DL483	DL357 tcp-6::mTn10 (class I mutant)	This study
DL632	MC4100 tcp-2::mTn10	This study
DL652	MC4100 tcp-1::mTn10	This study
CAG12206	HfrH nadA3502::Tn10 kan	29
CAG12204	KL227 btuB3192::Tn10 kan	29
CAG12203	KL208 zbc-3105::Tn10 kan	29
CAG12202	KL96 trpB3193::Tn10 kan	29
CAG18531	MG1655 zbh-3108::Tn10 kan	29
CAG12130	MG1655 zcb-3111::Tn10 kan	29
CAG18613	MG1655 zcc-3112::Tn10 kan	29
CAG18703	MG1655 putP5::Tn5	29
CAG12124	MG1655 zce-3113::Tn10 kan	29
CAG18516	MG1655 zcf-3114::Tn10 kan	29
CAG18551	MG1655 zch-3117::Tn10 kan	29
Bacteriophages		
λ246	papBAp-lac fusion phage	4
λ1098	ptac-transposase mini-tet transposon	30
P1L4	Virulent phage P1	L. Caro
T4GT7	Phage T4 mutant	31
Plasmids		
pREG153	R388 replicon containing bla and cos	14
pDAL278B	pREG153::mTn10	This study

[a] Resistance determinants: kan, kanamycin; tet, tetracycline.

that initiated pilin transcription at 23°C. Analysis of one class of mutants identified a locus that was involved in the thermoregulation of pap pilin transcription that we designated tcp, for thermoregulatory control of pap. The tcp locus appeared to be distinct from the recently described E. coli homolog to the S. flexneri virR gene, which is located at about 27.5 min on the E. coli chromosome (12).

MATERIALS AND METHODS

Strains and media. The bacterial strains and bacteriophages used in this study are listed in Table 1. Luria-Bertani (LB) broth, LB agar, M9 minimal broth, and M9 minimal agar were prepared as described previously (20). When used, the following supplements were at the indicated final concentrations: lactose, 0.2% (wt/vol); maltose, 0.2% (wt/vol); glycerol, 0.2% (vol/vol); ampicillin, 50 μg/ml; kanamycin, 25 μg/ml; tetracycline, 15 μg/ml; streptomycin, 100 μg/ml;

5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), 40 μg/ml. All M9 solid media contained X-gal.

Construction of strain DL357. Strain DL357 was constructed by lysogenizing MC4100 recA1 (R. Isberg) with the phage λ246 (4). Phage λ246 contains a papBAp-lac fusion in which the lac genes are under the control of the papBAp pilin promoter. The λ246 phage also contains the coding sequences for the papI and papB regulatory genes.

Tn10 mutagenesis of DL357. Bacterial strain DL357 was mutagenized with a mini-Tn10 (mTn10) carried on phage λ1098. The mutagenesis was carried out as described previously (30), with the following modifications. After a 90-min incubation at 37°C, dilutions of the mixtures were plated on M9 minimal medium containing lactose, X-gal, and the antibiotics tetracycline and kanamycin and were incubated overnight at 42°C. Colonies on the plates were harvested in 2 ml of λ diluent (10 mM Tris base, 10 mM MgSO₄) containing 50 mM NaCl and 0.01% gelatin. Dilutions of the resuspension were plated on M9-lactose-kanamycin-tetracycline medium and subsequently incubated at 23°C.

P1 transduction. The preparation of P1 lysates and P1 transductions were carried out as described previously (20).

UV induction of λ246. The UV induction of λ246 was carried out as described previously (28).

Lysogenization of UV-induced phage. A 5-ml overnight culture of the recipient E. coli strain was made in LB with 0.2% maltose. The culture was centrifuged and the pellet was suspended in 2.5 ml of 10 mM MgSO₄. A total of 100 μl of E. coli recipient was mixed with 100 μl of phage lysate from UV-induced bacteria and incubated at room temperature for 20 min. Following the addition of 2 ml of LB-maltose, cultures were incubated at 37°C for 1 to 2 hs. Portions (0.1 ml) were plated on M9 minimal medium containing glycerol and kanamycin.

DNA probe isolation. Plasmid pDAL278B (Table 1), which contains a mTn10 insertion, was used to isolate a mTn10 DNA probe. Digestion of pDAL278B with restriction endonucleases EcoRI and HindIII yielded a 868-base-pair DNA fragment internal to the mTn10 element (30). This DNA fragment was purified from a 1% agarose gel by a method described previously (2) and nick translated with [α-³²P]dCTP as described by Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). The mTn10 DNA probe specific activities were in the range of 2 × 10⁷ to 5 × 10⁷ cpm/μg.

The λ246 probe used to visualize pap and lambda phage DNA sequences was prepared as follows. DNA was purified from intact λ246 phage as described previously (32). The isolated DNA was nick translated with [α-³²P]dCTP as described by Bethesda Research Laboratories.

Southern blotting. Chromosomal DNA was isolated and digested with restriction endonucleases as described previously (2, 16). The DNA was concentrated by ammonium acetate-ethanol precipitation at 4°C. The DNA samples were loaded onto a 0.6% agarose gel, and electrophoresis was carried out for 3.5 h at 90 V. Transfer of DNA from the agarose gel to nitrocellulose paper was carried out as described previously (16).

Calculation of phase transition rates. The phase transition rates were determined as described previously (4). Each transition rate was based on data acquired from two to five colonies.

Mapping of genes using Hfr, T4GT7, and P1 vehicles. The mapping of the mTn10 mutations in DL632 and DL652 was performed as described previously (29). Portions were removed from the Hfr mating mixtures at 20 and 35 min. The portions were diluted in LB and vortexed to disrupt mating

TABLE 2. Effect of temperature on phase transition frequencies observed for *E. coli pap'-lacZYA* fusion lysogens
in both wild-type and mutant backgrounds

| *E. coli* *lacZYA* Φ lysogen | Temp (°C)[a] | | Lac phenotype, carbon source[b] | Observed Lac⁺ ↔ Lac⁻ switch frequency | | |
	1°	2°		Total no. of colonies counted	No. of Lac⁺/no. of Lac⁻ colonies	Weighted avg of frequencies[c]
DL357	37	37	Lac⁺, M9-glycerol	11,271	2,812/8,459	$\alpha = 3.32 \times 10^{-2}$
DL478				2,073	1,273/800	$\alpha = 1.86 \times 10^{-2}$
DL479				2,998	1,436/1,562	$\alpha = 2.44 \times 10^{-2}$
DL357	37	37	Lac⁻, M9-glycerol	31,090	21/31,069	$\beta = 2.75 \times 10^{-5}$
DL478				3,791	47/3,744	$\beta = 8.51 \times 10^{-4}$
DL479				2,890	21/2,869	$\beta = 4.65 \times 10^{-4}$
DL478	23	23	Lac⁺, M9-glycerol	6,376	398/5,978	$\alpha = 4.22 \times 10^{-2}$
DL479				5,406	1,340/4,066	$\alpha = 3.44 \times 10^{-2}$
DL478	23	23	Lac⁻, M9-glycerol	8,208	11/8,197	$\beta = 6.16 \times 10^{-5}$
DL479				34,805	282/34,523	$\beta = 3.50 \times 10^{-4}$

[a] Primary (1°) refers to the initial plate from which the colony was picked; secondary (2°) refers to the plates that the colony was transferred to for quantitation of switch frequencies. All plates were M9-glycerol containing X-gal.

[b] Carbon source refers to that included in the solid media that were used to inoculate parent and progeny colonies.

[c] The weighted average of the switch frequency was calculated as described in the text and takes into account the number of progeny examined for each experiment used to calculate the average. The Lac⁺ to Lac⁻ switch frequency is designated α, and the Lac⁻ to Lac⁺ switch frequency is designated β.

pairs. After incubation at 37°C for 1 h, dilutions from each time point were plated on LB medium containing streptomycin and kanamycin. Kan^r colonies were picked from the plates and incubated on LB-tetracycline to determine whether recombinational replacement of mTn*10* DNA sequences occurred. Phage T4GT7 and phage P1 transductants were selected on LB-streptomycin-kanamycin plates. Transductants were subsequently screened on LB plates containing tetracycline to determine tetracycline sensitivity.

The empirical equation $F = (1 - D/L)^3$ (29), where F is the cotransduction frequency, L is the length of chromosomal DNA packaged by P1 (adjusted to 1.8 min to account for the resistance marker), and D is the distance (in minutes), was used to determine the distance of the Tn*10* mutation from the respective Tn*10 kan* and Tn*5* markers.

RESULTS

The goal of this study was to identify the chromosomal gene(s) that is required for the thermoregulation of *pap* pilin gene transcription. For this analysis *E. coli* DL357, which carries the prophage λ246 (4), was used. Phage λ246 contains a *papBAp-lac* fusion in which the *lac* genes are under the control of the *papBAp* pilin promoter. The λ246 phage also contains the coding sequences of the *papI* and *papB* regulatory genes. At 37°C, pilin transcription is subject to a phase variation mechanism in which asymmetric phase variation rates are displayed. The Lac⁺ → Lac⁻ transition rate is 3.32 × 10⁻², while the Lac⁻ → Lac⁺ transition rate is 2.75 × 10⁻⁵ (Table 2). Incubation of this strain at 23°C results in the abrogation of phase variation, as evidenced by a uniform Lac⁻ colony phenotype (Fig. 1A and B). To identify the gene(s) involved in the thermoregulation of pilin gene transcription, strain DL357 (Table 1) was subjected to mTn*10* mutagenesis with the conditionally lytic phage λ1098 (30). Following incubation with phage λ1098, bacteria were inoculated onto M9-lactose-tetracycline-kanamycin medium to select for the presence of both mTn*10* and *papBAp-lac* DNA sequences. This screen was designed to allow us to identify negative regulators of pilin transcription. Positive regulators of transcription would not be identified by this protocol. After 5 days of incubation at 23°C, many small Lac⁻

colonies were observed, in addition to a few large Lac⁺ colonies. The appearance of small Lac⁻ colonies that were able to grow on lactose medium was the result of a low level of β-galactosidase expressed at this low temperature (data not shown). The large Lac⁺ colonies, which appeared to have lost thermoregulation of *pap* pilin transcription, were chosen for further study.

Nineteen Lac⁺ colonies were chosen for analysis, each of which was derived from a separate mTn*10* mutagenesis pool, and were purified by serial passage on M9-lactose-tetracycline-kanamycin plates at 23°C. Subsequently, the mutant colonies were incubated on M9 minimal medium containing glycerol, tetracycline, and kanamycin; and the Lac colony phenotype was observed at both 23 and 37°C. Two classes of mutants were obtained: class I mutants were subject to phase variation at both 23 and 37°C (Fig. 1C through F; note that both Lac⁺ and Lac⁻ colony phenotypes were observed at 23°C), whereas class II mutants displayed only a Lac⁺ colony phenotype at both 23 and 37°C.

To determine whether the thermoregulatory mutant phenotypes were the result of a single mTn*10* chromosomal insertion, mTn*10* mutations were transferred from their original *recA1* backgrounds to *E. coli* DL379 (Table 1) by phage P1 transduction. Strain DL379 is a *recA⁺* phage λ246 lysogen. By analyzing the Lac colony phenotypes of the P1 transductants, we determined whether the mutant thermoregulatory phenotypes were the result of mTn*10* insertions. Only 7 of 19 of the mTn*10* mutations were linked to the mutant thermoregulatory phenotype. Five of the linked mTn*10* mutations were class I mutants, while the remaining two were class II mutants.

The phage P1 transductants obtained as described above were used to determine whether any of the mTn*10* insertions were located within the λ246 phage. Mutant strains were exposed to UV light, and the resulting phage λ246 lysates were used to lysogenize strain MC4100 *recA1*. Lysogens were serially passed on M9-glycerol-kanamycin medium to determine the Lac phenotypes of the colonies after incubation at either 23 or 37°C. The mutations for the two class II mutants were linked to phage λ246. The mutations for the
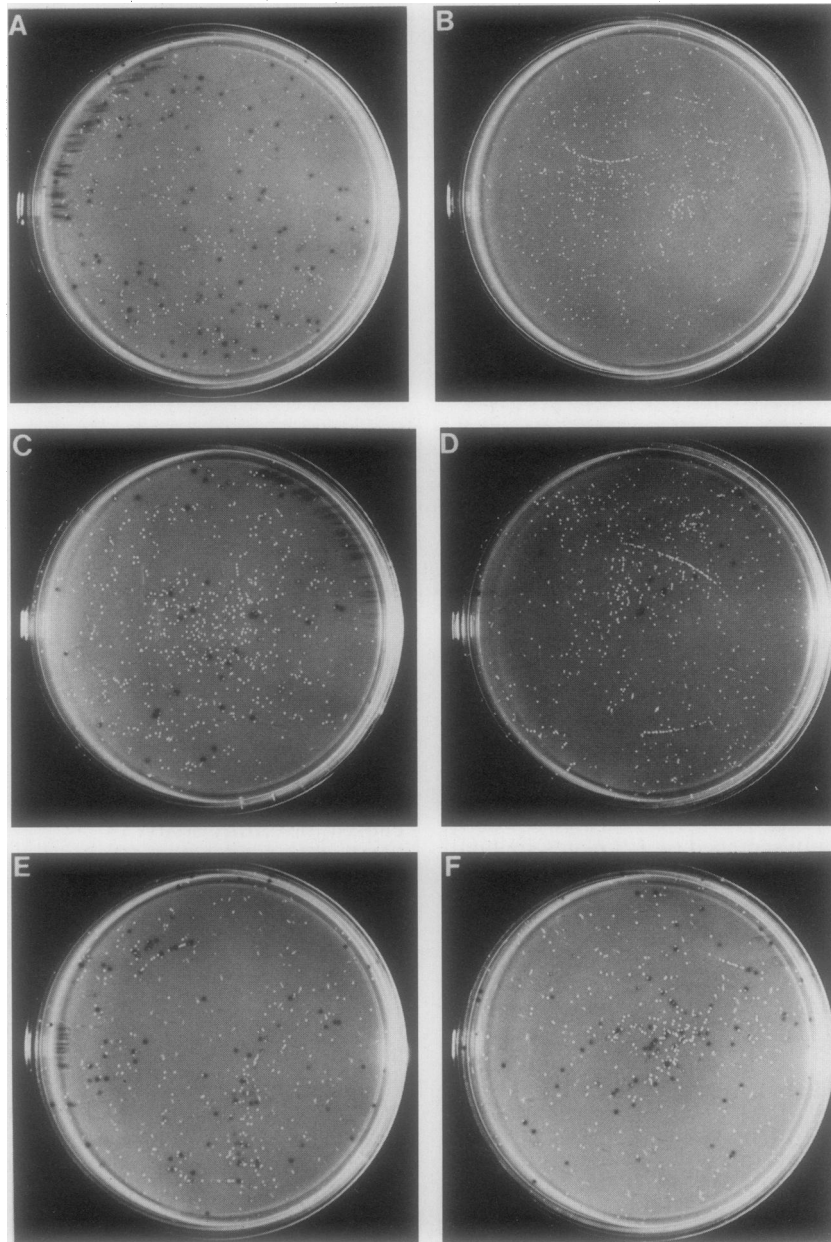
FIG. 1. Analysis of Lac phenotypes of strain DL357 and thermoregulatory mutant strains DL478 and DL479. A single colony (Lac⁻ for DL357 at 23°C; Lac⁺ for the remaining colonies) of each strain was isolated from M9-glycerol plates incubated at either 23 or 37°C. Bacteria were subsequently inoculated onto M9-glycerol plates and incubated at the same temperature that was used for the initial inoculations. Plates were incubated for 36 h at 37°C or for 5 days at 23°C prior to being photographed. (A) DL357, 37°C; (B) DL357, 23°C; (C) DL478, 37°C; (D) DL478, 23°C; (E) DL479, 37°C; (F) DL479, 23°C.

five class I mutants were not linked to λ246, suggesting that the mTn10 insertions were within a chromosomal gene(s).

To determine whether the mTn10 elements in the five class I thermoregulatory mutants were located near one another, Southern blotting was performed. Chromosomal DNAs were isolated from each of the class I mutants (strains DL478, DL479, DL480, DL481, and DL483; see Table 1) and were digested with the restriction enzyme SalI. Because the mTn10 element does not contain any SalI restriction sites, each mTn10 should be present within a single SalI restriction fragment. To identify mTn10 DNA sequences, hybridization was performed with an α-³²P-labeled, 868-base-pair EcoRI-

HindIII DNA fragment contained within mTn10 (see Materials and Methods). All five mTn10 insertions in the class I mutants were located within a 5.9-kilobase DNA fragment, suggesting that the mTn10 mutations are in the same gene or closely linked genes (Fig. 2A, lanes 2, 3, 4, 5, and 7). For comparison, Southern blot analysis of one of the class II mutants (DL482) is also shown (Fig. 2A, lane 6). We propose that the locus defined by the class I mutants be designated tcp, for thermoregulatory control of pap.

Chromosomal DNAs from the thermoregulatory class I mutants were also digested with EcoRI and hybridized with the same mTn10 DNA probe. Because the mTn10 DNA

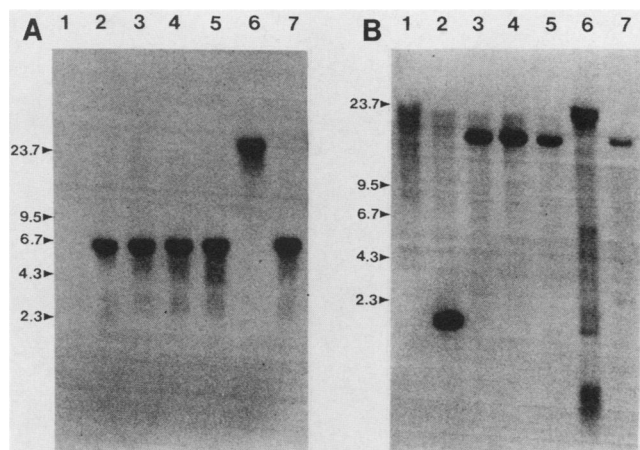FIG. 2. Southern blot hybridization analysis of mTn*10* insertions in the *E. coli* thermoregulatory mutants. The mTn*10* insertions were visualized with an [α-^{32}P]dCTP-labeled, 868-base-pair *Eco*RI-*Hin*dIII fragment internal to the mTn*10*. (A) Chromosomal DNA samples digested with the restriction endonuclease *Sal*I. (B) Chromosomal DNA samples digested with the restriction endonuclease *Eco*RI. Chromosomal DNA was isolated from the following strains: DL357 (lanes 1), DL478 (lanes 2), DL479 (lanes 3), DL480 (lanes 4), DL481 (lanes 5), DL482 (lanes 6), and DL483 (lanes 7). Numbers to the left of the gels are in kilobases.

sequence contains a single *Eco*RI site and because a *Eco*RI-*Hin*dIII DNA fragment was used as a probe, only one of the two *Eco*RI DNA fragments containing mTn*10* was detected on Southern blots. Our results showed that the mTn*10* was present on a 1.7-kb *Eco*RI DNA fragment in the mutant DL478 (Fig. 2B, lane 2), whereas mTn*10* was located on a 17-kb *Eco*RI DNA fragment in the remaining four mutants (Fig. 2B, lanes 3, 4, 5, and 7). For comparison, Southern blot analysis of strain DL482 is also shown (Fig. 2B, lane 6). The hybridization signal seen in parent strain DL357 (Fig. 2B, lane 1) was not due to a mTn*10* insertion. The blot in Fig. 2B was previously probed at a lower stringency and stripped before it was hybridized with the mTn*10* probe. It appears that incomplete stripping caused the high background in this and other lanes. We did not detect hybridization of mTn*10* DNA sequences with the parental strain DL357, based on the Southern blot shown in Fig. 2A, lane 1. The blots shown in Fig. 2 were stripped of the α-^{32}P-labeled mTn*10* DNA probe and were hybridized with α-^{32}P-labeled λ246 DNA (data not shown). No new λ DNA fragments containing mTn*10* were detected in the class I thermoregulatory mutants compared with those found in strain DL357, confirming that the mTn*10* mutations were not within the λ246 phage.

The *E. coli* chromosomal locations of the mTn*10* mutations in strains DL478 and DL479 were initially determined by Hfr mapping (29). The Hfr strains used contained Tn*10 kan* markers located 20 min from the origin of transfer. The Hfr strains were mated to *E. coli* DL632 (Table 1), which is a strain MC4100 P1 transductant that contains the *tcp-2*::mTn*10* mutation from DL479. Tet^s colonies arising from these matings represent those events in which mTn*10* was lost through recombination with wild-type DNA sequences. Strain CAG12204 did not yield any Tet^s colonies, while strains CAG12206, CAG12203, and CAG12202 yielded significant numbers of Tet^s colonies. Data accumulated from the Hfr strain matings suggested the mTn*10* mutation is located between 17 and 28 min on the chromosome. Further transductional analysis with phage T4GT7 (31) indicated that

the mTn*10* element is located between 21 and 24 min on the *E. coli* chromosome (data not shown).

Fine mapping of the *tcp* mutants was performed with phage P1, which transduces about 2 min of the chromosome (29). Phage P1 lysates were made from strains containing Tn*10 kan* or Tn*5* markers between 21.00 and 25.25 min on the chromosome. Strain DL632 transductants were selected on LB-streptomycin-kanamycin plates, and the colonies were screened for tetracycline sensitivity. Phage P1 lysates from strains CAG18613 and CAG12124, which contained Tn*10 kan* markers at 22.25 and 24.25 min, respectively, produced Tet^s colonies (Fig. 3). Transductants from the lysate made on strain CAG18703, which carries transposon Tn*5* at 22.75 min, exhibited the largest number of Tet^s colonies. The P1 transductions with lysates made from strains CAG12130 and CAG18516, containing Tn*10 kan* at 21.00 and 25.25 min, respectively, yielded only Tet^r transductants (Table 1 and Fig. 3).

By using the cotransduction frequencies presented in Fig. 3, the mTn*10* mutation in strain DL632 was mapped to approximately 23.4 min on the *E. coli* chromosome (see Materials and Methods). Similarly, the *tcp-1*::mTn*10* mutation in *E. coli* strain DL652, a P1 transductant derived from DL478, was mapped and found to have a location similar to that in strain DL632.

The class I thermoregulatory mutants described alternated between phase-off (Lac$^-$) and phase-on (Lac$^+$) pilin transcription states at both 37 and 23°C. To determine whether the phase transition rates displayed by these mutant *E. coli* strains were similar to those of the parental strain DL357, we measured phase transition frequencies for thermoregulatory mutant strains DL478 and DL479. Strains DL478 and DL479 were chosen because the mTn*10* elements were located on different *Eco*RI DNA fragments in the two mutants. Single Lac$^+$ or Lac$^-$ colonies were isolated from M9-glycerol-kanamycin plates for strain DL357 and from M9-glycerol-tetracycline-kanamycin plates for strains DL478 and DL479. Dilutions of the bacteria in M9 minimal salts were inoculated onto M9-glycerol plates containing the appropriate antibiotics to determine the frequency at which the strains alternated between the Lac$^+$ and Lac$^-$ states. The initial temperature at which colonies were chosen and the subsequent incubation temperature were varied to monitor the effect of temperature on phase transition frequencies.

As shown in Table 2, when colonies were isolated at 37°C and subsequent incubation was at 37°C, the Lac$^+$ → Lac$^-$ phase transition frequencies were similar for all three strains. Interestingly, the Lac$^+$ → Lac$^-$ frequencies obtained at 23°C for the two thermoregulatory mutants DL478 and DL479 were similar (within a twofold range) to the frequencies obtained at 37°C for strains DL357, DL478, and DL479. These results indicated that the mTn*10* insertions did not alter the Lac$^+$ → Lac$^-$ transition frequencies at either 23 or 37°C.

The Lac$^-$ → Lac$^+$ phase transition rate, however, was dramatically affected by the mTn*10* mutations. While the Lac$^-$ → Lac$^+$ phase transition frequencies obtained at 37°C for strains DL478 and DL479 were similar (within a twofold range), they were significantly higher (31- and 17-fold, respectively) than that for strain DL357. Thus, although the mutations were isolated based on the loss of *pap* pilin transcriptional thermoregulation, they also affected the phase variation phenotype. Lowering of the temperature to 23°C had a differential effect on the Lac$^-$ → Lac$^+$ transition frequencies of *E. coli* DL478 and DL479. The Lac$^-$ → Lac$^+$ phase transition rate of strain DL478 was reduced 14-fold,
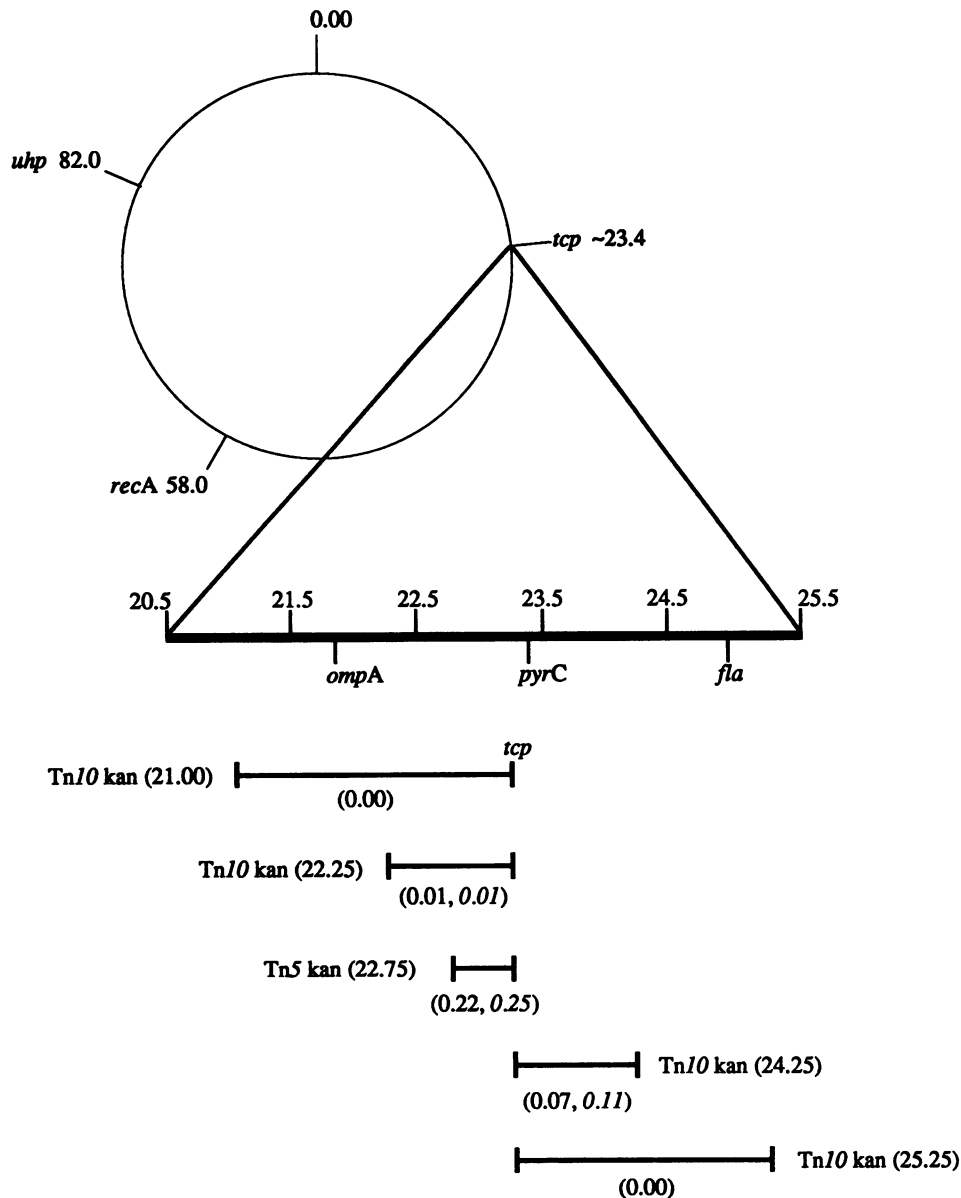
FIG. 3. Mapping of the *tcp* gene. Mapping of the gene was performed as described in the text. The dark bars indicate the approximate distance (in minutes) between the *tcp* locus and neither Tn*10* *kan* or Tn*5*. Numbers on the left beneath the dark bars denote the phage P1 cotransduction frequencies obtained between transposon markers and the *tcp-2* mTn*10* insertion within strain DL632. The italicized numbers denote the phage P1 cotransduction frequencies obtained between transposon markers and the *tcp-1* mTn*10* insertion within strain DL652.

whereas incubation at 23°C had little, if any, effect on the transition rate for strain DL479 (Table 2). Thus, although the two mutants both displayed much higher phase transition rates than that of strain DL357 at 37°C, only mutant strain DL479 maintained a significantly higher phase transition rate at 23°C.

DISCUSSION

In this study we identified a genetic locus, *tcp*, that appears to play an important role in the thermoregulation of *pap* pilin transcription. Transposon mTn*10* insertions within this locus resulted in an abrogation of *pap* thermoregulation (Fig. 1). Based on genetic mapping results, *tcp* is located at

approximately 23.4 min on the *E. coli* chromosome. Interestingly, *E. coli* strains containing the *tcp*::mTn*10* insertions displayed a significantly higher *pap* pilin phase-off → phase-on transition frequency at 37°C than that of strain DL357 (Table 2). These results indicated that *tcp* may play an inhibitory role in *pap* pilin phase variation. Although the mechanism(s) by which the *tcp* locus might function to affect *pap* pilin thermoregulation is not known, one possibility is that *tcp* encodes an inhibitory protein that interacts with the *papBAp* promoter region. At 23°C, thermoregulation could be effected by an increase in *tcp* transcription or by a temperature-induced activation of the Tcp protein.

The phase transition rates displayed by strains containing the mutant *tcp-1*::mTn*10* (strain DL478) and *tcp-2*::mTn*10*

(strain DL479) alleles were different. Based on Southern blot analysis (Fig. 2), it appears that the mTn*10* elements in these two mutant strains are closely linked (within 5 kb) but are located in different chromosomal sites. Whereas both thermoregulatory mutants displayed elevated phase-off (Lac⁻) → phase-on (Lac⁺) transition rates at 37°C, only strain DL479 maintained an elevated phase-off → phase-on transition rate at 23°C (Table 2). Although it is not clear why these two *E. coli* mutant strains were phenotypically different, it is possible that the mTn*10* mutations were located in different genes of a *tcp* operon. An alternative hypothesis is that both thermoregulatory mutants contain mTn*10* within a single *tcp* gene, with each mTn*10* insertion causing a different phase variation phenotype.

In this study, we isolated 19 thermoregulatory mutants that fell into one of two classes based on *pap* pilin phase variation phenotypes. Interestingly, all of the class I mutants appeared to contain mTn*10* insertions within the *tcp* locus, as evidenced by Southern blot analysis (Fig. 2). Two of the class II mutants also contained mTn*10* insertions that were linked to the expression of a mutant thermoregulatory phenotype. The insertions in the class II mutants mapped to the λ246 *papBAp-lac* fusion phage. The remaining 12 mutants did not display either a class I or a class II phenotype. These mutations were not linked to mTn*10*, suggesting that these phenotypes were due to a spontaneous mutation during selection. Similarly, we isolated 11 spontaneous thermoregulatory mutants using the M9-lactose selection procedure described above. Five of these mutants displayed a class I phenotype, and six mutants displayed a class II phenotype. All of the spontaneous class I mutations mapped to non-phage portions of the chromosome, and thus were not located within *pap* DNA sequences. These results are consistent with our results obtained using mTn*10* since the mutations within both transposon-induced and spontaneous class I mutants are located chromosomally, outside of the *papBAp-lac* fusion phage. We are now trying to determine whether the mutations within the spontaneous class I mutants are located within *tcp*. For the spontaneous class II mutants, all of the mutations mapped to the *papBAp-lac* fusion phage, as did the mTn*10* insertions in transposon-induced class II mutants. The constitutive pilin transcription phenotypes exhibited by the class II mutants could be caused by mutations that alter the pilin promoter activity or, in the case of mTn*10* insertions, by the introduction of a transposon promoter that drives *lac* transcription. In the transposon-induced class II mutants that we analyzed, the mTn*10* insertion was not within the 1.6-kb region of *pap* DNA contained in the λ246 *papBAp-lac* fusion phage (data not shown). This suggests that constitutive pilin transcription in these mutants is not the result of an insertion of mTn*10* in a DNA-binding site for a repressor of pilin transcription.

Previously, it was found that DNA sequences within the *papI-papB* region are necessary to obtain the thermoregulatory phenotype (10). In addition, evidence was presented suggesting that thermoregulation of pilin transcription occurs in the absence of either the PapI or the PapB protein. Interestingly, it was found that in the presence of a multicopy plasmid containing *papI*, thermoregulation was abolished. These results suggested that a non-*pap*-encoded protein(s) is involved in thermoregulation and that this protein(s) might act at the level of PapI expression. Our finding that a non-*pap* locus plays a role in the thermoregulation of *pap* pilin transcription is therefore consistent with these results.

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