JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 179–185
0021-9193/86/100179-07$02.00/0

Vol. 168, No. 1

Metastable Regulation of Type 1 Piliation in *Escherichia coli* and Isolation and Characterization of a Phenotypically Stable Mutant

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Received 14 April 1986/Accepted 30 June 1986

Type 1 piliation in *Escherichia coli* exhibits phase variation due to the inversion of a small, ca. 300-base-pair, element that regulates *pilA* (*fimA*), the gene that encodes the structural subunit of pili (Abraham et al., Proc. Natl. Acad. Sci. USA 82:5724–5727, 1985). We have used the inversion as an assay to characterize a stably piliated mutant. The mutant strain did not exhibit the *pilA* ON and *pilA* OFF colonial variants characteristic of the wild type; rather, every clone produced a level of *pilA* expression intermediate between ON and OFF wild-type populations. The mutant phenotype was conferred by a lesion at a previously undescribed locus between *hemA* and *trpA*, which we have termed *pilG*. Examination of the *pilA* promoter region in four *pilG* mutant populations indicated that the phenotypic stability conferred by the *pilG* mutation was not due to an inability to carry out the inversion. Rather, all *pilG* mutant populations consisted of approximately equal mixtures of ON and OFF individuals. We suggest that *pilG* mutants may undergo such rapid switching of the *pilA* promoter that populations exhibit an intermediate level of *pilA* expression and phenotypic stability.

Type 1 piliation in *Escherichia coli* is subject to metastable expression which is regulated at the level of transcription (4, 18). The molecular mechanism for this variation appears to be due to the regulation of *pilA* (*fimA*) (1, 4, 18), the structural gene encoding pilin located at approximately 98 min on the *E. coli* genetic map (2, 17, 18). Abraham et al. (1) have shown recently that an inversion of a 314-base-pair (bp) segment of DNA encompassing the *pilA* promoter region accompanies a switch in the expression of the *pilA* gene. The small size and the lack of an open reading frame in the invertible region (1) preclude the invertible element from encoding an enzyme responsible for catalyzing or regulating the inversion. Thus, the genes for putative effector molecules which influence the inversion would be located elsewhere in the *E. coli* chromosome.

We have previously characterized two types of mutants which have lesions that influence the transcriptional regulation of *pilA*. One type of lesion maps to a gene, *hyp*, that is located next to *pilA* and encodes a repressor of *pilA* transcription (4, 15, 16). Thus, *hyp* mutants are hyperpiliated (15, 16) and show a higher level of expression of *pilA* when *pilA* transcription is measured by using a *pilA-lacZ* fusion (18). However, the *hyp* gene product is not required for metastable expression of *pilA* (18). The second type of lesion is a small deletion, encompassing the invertible region, which encodes the *pilA* promoter region (15, 17, 18; also see Discussion). Such deletion mutations lower drastically the level of piliation, but low amounts of pilin are still produced (15, 16). A third type of lesion, described by Freitag et al. (5), defines a locus near the chromosomal *pil* region (2, 5) that appears to abrogate metastable variation. Thus, mutants having this third type of lesion are locked into one phase or the other (5).

We report here a description of the metastable inversion that controls *pilA* transcription. We then relate experiments in which the inversion was used to characterize a mutant having a lesion that increases the orientational instability of the invertible region. Interestingly, the mutant was first noticed by virtue of its phenotypic stability, which appears, in light of the results presented here, to be due to a perpetually mixed population of ON and OFF individuals, rather than to the stability of the *pilA* promoter, as the phenotype originally suggested.

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MATERIALS AND METHODS

Bacterial strains, bacteriophage strains, plasmids, and media. The bacterial strains used in this study were all *E. coli* K-12 derivatives. These strains are listed in Table 1, along with the bacteriophage strains used. Previously described Pil plasmids used in this study are also listed in Table 1. Culture media consisted of L-broth, L-agar (13), MacConkey agar (Difco Laboratories, Detroit, Mich.), tetrazolium agar (20), and minA agar (13). Antibiotic additions were as previously described (16), unless otherwise noted.

Genetic techniques. Random transposon Tn*10* insertions were isolated in strain ORN117 by infecting overnight cultures with λ::Tn*10* (Table 1) at a multiplicity of infection of ca. 1 and plating dilutions on L-agar plates containing 20 μg of tetracycline per ml. Four plates, each containing ca. 300 Tet^r colonies, were scraped, and a P1 lysate was made from each plate. P1 transduction was carried out by standard procedures (13).

A Tn*10* insertion linked to *pilG* was located on the *E. coli* chromosome by interrupted mating experiments. Logarithmically growing Hfr donor and recipient cultures were mixed in a ratio of 20:1 (recipient to donor) in a total volume of 1.0 ml of L-broth at 37°C. Mating was carried out for 30 min or interrupted at 5-min intervals. In either case, mating was stopped by making a 10-fold dilution of a portion of the mating mixture and subjecting the diluted sample to vigorous shaking on a gyratory mixer for 30 s. Dilutions of the interrupted mating mixture were plated on minimal medium containing the appropriate energy and carbon sources to select recombinants having received nutritional markers located near the origin of transfer. Samples were also plated on L-agar containing 20 μg of tetracycline per ml. F′ matings were carried out as for Hfr matings, except that the mating time was extended to 3 h.

TABLE 1. Bacterial and bacteriophage strains and plasmids

Bacterium, bacteriophage, or plasmid	Description	Source or reference
E. coli strains		
ORN105	*thr leu proA2* Δ(*argF-lac*)*U169 galK his argE rpsL supE mtl xyl recBC sbcB* Pil$^+$ Tet^r (has Tn*10* between *hsd* and *serB*; exhibits metastable expression of piliation)	18
ORN115	*thr-1 leuB thi-1* Δ(*argF-lac*)*U169 malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA2 supE44 pilG* Pil$^+$ (does not exhibit metastable expression of piliation)	18
ORN117	Same as ORN105, except ϕ(*pilA'-kan-lacZYA*) (exhibits metastable expression of *pilA*)	18
ORN116	Same as ORN115, except ϕ(*pilA'-kan-lacZYA*) (does not exhibit metastable expression of *pilA*)	18
ORN125	Same as ORN116, only *pilG*$^+$ *zcg*::Tn*10* Tet^r (exhibits metastable expression of *pilA*)	P1 transduction from ORN117::Tn*10* pool
ORN126	Same as ORN116, only *zcg*::Tn*10* (does not exhibit metastable expression of *pilA*)	P1 transduction from ORN125
KL208	Hfr (*relB* < *recE*)[a] *relA1*? λ$^-$ (point of origin ca. 32 min)	9
ORN128	Same as KL208, except *zcg*::Tn*10*	P1 transduction from ORN125
JC1552	F$^-$ *leuB trp-31 hisG metB1 lacY1 gal-6 malA1 xyl-7 mtl-2 rpsL104 fhuA2 tsx-1* λ^r λ$^-$ *supE44*	9
SS320	*azi-9 pro-48 lacZ118 lacI22 trpA9605 his-85 gyrA19 rpsL171 metE70 trpR55* λ$^-$ *pilG*$^+$	B. Bachmann
ORN129	Same as SS320, except *zcg*::Tn*10*	P1 transduction from ORN125
JK268	*dadR1* (*dadA*) *trpE6 trpA62 tna-5 purB58 pilG*$^+$	6
ORN130	Same as JK268, except *zcg*::Tn*10*	P1 transduction from ORN125
ORN131[b]	Same as ORN125, only *trpA62*	P1 transduction from ORN130
ORN132[b]	Same as ORN126, only *trpA62*	P1 transduction from ORN126 and JK268
S730	*purB51 hemA30 trp-45 his-68 tyrA2 pur-65 thi-1 lacY1 gal-6 malA1 xyl-7 mtl-2 rpsL125 fhuA tsx-70* λ^r λ$^-$ *supE44*	19
KL702	F'125 (*pyrD trp recE*)/*pyrD34 trp-45 his-68 recA1 thi-1 galK35 malA1* (λ^r) *xyl-7 mtl-2 rpsL118* λ$^-$	10
Bacteriophages		
P1	*vir*	Laboratory collection
λ::Tn*10*	*b*221 *c*I857::Tn*10* *O*uga261	N. Kleckner
Plasmids		
pORN123	pBR322 Δ*Pvu*II	18
pORN119	*pilA' hyp'*::IS*50'* pORN123 Tc^r	18
pORN111	*pilA'* Δ*pilAp hyp'*::IS*50'* pORN123 Tc^r	Subclone from pORN103 (16)

[a] <, Direction of transfer; parentheses indicate that *relB* and *recE* are wild-type alleles transferred last and first, respectively.
[b] May also be *trpE6*.

Receipt of the *pilG*$^+$ allele in P1 transduction experiments was scored by streaking Tet^r transductants onto lactose-MacConkey plates containing tetracycline. Transductants that had received the *pilG*$^+$ allele along with Tn*10* exhibited red (Lac$^+$) and white (Lac$^-$) colonies, compared with the uniformly pink colonies exhibited by Tet^r transductants, which still possessed the mutant *pilG* allele. (See Fig. 3 for comparison of the colonial phenotype of strains ORN125 and ORN126 on lactose-MacConkcy agar.) There was no difference observed in the cotransduction frequencies between receipt of the wild-type and mutant *pilG* alleles, indicating that the locus had no effect on cell viability. Receipt of a mutant *trpA* allele was scored by patching Tet^r transductants onto minA agar containing all required amino acids except tryptophan. The Dad$^-$ phenotype was scored by the ability of Tet^r transductants to grow on minimal medium containing D-threonine and D-leucine to fulfill the auxotrophic requirements for those two amino acids. Concentrations of the D-amino acids were the same as for the L-amino acids (13). The *hemA*$^+$ allele (2) was scored in Tet^r transductants by patching colonies onto L-agar plates. Control plates consisted of L-agar plates that had been spread with 0.1 ml of a 10-mg/ml solution of delta-aminolevulinic acid (19).

Recombinant DNA techniques and DNA hybridization. Conditions for restriction endonuclease digestion, agarose gel electrophoresis, and isolation and ligation of DNA fragments have been described previously (15–17). Chromosomal DNA was isolated as previously described (7, 8) and digested to completion with *Taq*I or *Hin*fI restriction endonuclease under conditions suggested by the supplier (Bethesda Research Laboratories). Digested chromosomal DNA fragments were separated by acrylamide gel electrophoresis (4% acrylamide) by the standard procedures (11). DNA fragments were denatured in situ (21) and transferred to nylon mesh (Nytran, 0.45 μm; Schleicher & Schuell) by the dry transfer method outlined by Maniatis et al. (11). Subsequent hybridization conditions followed those specified by the manufacturer of Nytran (Schleicher & Schuell).

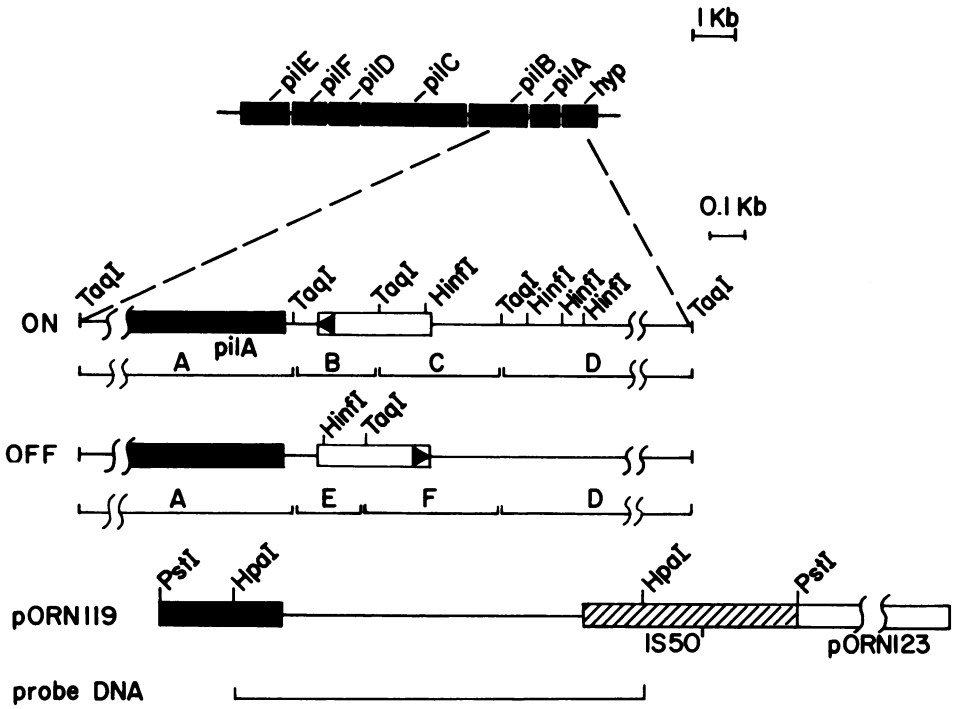

FIG. 1. Invertible segment of the *pilA* promoter. The *pil* region is shown at the top of the figure. The size and arrangement of the genes of the *pil* region are shown by solid boxes. The genes have all been described previously (12, 16), except for *pilF*, which encodes an 18-kilodalton product that regulates pilus length (Maurer and Orndorff, submitted). The region immediately upstream from *pilA* is shown in the ON and OFF phases. The open box with the arrowhead depicts the size and orientation of the promoter region. The positions of the *Hin*fI and *Taq*I sites show the asymmetry of the sites with respect to the inverted region. The lettered segments in the ON and OFF diagrams depict the *Taq*I restriction fragments that were detected by the probe DNA from pORN119, shown at the bottom of the figure (segments A and D are not drawn to scale). A Southern blot showing the *Taq*I restriction fragments corresponding to the lettered segments is presented in Fig. 4B.

Radiolabeled probe DNA was prepared by nick translation as described by Moseley et al. (14).

Phenotypic detection of a change in phase of *pilA* expression. Strain ORN105 undergoes wild-type phase variation, producing piliated and nonpiliated cells (12, 18). This variation correlates with the transcriptional state of *pilA* as detected in strain ORN117 with a *pilA*-kanamycin resistance gene (*kan*)-*lacZYA* fusion (18). Thus, *pilA* ON and *pilA* OFF clones (colonies) are detected by their Lac^+ (ON) or Lac^- (OFF) phenotype on lactose-MacConkey agar plates (18).

DNA isolation from clonal populations. DNA derived from clonal populations refers to DNA extracted from the progeny of a single colony exhibiting a single Lac phenotype (i.e., a nonsectored colony). Colonies were picked and grown for 6 h at 37°C in L-broth, and the DNA was extracted and prepared for Southern blot analysis as described above. The short growing time was used as a precaution to obtain a population reflective of the phenotype of the original colony. Nevertheless, the resultant cultures were always plated on indicator media to assess the homogeneity of the population at the time of DNA extraction.

Assay of beta-galactosidase activity. Beta-galactosidase activity in metastable populations was measured as described previously (18).

RESULTS

Detection of inversion of the *pilA* promoter. The invertible nature of the *pilA* promoter was initially suspected because of the high frequency of spontaneous deletions of a ca. 300-bp segment just upstream of the *pilA* gene (15–18). Restriction endonuclease mapping of the deleted region with subclones pORN111 and pORN119 revealed that a *Taq*I and an *Hin*fI site were located asymmetrically in the deleted region (open box in Fig. 1). We reasoned that if an inversion of the 300-bp region were occurring, then an examination of chromosomal DNA from ON and OFF populations, cut with *Taq*I or *Hin*fI and probed with a radiolabeled segment of DNA encompassing the promoter region (Fig. 1), should reveal a reversible change in the hybridization pattern in ON and OFF populations. The change in the banding pattern observed from sequential ON-OFF-ON-OFF populations of strain ORN125 digested with either *Taq*I or *Hin*fI suggested the rearrangement of the ca. 300-bp region shown in Fig. 1. (The results of the actual hybridization experiments for *Taq*I-cut chromosomal DNA are shown in Fig. 4B.)

Isolation of a *pilG* mutant. A strain (ORN115) having a lesion in *pilG* was detected accidentally during routine screening of piliated strains by our inability to isolate an OFF (nonpiliated) clone (12). Introduction of a *pilA-kan-lacZYA* fusion by P1 transduction into strain ORN115 from a donor (strain ORN117) that exhibited wild-type phase variation revealed that all the Kan^r transductants of ORN115 (represented by strain ORN116) exhibited a constant, intermediate level of beta-galactosidase activity, in contrast to the donor, in which the transcription of *pilA* was metastable. This difference was readily apparent when colonies of ORN116 and ORN117 were examined on lactose-Mac-

TABLE 2. Colonial phenotype and level of *pilA* transcription of *pilG* mutant clones[a]

Strain	Variation in colonial phenotype	Colonial phenotype[b]	Relative beta-galactosidase activity[c]
ORN117 (*pilG*$^+$)	Yes	ON	0.910
		OFF	0.070
ORN126 (*pilG*)	No	Intermediate	0.504
ORN125 (*pilG*$^+$)	Yes	ON	0.750
		OFF	0.020

[a] Colonial phenotype was judged on lactose-MacConkey agar plates. Variation in colonial phenotype was demonstrated by the ability of individual clones to give rise to phenotypically ON and OFF colonial variants.

[b] The ON, OFF, and intermediate designations refer to the degree of red coloration in the colonies: ON, red: OFF, white: intermediate, pink.

[c] Beta-galactosidase activity was measured in logarithmically growing cells as described previously (18). The values represent the relative activity per cell as measured against strain ORN115 (0). When beta-galactosidase activities are given for metastable populations, the values were calculated by taking into account the percentage of Lac$^+$ and Lac$^-$ cells in the population (18). One unit of beta-galactosidase activity was equivalent to about 167 Miller units (13).

Conkey agar. Colonies of ORN116 were pink, which contrasted to the red and white colonial types of strain ORN117. These results indicated two things: (i) the phenotypic stability of piliation on ORN115 was reflected in the apparent stability of *pilA* expression, and (ii) the genetic character conferring this stable phenotype was not closely linked to *pilA*. (*pilA* is located at ca. 98 min on the *E. coli* genetic map [2, 18].)

Introduction of a wild-type *pilG* allele into strain ORN116. Random Tn*10* insertions were obtained in a *pilA* ON (Lac$^+$) population of strain ORN117 (*pilG*$^+$), and a P1 transducing lysate was made as described in Materials and Methods. The transducing lysates were used to infect strain ORN116 (*pilG*). Tet^r transductants were selected on lactose-MacConkey agar and scored for the dark red colonial phenotype that would suggest that the recipient could now undergo phase variation. One such transductant was isolated and was shown subsequently to undergo phase variation of *pilA* expression at a rate and magnitude similar to the donor strain, ORN117 (18). The linkage of the Tn*10* insertion to the *pilG*$^+$ allele was confirmed by P1 transduction of the wild-type *pilG* allele from this transductant to strain ORN116, forming strain ORN125. The same Tn*10* insertion (hereafter designated *zcg*::Tn*10*) was positioned next to the mutant allele of *pilG* in strain ORN116 (forming strain ORN126). Thus, two strains (ORN125 and ORN126) were constructed that were isogenic save for the *pilG* locus (Table 2).

Location of the *zcg*::Tn*10* insertion on the *E. coli* chromosome. The chromosomal location of the *zcg*::Tn*10* insertion was first estimated by using a kit of seven Hfr strains (kindly provided by B. Bachmann). The *zcg*::Tn*10* insertion was introduced into each of the seven Hfr strains by P1 transduction, and 30-min matings were carried out with appropriate recipient strains. For each Hfr mating, recombinant selection was usually based on the use of several individual nutritional markers near the origin of transfer. Recombinants receiving a particular marker were then scored for tetracycline resistance. This process led to the localization of the *zcg*::Tn*10* insertion near the *trpA* gene. Interrupted matings between strain ORN128 and JC1552 revealed that the *zcg*::Tn*10* insertion in ORN128 was located counterclockwise from *trpA*.

P1 transduction from strain ORN129 (Trp$^-$ PilG$^+$ Tet^r) and strain ORN130 (Trp$^-$ PilG$^+$ Tet^r Dad$^-$) to strain ORN116 (Trp$^+$ PilG$^-$ Tet^s Dad$^+$) and P1 transduction from strain ORN125 (Trp$^+$ Tet^r HemA$^+$) to strain S730 (Trp$^-$ Tet^s HemA$^-$) with selection for Tet^r and scoring for the appropriate Trp, PilG, Dad, or HemA phenotype revealed that the *pilG* locus was located between *hemA* and *trpA*. This location was further indicated by P1 transduction of the *pilG* allele from strain ORN116 (Trp$^+$ PilG$^-$ Tet^s) to strain ORN131 (Trp$^-$ PilG$^+$ Tet^r) with selection for Trp$^+$ and scoring for PilG$^+$ and Tet^r (Table 3). A summary of the linkage of the various markers is shown in Fig. 2.

The mutant *pilG* allele in strain ORN132 (*trpA pilG zcg*::Tn*10*) was complemented in *trans* by F'125 (*pilG*$^+$ *trpA*$^+$) from strain K702. ORN132(F'125) transconjugates were selected by their Trp$^+$ phenotype (counterselection for the K702 donor strain was accomplished by omitting histidine from the selection plates). Several transconjugate clones were tested for tetracycline resistance and for the ability to carry out phase variation. All Trp$^+$ Tet^r transconjugants exhibited colonial phase variation identical to that of strain ORN126 (*pilG*$^+$).

Effect of the *pilG* mutation on *pilA* expression. The role of a *pilG* lesion in producing the stable intermediate level of beta-galactosidase activity was examined by comparing four *pilG*$^+$ and four *pilG* clonal populations (Fig. 3). Examination of the orientation of the *pilA* promoter in four clones of strain ORN125 (*pilG*$^+$), consisting of ON-OFF-ON-OFF sequential isolates, and four random sequentially isolated clones of ORN126 (*pilG*) revealed that each *pilG* mutant clone exhibited a banding pattern consistent with an approximately equal mixture of ON and OFF individuals (Fig. 4A). In contrast, each wild-type clone consisted of individuals of predominantly one orientation or the other (Fig. 4B). Accordingly, P1 transduction of the *pilA-kan-lacZYA* region from clones of strain ORN126 (*pilG*) to strain ORN105 (*pilA*$^+$ *pilG*$^+$) produced approximately equal numbers of ON (Lac$^+$) and OFF (Lac$^-$) transductants, whereas at least 90% of the transductants arising from receipt of the *pilA-kan-lacZYA* region from ON or OFF clones of ORN125 (*pilG*$^+$) exhibited the Lac phenotype of the donor population (Table 4).

TABLE 3. P1 cotransduction frequencies involving *zcg*::Tn*10* and *pilG*[a]

Cross	Donor strain	Recipient strain	Selected marker	No. scored	No. receiving unselected marker(s):								
					pilG	*trpA*	(*trpA-pilG*)	*dadA*	(*dadA-pilG*)	*hemA*	(*trpA-hemA*)	*zcg*	(*zcg-pilG*)
A	ORN129	ORN116	*zcg*::Tn*10*	181	49	16	16	—[b]	—	—	—	NA[c]	NA
B	ORN130	ORN116	*zcg*::Tn*10*	212	55	18	18	17	2	—	—	NA	NA
C	ORN125	S730	*zcg*::Tn*10*	375	—	13	—	—	—	307	8	NA	NA
D	ORN116	ORN131	*trpA*	156	53	NA	NA	—	—	—	—	21	21

[a] Crosses were performed as described in the text. The number scored represents the total from two separate experiments.

[b] —, Marker not scored in that particular cross.

[c] NA, selected locus in a particular cross.

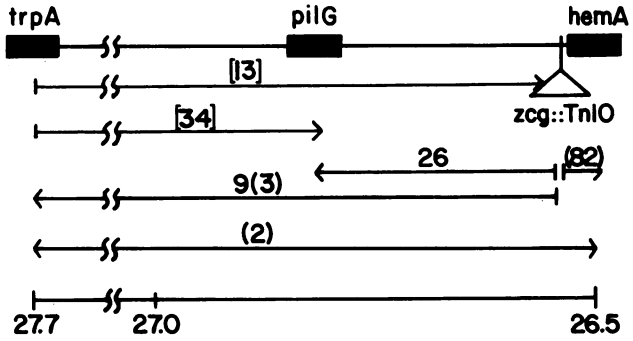

FIG. 2. Chromosomal position of the *pilG* locus determined by P1 cotransduction. Cotransduction frequencies, in percent, are shown above each arrow. The arrowhead points in the direction of the unselected marker. When two numbers are given, the first number refers to the average frequency obtained in crosses A and B (Table 3). The second number (in parentheses) refers to values calculated in cross C (Table 3). Numbers in brackets refer to the cotransduction frequencies obtained in cross D (Table 3). The map units shown at the bottom of the figure were extrapolated from positions and distances shown in edition 7 of the *E. coli* genetic map (2).

DISCUSSION

A small invertible region of DNA encompassing the *pilA* promoter region has been found that appears to regulate *pilA* transcription (1). Using the inversion as an assay, we describe and map a new locus, *pilG*. Mutants having a lesion in *pilG* exhibited phenotypically stable expression of *pilA*. However, the phenotypic stability exhibited by the mutants was not reflective of genotypic stability of the *pilA* promoter. Rather, all *pilG* mutant populations appeared to consist of approximately equal mixtures of ON and OFF individuals.

The importance of the 300-bp segment of DNA immediately upstream from *pilA* in the control of piliation has been reported by Abraham et al. (1) and was initially suggested to us by the high frequency of precise excision of this region in hyperpiliated mutants (15–17). Mutants having this deletion were found to have very low levels of piliation (15, 16, 18). (This region was previously referred to as a 246-bp region [17, 18] and a 250-bp region [15, 16]. The ca. 50-bp discrepancy arose from an early mismapping of the *Taq*I site in the invertible segment.) Careful mapping of restriction endonuclease sites in the deleted region revealed the presence of two asymmetrically located *Taq*I and *Hin*fI sites that were suitable for determining whether the region inverted during a phase switch. Digestion of chromosomal DNA from populations exhibiting the *pilA* ON and OFF phenotype and the use of probe DNA encompassing the *pilA* promoter region revealed the reversible inversion of the 300-bp region and correlated the inversion to a change in the transcriptional mode of *pilA*.

Although the element described by Abraham et al. and the element herein described came from different strains of *E. coli* (1, 16), the nucleotide sequences appear to be, with minor differences, the same (1, 17; unpublished observations). However, the results reported here are somewhat different from those of Abraham et al., in that we observed no multiple copies of the upstream region of *pilA*. Thus, we saw no multiple banding in Southern blots of chromosomal digests (1).

As was suggested by Abraham et al. (1), we infer that the inversion of the *pilA* promoter is responsible for the metastable expression of *pilA*. Thus, in the ON phase, the promoter would be oriented in the direction of transcription of *pilA*; in the OFF phase, the promoter would be oriented opposite the direction of *pilA* transcription.

We used the difference in the hybridization pattern of ON and OFF populations to help characterize a new locus, *pilG*. The *pilG* locus was defined by a single lesion (presumably prototypic) which produced clones (colonies) showing no variation in *pilA* expression. This is in contrast to the wild-type situation, in which ON (Lac^+) and OFF (Lac^-) clones (colonies) are readily observed. In addition to not exhibiting normal colonial variation, *pilG* mutant clones exhibited an intermediate level of beta-galactosidase activity, producing pink colonies on lactose-MacConkey plates rather than the red or white colonies of the wild type.

The genetic character conferring the *pilG* phenotype was located by (i) tagging the wild-type locus with a Tn*10* transposon (*zcg*::Tn*10*), (ii) determining the location of the transposon by employing interrupted mating, and (iii) using P1 transduction to establish the order of the *pilG* and *zcg*::Tn*10* loci to known chromosomal markers. The results of this procedure indicated that *pilG* was located between *trpA* and *hemA* on the *E. coli* K-12 map (2). The *pilG* allele could be complemented in *trans* by F'125, which encodes the region between *trpA* and *hemA* (10). Our observation of *trans* complementation indicated the recessive nature of the

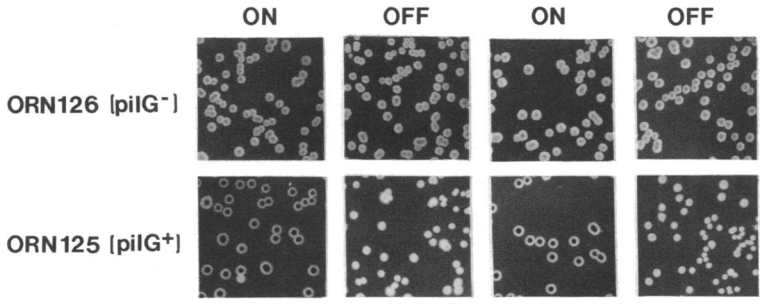

FIG. 3. Colonial phenotype of sequential isolates of strains ORN126 (*pilG*) and ORN125 (*pilG*$^+$). Sequential isolates of ORN126 and ORN125 were obtained by picking and streaking a single colony. After overnight growth, the colonies were examined for the ON or OFF phenotype, and a second colony was streaked for isolation. This process was repeated until four isolates were obtained. Cells comprising a portion of each isolated colony were grown briefly as described in the text, the DNA was extracted, and the orientation of the *pilA* promoter region was determined. Each panel represents the colonial phenotype of the progeny of the parental clone. The pink (gray) colonial phenotype of ORN126 on lactose-MacConkey plates in the upper panels is contrasted with the red (black) and white phenotypes of strain ORN125 in the lower panels. The position of the *pilA* promoter for each of the eight populations shown is presented in Fig. 4.

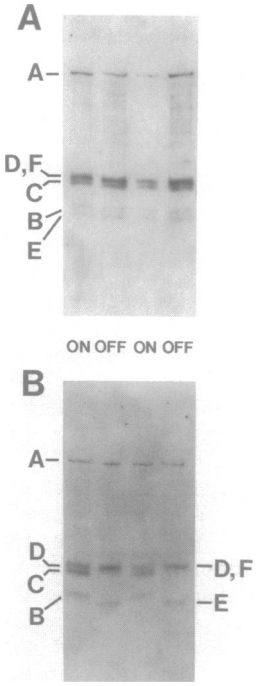

FIG. 4. Southern blot analysis of sequential isolates of strains ORN126 (*pilG*) and ORN125 (*pilG*$^+$). (A) Banding pattern of *Taq*I-digested DNA from random, sequentially isolated populations of strain ORN126 probed with the *Hpa*I fragment from pORN119 (Fig. 1). Each lane is labeled ON or OFF to denote the phenotype of the wild-type ORN125 (shown in the lower panel). Bands in each lane are labeled with a letter to denote a particular restriction endonuclease fragment shown in Fig. 1. (B) Banding pattern of *Taq*I-digested DNA from sequentially isolated ON and OFF populations of strain ORN125 probed with the *Hpa*I fragment from pORN119 (Fig. 1). Bands in each lane are labeled with a letter corresponding to a particular restriction endonuclease fragment shown in Fig. 1.

mutant *pilG* allele and also should facilitate further characterization of the *pilG* locus.

Both the physical and genetic evidence that we have gathered suggests that the *pilG* mutation produces clones consisting of both ON and OFF individuals, rather than each individual's having a stable level of *pilA* expression. This was made evident through the use of probe DNA, which showed that clonal populations of *pilG* mutants have both orientations of the *pilA* promoter, and through the use of genetic transduction, which showed that each *pilG* mutant clone contained approximately equal numbers of individuals capable of donating *pilA* in the ON or the OFF mode.

To account for the PilG$^-$ phenotype, we suggest that the *pilG* locus encodes an inhibitor of *pilA* promoter inversion. Thus, *pilG* mutants carry out the inversion at a much higher rate than the wild type, resulting in clones (colonies) that are perpetually mixed. Although attempts to quantitate the proposed increase in the rate of *pilA* promoter inversion in *pilG* mutants have not been successful, available experimental evidence suggests that our lack of success may be due to the rapidity with which the populations reach an equilibrium mixture of ON and OFF individuals (unpublished observations).

An unexpected feature of *pilG* mutants was that the PilG$^-$ phenotype could be suppressed by an insertion mutation in *hyp*, a gene that encodes a repressor of *pilA* transcription (15, 18). That is, *pilG hyp* double mutants exhibited wild-type phenotypic and genotypic variation in *pilA* expression, except that because of the loss of repressor activity, the level of *pilA* transcription was higher in these double mutants in the ON phase (18). Interestingly, the *hyp* gene is not required for inversion of the *pilA* promoter, and initial observations suggested no effect of the loss of *hyp* on the frequency of inversion (18). However, we have recently determined that *hyp* mutants have about a 10-fold-higher rate of switching in the ON to OFF direction. (Our initial observations failed to take into account that very few hyperexpressing individuals in a predominantly OFF clone [colony] will make the colony appear phenotypically ON.) Also, P. Klemm (8a), using a much different experimental system, has obtained results that suggest that inversion frequency is affected by the *hyp* (*fimE*) gene. We conclude from the foregoing that both *hyp* and *pilG* may play some role in the metastable inversion of the *pilA* promoter, even though neither gene is required for inversion.

The *pilG* locus appears to be distinct from a locus described by Freitag et al. (5) that is required for phase variation. This is because the *pilG* locus confers a much different phenotype and is located at a different site on the *E. coli* chromosome. Also, *pilG* appears to be distinct from *flu*, a metastable gene controlling cell surface properties (3). Again, the phenotypic properties and map position of the two loci are quite distinct.

Our finding that mutants can be isolated that are phenotypically stable yet undergo genotypic phase variation underscores the importance of distinguishing between mutants that do not undergo phase variation and those that do not exhibit it. Also, the characterization of loci such as *pilG* may help explain the widely different basal rates of phase switching within a given metastable system exhibited by various strains of *E. coli* and *Salmonella typhimurium*.

TABLE 4. P1 transduction of the *pilA-kan-lacZYA* region from ORN125 (*pilG*$^+$) and ORN126 (*pilG*) clones to ORN105 (*pilA*$^+$ *pilG*$^+$)[a]

Donor strain[b]	Colonial phenotype of donor strain	No. of Kan^r transductants of ORN105 scored as:		% Lac$^+$ transductants
		Lac$^+$	Lac$^-$	
ORN125	ON	126	7	95
ORN125	OFF	18	169	10
ORN126	Intermediate	40	33	54
ORN126	Intermediate	42	45	48

[a] Individual colonies of the donor strains were picked, grown briefly in L-broth, and infected with P1. Transducing lysates from each of the donor strains were used to infect ORN105. ORN105 transductants having received the *pilA-kan-lacZYA* region were selected on lactose-MacConkey agar plates containing kanamycin, and the Lac phenotype of the colonies was scored.

[b] A stock culture of strain ORN125 was streaked on lactose-MacConkey agar, and a Lac$^+$ and a Lac$^-$ colony were picked to represent the ON and OFF phenotype. Two well-isolated colonies of strain ORN126 were used as representatives of the intermediate phenotype.

ACKNOWLEDGMENTS

We thank Barbara Bachmann for strains and R. Isberg for a critical reading of the manuscript.

This work was supported by North Carolina fund project number 197227 and by Public Health Service grant 1RO1 AI-22223-01 from the National Institute of Allergy and Infectious Diseases.

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