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Rapid Site-Specific DNA Inversion in *Escherichia coli* Mutants Lacking the Histonelike Protein H-NS

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Escherichia coli pilG **mutants are thought to have a dramatically higher DNA inversion rate as measured by the site-specific DNA inversion of the type 1 pili *pilA* promoter. DNA sequence of the *pilG* gene confirmed its identity to the gene encoding the bacterial histonelike protein H-NS. Unlike other histonelike protein complexes that enhance site-specific DNA recombination, the H-NS protein inhibited this process. This inhibition was indicated by the increased inversion rate of the *pilA* promoter region effected by two different mutant *pilG* alleles. One of these alleles, *pilG1*, conferred a mutant phenotype only at low temperature attributable to a T-to-G transversion in the -35 sequence of the *pilG* promoter. The other allele, *pilG2-tetR*, was an insertion mutation in the *pilG* coding region that conferred the mutant phenotype independent of temperature. We measured an approximately 100-fold-increased *pilA* promoter inversion rate in the mutant by exploiting the temperature-dependent expression of *pilG1* and using a novel rapid-population-sampling method. Contrary to one current view on how the H-NS protein might act to increase DNA inversion rate, we found no evidence to support the hypothesis that DNA supercoiling affected *pilA* promoter inversion.**

A number of site-specific recombination systems in procaryotes have been described in which the inversion of a DNA segment is responsible for alternating expression of one or more genes. The classic example of this type of gene regulation is the Hin-mediated H-segment inversion in *Salmonella typhimurium* dictating the antigenic type of flagellin expressed (42). Other Hin-related inversion systems have been described, including Gin-mediated G-loop inversion in Mu (18) and Cin-mediated C-loop inversion in bacteriophage P1 (20). The DNA inversion in these two systems determines expression of alternate tail fiber antigens, thereby dictating the host range of these phage. The Hin, Gin, and Cin invertases that promote the site-specific DNA recombination in these systems are functionally similar. Each of these invertases will effectively promote the inversion of the H, G, and C segments under appropriate conditions (20, 23, 28).

Type 1 pilus expression in *Escherichia coli* is regulated by the inversion of a 314-bp DNA segment containing the promoter for *pilA* (*fimA*), the gene encoding the type 1 pilin monomer (1, 24, 43). When the *pilA* promoter segment is in one orientation (ON), *pilA* is transcribed and the *E. coli* cells are type 1 piliated. With the promoter in the other orientation (OFF), there is no *pilA* transcription and the cells are nonpiliated. Though the mechanism of *pilA* promoter inversion is not completely understood, it is clear that this process is significantly different from the Hin-related inversion systems. The 9-bp inverted repeats flanking the invertible *pilA* promoter segment share no homology with the sequences that bind the Hin-related invertases (24). Also, the Hin protein does not promote *pilA* promoter inversion (12). The *fimB* and *pilH* (*fimE*, *hyp*) genes encode proteins reported to mediate *pilA* promoter inversion (24, 40, 43). Interestingly, these two proteins have significant amino acid homology with the portion of phage lambda integrases possessing recombination activity (2, 10).

Although each of these described inversion systems employs one or more specific enzymes for inversion, several of the systems have been shown to require a histonelike protein complex for efficient activity: HU and Fis are required for efficient H-segment inversion in *S. typhimurium* (21) and G-segment inversion in phage Mu (26, 45). Fis alone appears sufficient to enhance the C segment inversion in P1 phage (15). There are two potential binding sites for the histonelike protein complex integration host factor (IHF) flanking the left inverted repeat of the *pilA* promoter segment (10). Though the mechanism of action is unknown, it was subsequently demonstrated that IHF complex is required for efficient site-specific recombination of the invertible type 1 pilus *pilA* promoter segment (8, 10).

We recently described a mutant that exhibited a phenotype consistent with increased *pilA* promoter inversion frequency (43). In this mutant, we could isolate only populations of cells containing approximately equal mixtures of *pilA* promoter ON and OFF orientations (43). The lesion producing this phenotype defined a locus, termed *pilG*, which mapped to approximately 27 min on the *E. coli* chromosome. Thus, the locus was unlinked to the type 1 pilus gene cluster located at approximately 98 min. Subsequently, other investigators have noted that a number of lesions influencing the transcription of unlinked genes in *E. coli* and *Shigella flexneri* (7, 32) map to the vicinity of *pilG*. In *E. coli*, these genes include *bglY* (29), *osmZ* (16), and *drdX* (13).

Mutations in the *bglY* gene result in two described phenotypes. The first phenotype is activating transcription of the normally cryptic *bglCBS* operon (6). The second is increased generation of chromosomal deletions (29). The *osmZ* gene, which is allelic to *pilG* (16, 19), is involved in the transcriptional regulation of genes that respond to osmotic shock (16). More specifically, mutants in *osmZ* result in constitutive transcription of *proU*, a gene normally expressed only under conditions of high osmolarity. The suggested mechanism for

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TABLE 1. Bacteria, bacteriophage, and plasmids

Bacterium, bacteriophage, or plasmid	Description	Source or reference
Bacteria		
JC7623	*thr leu proA2 lacY1 galK his argE rpsL supE mtl syl recBC sbcB* Pil$^-$	17
MC1000	*araD139* Δ(*araABOIC leu*)*7697* Δ(*lacIPOZY*)X74 *galU galK rpsL thi*	5
JTT1	*pyrF gal-25 nirA strA195*	44
RS2	Same as JTT1 except *top-10*	44
LE392	*supR supE hsdR galK trpR metB lacY tonA*	Laboratory collection
ORN105	*thr leu proA2* Δ(*argF-lac*)U169 *galK his argE rpsL supE mtl xyl recBC sbcB* (has Tn*10* between *hsd* and *serB*)	38
ORN114	MC1000 *pilH*::Tn*5*-132	38
ORN116	*thr-1 leuB thi-1* Δ(*argF-lac*)U169 *malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA2 supE44* Φ(*pilA'-lacZYA-kan*) *pilG*	38
ORN131[a]	*thr-1 leuB thi-1* Δ(*argF-lac*)U169 *malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA2 trpA62 supE44* Φ(*pilA'-lacZYA-kan*) *zcg*::Tn*10*	43
ORN132[a]	Same as ORN131 except *pilG1*	43
ORN154	*thr leu proA2* Δ(*argF-lac*)U169 *galK his argE rpsL supE mtl xyl recBC sbcB pilC*::Tn*5*	P1 transduction from ORN106 to ORN105 (33)
ORN185	Same as ORN131 except Trp$^+$ Tet^s	P1 transduction from ORN105
ORN187	Same as ORN116 except *pilH*::Tn*5*-132	P1 transduction from ORN114 to ORN116
THK5	Same as ORN131 except *recA13*	—[b]
THK6	Same as ORN132 except *recA13*	—[b]
THK26	Same as JC7623 except *tetR* gene adjacent to *pilG*$^+$	Linear transformation of JC7623 with *Sal*I-cut pTHK111
THK28	Same as ORN154 except *pilG2-tetR*	See Results and Fig. 4
THK30	Same as ORN185 except *pilG2-tetR*	See Results and Fig. 4
THK31	Same as ORN116 except *tetR* adjacent to the *pilG*$^+$ allele	P1 transduction from THK26
THK32	Same as ORN116 except *tetR* adjacent to the *pilG1* allele	P1 transduction from THK26
Bacteriophages		
P1	*vir*	R. Isberg
Lambda	Kohara phage 250	27
Plasmids		
pBR322	Ap^r Tc^r ColE1	4
pSH2	Contains all genes of the *pil* region	37
pORN124[c]	Cm^r Sp^r mini-F	30
pTHK110	pBR322 *pilG*	See Results and Fig. 4
pTHK111	pTHK110 except *tetR* gene in the *Xho*I site	See Results and Fig. 4
pTHK113	pBR322 *pilG*	See Results and Fig. 4
pTHK114	pTHK113 except *pilG2-tetR*	See Results and Fig. 4
pTHK116	pBR322 *pilG1* (same as pTHK113 only carrying the *pilG1* mutant allele)	Derived by cloning ca. 2-kb *Eco*RI-*Sal*I fragments from THK6
pTHK117	pBR322 *pilG* (carries the *Stu*I-*Eco*RI fragment from pTHK113)	See Results and Fig. 4
pTHK119	pTHK117 except pORN124 replicon (single copy)	—[c]

[a] May also be *trpE6*.

[b] The *recA* allele was added to a Srl$^-$ derivative of ORN116 (*srl*::Tn*10*) that had been constructed by Bochner selection (3) to produce ORN116 (*srl* Tet^s). The region from *zcg*::Tn*10* through *trp* was then added to this strain by transduction from ORN131 or ORN132. Introduction of the wild-type *srl* allele adjacent to *recA13* allele completed the construction.

[c] pORN124 was made from pKP1013 by removing one of the two *Eco*RI sites so that pORN124 has a unique *Eco*RI site in the chloramphenicol resistance gene.

osmZ activity is altered DNA supercoiling, in that *osmZ* mutants exhibited increased plasmid linking number (16, 19). This phenomenon is the same as that seen in a *topA* mutant (defective in topoisomerase I activity). The *drdX* gene is involved in the temperature-dependent transcriptional regulation of the *pap* operon, which encodes products necessary for P piliation (13). This operon, normally transcribed at 37 but not 25°C, was transcribed at both temperatures in a *drdX* mutant. The DNA sequence of the *drdX* gene revealed that it was nearly identical to *hns* (13), the gene encoding the *E. coli* histonelike protein H-NS (41), and encoded a product of exactly the same predicted amino acid sequence as the H-NS protein. DNA sequence of the *S. typhimurium osmZ* gene revealed that it also encodes a protein that, except for seven amino acids, is the same as H-NS (19).

The described effects of mutations in the *bglY*, *osmZ*, and *drdX* genes involve altered transcriptional regulation and increased frequency of chromosomal deletions. The suggested mechanism of the pleiotropic effects observed in these mutants is through altered DNA topology (7, 16, 19). In this report, we present evidence that there is a dramatic increase in the *pilA* promoter inversion rate in *pilG* mutants, indicating that the *pilG* product inhibits *pilA* promoter inversion. This increased inversion rate is most likely due to the absence of the *pilG* product, the histonelike protein H-NS. Further, our results do not support the supposition that the mechanism by which H-NS influences *pilA* promoter inversion is via a generalized effect on DNA supercoiling.

MATERIALS AND METHODS

Bacterial and bacteriophage strains, plasmids, and media. The bacterial strains (all *E. coli* K-12 derivatives), bacteriophage strains, and plasmids used or isolated in this investi-

gation are listed in Table 1. Media consisted of L agar, L broth, and MacConkey agar (Difco Laboratories, Detroit, Mich.); antibiotics were added as previously described (36). Minimal medium was minA medium supplemented with amino acids as described by Miller (35).

Genetic techniques. Generalized transduction using P1 *vir* was carried out as described by Miller (35). P1 phage were also used to obtain the switching frequency of the *pilA* promotor region by briefly growing the phage on samples of cells. In this case, phage were grown by adding 10 μl of a P1 phage stock lysate (5×10^9 PFU/ml) to 100 μl of culture. Following a 20-min adsorption period, the suspension was diluted to 1 ml with prewarmed L broth (37°C) and grown, with shaking, for 1.5 to 2 h at 37°C. At the end of this time period, 2 drops of chloroform was added to the culture and the suspension was cleared by a brief centrifugation. The donor cells used to determine the *pilA* promoter inversion rate were maintained by serial dilution to obtain samples up to 300 generations following the initial inoculation. Lambda phage were grown in liquid culture as described by Maniatis et al. (31). Linear DNA was introduced into the *E. coli* chromosome by electroporation into strain ORN154 (*recBC sbcB*), using the procedure recommended by the manufacturer (Bio-Rad, Rockville Centre, N.Y.) for electroporating closed circular DNA into *E. coli*.

Recombinant DNA techniques. Conditions for restriction endonuclease digestion, agarose gel electrophoresis, isolation and ligation of DNA fragments, and Southern blotting were as described previously (36, 37). DNA sequencing of the wild-type and *pilG1* alleles was carried out by using the 1.8-kb *Eco*RI-*Sal*I fragments from pTHK113 and pTHK116 (see Results) that had been cloned into M13 mp18 and mp19. Dideoxy-chain termination sequencing was performed with Sequenase (U.S. Biochemical, Cleveland, Ohio), using the procedure suggested by the manufacturer.

Analysis of DNA supercoiling. Plasmids used to examine factors affecting DNA supercoiling were propagated in *pilG* mutant and parental strains. Bacteria were grown in L broth with shaking. Different growth times and temperatures were examined for their influence on supercoiling. The use of antibiotics to maintain the plasmids was scrupulously avoided. To analyze the degree of DNA supercoiling, the isolated plasmids were resolved electrophoretically in 1% agarose gels containing 2.5 μg of chloroquine per ml of Tris-borate-EDTA buffer (31). Following electrophoresis, the chloroquine was removed from the gel by using 10 mM $MgSO_4$ for 4 h, and then the gel was counterstained with ethidium bromide for 1 h.

RESULTS

Quantitation of the *pilA* promoter inversion rate. The *pilA* promoter inversion rate was calculated by using *E. coli* strains containing a *pilA'-lacZ-kan* fusion (Fig. 1). In the parental strain (ORN131) containing the wild-type *pilG* allele, this fusion results in red and white colonies on lactose MacConkey agar, depending on the *pilA* promoter orientation (38; Fig. 2). That is, *pilA* ON colonies are red (Lac^+), and *pilA* OFF colonies are white (Lac^-). Such colonies are generated because the inversion rate in the parental strain (approximately 10^{-4} per cell per generation) results in colony populations having, predominantly, the promoter orientation of the cell that started the colony. The frequency of Lac expression changes in strains containing this *pilA'-lacZ-kan* fusion is essentially the same as the frequency of type 1 pilus expression changes observed in identical genetic backgrounds (38). Consequently, Lac expression changes in *pilA'-LacZ-kan*-containing strains provides an accurate assay for *pilA* expression changes. Colonies formed by *E. coli* ORN132, identical to ORN131 except having the mutant *pilG1* allele, produce colonies that are uniformly pink when grown at 31°C on lactose MacConkey agar. These pink colonies consist of approximately equal numbers of ON and OFF individuals, as determined by Southern blot analysis of the *pilA* promoter orientation in this strain (43). However, this *pilG1* strain exhibits the wild-type phenotype (red and white colonies on lactose MacConkey) when grown at 42°C (Fig. 2). We refer to the conditional character of the *pilG1* lesion as cold sensitive (i.e., the mutant phenotype is exhibited at the lower temperature).

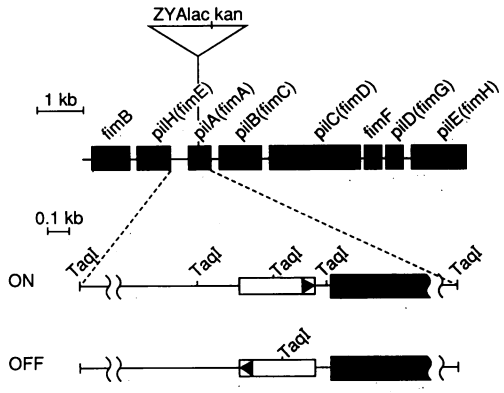

FIG. 1. Genes of the *pil* region at approximately 98 min on the *E. coli* genetic map. The expanded area shows the region of *pilA* promoter inversion. The *pilA* gene encodes the structural protein for type 1 pili and is shown containing a *lacZ-kan* transcriptional fusion. Inversion of the promoter (ON to OFF) results in an approximately 10-fold change in β-galactosidase activity, which is easily detectable phenotypically on lactose MacConkey agar plates (38) and physically by using the asymmetric *Taq*I restriction endonuclease sites. The *fimB* and *pilH* (*fimE*) genes are involved in transcriptional regulation of *pilA* and promoter inversion (24, 37). The remaining genes are involved either in pilus assembly, proper pilus morphology, or receptor binding (25, 33). The genetic mnemonics of Klemm and Christiansen (25) and Orndorff et al. (38) are used to denote the genes.

It was hypothesized that the pink colonial phenotype in the *pilG1* strain at 31°C was due to an increased inversion frequency of the *pilA* promoter region; however, the DNA inversion frequency in the *pilG1* strain was too great to measure by standard mutation rate analysis (43). Therefore, P1 phage were used to capture the *pilA* promoter region at different time points during the growth of the *pilG1* mutant at 31°C (Fig. 3). The orientations of the captured promoter regions were then determined. Initially, several red or white colonies were picked from strains ORN131 and ORN132 which had been grown on lactose MacConkey agar plates at 42°C. The colonies were resuspended in broth, and the suspensions were shifted to 31°C. P1 phage were grown briefly (1 to 2 h) on an initial sample (time 0) and on subsequent samples over the next 300 to 600 generations. The resulting P1 lysates were used to transduce *E. coli* ORN105 (*pilG*$^+$ *pilA*$^+$ *lacU*169 Kan^s) (38) to kanamycin resistance. Transductants were selected on lactose MacConkey agar containing kanamycin and scored for their Lac phenotype. The colony phenotype (Lac^+ or Lac^-) of a given recipient (now having the *pilA'-lacZ-kan* fusion) had a greater than 90% chance of displaying the *pilA* promoter

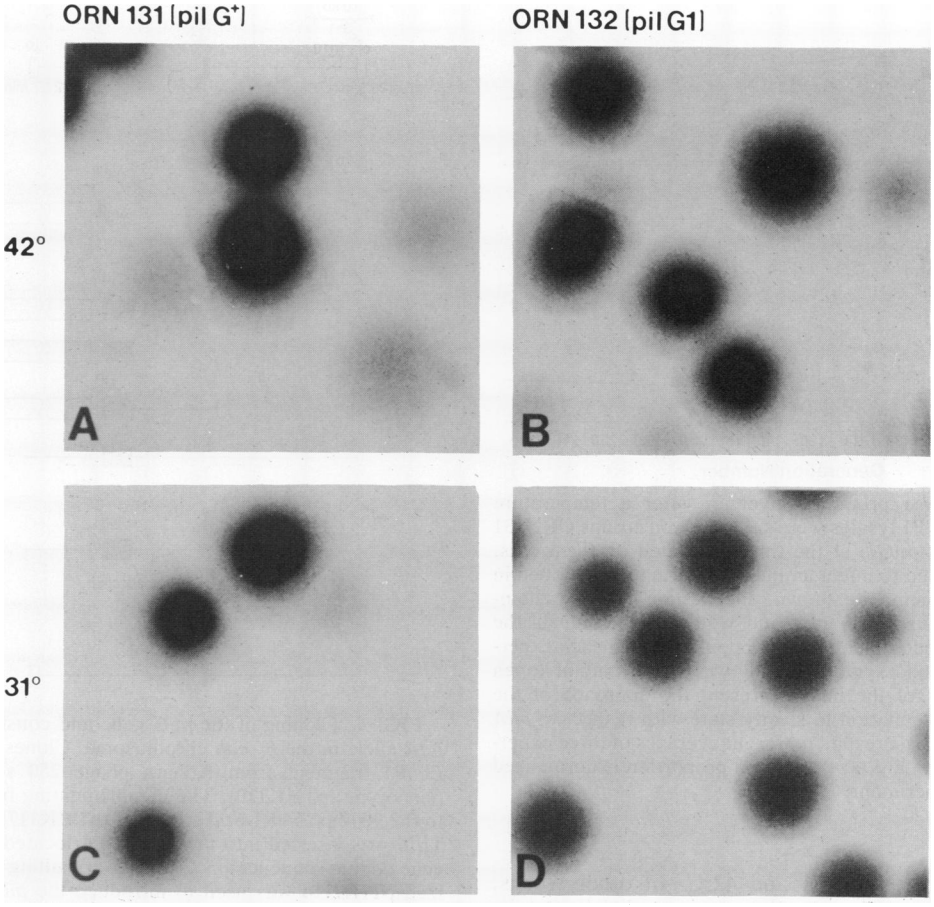

FIG. 2. Lac colonial phenotype of the wild-type ORN131 strain and the temperature-dependent nature of the *pilG1* mutant phenotype displayed by strain ORN132. The wild-type strain differed little in its inversion rate with temperature as denoted by the red (black) and white (grey) colonies on lactose MacConkey agar at 31 and 42°C (A and C). However, the mutant, while displaying a near-wild-type DNA inversion rate at 42°C, exhibited rapid inversion at 31°C, producing the characteristic uniform pink (dark grey) colony phenotype (B and D).

region orientation of the donor at the time it was packaged in the P1 phage (43). Thus, the proportion of Lac^+ and Lac^- transductants mirrored the proportion of Lac^+ and Lac^- individuals in the mutant population at the time the P1 phage were grown on them. Since *pilA* promoter inversion occurs at a low frequency in the $pilG^+$ background, inversions that occurred following transduction of the *pilA'-lacZ-kan* allele would not contribute significantly to the distribution of Lac^+ and Lac^- phenotypes observed in the recipient population. By comparing the inversion rates of mutant and wild-type strains (ORN131 and ORN132), we found that the promoter inversion rate was, on average, 100 times greater in the *pilG1* mutant (Table 2). Stated another way, *pilA* promoter orientation reached steady state within 20 generations in the *pilG1* mutant population. However, even after 300 generations, the *pilA* promoter orientation had not achieved steady state in the wild-type *pilG* population.

Isolation of the wild-type *pilG* allele and construction of a strain carrying a *pilG2-tetR* insertion. By using earlier *pilG* mapping and complementation data (43), the wild-type *pilG* allele was cloned from the *E. coli* genomic library generated by Kohara et al. (27) (Fig. 4). Fragments of *Eco*RI-digested Kohara phage 250 were ligated into the *Eco*RI site of pBR322. Clones containing the 10-kb *Eco*RI fragment from the phage insert, termed pTHK110, suppressed the mutant *pilG1* phenotype in strain THK6 (*pilG1*). That is, THK6 containing pTHK110 generated both red (Lac^+) and white (Lac^-) colonies when grown at 31°C on lactose MacConkey agar, whereas THK6 alone generated only pink colonies under the same conditions. The mutant *pilG1* phenotype was also suppressed by this 10-kb fragment on a single copy vector, pORN124 (pTHK119; Table 1). Deletion and insertion analysis aided in reducing the size of the complementing fragment to the 900-bp *Stu*I-*Eco*RI fragment (pTHK117) (Fig. 4). The *tetR* gene from Tn*10* (22) was introduced into the *Hpa*I site of pTHK113, resulting in pTHK114 (Fig. 4). The pTHK114 clone did not suppress the mutant *pilG1* phenotype. Subsequent DNA sequence analysis confirmed that the *tetR* gene was inserted into the *pilG* coding region in pTHK114. Linearized pTHK114 was used to introduce the insertion mutation into the chromosome of ORN154 (*recBC sbcB*), creating THK28 (Fig. 4). This insertion allele, *pilG2-tetR*, was moved via P1 transduction into a strain containing the *pilA'-lacZ-kan* fusion (ORN185), thus creating strain THK30 (Fig. 4). Both genetic and physical measurements supported the uncomplicated introduction of the *pilG2-tetR* allele as shown. Strain THK30, with the *pilG2-tetR* lesion in the chromosome, exhibited the same phenotype with respect to *pilA* promoter inversion frequency as the mutant carrying the *pilG1* allele at 31°C except that the rapid promoter

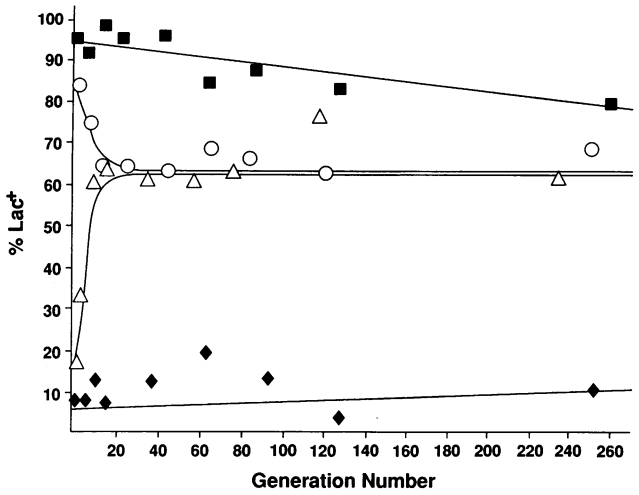

FIG. 3. Rate of *pilA* promoter inversion after a temperature switch to 31°C. Rapid P1 lysates were generated on strains ORN131 (*pilG*$^+$) and ORN132 (*pilG1*) at the times indicated on the *x* axis. These lysates were used to infect an indicator strain as described in the text. Two experiments are shown, one starting with a predominantly Lac$^+$ population of mutant and wild-type individuals the other starting with predominantly Lac$^-$ populations. In each experiment, a wild-type (solid symbols) is shown with a mutant (open symbols). The slopes of the lines represent the approach of the temperature-shifted population to steady state with respect to *pilA* promoter orientation. The results represent averages of three experiments in which the initial Lac expression proportion is normalized to that of a single experiment.

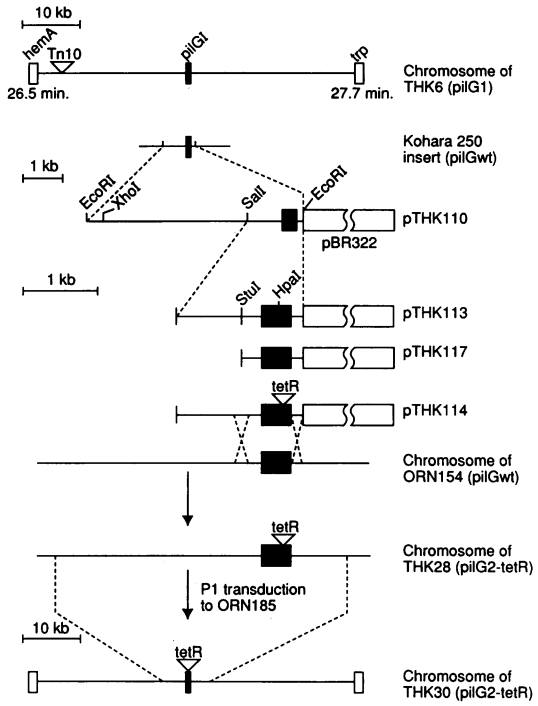

FIG. 4. Cloning of the *pilG* gene and construction of the *pilG2-tetR* allele in the *E. coli* chromosome. Clones containing the 10-kb *Eco*RI fragment from Kohara phage 250 suppressed the *pilG1* phenotype (pTHK110). The complementing fragment was reduced to the 900-bp *Eco*RI-*Stu*I fragment (pTHK117). The *tetR* gene from Tn*10* was inserted into the *Hpa*I site (located 110 bp into the *pilG* gene coding sequence) in pTHK113, resulting in pTHK114. Linearized pTHK114 was used to introduce the *pilG* insertion mutation into the chromosome of ORN154 creating strain THK28. This mutant allele was transduced into a strain containing the *pilA'-lacZ-kan* fusion, creating strain THK30. The *pilG* gene is transcribed from right to left.

inversion occurred at both 31 and 42°C. In other words, transduction of the *pilG2-tetR* allele into a *pilA'-lacZ-kan*-containing strain resulted in pink colonies regardless of growth temperature.

Defect in the cold-sensitive *pilG1* allele. The mutant *pilG1* allele was isolated from strain THK6 (Fig. 4) by cloning 1.8-kb size-fractioned *Eco*RI-*Sal*I-digested chromosomal DNA into pBR322 and scoring for hybridization with the parental *pilG* allele. Both the *pilG* and *pilG1* genes were sequenced by the dideoxy-chain termination method. Sequencing confirmed the identity of the wild-type *pilG* allele with *hns* (41), *drdX* (13), *E. coli* GM301 *osmZ* (34), and *S. typhimurium osmZ* (19). As with *drdX* and *E. coli osmZ*, the *pilG* coding sequence contained a T instead of a C at position 969 and an A instead of a G at position 1032 with respect to the published *hns* sequence (41 [GenBank accession number X07688]). These base transitions do not change the amino acid sequence predicted from the *hns* open reading frame. The *pilG* gene does not contain the two additional single base differences noted in the *E. coli osmZ* gene that are located between the *hns* promoter and open reading frame (34). The *pilG1* allele was identical to the wild-type *pilG* gene with the exception of a T-to-G transversion in the consensus -35 promoter sequence (position 792 in the *hns* gene sequence). Interestingly, the *pilG1* allele contained on pBR322 (pTHK116) suppressed the *pilG1* phenotype at 31°C in strain THK6. This result suggested that the cold-sensitive phenotype could be eliminated by increasing the mutant allele copy number.

TABLE 2. Mutant (*pilG1*) and wild-type switching rates

Strain	Rate[a]	
	Lac$^+$→Lac$^-$	Lac$^-$→Lac$^+$
ORN131 (wild type)	6.3×10^{-4}	1.5×10^{-4}
ORN132 (*pilG1*)	1.5×10^{-2}	3.2×10^{-2}

[a] Calculated from the slopes of the lines shown in Fig. 3 and expressed as switches per cell per generation.

Effect of *pilG* lesions on DNA supercoiling. Because of the allelic nature of *osmZ* with *pilG* (19, 34) and the described effects on DNA supercoiling attributed to *osmZ* (7, 16, 19), we investigated the possible correlation of rapid inversion rate to the level of DNA supercoiling. Reporter plasmid pSH2 (36) was introduced into strains containing different *pilG* alleles. This plasmid contained the type 1 pilus gene cluster including the invertible element so that any specific affinity that the *pilG* product may have had with this DNA segment could have been noted. Neither of the mutant *pilG* alleles had any effect on the reporter plasmid linking number when compared with the wild type (Fig. 5). This observation was the same regardless of growth temperature or reporter plasmid used in the assay (i.e., pBR322 and pACYC184 as well as pSH2 were tested). It was inferred that *pilG* lesions that increased promoter inversion rate did not exert a global effect on DNA supercoiling. Conversely, a *topA* lesion, which dramatically affected DNA supercoiling (Fig. 5), produced no discernible change in inversion frequency as

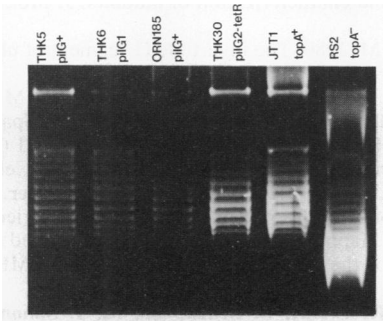

FIG. 5. Effects of the *pilG1* and *pilG2-tetR* alleles on DNA supercoiling. Plasmid pSH2, containing the genes encoding type 1 pili and the invertible *pilA* promoter, was propagated at 31°C overnight in strains isogenic except for the *pilG* allele and in strains JTT1 and RS2, which are isogenic except for a lesion in *topA* (44). Plasmid DNA isolated from the indicated strains was electrophoresed in a 1% agarose gel containing 2.5 μg chloroquine per ml of buffer. The results demonstrate that plasmid isolated from RS2 (*topA*) was more tightly supercoiled and therefore migrated faster than the more relaxed plasmid isolated from strain JTT1. Note that the degree of supercoiling is the same for plasmids isolated from THK5 and THK6 (isogenic except that THK6 is *pilG1*) as well as for plasmids isolated from ORN185 and THK30 (isogenic except that THK30 is *pilG2-tetR*).

measured by Lac expression changes (data not shown) when *pilA'-lacZ-kan* fusions were introduced into otherwise isogenic *topA* mutant and wild-type strains (RS2 and JTT1) (44) via P1 transduction.

DISCUSSION

We have described the properties of two *pilG* mutant alleles, *pilG1* and *pilG2-tetR*, which greatly increased the site-specific inversion rate of the 314-bp *pilA* promoter region in *E. coli*. DNA sequence analysis demonstrated that the *pilG* gene encoded the previously described DNA-binding protein H-NS (41).

The increased *pilA* promotor inversion rate in the *pilG1* mutant was unprecedented. The mutant population reached a steady state with respect to promoter orientation (i.e., equal numbers of ON and OFF individuals) within approximately 20 generations. In contrast, steady state in the wild type was not achieved even after 300 generations. These results correspond with the uniform colonial appearance of the mutants grown on lactose MacConkey agar. Since the colonial population should be at steady state before 1% of the final colony population was achieved, the population would be essentially an equal mixture of *pilA* ON and *pilA* OFF individuals before the colonies were visible. Thus, the uniform colonial appearance of *pilG* mutants belies the hyperinstability of each member of the colony.

The cold-sensitive phenotype produced by the *pilG1* allele was instrumental in allowing promoter inversion rate measurements. Implicit in the use of this temperature-dependent phenotype was the assumption that little or no product of the *pilG1* allele was produced at 31°C. The only difference between the wild-type *pilG* and mutant *pilG1* genes was a T-to-G transversion in the -35 region of the *pilG1* consensus promoter sequence. It is possible that the *pilG1* promoter is less efficient at binding RNA polymerase at reduced temperature, resulting in lower cellular levels of H-NS at 31 than at 42°C. This hypothesis is consistent with our observation that the *pilG2-tetR* insertion mutation, causing either no H-NS expression or expression of nonfunctional H-NS, resulted in rapid *pilA* promoter inversion independent of growth temperature. Also, apparently the effect of the *pilG1* promoter mutation could be alleviated by increasing the copy number of the *pilG1* gene. This increased copy number could, in theory, result in restoring near-normal levels of H-NS in the cell. To our knowledge, this is the first reported situation in which a cold-sensitive phenotype was correlated with a lesion 5′ to the coding region.

The mechanism by which the absence of the *pilG* product (H-NS) increases the inversion rate is not clear. H-NS shares many properties with eucaryotic histone proteins (11, 14, 39). These properties include binding to double-stranded DNA more avidly than to single-stranded DNA, increasing DNA thermal stability (11), protecting DNA from nuclease digestion (39), and inhibiting transcription (14). Other members of this class of bacterial histonelike proteins have been shown to be required for efficient site-specific DNA recombination to occur in a number of well-described systems. Included in this group of site-specific recombination systems is the type 1 pilus *pilA* promoter region that requires the histonelike protein complex IHF for efficient promoter inversion to take place (8, 10). In contrast to the requirement of IHF for *pilA* promoter inversion, we have shown that H-NS effectively acts to inhibit this same promoter inversion.

Work by others (16, 19) indicated that *pilG* encoded a product that influenced DNA supercoiling and suggested that this property ultimately regulated inversion. However, we found no evidence that the *pilG1* and *pilG2-tetR* lesions described here resulted in global alterations in DNA supercoiling. Also, a *topA* lesion resulting in increased plasmid DNA supercoiling did not appreciably affect *pilA* promoter inversion frequency. The apparent discrepancy between the work presented here and that reported earlier (16, 19) might be explained by differences in mutant *pilG* and *osmZ* alleles or the presence of multiple allelic forms of the gene reported in certain strains (13) (our parental strain has a single copy of *pilG*).

Given the reported DNA-binding properties of H-NS, it is possible that this protein has localized effects on DNA strand fluidity (9, 29), perhaps inhibiting the formation of secondary DNA structures that form the substrate for proteins more specifically involved in *pilA* promoter inversion. It has also been established that H-NS effects the transcriptional regulation of a number of unlinked genes (7, 13, 16, 32). Consequently, it is equally possible that reduced levels of H-NS cause increased expression of the *fimB* and *pilH* (*fimE*) gene products which are responsible for promoting the *pilA* promoter inversion event (24, 40). Altered levels of these proteins might result in the rapid *pilA* promoter inversion observed in *pilG* mutants. Lesions in either the *pilH* or *fimB* gene suppress the rapid inversion in *pilG* mutants. The *pilA* promoter inverts at near-parental frequency in strains containing both the *pilG1* allele and an insertion mutation in *pilH* (43). Also, *pilG1* and *pilG2-tetR* mutants with an insertion mutation in *fimB* showed no *pilA* promoter inversion at all (our unpublished results). Consequently, despite some uncertainty of the molecular mechanism by which lesions in *pilH* and *fimB* suppress rapid inversion in the double mutants, it is evident that *pilG* mutants do not carry out the inversion by a mechanism that supersedes or otherwise bypasses the normal inversion process, but rather enhances the inversion rate.

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