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An AT-Rich Tract Containing an Integration Host Factor-Binding Domain and Two UP-Like Elements Enhances Transcription from the *pilEp*$_1$ Promoter of *Neisseria gonorrhoeae*

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The *pilE* gene of *Neisseria gonorrhoeae* is transcribed from a σ^{70} promoter (*pilEp*$_1$) with an AT-rich tract extending 65 nucleotides upstream of the −35 box. Within this region is an integration host factor (IHF)-binding core consensus sequence. We have performed a detailed analysis to determine which upstream sequences are required for efficient transcription from *pilEp*$_1$ in *N. gonorrhoeae*. Deletion of sequences upstream of the AT-rich tract had no effect on the level of transcription. However, the IHF-binding core consensus sequence and the AT-rich sequence further upstream were both required for enhanced levels of transcription from this promoter in both *N. gonorrhoeae* and an *Escherichia coli* strain producing IHF. In addition, an UP-like element positioned between the −35 box and the IHF-binding site was required for maximal transcription. The AT-rich region upstream of the IHF-binding core consensus sequence can also act as an UP-like element when appropriately repositioned upstream of the −35 box.

The production of type 4 pili has been described in over 15 species of gram-negative bacteria, most of which are potential human, animal, or plant pathogens. The presence of type 4 pili has been associated with colonization of eukaryotic cells and twitching motility (41), social gliding motility in *Myxococcus xanthus* (46), and conjugation associated with plasmid R64 (23).

Despite numerous reports documenting the identification of type 4 pilin gene homologs, only a few studies on the regulation of pilin synthesis have appeared in the literature. In *Pseudomonas aeruginosa*, the promoter responsible for transcription of the pilin subunit gene (*pilA*) is σ^{54} dependent (21), and expression is subject to regulation by a two-component system encoded by *pilR* and *pilS* (19). Homologs of *pilR* and *pilS* have been identified in *M. xanthus* in a contiguous cluster with the pilin subunit gene, *pilA* (46). However, in neither of these systems has the environmental signal detected by the sensor protein, PilS, been determined.

The production of bundle-forming pili (Bfp) by enteropathogenic strains of *Escherichia coli* is subject to transcriptional regulation involving environmental signals which are potentially relevant to the small intestine (31). However, in contrast to the situation in *P. aeruginosa* and *M. xanthus*, the *bfpA* gene (encoding the pilin subunit) is transcribed from a σ^{70}-dependent promoter with an upstream AT-rich tract. In addition, there is a requirement for an activator protein, BfpT, which is a member of the AraC family of transcriptional activators (42). Transcription of the type 4 pilin subunit gene (*tcpA*) in *Vibrio cholerae* is similarly subject to positive regulation by an AraC-type activator protein (ToxT) via a σ^{70}-dependent promoter with an upstream AT-rich region (5). As would be expected, in the absence of the appropriate activator protein, the level of expression of these pilin subunit genes is extremely low when cloned in laboratory strains of *E. coli*.

The obligate human pathogens *Neisseria gonorrhoeae* and *Neisseria meningitidis* are both dependent on the production of type 4 pili for the initial colonization of mucosal surfaces, a prerequisite for subsequent infection (22). The region upstream of the gonococcal pilin subunit gene, *pilE*, shares features with both the *P. aeruginosa pilA* and the *E. coli bfpA* genes. Three promoter consensus sequences have been identified upstream of *pilE* (11). *pilEp*$_1$ and *pilEp*$_2$ are σ^{70}-dependent promoters, and both are functional when the *pilE* gene is expressed in *E. coli*. The third promoter, *pilEp*$_3$, is σ^{54} dependent and overlaps *pilEp*$_1$. This promoter is functional in a *P. aeruginosa* background expressing PilR and PilS (7, 11). However, due to the fact that neither *N. gonorrhoeae* nor *N. meningitidis* has an intact *rpoN* gene, the *pilEp*$_3$ promoter is nonfunctional in these species (26). In fact, it is clear that in *N. gonorrhoeae* (at least when grown under standard laboratory conditions), *pilE* transcription requires only P1 (11).

Interestingly, as was observed in the cases of the *bfpA* and *tcpA* promoters, the sequences directly upstream of *pilEp*$_1$ in both neisserial species are highly AT rich. AT-rich sequences upstream of several *E. coli* and bacteriophage promoters have been shown to stimulate transcription from those promoters by RNA polymerase (RNAP) in the absence of any other protein factors (14, 32, 33). Such AT-rich sequences, known as UP elements, are generally located within the −40 to −60 region with respect to the transcription start point (TSP) and act as recognition sites for the RNAP α subunit (33). In particular, the C-terminal 85 amino acids of the α subunit (αCTD) make specific protein-DNA interactions with the UP element, resulting in factor-independent transcriptional activation (33). αCTD can also make protein-protein interactions with certain activator proteins in the absence of an UP element, thus enhancing transcription by increasing the efficiency of DNA binding by RNAP (6).

Recently, it was reported that gonococcal integration host factor (IHF) binds to a region upstream of the *pilEp*$_1$ promoter and is a transcriptional cofactor of *pilE* (18). This conclusion was based on the observations that purified gonococcal IHF subunits bind to sequences upstream of the *pilE* promoters and that deletion of 70 bp (from −60 to −130), including both the IHF-binding site and further upstream sequence, resulted in a

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TABLE 1. Oligonucleotides used in this study for the construction of deleted and mutated $pilEp_1$::*cat* reporter derivatives

No.	Construct	Sequence
1467	Δ −111	5′ CTTCCGGATCCCACACCCACC 3′
5828	Δ −90	5′ TACAAAATAAAAACAATTATATAG 3′
4856	Δ −82	5′ TAAAAACAATTATATAGAG 3′
4857	Δ −59	5′ CGCATAAAATTTCACCTC 3′
3997	Δ −52	5′ AACGGATCCAATTTCACCTC 3′
384	Δ −37	5′ CATAAAATGATCACGAAT 3′
6161	IHF mut	5′ ATAAAAAGTATTATACAGAGATAAA CGC 3′
6162	IHF mut	5′ GTTTATCTCTGTATAATACTTTTTATT TTG 3′
5935	UP mut	5′ GGACTGAGGTGAAACTCTATGCGTTT ATC 3′
5936	UP mut	5′ CGCATAGAGTTTCACCTCAGTCCATAA AATG 3′
5817	Δ −38−77	5′ CTAAAAGAAAATACAAAATAAAAACA TAAAATGATCACG 3′
5816	P_L-$pilEp_1$	5′ CAAAAAATAAATTCATATAAAAAACA TAAAATGATCACG 3′

10-fold reduction in *pilE*-specific mRNA. It was suggested that a potential role for IHF in this system was to stabilize a bend in the DNA so as to enhance the protein-protein interaction between RNAP and a putative activator protein.

In this study we have performed a detailed analysis to determine whether upstream sequences, in addition to the IHF-binding site, are required for efficient transcription from $pilEp_1$ in *N. gonorrhoeae* and *E. coli*. Results indicate that the IHF-binding core consensus and 5′-proximal AT-rich sequences are associated with significant transcriptional activation of this promoter and that sequences upstream of the AT-rich tract do not further enhance transcription. An UP-like element positioned between the −35 box and the IHF-binding domain was also found to be required for maximal $pilEp_1$ activity. An additional UP-like element was identified upstream of the IHF-binding core consensus sequence.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strain used in all cloning experiments was DH5α [F^- *endA1 thi-1 hsdR17 supE44 relA1* Δ*lacU169* (ϕ80 Δ*lacZ*M15)]. The *E. coli* strains SØ1718 and SØ1753 (an *ihfA*::Tn*10* derivative of SØ1718), used to assess the role of IHF in $pilEp_1$ transcription, were kindly donated by Per Klemm. The *N. gonorrhoeae* strain used was MS11-A (36). The $pilEp_1$::*cat* reporter plasmid used to generate the deletant reporter constructs was pJKD1304, a derivative of pJKD862 with the $pilEp_2$ and $pilEp_3$ promoters inactivated by site-directed mutamutagenesis (11).

Media and culture conditions. The growth conditions for *E. coli* and gonococcal strains have been described previously (11). Gonococcal transformations were performed as previously described (3). Chloramphenicol-resistant transformants were selected on GC agar plates containing either 7 or 8 μg of chloramphenicol ml^{-1}, depending on the expected chloramphenicol acetyltransferase (CAT) level.

Recombinant DNA techniques. DNA manipulations were performed according to standard procedures (34). DNA sequencing and synthesis of oligonucleotide primers were performed as described previously (11). The oligonucleotide primers used to generate the upstream deletion derivatives and fusion derivatives and for site-directed mutagenesis are shown in Table 1. For construction of the deletion and fusion derivatives, DNA fragments with the appropriate $pilEp_1$-containing regions were amplified by PCR, using the appropriate primer in conjunction with M13 reverse primer and with the plasmid pJKD1304 as a template. This required 30 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, followed by 1 cycle of 1 min at 94°C, 1 min at 50°C, and 5 min at 72°C in an FTS-1 thermal sequencer (Corbett Research). Site-directed mutagenesis by PCR was performed essentially as described previously (11). The amplified fragments were cloned into pUC18 digested with *Hinc*II, and the nucleotide sequences were determined for the regions upstream of *cat*. Promoter-containing *Ava*I/*Aoc*I fragments were subcloned into pJKD862, replacing the wild-type promoter-containing fragment. The $pilEp_1$::*cat* cassettes thus generated were subcloned on *Bam*HI fragments into the single *Bgl*II site of pJKD1854, a derivative of pJKD1250 (11) containing a 2.9-kb fragment internal to the gonococcal *iga* gene, with the *Sph*I/*Bam*HI fragment from pJKD1499 (11) as a source of the gonococcal transformation uptake signal. Transformation of *N. gonorrhoeae* MS11-A with linearized plasmid DNA resulted in recombination of the $pilEp_1$::*cat* reporter cassettes into the *iga* gene such that transcription of *cat* was in the direction opposite to transcription of *iga*. These recombinant plasmids were also transformed into *E. coli* strains SØ1718 and SØ1753.

Determination of CAT levels in bacterial cell extracts. Cell extracts of *E. coli* and gonococcal strains were prepared by the freeze-thaw method according to the manufacturer's instructions supplied with the CAT enzyme-linked immunosorbent assay kit (Boehringer Mannheim). *E. coli* cells were harvested from cultures grown for 16 h on L agar plates supplemented with ampicillin (50 mg/μl). Gonococcal cells were harvested from GC agar plates incubated for 20 h at 37°C in the presence of 5% CO_2. The determination of CAT levels in these extracts was performed as described previously (11).

RESULTS

***$pilEp_1$* transcription is enhanced by upstream sequences in *N. gonorrhoeae*.** We have shown previously that a $pilEp_1$::*cat* fusion (in which the P2 −10 and P3 −24 boxes were altered via site-directed mutagenesis), when recombined into the gonococcal chromosome, gave rise to levels of CAT similar to those of a reporter with all three wild-type promoter sequences (11). We were interested in determining whether transcription from $pilEp_1$ was dependent on additional sequences upstream of the poorly conserved −35 box (TAAAAT). As shown in Fig. 1A, the sequence upstream of the $pilEp_1$ TSP contains an 80% AT-rich tract extending from −30 to −100, within which a region protected by IHF has been mapped via DNase I footprinting (18). This protected region was originally proposed to contain two putative 13-bp IHF-binding core consensus sequences (domains 1 and 2) which partially overlap and are on opposite sides of the DNA helix. However, the sequence of domain 2 is closest to the *E. coli* consensus, WATCAANN NNTTR (9), and the DNase 1 footprint (18) is consistent with IHF binding preferentially to domain 2. Consequently, this is the sequence which is designated the putative IHF-binding core consensus sequence in this study, as shown in Fig. 1A. Deletions were generated from a $pilEp_1$::*cat* fusion to remove the sequence upstream of the AT-rich tract (Δ −111), the AT-rich sequence upstream of the primary IHF-protected domain (Δ −90), the sequence upstream of the putative IHF-binding core consensus sequence (Δ −82), all of the sequence protected by IHF (Δ −59), and additional sequence between this region and the poorly conserved $pilEp_1$ −35 box (Δ −37). Three base substitutions were also introduced at conserved positions within the putative IHF-binding core consensus sequence by site-directed mutagenesis (Fig. 1B). These substitutions were expected to have a severe effect on IHF binding, based on previous studies (14, 17), and the resulting construct was designated IHF mut. The new upstream regions generated by cloning the appropriate fragments into pUC18, and subsequently into pJKD1854, are shown in Fig. 1B.

The wild-type, deletant, and mutated reporter cassettes were recombined into the chromosomal *iga* gene of *N. gonorrhoeae* MS11-A. Cell extracts were prepared from the recombinant gonococcal strains, and CAT levels were determined. The results shown in Fig. 2 clearly indicated that deletion of the region upstream of the AT-rich tract (Δ −111) had no significant effect on $pilEp_1$ transcription as measured by CAT levels. However, deletion of a further 21 nucleotides (Δ −90) was associated with a fourfold reduction in transcriptional activity. Deletion of the entire AT-rich region upstream of the 13-bp core consensus sequence (Δ −82) resulted in a level of transcription similar to that obtained when the entire IHF-binding domain was deleted (Δ −59) or mutated (IHF mut). A further threefold reduction in CAT levels was observed upon replacement of the AT-rich sequence upstream of nucleotide −37

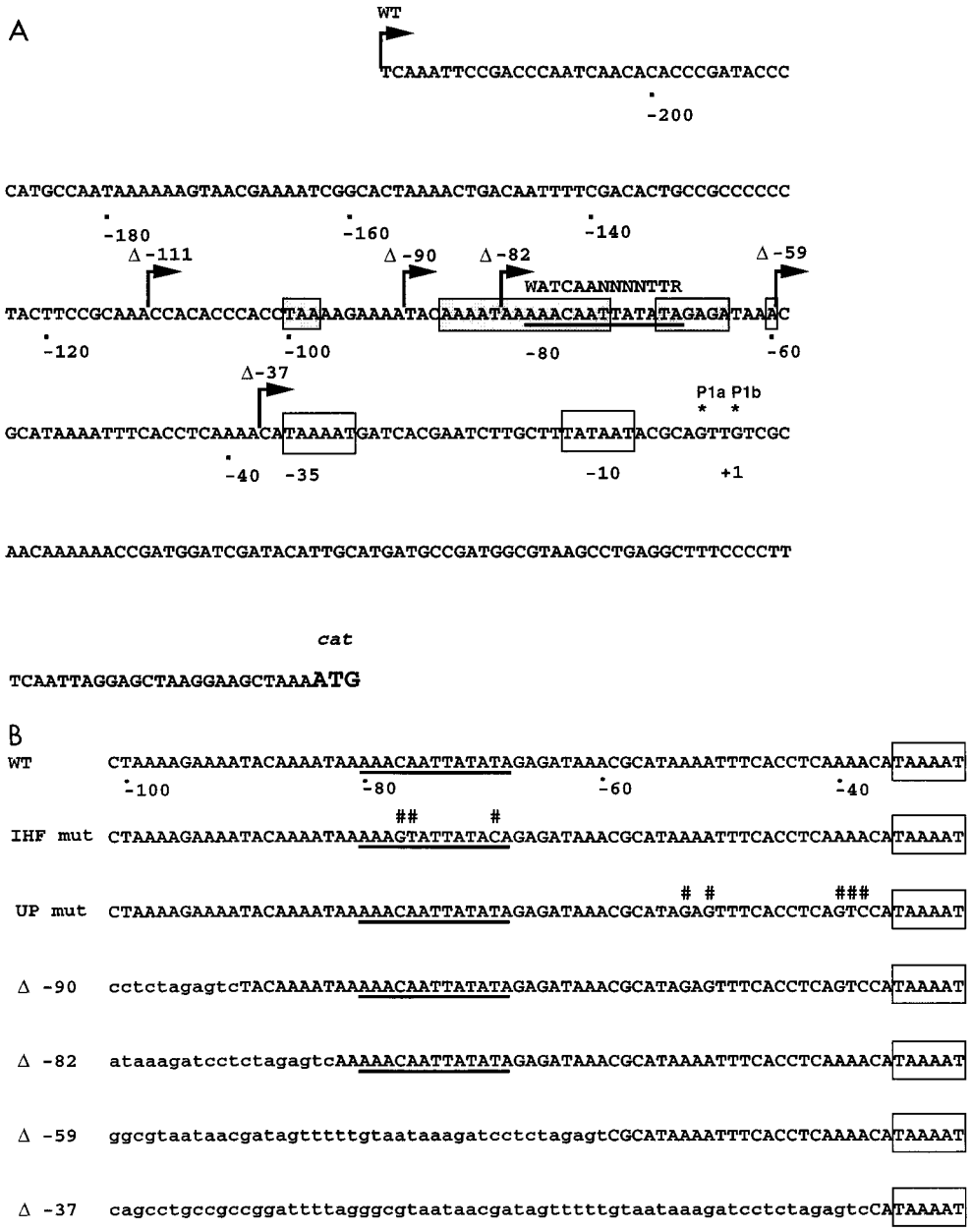

FIG. 1. Construction of deletant and mutant derivatives of a *pilEp*$_1$::*cat* reporter cassette. (A) Nucleotide sequence of the region containing the *pilEp*$_1$ promoter (open boxes) fused to the *cat* gene in the wild-type (WT) reporter cassette. The nucleotides contained within the shaded boxes are protected by IHF (18), with the solid underline indicating the putative 13-bp core consensus sequence previously designated domain 2 (18). The *E. coli* core consensus sequence (9) is shown above for comparison. Bent arrows indicate the sequences contained within each of the deletant derivatives (Δ −111, Δ −90, Δ −82, Δ −59, and Δ −37). Asterisks indicate the two TSPs (Pla and P1b) associated with *pilEp*$_1$ (11). The large ATG defines the start codon of *cat.* (B) The sequences from −30 to −101 for the WT *pilEp*$_1$::*cat* reporter, the mutated reporter derivatives (IHF mut and UP mut), and each of the deletant reporter constructs (Δ −90, Δ −82, Δ −59, and Δ −37) following subcloning into the unique *Bgl*II site within the *iga* gene fragment in pJKD1854. Nucleotides represented in lowercase letters are vector- or *iga*-derived. The putative IHF-binding core consensus sequences are underlined. #, a nucleotide substitution generated by site-directed mutagenesis. The −35 regions are boxed.

with a vector- or *iga*-derived sequence. A possible explanation for this observation is that the sequence between −37 and −59 functions as an UP element, enhancing the basal level of transcription from this promoter. Although no consensus sequence has yet been determined for an UP element in *E. coli*, the αCTD is thought to interact with DNA as a twofold symmetric dimer, recognizing two distinct regions within the UP element (12). Furthermore, DNA flexibility is thought to be crucial for the role of an UP element (28). Comparison of the −37 to −60 region upstream of *pilEp*$_1$ with several well-characterized UP elements (data not shown) suggested that one or both of the two poly(A) tracts centered at −39.5 and −52.5, which were disrupted in Δ −37 (Fig. 1B), may be important for transcriptional enhancement. Consequently, five base substitutions were introduced by site-directed mutagenesis (at positions −38, −39, −40, −51, and −53) to specifically disrupt the poly(A) tracts (Fig. 1B). The activity of *pilEp*$_1$ with this mutant upstream region substituted in the absence of the IHF-binding domain was comparable with that of Δ −37 (data not shown). In the presence of the IHF-binding domain (UP mut), pro-

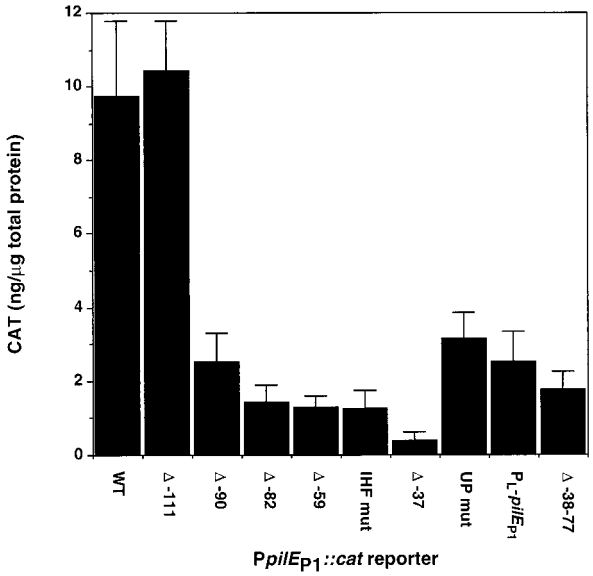

FIG. 2. Effect of upstream sequences on *pilEp*$_1$ transcription in *N. gonorrhoeae*. CAT levels were measured in cell extracts prepared as described in Materials and Methods from gonococcal reporter strains containing *pilEp*$_1$::*cat* cassettes with wild-type (WT) or substituted upstream sequences (explained in the legends to Fig. 1 and 4). The results shown are averages of at least four separate experiments, and error bars represent 1 standard deviation.

moter activity was shown to be threefold lower than that of the wild-type reporter (Fig. 2), confirming that the sequence located between the IHF-binding domain and the *pilEp*$_1$ −35 box is important for promoter activity in *N. gonorrhoeae*.

An intact *ihfA* gene is required for maximal transcription from *pilEp*$_1$ in *E. coli*. Our results are consistent with those of a previous study (18), where a 10-fold reduction in *pilE*-specific mRNA was observed when a 70-nucleotide deletion was made upstream of nucleotide −59. On the basis that this deletion removed the IHF-binding domain, it was concluded that IHF was required for optimal *pilE* transcription. Despite the fact that the gonococcal *ihfA* and *ihfB* genes (encoding the IHF α and β subunits) have been cloned, it has not been possible to isolate a mutant with a mutation in either of these genes (18a). Therefore, it was necessary to evaluate the role of IHF in the enhancement of *pilEp*$_1$ transcription in an *E. coli* background. To this end, pJKD1854-derived plasmids containing the wild-type *pilEp*$_1$::*cat*, deletant derivatives Δ −111, Δ −90, and Δ −59, and the IHF mut and UP mut reporters were transformed into the *E. coli* strains SØ1718 and SØ1753 (an *ihfA*::Tn*10* derivative of SØ1718). Cell extracts were prepared and assayed for CAT. The results (Fig. 3) indicated that the levels of *pilEp*$_1$ transcription obtained in the *ihfA* mutant background were three- to fourfold lower for the wild-type and Δ −111 reporters than the levels measured in the wild-type *E. coli* background. However, when the IHF-binding domain upstream of *pilEp*$_1$ was partially deleted (Δ −90) or completely deleted (Δ −59), or when the core consensus sequence was mutated (IHF mut), this difference was negated. It has been well documented that certain IHF-binding core consensus sequences require additional 5′-proximal bases, with a high AT content, for efficient binding of IHF to occur (17). The fact that CAT levels obtained for the Δ −90 reporter were significantly reduced relative to those of the wild-type and Δ −111 reporters in both *N. gonorrhoeae* and *E. coli* indicated that the additional AT-rich sequence upstream of the core consensus sequence is absolutely required for IHF to bind and/or enhance transcription from *pilEp*$_1$. The observation that deletion of sequences upstream of the IHF-protected domain (Δ −111) similarly had no effect on *pilEp*$_1$ transcription in either *N. gonorrhoeae* or *E. coli* indicated that the role of IHF in the enhancement of transcription from this promoter is unlikely to be associated with the binding of a neisseria-specific activator protein to sequences further upstream.

It also appears from the data presented in Fig. 3 that the UP-like element directly upstream of the *pilEp*$_1$ −35 box plays a significant role in the transcriptional enhancement of this promoter in an *E. coli* background. In fact, mutation of the UP-like element (UP mut) resulted in an eightfold reduction in promoter activity in the *ihfA*$^+$ background and a similar reduction in the *ihfA* mutant. This effect is apparently greater than that observed in *N. gonorrhoeae*, where mutation of the UP-like element resulted in only a threefold reduction in promoter activity. However, in a gonococcal background, deletion of the IHF-binding domain had a more deleterious effect on *pilEp*$_1$ transcription (eightfold reduction) than that observed in *E. coli* (three- to fourfold reduction). These observed differences in the relative roles of the IHF-binding site and UP-like element may be a reflection of the fact that, in *E. coli*, the reporters were present on multicopy plasmids, while they were integrated into the chromosome as single copies in *N. gonorrhoeae*, potentially resulting in differences in the levels of DNA supercoiling. However, in both cases the two elements appear to be functionally independent.

The AT-rich sequence upstream of the IHF-binding core consensus sequence is capable of enhancing *pilEp*$_1$ transcription. The AT-rich sequence 5′ to the IHF-binding core consensus sequence upstream of *pilEp*$_1$ is reminiscent of a sequence similarly located upstream of the bacteriophage λ early P$_L$ promoters (13, 14), as shown in Fig. 4. Transcription of λ P$_L$1 is activated by IHF, in the presence of an intact αCTD (13). In contrast to *pilEp*$_1$, the DNA between the −35 box and the IHF-binding site upstream of λ P$_L$1 is not particularly AT rich, and a role for this region as an UP element has not been

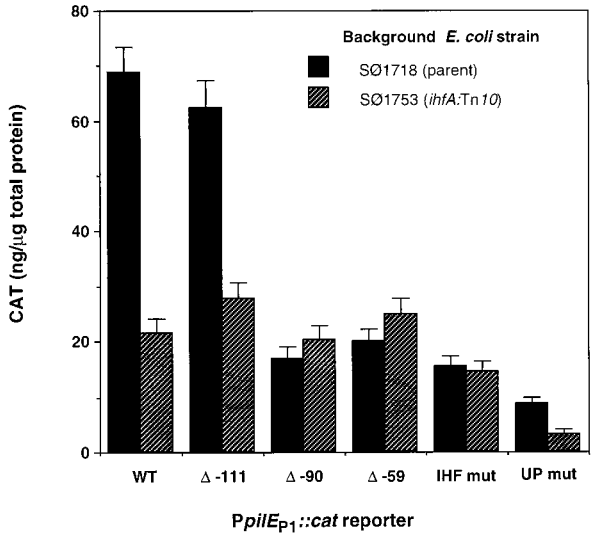

FIG. 3. Effect of an *ihfA* mutation on *pilEp*$_1$ transcription in *E. coli*. CAT levels were measured in cell extracts prepared as described in Materials and Methods from *E. coli* SØ1718 (parent strain) and SØ1753 (*ihfA*::Tn*10*) transformed with pJKD1854-derived plasmids containing *pilEp*$_1$::*cat* cassettes with and without upstream deletions or mutations (Fig. 1). The results shown are averages of at least four separate experiments, and error bars represent 1 standard deviation.

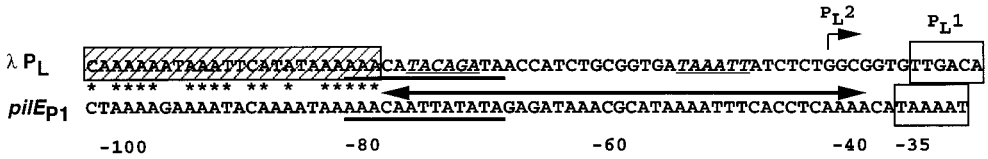

FIG. 4. Comparison of the sequence upstream of *pilEp*$_1$ with the sequence upstream of the λ P_L1 promoter (14). The −35 regions of *pilEp*$_1$ and λ P_L1 are contained within the open boxes. The −10 and −35 sequences associated with λ P_L2 are in italics and underlined, with the TSP denoted by the bent arrow. The nucleotides contained within the hatched box have been shown to function as an UP element through the binding of the α subunit of RNAP. Asterisks denote identical nucleotides shared between the *pilEp*$_1$ and λ P_L sequences within this region. IHF-binding core consensus sequences are underlined in boldface. Nucleotides below the double-headed arrow were deleted in the construction of the Δ −38−77 and P_L-*pilEp*$_1$ reporter derivatives, resulting in the relocation of either the gonococcal putative UP element or the λ P_L UP element directly upstream of nucleotide −37.

reported. However, an αCTD-binding site has been identified upstream of the IHF core consensus sequence, within the IHF-protected region. This region has been shown to function as an UP element for the λ P_L2 promoter (in the absence of IHF) and, when placed directly upstream, of λ P_L1 or P_{lac} (14). The role of this UP element, when located at the normal position (−79 to −102 with respect to the P_L1 TSP), in the IHF-mediated activation of P_L1 has not been confirmed. However, it has been suggested that the change in DNA conformation induced by IHF binding could increase the affinity of RNAP for P_L1 through direct contact between αCTD and this UP element (14).

Comparison of the sequences directly upstream of the *pilEp*$_1$ and λ P_L1 IHF-binding core consensus sequences reveals that 17 of the 24 nucleotides are identical (Fig. 4). Based on this similarity, a *pilEp*$_1$::*cat* reporter was constructed in which the sequence from −78 to −101 was fused by PCR, directly upstream of nucleotide −37, thus relocating the putative UP element immediately upstream of the *pilEp*$_1$ −35 box (Fig. 4). The effect of creating this fusion was the same as that of deleting the sequence from nucleotides −38 to −77, including the IHF-binding core consensus sequence. The *pilEp*$_1$::*cat* reporter thus generated was designated Δ −38−77. Similarly, the 21-nucleotide λ P_L UP element was fused upstream of *pilEp*$_1$ (P_L-*pilEp*$_1$), locating the sequence at the same position relative to the *pilEp*$_1$ −35 box as it is located upstream of λ P_L2 (14). The results of CAT enzyme-linked immunosorbent assays performed on gonococcal strains containing these reporters are shown in Fig. 2. It can be seen from this data that the λ P_L UP element can enhance transcription from *pilEp*$_1$ approximately sevenfold with respect to the basal level associated with Δ −37. The Δ −38−77 construct gave rise to levels of CAT comparable with those of Δ −82, Δ −59, and IHF mut constructs (each of which contain the wild-type −37 to −59 sequence), indicating that the sequence from −78 to −101 upstream of *pilEp*$_1$ is also able to enhance transcription from that promoter when relocated directly upstream of the −35 box, but to a lesser extent than the λ P_L UP element.

DISCUSSION

Previous work in this laboratory has clearly shown that, of the three promoter consensus sequences identified upstream of the *pilE* gene, the only one which is functional in *N. gonorrhoeae* during growth in vitro is the σ^{70}-dependent promoter *pilEp*$_1$ (11). Disruption of the *pilEp*$_1$ −10 box via site-directed mutagenesis resulted in insignificant levels of transcription from the *pilE* upstream region. A gonococcal P_n strain (with a deletion encompassing the promoter and 5′ end of the chromosomal *pilE* gene) containing *pilE* on a multicopy plasmid, with only *pilEp*$_2$ and *pilEp*$_3$ intact, did not express pili. On the other hand, a similar strain with *pilEp*$_1$ as the only intact promoter was highly piliated (our unpublished observations). Perhaps in an ancestor of the pathogenic neisseriae, transcription of the *pilE* gene was σ^{54} dependent, but it has since evolved to become σ^{70} dependent. It is unclear whether this was a consequence of the deletion in *rpoN* (25) and/or the gonococcal *pilR* and *pilS* homologs (8) or whether these occurred later. It is intriguing that both the σ^{54} promoter upstream of *pilE* and a sequence capable of functioning as an activator-binding site have been conserved in all gonococcal strains examined to date, and in some meningococcal strains, despite the fact that they are apparently no longer functional. However, this could be merely an indication of a relatively recent evolutionary event.

Prior to the demonstration that the pathogenic neisseriae contain an inactive *rpoN* gene, studies on the regulation of *pilE* were performed with the aim of identifying genes encoding putative transcriptional regulators (38). Using a *pilEp*::*cat* transcriptional fusion as a reporter, two divergently transcribed genes, designated *pilA* and *pilB* (not homologs of the *P. aeruginosa pilA* and *pilB* genes), were cloned in *E. coli* (38). PilA and PilB were reported to function as a two-component regulatory system controlling the transcription of *pilE* via the σ^{54} promoter (38–40), despite the fact that significant amino acid similarity was identified between PilA and FtsY (39). In *E. coli*, FtsY has been shown to be the functional homolog of the eukaryotic "docking protein" (26), the receptor for the signal recognition particle. The signal recognition particle is essential for the biogenesis of a subset of inner membrane proteins (37, 43). PilB, on the other hand, has recently been shown to function as a peptide methionine sulfoxide reductase (45). It is intriguing that purified PilA protein has been shown to bind to a DNA fragment containing the *pilE* promoter. Gel mobility shift assays performed on fragments containing the *pilE* promoters identified several regions necessary for this binding (2). In particular, a fragment lacking the sequence from −125 to −183 was no longer able to bind PilA. However, in this study, deletion of a sequence upstream of nucleotide −111 had no effect on transcription from *pilEp*$_1$, suggesting that this region does not contain an essential activator-binding site, and thus that PilA is not a transcriptional activator of *pilE*. Indeed, much of the data accumulated in relation to the putative role of PilA in gonococcal piliation and viability (24, 39, 40) can be explained on the basis that this protein is the gonococcal homolog of FtsY and therefore more likely to be involved in regulation at a posttranscriptional level.

The role of IHF in the transcriptional enhancement of *pilE* may originally have been architectural, i.e., in the bending of the DNA between the σ^{54}-dependent promoter and the activator-binding site so as to enhance the protein-protein interactions between the RNAP and the bound activator. Several examples of such an indirect role for IHF in the activation of σ^{54} promoters in *E. coli* and other bacterial species have been documented (16). There are also examples of IHF playing an

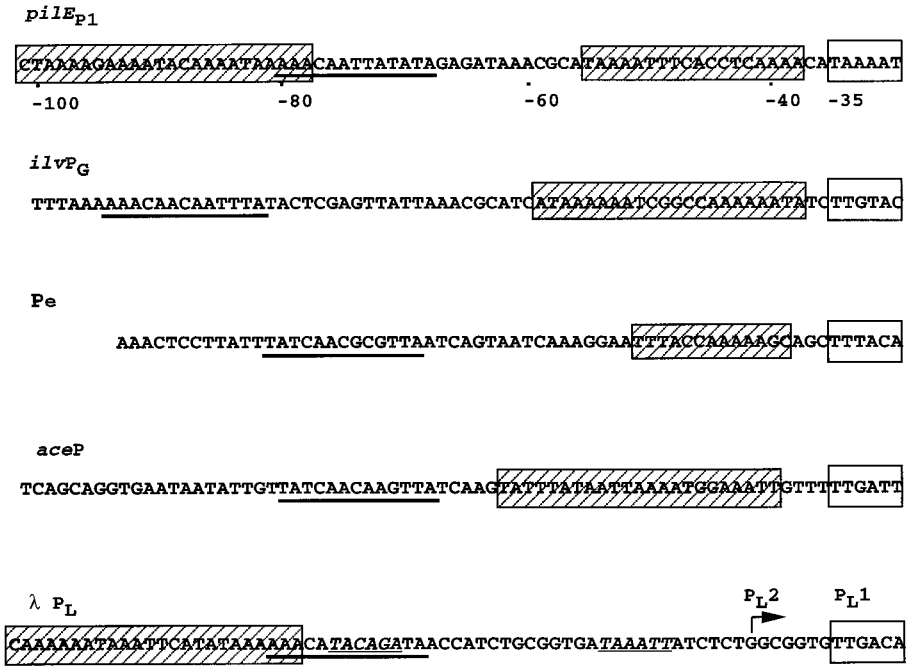

FIG. 5. Comparison of the sequence upstream of and including the $pilEp_1$ −35 box with the corresponding sequences of a series of IHF-activated promoters described in *E. coli* and bacteriophages. $ilvp_G$ is located upstream of the *E. coli ilvGMEDA* operon (30), Pe is an early promoter of phage Mu (44), *acep* is located upstream of the operon encoding the enzymes for acetate utilization in *E. coli* (28), and λ P_L is an early promoter of phage λ (13, 14) containing the P_L2 promoter (italicized and underlined) in addition to the P_L1 −35 box. The IHF-binding core consensus sequences are underlined in boldface, the −35 regions are defined by open boxes, and sequences shown to be associated with transcriptional enhancement, or confirmed UP elements, are contained within hatched boxes.

architectural role in the activation of a σ^{70} promoter in *E. coli* (35) and an AlgU-dependent promoter in *P. aeruginosa* (10).

The results presented in this study suggest that IHF plays a direct role in the transcriptional activation of the $pilEp_1$ promoter, i.e., no additional activators appear to be required. To our knowledge, this is the first example of such a role for IHF to be described in a bacterial species other than *E. coli*. The importance of the AT-rich region, 5′ to the IHF-binding core consensus sequence, for the $pilEp_1$ transcription-activating role of IHF was also demonstrated in this study. Only a subset of *E. coli* IHF-binding sites have such additional domains (15). However, placement of an AT-rich element upstream of a core consensus element can significantly increase the affinity of that element for IHF-binding (17).

The introduction of mutations at nucleotides −90, −93, and −98 within the IHF-protected region upstream of λ P_L resulted in a threefold decrease in IHF binding (14). We observed that replacement of the two poly(A) tracts immediately 5′ to nucleotide −90, upstream of $pilEp_1$, with vector-derived sequence resulted in a fourfold reduction in P1 transcriptional activity. One possible explanation for this result is that the conformation and flexibility of the DNA in the vicinity of the IHF core consensus sequence plays an important role in determining the efficiency of IHF binding (15). Further experiments are required to determine the likelihood of this explanation.

In addition to the domain bound by IHF, the AT-rich region upstream of $pilEp_1$ has been shown to contain two UP-like elements. The first of these, positioned within the −37 to −59 region, enhances $pilEp_1$ transcription three- to fourfold in *N. gonorrhoeae*, independently of the IHF-mediated activation. Similar elements have been described directly upstream of several *E. coli* and bacteriophage promoters, including ones which are directly activated by IHF. Figure 5 shows an alignment between the sequence upstream of $pilEp_1$ and the equivalent sequences upstream of several promoters which have been shown to be directly activated by IHF. In the case of the *E. coli ilvGMEDA* operon, it was suggested that the formation of an IHF-DNA nucleoprotein structure caused a conformational change in the DNA helix at the promoter and that this enhanced transcription initiation via increased open complex formation in a manner which was sensitive to DNA supercoiling (30). In addition to the contribution of the IHF-induced bend to transcriptional activation, the AT-rich sequence centered at nucleotide −50 significantly enhanced $ilvp_G$ transcriptional levels through the formation of an intrinsic DNA bend (29). The transcriptional activation properties of the IHF-binding domain and the region associated with the intrinsic bend were functionally independent in the case of the $ilvp_G$ promoter (30). On the other hand, IHF-mediated activation of the early promoter of bacteriophage Mu (Pe) is thought to be associated with improved binding of αCTD to an UP element located between −39 and −51 (44). An αCTD-binding UP element has likewise been identified between the IHF-binding site and the promoter region upstream of the *E. coli* acetate operon, *aceBAK* (28). However, the functional independence or interaction of the two elements was not reported in this study. It appears, on the basis of the alignments presented in Fig. 5, that $pilEp_1$ shares the AT-rich sequence within the −40 to −60 region, characteristic of the Pe, *acep*, and $ilvp_G$ promoters. Clearly this region enhances transcription from $pilEp_1$, but the mechanism involved has yet to be confirmed. The most likely explanations, based on the sequence similarities, are that the region functions (i) as a binding site for αCTD (or some other factor) or (ii) by introducing an intrinsic DNA bend upstream of the promoter, which in turn enhances binding of RNAP or alters the kinetics of open complex formation. The observation that the level of activation associated with the

presence of the IHF-binding domain (eightfold in *N. gonorrhoeae* and three- to fourfold in *E. coli*) was the same in the presence and absence of the wild-type UP-like element indicated that the two regions are functionally independent.

The second region capable of enhancing $pilEp_1$ transcription is positioned between −78 and −101, upstream of the IHF-binding core consensus sequence but within the IHF-protected domain. This element shares 17 identical nucleotides out of 24 with a similarly placed UP element within the λ P_L upstream region.

It remains to be directly demonstrated that one or both gonococcal UP-like elements enhance transcription through binding to the RNAP α subunit. Extensive analysis of the *E. coli* RNAP α subunit (4, 12) have suggested that interaction of the CTD with the $rrnBp_1$ UP element involves dimerization and binding to two distinct regions (centered at −42 and −52) within the UP element. Recent evidence suggests that in the presence of an activator (e.g., cyclic AMP receptor protein [CRP]) bound upstream of a promoter, the two α subunits are capable of binding different DNA sites as monomers (27). It was shown in this study that the β′-associated α subunit was capable of contacting a site as far upstream as −96 in the presence of a CRP dimer bound at −74.5 with respect to the TSP. It is intriguing that the locations of these contact and binding sites are remarkably similar to the locations of the centers of the $pilEp_1$ upstream UP-like element and IHF-binding core consensus sequence.

The nucleotide sequence of the gonococcal *rpoA* gene (encoding the α subunit of RNAP) was identified following a BLAST search (1) of the genomic sequence data for strain FA1090, released on the University of Oklahoma *N. gonorrhoeae* Genome Database (32a). This sequence was translated, and an alignment was generated between the deduced amino acid sequence and the amino acid sequence of the *E. coli* α subunit (20). It was clear from this alignment that the amino acid sequence of the C-terminal end of the gonococcal α subunit is very similar to the equivalent region of the *E. coli* protein, with 60 of 85 amino acids identical (data not shown). Seven amino acids within two domains of the *E. coli* αCTD have been shown to be crucial for DNA binding to the *rrnB* UP element (12). All of them are conserved in the gonococcal α subunit (data not shown). Thus, it is likely that similar interactions occur between gonococcal UP elements and αCTD.

Work is currently under way in our laboratory to determine the relative roles and interactions of IHF, the UP-like elements, and the α subunit of RNAP in the transcriptional activation of the gonococcal *pilE* gene. The potential role of DNA supercoiling in the regulation of this important gene is also under investigation.

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