An AT-Rich Tract Containing an Integration Host Factor-Binding Domain and Two UP-Like Elements Enhances Transcription from the pilEp1 Promoter of Neisseria gonorrhoeae

JANET A. M. FYFE* AND JOHN K. DAVIES

Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia

Received 16 December 1997/Accepted 20 February 1998

The pilE gene of Neisseria gonorrhoeae is transcribed from a σ70 promoter (pilEp1) 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 pilEp1 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 σ54 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 σ54-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 σ70-dependent promoter with an upstream AT-rich region (5).

* Corresponding author. Mailing address: Department of Microbiology, Monash University, Wellington Rd., Clayton, Victoria 3168, Australia. Phone: 61 3 9905 4809. Fax: 61 3 9905 4811. E-mail: Janet.Fyfe@med.monash.edu.au.

Copyright © 1998, American Society for Microbiology


10021-9193/98/$04.00 + 0

Downloaded from http://jb.asm.org/ on October 14, 2017 by guest
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 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 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 hsdR17 supE44 relA1 ΔlacU169 (Δ80 ΔlacZ1M15). The E. coli strains SØ1718 and SØ1753 (an ihf::Tn10 derivative of SØ1718), used to assess the role of IHF in pilEp transcription, were kindly donated by Per Klemm. The N. gonorrhoeae strain used was MS11-A (36). The pilEp::cat reporter plasmid used to generate the deletant reporter constructs was pJKD1304, a derivative of pJKD862 with the pilEp and pilEp 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 pilEp1-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 HindIII and the nucleotide sequences were determined for the regions upstream of pilEp::cat. Primer-containing AvaI/AoeI fragments were subcloned into pJKD862, using the wild-type promoter-containing fragment as a vector. The pilEp::cat cassette thus engineered were subcloned on BamHI fragments into the single BglII site of pJKD1854, a derivative of pJKD1250 (11) containing a 2.9-kb fragment internal to the gonococcal iga gene, with the SphI/BamHI 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::cat cassette into the iga gene such that transcription of cat was in the direction opposite to transcription of iga. These recombinant plasmids were then 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 ml−1). Gonococcal cells were harvested from GC agar plates incubated for 20 h at 37°C in the presence of 5% CO2. The determination of CAT levels in these extracts was performed as described previously (11).

RESULTS

pilEp1 transcription is enhanced by upstream sequences in N. gonorrhoeae. We have shown previously that a pilEp::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 pilEp1 was dependent on additional sequences upstream of the poorly conserved −35 box (TAAAT). As shown in Fig. 1A, the sequence upstream of the pilEp1 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 NNTTNR (9), and the DNase I 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::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 pilEp1 −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 pilEp1 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
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 pilEp₁ 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 pilEp₁ 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. 1.** Construction of deletant and mutant derivatives of a pilEp₁::cat reporter cassette. (A) Nucleotide sequence of the region containing the pilEp₁ 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 (P₁a and P₁b) associated with pilEp₁ (11). The large ATG defines the start codon of cat. (B) The sequences from −30 to −101 for the WT pilEp₁::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 BglII 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.
enhancement of transcription. Therefore, it was necessary to evaluate 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 pilEp1−35 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 pilEp1 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 pilEp1 transcription. The AT-rich sequence 5′ to the IHF-binding core consensus sequence upstream of pilEp1 is reminiscent of a sequence similarly located upstream of the bacteriophage λ early P1 promoters (13, 14), as shown in Fig. 4. Transcription of λ P1,1 is activated by IHF, in the presence of an intact cCTD (13). In contrast to pilEp1, the DNA between the −35 box and the IHF-binding site upstream of λ P1,1 is not particularly AT rich, and a role for this region as an UP element has not been previously reported. The observation that deletion of sequences upstream of the IHF-protected domain (Δ−111) similarly had no effect on pilEp1 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.

An intact ihfA gene is required for maximal transcription from pilEp1 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 pilEp1 transcription in an E. coli background. To this end, pKD1854-derived plasmids containing the wild-type pilEp1::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 SO1718 and SO1753 (an ihfA::Tn10 derivative of SO1718). Cell extracts were prepared and assayed for CAT. The results (Fig. 3) indicated that the levels of pilEp1 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 pilEp1 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 pilEp1.

FIG. 3. Effect of an ihfA mutation on pilEp1 transcription in E. coli. CAT levels were measured in cell extracts prepared as described in Materials and Methods from E. coli SO1718 (parent strain) and SO1753 (ihfA::Tn10) transformed with pKD1854-derived plasmids containing pilEp1::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.
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 P2 promoter (in the absence of IHF) and, when placed directly upstream, of λ P0 or Plac (14). The role of this UP element, when located at the normal position (−79 to −102 with respect to the P1 TSP), in the IHF-mediated activation of P1 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 P1 through direct contact between αCTD and this UP element (14).

Comparison of the sequences directly upstream of the pilEp1 and λ P1 IHF-binding core consensus sequences reveals that 17 of the 24 nucleotides are identical (Fig. 4). Based on this similarity, a pilEp2-cat reporter was constructed in which the sequence from −78 to −101 was fused by PCR, directly upstream of nucleotide −37, still relocating the putative UP element immediately upstream of the pilEp1 −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 pilEp2-cat reporter thus generated was designated Δ −38−77. Similarly, the 21-nucleotide λ P2 UP element was fused upstream of pilEp1 (PpilEp1), locating the sequence at the same position relative to the pilEp1 −35 box as it is located upstream of λ P2 (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 P2 UP element can enhance transcription from pilEp1 approximately sevenfold with respect to the basal level associated with Δ −37. The Δ −38−77 construct gave rise to levels of CAT comparable to 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 pilEp1 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 P1 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 σ54-dependent promoter pilEp1 (11). Disruption of the pilEp1 −10 box via site-directed mutagenesis resulted in insignificant levels of transcription from the pilEp1 upstream region. A gonococcal pilE strain (with a deletion encompassing the promoter and 5’ end of the chromosomal pilE gene) containing pilE on a multicopy plasmid, with only pilEp1 and pilEp2 intact, did not express pili. On the other hand, a similar strain with pilEp1 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 σ54 dependent, but it has since evolved to become σ70 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 σ54 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 transferred 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 σ54 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 pilEp1, 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 σ54-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 σ54 promoters in E. coli and other bacterial species have been documented (16). There are also examples of IHF playing an
architectural role in the activation of a σ70 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 pilEp1 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 pilEp1 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 Pl 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 pilEp1, 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 pilEp1 has been shown to contain two UP-like elements. The first of these, positioned within the −37 to −59 region, enhances pilEp1 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 pilEp1 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 ilvG-MEDA 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 ilvG 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 pilEp1 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, acep (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 pilEp1 shares the AT-rich sequence within the −40 to −60 region, characteristic of the Pe, acep, and ilvG promoters. Clearly this region enhances transcription from pilEp1, 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 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 P1 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 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, 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 gonococcal genome 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.

ACKNOWLEDGMENTS

We thank Per Klemm for kindly providing bacterial strains. We are also grateful to Jim Pittard and Ji Yang for helpful discussions during the preparation of the manuscript.

This work was supported by a project grant from the Australian National Health and Medical Research Council.

REFERENCES

30. Paredes, R. S., and G. W. Hatfield. 1996. Transcriptional activation by pro-


