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Neisseria meningitidis C114 Contains Silent, Truncated Pilin Genes That Are Homologous to *Neisseria gonorrhoeae pil* Sequences

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Neisseria meningitidis **pili can be classified into two groups: those (referred to here as class I pili) which are similar to gonococcal pili in that they react with monoclonal antibody SM1 and those that are dissimilar to gonococcal pili in that they lack the SM1-reactive epitope (class II pili). Pilus expression in *N. meningitidis* C114, a class II pilus-producing isolate, was investigated. The sole genomic segment of this strain that bore extensive homology with the *pilE* locus of *Neisseria gonorrhoeae* P9 was cloned in *Escherichia coli*. The production of the pilus structural subunit (pilin) from this meningococcal segment could not be detected by immunological and coupled in vitro transcription-translation analyses. Nucleotide sequence analysis revealed the presence in the C114 genome of two variant, tandemly arranged pilin genes (copies 1 and 2). Copies 1 and 2 are partial pilin genes that constitute part of a silent meningococcal pilin gene (*pil* gene) region, designated *pilS*. Both copies are truncated, corresponding to variable domains of the gonococcal *pilE* gene but lacking homologous N-terminal coding sequences. Located within sequences surrounding copies 1 and 2 were several classes of repeated elements that are associated with *pil* loci in *N. gonorrhoeae*.**

Neisseria meningitidis is a major cause of meningitis worldwide. Several meningococcal components have been implicated in pathogenesis, including filamentous protein appendages, called pili, that can be expressed both in laboratory culture and during natural infection (26, 32). Meningococcal pili exhibit interstrain differences with respect to the physical and immunological properties of their structural subunit, pilin (17, 32, 34). In these and certain other respects, pilus expression in *N. meningitidis* and *Neisseria gonorrhoeae* is similar. The pili of both organisms promote attachment to human epithelia, and pilus expression in each case can be attenuated (an example of pilus phase variation), or an immunologically novel pilus type can be elaborated (pilus antigenic variation) (2, 5, 9, 17, 23, 31, 33, 36). Although gonococcal pilus phase variation can be either reversible or irreversible, it is not known whether the same applies in *N. meningitidis*. Indeed, compared with gonococcal pilus expression, pilus expression in *N. meningitidis* is generally less well characterized.

Gonococcal pilus phase and antigenic variation are effected by altering the expression and/or nucleotide sequence of *pilE*, the structural gene for pilin (5, 12, 30). Irreversible pilus phase variation in *N. gonorrhoeae* results from deletions at the full genomic complement of (one or two) *pilE* loci (12, 14, 23). Chromosomal deletions that may be analogous have also been observed for *N. meningitidis* in association with piliated-to-nonpiliated phase transition (17). Reversible gonococcal pilus phase variation apparently follows partial pilin gene replacement at *pilE*, and a similar mechanism may operate in pilus antigenic variation (30). Both events generate a new *pilE* gene whose translation product either is not assembled into mature pili (resulting in a nonpiliated phenotype) or is antigenically variant, endowing pili with new physical and antigenic properties (5, 15, 30). Gonococcal pilus antigenic variation has been shown to follow a gene conversion event involving *pilE* and at least one of a considerable repertoire of silent pilin (*pilS*) gene segments (4, 14, 24).

Despite antigenic diversity, pilin monomers from all piliated gonococci analyzed to date share a common N-terminal domain within which is located an invariable epitope recognized by monoclonal antibody SM1 (11, 15, 18, 34). This epitope has been localized to a position between amino acids 48 and 53 on mature pilin (15). The majority of fresh, piliated isolates of *N. meningitidis* possess pili (referred to here as class I pili) that also react with antibody SM1 (17, 32, 34). However, a second class of meningococci exists whose members elaborate SM1-nonreactive pili (referred to as class II pili) (17, 33, 34). Nevertheless, all meningococcal isolates tested harbor genomic sequences that are homologous to gonococcal *pilE* probes (1, 17).

It is possible that the genomic sequences of class II-piliated meningococci that are homologous to *N. gonorrhoeae pilE* probes encode the N-terminal domains of their respective pilins: the N-terminal amino acid sequences of several meningococcal pilins are conserved with respect to the analogous domain of gonococcal pilin (26). However, the N termini of class I and II pilins are immunologically, and therefore probably structurally, distinct. To investigate this potential dichotomy, we have characterized pilus expression in *N. meningitidis* C114, a class II-piliated meningococcal isolate (17). This paper reports the cloning and expression and nucleotide sequence analyses of a pilin gene region of *N. meningitidis* C114.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. *N. meningitidis* C114, a class II-piliated clinical isolate, has been described in detail elsewhere (17). *Escherichia coli* GC1 (12) was used for preliminary analysis of cloned DNA. Subsequent analysis of meningococcal sequences was performed with *E. coli* DH1 [F^- *recA1 hsdR17* (r_K^- m_K^+) *endA1 gyrA96 thi-1 supE44* λ^-] (6) and JM103 (for the propagation of bacteriophage M13 and recombinant derivatives) (16) as hosts.

Reagents, enzymes, and isotopes. All chemicals were of reagent grade or purer. Restriction endonucleases, T4 DNA

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ligase, nuclease BAL 31, calf intestinal alkaline phosphatase, and DNA polymerase I (large fragment) were obtained from Boehringer Mannheim. M13 universal sequencing primer, [α-^{35}S]dATP (>600 Ci · mmol^{-1}; >22 TBq · mmol^{-1}), [α-^{32}P]dCTP (approximately 3,000 Ci · mmol^{-1}; 110 TBq · mmol^{-1}), [^{35}S]methionine (>800 Ci · mmol^{-1}), and ^{125}I-labeled *Staphylococcus aureus* protein A (>30 mCi · mg^{-1}; >1.1 GBq · mg^{-1}) were supplied by Amersham. Coupled transcription-translation and random hexanucleotide labeling kits were supplied by P&S Biochemicals, Ltd. Polyclonal antiserum, raised against purified *N. meningitidis* C114 pili (17), was generously supplied by C. A. Hart.

Medium and growth conditions. Culture conditions for *E. coli* were as described by Nicolson et al. (14). *N. meningitidis* C114 was grown at 37°C for 16 to 20 h on CTA medium (17) in the presence of 5 to 7% (vol/vol) CO_2. The presence of piliated cells in the resultant lawns was confirmed by electron microscopy.

Cloning of *N. meningitidis pil* sequences. Procedures for the isolation and cloning of DNA have been described elsewhere (10, 14, 17). Total genomic DNA from *N. meningitidis* C114 was partially digested with *Cla*I. The resulting fragments were ligated to *Cla*I-linearized pBR322 DNA (3) that had subsequently been treated with alkaline phosphatase. Ligated DNA molecules were used to transform *E. coli* GC1 to ampicillin resistance. *E. coli* GC1 was chosen as the host in this experiment, because it has previously been shown to be proficient in expression of gonococcal pilin genes on primary passage (12, 14). Approximately 14,000 ampicillin-resistant, tetracycline-sensitive transformant colonies were screened by the colony hybridization method of Hanahan and Meselson (7) for possession of DNA sequences homologous to the *pilE* gene of *N. gonorrhoeae* P9-2. The 1.2-kilobase (kb) *Xba*I-*Pvu*II fragment of recombinant plasmid pLV260 (14) used as probe contains all but 1 base pair (bp) of the P9-2 *pilE* gene (18).

Analysis of cloned *N. meningitidis pil* sequences. Standard DNA manipulations used in these analyses have been described previously (10, 14, 15). Linear gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Southern hybridization, and Western blotting (immunoblotting) protocols have been described elsewhere (14). A coupled transcription-translation system containing [^{35}S]methionine was used for in vitro analysis of plasmid-encoded proteins by the procedure of Pratt (20). Labeled polypeptides were fractionated by linear gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (in a gel containing 10 to 17.5% [wt/vol] acrylamide) and were analyzed autoradiographically (20).

DNA sequencing. A series of restriction fragments derived from the 1.3-kb *Pvu*II-*Cla*I fragment of pLV610 (containing the cloned meningococcal *pil* region; Fig. 1) were cloned into the M13 vectors mp18 and mp19 (16). DNA sequences were determined by the dideoxy chain termination method of Sanger et al. (21) by the strategy detailed in Fig. 1B. Both strands were sequenced, including overlaps across all internal restriction sites. Sequence information was processed using the DBUTIL computer program of Staden (25) and the IBM-PC program of Schwindinger and Warner (22).

RESULTS

Cloning and preliminary analysis of an *N. meningitidis* C114 *pil* region. A 1.2-kb *Xba*I-*Pvu*II fragment, containing all but 1 bp of the *pilE* gene of *N. gonorrhoeae* P9-2 (14, 18), was used to identify *E. coli* transformants that contained *N. meningitidis* C114 *pil* sequences from a meningococcal gene library constructed in pBR322. Ten positive clones were obtained in this way, and each possessed a *pilE*-homologous recombinant plasmid with a DNA insert of one of three distinct sizes (data not shown). Four such recombinant plasmids (designated pLV600, pLV610, pLV620, and pLV630) included representatives of each size class and were analyzed by restriction enzyme mapping (Fig. 1A). Plasmids pLV610 and pLV630 possessed apparently identical restriction maps. All *pilE*-homologous recombinant plasmids analyzed by restriction mapping possessed a consensus *Cla*I fragment of approximately 3.1 kb (Fig. 1A). The orientation of this fragment with respect to pBR322 sequences was different in pLV620 compared with plasmids pLV600 and pLV610. The relative positions of the three *Cla*I fragments on the meningococcal insert of pLV620 were determined by BAL 31 nuclease deletion analysis of *Xba*I-linearized pLV620 DNA (data not shown).

Southern hybridization analysis of total DNA that had been digested with *Cla*I, with *Cla*I plus *Xba*I (Fig. 2), or with other restriction endonucleases (data not shown) revealed that the chromosome of strain C114 possessed a single *pilE*-homologous locus. The same analyses showed that *Cla*I fragments of meningococcal origin that were represented in recombinant plasmid pLV600 were not contiguous on the chromosome of strain C114. The arrangement of these *Cla*I segments in pLV600 presumably reflects their juxtaposition in vitro. However, Southern hybridization data were consistent with the observed arrangements of restriction fragments on the meningococcal inserts of plasmids pLV610 and pLV620. Southern hybridization analysis further indicated that the unique *pilE*-homologous *Cla*I fragment of *N. meningitidis* C114 was identical or very similar to the consensus 3.1-kb *Cla*I fragment of plasmids pLV610 (Fig. 2), pLV600, pLV620, and pLV630 (data not shown). This implied that *pilE*-homologous plasmids had not undergone gross rearrangements, either in vitro or in *E. coli*, during isolation and analysis.

Analysis of expression of cloned *N. meningitidis pil* genes. Western analysis using a polyclonal antiserum raised against purified pili from *N. meningitidis* C114 (17) suggested that plasmid pLV610 did not encode an immunoreactive pilin that was expressed in *E. coli* GC1 (data not shown) or DH1 (Fig. 3A). The same result was obtained with plasmids pLV600 and pLV620 (data not shown). These findings were confirmed by dot-blot analysis of recombinant *pil* clones with the same polyclonal antiserum (data not shown). However, in vitro analysis of proteins encoded by plasmids pLV600, pLV610, and pLV620 indicated that the transcriptional-translational machinery of *E. coli* could function with meningococcal genes as substrates (Fig. 3B). Each recombinant plasmid programmed synthesis of a protein of about 13.6 kilodaltons that was not encoded by pBR322 (Fig. 3B). It was therefore likely that the structural gene of this protein was located on the consensus 3.1-kb meningococcal *Cla*I fragment. However, no strongly labeled polypeptide corresponded in the apparent M_r (17,100) to that of *N. meningitidis* C114 pilin (Fig. 3B) (17). In analogous in vitro analyses of cloned *N. gonorrhoeae pilE* loci, proteins corresponding in M_r to pilin are strongly labeled (18) and gonococcal pilin is synthesized in *E. coli* DH1 in vivo to levels of approximately 1% of the total protein (unpublished data). The presence of expressed *N. meningitidis* proteins of 33.3 or 34.7 kilodaltons (Fig. 3B) could have represented a pilin dimer that was not immunoreactive (although neither of these protein species had exactly twice the apparent M_r of *N. meningitidis*

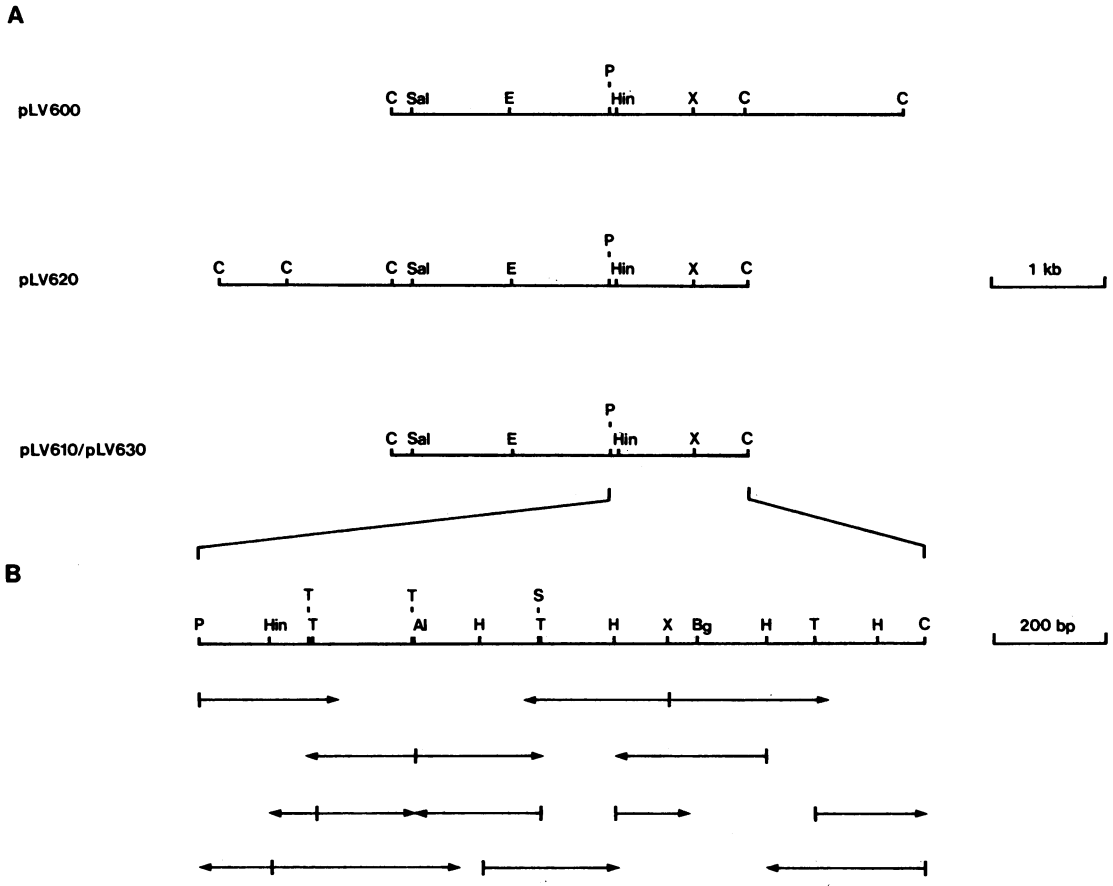
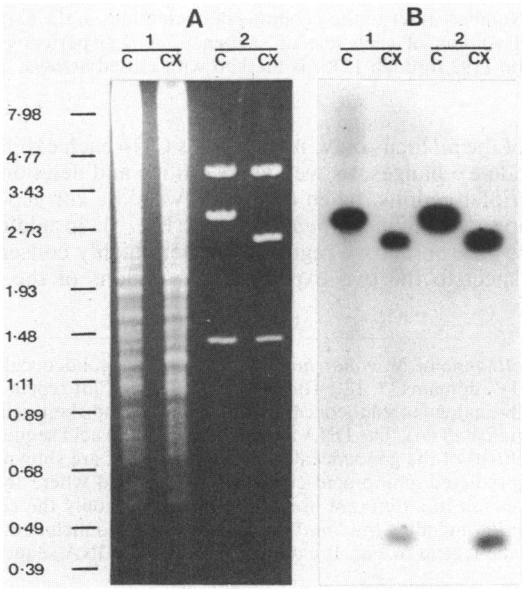


FIG. 1. (A) Restriction cleavage maps of the sequences derived from *N. meningitidis* C114 that are represented in recombinant, *pilE*-homologous plasmids. The map of plasmid pLV630 is apparently identical to that of pLV610. Restriction enzyme abbreviations: C, *Cla*I; E, *Eco*RV; Hin, *Hin*dIII; P, *Pvu*II; Sal, *Sal*GI; X, *Xba*I. (B) More-detailed restriction map of the sequenced 1.3-kb *Pvu*II-*Cla*I fragment of plasmid pLV610. Arrows designate the sequencing strategy and direction used. Additional abbreviations: Al, *Alu*I; Bg, *Bgl*I; H, *Hae*III; S, *Sau*3A1; T, *Taq*I.



C114 pilin). *N. meningitidis* C114 pilin synthesis could, however, be negatively regulated, possibly in a fashion analogous to repression of *Vibrio cholerae* pilin synthesis by ToxR (13). Such repressor activity, if expressed in *E. coli*, could mask the presence of a meningococcal *pilE* gene in recombinant clones. Alternatively, the cloned *pil* region studied here may be silent in *N. meningitidis* C114, the class II pili produced by this strain being encoded by a separate, nonhomologous pilin gene.

Further localization and sequence analysis of the *N. meningitidis* C114 *pil* locus. Recombinant plasmid pLV610, independently digested with different combinations of restriction endonucleases, was subjected to Southern hybridization analysis using the 1.2-kb *Xba*I-*Pvu*II fragment containing all but 1 bp of the *pilE* gene of *N. gonorrhoeae* P9-2 (18) as the

FIG. 2. High-stringency Southern hybridization analysis of the recombinant, *pilE*-homologous plasmid pLV610 and *N. meningitidis* C114 chromosomal DNA. The 1.2-kb *Xba*I-*Pvu*II fragment of *N. gonorrhoeae* P9-2, bearing most of the gonococcal *pilE* gene, was used as the probe. Plasmid pLV610 and meningococcal total DNA were each digested with *Cla*I (C) or *Cla*I plus *Xba*I (CX). Lane 1, *N. meningitidis* C114 total DNA; lane 2, pLV610 DNA. Panels A and B represent the same gel before (A) and after (B) Southern hybridization analysis. Size markers to the left of the gel are in kilobases.

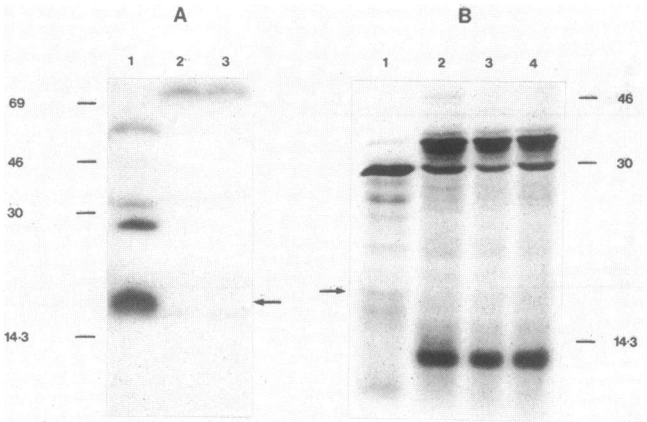


FIG. 3. Analysis of expression of cloned meningococcal *pil* sequences. (A) Western blot analysis of total protein from *N. meningitidis* C114 and *E. coli* harboring recombinant pLV610. The polyclonal rabbit antiserum used was prepared against purified pili from strain C114. Lanes contain total protein from *N. meningitidis* C114 (lane 1), *E. coli* DH1(pLV610) (lane 2), and *E. coli* DH1(pBR322) (lane 3). (B) Identification of [^{35}S]methionine-labeled cloned gene products synthesized in vitro. The plasmid DNA substrates were pBR322 (lane 1), pLV600 (lane 2), pLV610 (lane 3), and pLV620 (lane 4). Arrows in panels A and B depict the (predicted) position of native *N. meningitidis* C114 pilin (17.1 kilodaltons). Size markers to the side of each gel are in kilodaltons.

probe (data not shown). In this way, *pil* sequences were localized to a 1.15-kb *Hin*dIII-*Cla*I fragment that is resident upon the meningococcal insert of pLV610 (Fig. 1A). Southern hybridization patterns generated by probing restriction endonuclease fragments of total DNA from *N. gonorrhoeae* or *N. meningitidis* with either the 1.2-kb *Xba*I-*Pvu*II gonococcal *pilE* fragment or the 1.15-kb *Hin*dIII-*Cla*I fragment of pLV610 were indistinguishable (data not shown). Since the chromosome of *N. gonorrhoeae* contains *pilS* loci that lack N-terminal pilin coding sequences (4), this finding suggested that sequences corresponding to the variable region of the gonococcal *pilE* gene were present on the 1.15-kb *Hin*dIII-*Cla*I fragment of pLV610. This possibility was further investigated by using DNA sequence analysis.

The DNA sequence of the 1.3-kb *Pvu*II-*Cla*I fragment of recombinant pLV610, harboring the cloned *pil* region of *N. meningitidis* C114, is presented in Fig. 4. Comparison of the meningococcal *pil* locus with the sequence of the *pilE* gene of *N. gonorrhoeae* P9-2 enabled the identification of two sections (copies 1 and 2) that were strongly reminiscent of the gonococcal pilus structural gene (Fig. 5). Each copy included a coherent open reading frame that comprised a variant pilin gene copy truncated at its 5′ terminus and lacking coding information corresponding to the putative SM1-reactive epitope (Fig. 5) (15, 33). Copies 1 (nucleotides 436 through 639; Fig. 4) and 2 (nucleotides 872 through 1138;

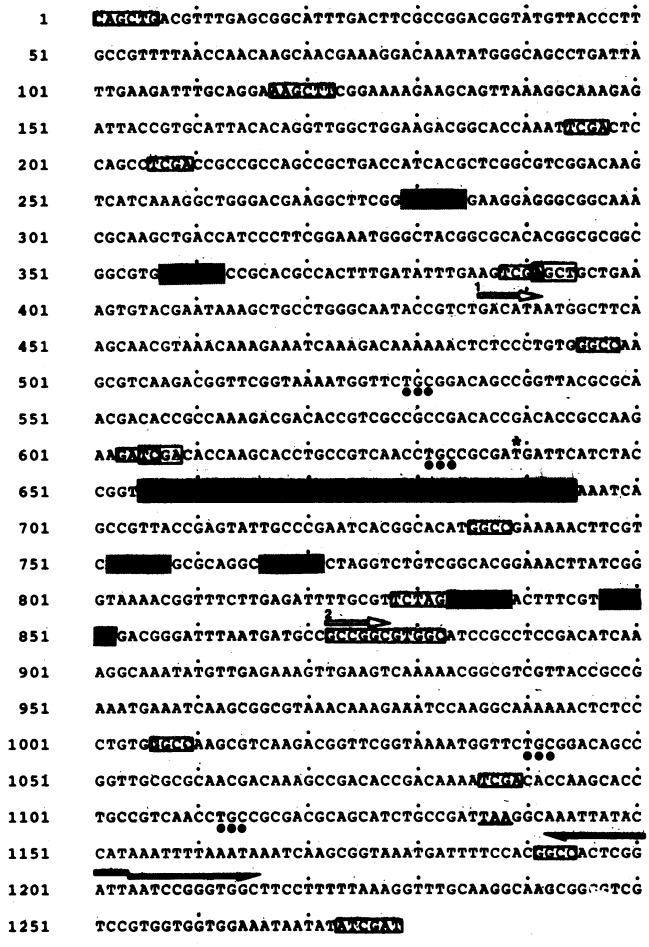
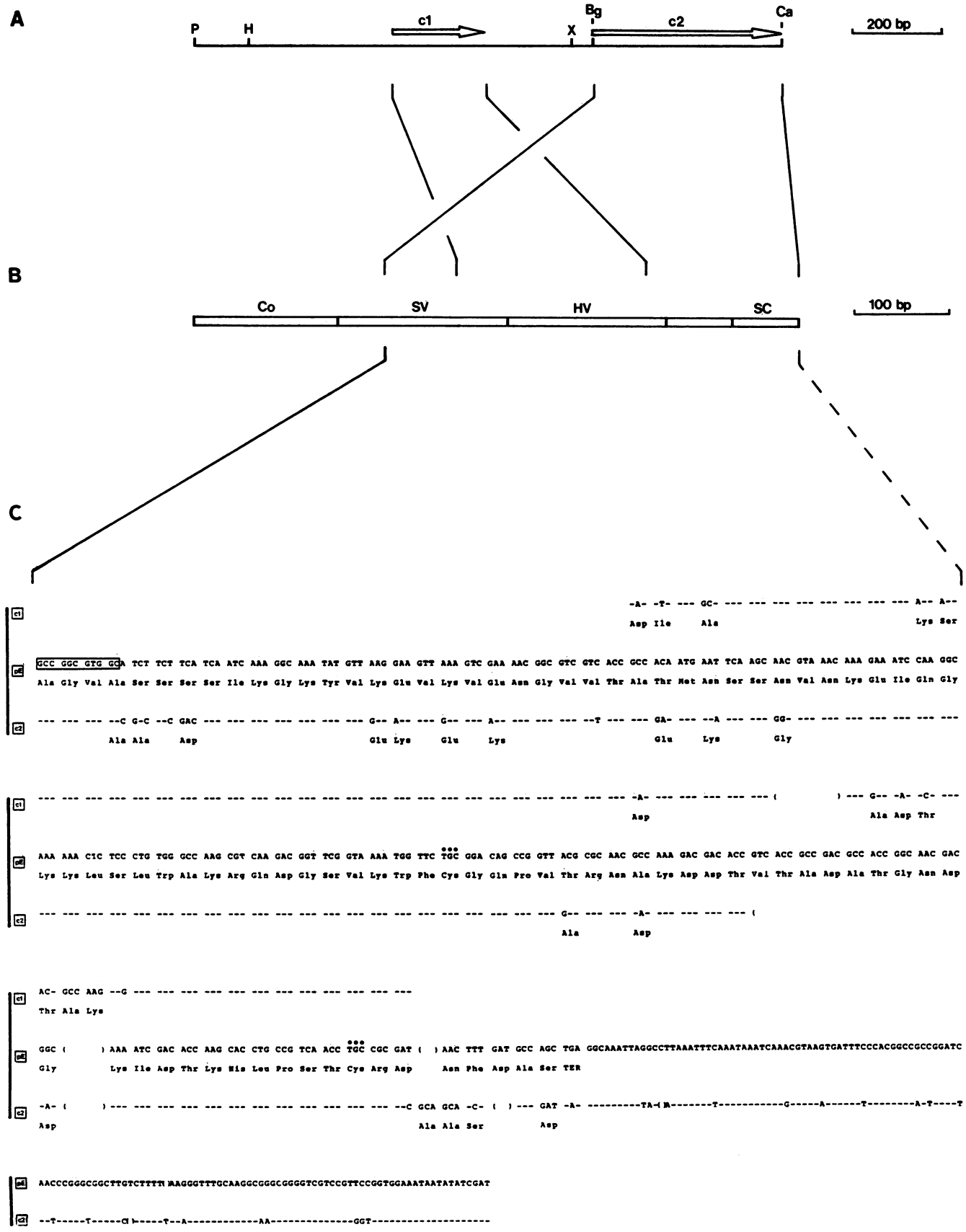


FIG. 4. DNA sequence analysis of the 1.3-kb *Pvu*II-*Cla*I fragment of recombinant pLV610. The sequence is shown 5′ to 3′ (left to right). The putative start positions of two pilin gene segments (copies 1 and 2) are marked with open arrows labeled 1 and 2, respectively. The 3′-terminal nucleotide of copy 1 (nucleotide 639) is indicated (*), and the stop codon terminating copy 2 (nucleotides 1136 through 1138) is underlined. The in-frame *cys* codons present in copies 1 and 2 (●) are indicated. The locations of elements similar to RS1 (▮) and RS3 (▒) (4) are marked. Selected restriction sites are boxed. Numbers refer to the positions of nucleotides in the extreme left-hand column of each line of sequence. A near-perfect palindrome (bp 1192 through 1215) is marked with closed arrows.

Fig. 4) of the *pil* locus of *N. meningitidis* C114 each exhibited nucleotide exchanges, as well as insertions and deletions of whole triplet codons, when compared with the corresponding region of *N. gonorrhoeae* P9 *pilE* (Fig. 5). In addition, each copy harbored two regions that were highly conserved with respect to the two expressed *cys* regions of the *pilE*

FIG. 5. Comparison of copies 1 and 2 of *N. meningitidis* C114 *pilS* with the *pilE* gene of *N. gonorrhoeae* P9-2 (18). The gonococcal *pilE* gene (B) is divided into its constant (Co), semivariable (SV), and hypervariable (HV) domains (5, 18). The *pilE*-linked *Sma*I-*Cla*I repeat (SC) is also illustrated. The extents of copy 1 (c1) and of copy 2 (c2) compared with analogous gonococcal *pilE* sequences and their relative positions on the meningococcal *pilE*-homologous 1.3-kb *Pvu*II-*Cla*I fragment are indicated (A). The DNA and predicted amino acid sequences of copies 1 and 2, compared with nucleotide and deduced amino acid sequences of part of the gonococcal *pilE* gene (pE) (18), are shown (C). Dashes indicate no sequence changes observed in these positions. Codon and predicted amino acid changes are indicated where found. Parentheses indicate nucleotide deletions. If the DNA sequence change did not result in a different predicted amino acid, only the codon change is shown. *cys* codons (●) are indicated. DNA sequences 3′ to copy 2 and *pilE* (including the *Sma*I-*Cla*I repeat) are also included. The unique *Bgl*I site of *pilE* is boxed. Abbreviations for restriction enzymes are as in the legend to Fig. 1, except for *Cla*I (Ca). DNA sequences are shown 5′ to 3′ (left to right).


A
P
H
c1
Bg
X
c2
Ca
200 bp
B
Co
SV
HV
SC
100 bp
C
c1
pE
c2

gene of *N. gonorrhoeae* P9 (Fig. 5) (15). Sequences of both copies that were located between the two *cys* region analogs of each exhibited considerable differences compared with the corresponding segment of the *pilE* gene of *N. gonorrhoeae* P9 (Fig. 5). Gonococcal pilus antigenic variants exhibit alterations of a similar type within analogous DNA segments of their respective *pilE* genes (5, 15).

Nucleotide sequences immediately upstream of and bridging copies 1 and 2 contained stop codons in all three translational reading frames. We therefore conclude that the *pil* locus analyzed here is not expressed in *N. meningitidis* C114 and that copies 1 and 2 each form part of a silent (i.e., *pilS*) locus. Unlike copy 1, the 3′ terminus of copy 2 was delineated by a stop codon (TAA) in the correct reading frame (Fig. 5). The DNA tract separating copies 1 and 2 contained a 40-bp sequence identical at all but three nucleotide positions to an RS1 element resident at the *pilS1* locus of *N. gonorrhoeae* $MS11_{ms}$ (Fig. 4) (4). In addition, the DNA segment between copies 1 and 2 contained two sets of invertedly repeated, RS3-like elements (Fig. 4) (4). Resident at the *pilS* locus of *N. meningitidis* C114 was a DNA segment bounded by a pseudo *Sma*I site (5′-TCCGGG-3′; nucleotides 1206 through 1211) at its 5′ terminus and a *Cla*I site (nucleotides 1273 through 1278) at its 3′ terminus (Fig. 4 and 5). This segment shares extensive homology with the *Sma*I-*Cla*I repeat of *N. gonorrhoeae* (11, 18).

The *pilS* locus of *N. meningitidis* C114 did not contain an arrangement of sequences that conform closely to the *E. coli* consensus promoter sequence (8) or indeed to other known *Neisseria* promoters, such as those of the opacity protein (27, 28), pilin (11), or immunoglobulin A protease genes (19).

DISCUSSION

The chromosome of *N. meningitidis* C114 harbors a silent locus, *pilS*, comprising two truncated, tandemly arranged variant pilin genes. Each pilin gene copy lacks an extensive fraction of 5′ sequence corresponding to coding information for the N-terminal portion of class I *N. meningitidis* pilin. The *pilS* locus is the only region of the chromosome of *N. meningitidis* C114 that possesses sequences bearing extensive homology to the *pilE* region of *N. gonorrhoeae* P9. Nevertheless, this meningococcal strain is piliated, producing class II pili. This study therefore provides indirect evidence that the *pilE* gene of *N. meningitidis* C114 differs significantly from that of *N. gonorrhoeae* and, ipso facto, class I-piliated meningococci. Presumably, such differences are also reflected in the primary structures of pilins elaborated by *N. meningitidis* C114 and *N. gonorrhoeae*. This is consistent with previously described, marked physical and immunochemical differences between gonococcal/meningococcal class I and class II meningococcal pili (17, 26, 33, 34). Indeed, amino-terminal amino acid sequences of several bacterial pilins are heterologous compared with analogous sequences in gonococcal pilin (35). It is as yet unknown, however, whether any of the pilin genes of such species are homologous to genomic sequences present in *N. meningitidis* C114 or other strains elaborating class II pili. We are currently attempting to clone the expressed C114 *pil* gene(s) to enable a direct comparison between the pilin components of class I and class II pili and between their respective *pilE* regions.

Several features of the *pilS* locus lead us to suppose that it might have played a role in pilus expression during the recent evolutionary history of strain C114. First, copies 1 and 2 of this meningococcal *pilS* locus do not contain

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5'- TTCCTTTTGGAAGGTTTTTGA -3'
5'- TTCCTTTTTAAAGGTTTGCAA -3'
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FIG. 6. Sequence homology between part of the Hin recognition site of *S. typhimurium* (the right-hand inverted repeat) (37) and the pseudo *Sma*I-*Cla*I repeat of *N. meningitidis* C114 (nucleotides 1216 through 1236; Fig. 4). The *S. typhimurium* sequence is shown at the top. Conserved nucleotides are boxed.

out-of-frame DNA sequence alterations compared with the *pilE* locus of *N. gonorrhoeae* P9. This suggests that copies 1 and 2 have been preserved and that they could have a function in pilus expression. Each copy may consist of one or more cartridges of pilin gene information that could participate in a gene conversion event, resulting in the expression of (all or part of) either copy. Pilin gene conversion occurs in *N. gonorrhoeae* $MS11_{ms}$ and may involve repeated elements resident at pilus gene loci (4, 24). Indeed, the arrangement of copies 1 and 2 on the *pilS* locus of *N. meningitidis* C114 is similar to that of copies 1 and 2 of the *N. gonorrhoeae* $MS11_{ms}$ *pilS1* locus, whose involvement in pilin gene conversion has been demonstrated (4). Second, several families of repeats that were initially identified in *N. gonorrhoeae* (4, 11), notably RS1-, RS3-, and *Sma*I-*Cla*I-like elements, are conserved at the *pilS* locus of *N. meningitidis* C114 (Fig. 4). Interspecific conservation of such elements at pilin gene loci is possibly an indication of their importance in pilus expression. The pseudo *Sma*I-*Cla*I repeat of *N. meningitidis* C114 harbors a sequence (bp 1216 through 1236; Fig. 4) that shares considerable homology both with the *Sma*I-*Cla*I repeat found at *pil* loci of *N. gonorrhoeae* (Fig. 5) (12, 18) and with part of the Hin (inverted repeat right) recombination site of *Salmonella typhimurium* (Fig. 6) (37). This meningococcal sequence is present at the 3′ terminus of a 24-bp near-perfect palindrome that is flanked at its 5′ end by part of a *Hae*III site (Fig. 4 and 5) and that might be involved in the initiation or resolution (or both) of recombination intermediates during gene conversion. However, since this arrangement of sequences is absent from the termini of the invertible H segment of *S. typhimurium* (37), it might alternatively represent a dormant transcriptional termination signal.

It is unclear as yet whether individual isolates of meningococci can express both class I and class II pili simultaneously. In our previous study of 15 isolates, we demonstrated that strains produced one or other but not both such pili (17). Nevertheless, class II-piliated isolates obviously retain part of the sequence information necessary for class I pilus production. Furthermore, it seems likely that the underlying genetic mechanisms of pilus phase and antigenic variation in *N. gonorrhoeae* and *N. meningitidis* are related.

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LITERATURE CITED

1. **Aho, E. L., G. L. Murphy, and J. G. Cannon.** 1987. Distribution of specific DNA sequences among pathogenic and commensal *Neisseria* species. Infect. Immun. **55**:1009–1013.
2. **Bergstrom, S., K. Robbins, J. M. Koomey, and J. Swanson.** 1986. Piliation control mechanisms in *Neisseria gonorrhoeae*. Proc. Natl. Acad. Sci. USA **83**:3890–3894.

3. **Bolivar, R., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, and H. W. Boyer.** 1977. Construction and characterisation of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Haas, R., and T. F. Meyer.** 1986. The repertoire of silent pilus genes in *Neisseria gonorrhoeae*: evidence for gene conversion. Cell **44**:107–115.
5. **Hagblom, P., E. Segal, E. Billyard, and M. So.** 1985. Intragenic recombination leads to pilus antigenic variation in *Neisseria gonorrhoeae*. Nature (London) **315**:156–158.
6. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
7. **Hanahan, D., and M. Meselson.** 1980. Plasmid screening at high colony density. Gene **10**:63–67.
8. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15**:2343–2361.
9. **Lambden, P. R., J. E. Heckels, H. McBride, and P. J. Watt.** 1981. The identification and isolation of novel pilus types produced by variants of *N. gonorrhoeae* P9 following selection *in vivo*. FEMS Lett. **10**:339–341.
10. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81**:6110–6114.
12. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *N. gonorrhoeae* involves chromosomal rearrangement. Cell **30**: 45–52.
13. **Miller, V. L., R. K. Taylor, and J. J. Mekalanos.** 1987. Cholera toxin transcriptional activator ToxR is a transmembrane DNA binding protein. Cell **48**:271–279.
14. **Nicolson, I. J., A. C. F. Perry, J. E. Heckels, and J. R. Saunders.** 1987. Genetic analysis of variant pilin genes from *Neisseria gonorrhoeae* P9 cloned in *Escherichia coli*: physical and immunological properties of encoded pilins. J. Gen. Microbiol. **133**: 553–561.
15. **Nicolson, I. J., A. C. F. Perry, M. Virji, J. E. Heckels, and J. R. Saunders.** 1987. Localization of antibody-binding sites by sequence analysis of cloned pilin genes from *Neisseria gonorrhoeae*. J. Gen. Microbiol. **133**:825–833.
16. **Norrander, J., T. Kempe, and J. Messing.** 1983. Improved M13 vectors using oligonucleotide-directed mutagenesis. Gene **26**: 101–106.
17. **Perry, A. C. F., C. A. Hart, I. J. Nicolson, J. E. Heckels, and J. R. Saunders.** 1987. Inter-strain homology of pilin gene sequences in *Neisseria meningitidis* isolates that possess markedly different antigenic pilus types. J. Gen. Microbiol. **133**:1409–1418.
18. **Perry, A. C. F., I. J. Nicolson, and J. R. Saunders.** 1987. Structural analysis of the *pilE* region of *Neisseria gonorrhoeae* P9. Gene **60**:85–92.
19. **Pohlner, J., R. Halter, K. Beyreuther, and T. F. Meyer.** 1987. Gene structure and extracellular secretion of *Neisseria gonorrhoeae* IgA protease. Nature (London) **325**:458–462.
20. **Pratt, J. M.** 1984. Coupled transcription and translation in prokaryotic cell-free systems, p. 179–209. *In* B. D. Hames and S. J. Higgins (ed.), Transcription and translation: a practical approach. IRL Press, Oxford.
21. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
22. **Schwindinger, W. F., and J. R. Warner.** 1984. DNA sequence analysis on the IBM-PC. Nucleic Acids Res. **12**:601–604.
23. **Segal, E., E. Billyard, M. So, S. Storzbach, and T. F. Meyer.** 1985. Role of chromosomal rearrangement in *N. gonorrhoeae* pilus phase variation. Cell **40**:293–300.
24. **Segal, E., P. Hagblom, H. S. Seifert, and M. So.** 1986. Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. Proc. Natl. Acad. Sci. USA **83**:2177–2181.
25. **Staden, R.** 1980. A new computer program for the storage and manipulation of DNA gel reading data. Nucleic Acids Res. **8**:3673–3694.
26. **Stephens, D. S., A. M. Whitney, J. Rothbard, and G. K. Schoolnik.** 1985. Pili of *Neisseria meningitidis*. Analysis of structure and investigation of structural and antigenic relationships to gonococcal pili. J. Exp. Med. **161**:1539–1553.
27. **Stern, A., M. Brown, P. Nickel, and T. F. Meyer.** 1986. Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation. Cell **47**:61–71.
28. **Stern, A., and T. F. Meyer.** 1987. Common mechanism controlling phase and antigenic variation in pathogenic neisseriae. Mol. Microbiol. **1**:5–12.
29. **Swanson, J., S. Bergstrom, O. Barrera, K. Robbins, and D. Corwin.** 1985. Pilus$^-$ gonococcal variants. Evidence for multiple forms of piliation control. J. Exp. Med. **162**:729–744.
30. **Swanson, J., S. Bergstrom, K. Robbins, O. Barrera, D. Corwin, and J. M. Koomey.** 1986. Gene conversion involving the pilin structural gene correlates with pilus$^+$ to pilus$^-$ changes in *Neisseria gonorrhoeae*. Cell **47**:267–276.
31. **Swanson, J., J. Kraus, and E. C. Gotschlich.** 1971. Studies on gonococcus infection. I. Pili and zones of adhesion: their relationship to gonococcal growth patterns. J. Exp. Med. **134**: 886–906.
32. **Tinsley, C. R., and J. E. Heckels.** 1986. Variation in the expression of pili and outer membrane protein by *Neisseria meningitidis* during the course of meningococcal infection. J. Gen. Microbiol. **132**:106–113.
33. **Trust, T. J., R. M. Gillespie, A. R. Bhatti, and L. A. White.** 1983. Differences in the adhesive properties of *Neisseria meningitidis* from human buccal epithelial cells and erythrocytes. Infect. Immun. **41**:106–113.
34. **Virji, M., and J. E. Heckels.** 1983. Antigenic cross-reactivity of *Neisseria* pili: investigations with type- and species-specific monoclonal antibodies. J. Gen. Microbiol. **129**:2761–2768.
35. **Yoshimura, F., T. Takasawa, M. Yoneyama, T. Yamaguchi, H. Shiokawa, and T. Suzuki.** 1985. Fimbriae from the oral anaerobe *Bacteroides gingivalis*: physical, chemical, and immunological properties. J. Bacteriol. **163**:730–734.
36. **Zak, K., J.-L. Diaz, D. Jackson, and J. E. Heckels.** 1984. Antigenic variation during infection with *Neisseria gonorrhoeae*: detection of antibodies to surface proteins in sera of patients with gonorrhoea. J. Infect. Dis. **149**:166–173.
37. **Zieg, J., and M. Simon.** 1980. Analysis of the nucleotide sequence of an invertible controlling element. Proc. Natl. Acad. Sci. USA **77**:4196–4200.