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Genetics

Pilus genes of *Neisseria gonorrheae*: Chromosomal organization and DNA sequence

(pathogenesis/phase variation/antigenic variation)

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ABSTRACT **We have mapped two regions of the *Neisseria gonorrheae* genome, *pilE1* and *pilE2*, which are involved in pilus expression. When the cells are in the piliated P^+ state, these two loci carry sequences necessary for pilin production. A silent locus, *pilS1*, also maps near *pilE1* and *pilE2*. *pilS1* contains structural gene information but lacks pilus promoter sequences. The pilus gene sequences in *pilE1* and *pilE2* are identical in strain MS11.**

The pilus is a predominant surface antigen of *Neisseria gonorrheae* and allows adhesion of the bacterium to various host cells (1–4). Pilus expression can be turned on and off at high frequency (phase variation), a condition that can be detected easily by differences in colonial morphology; piliated (P^+) cells are significantly more infective than nonpiliated (P^-) cells (5–7). Many pilin serotypes exist, and some gonococcal isolates can express more than one biochemically distinct pilin type (8–10). Recently, we have obtained evidence that a single clone expressing one pilin serotype can give rise to lines that express many antigenically variant pilin (unpublished data).

The fimbriate pilus structure is composed of identical subunits of ≈18,000 daltons. Pilin of different serotype all share a highly conserved strongly hydrophobic amino-terminal region, while their hydrophilic carboxyl-terminal domains are variable (11, 12). This latter region is the major target for the host immune response.

We have reported the isolation of two pilus gene copies from *N. gonorrheae* strain MS11 (13). A single gonococcal genome contains several pilus-related sequences, and transition from the P^+ to the P^- state, and vice versa, involves reversible reorganization of chromosomal elements (13). We report here the alignment of some of the pilus gene copies in a chromosomal map and an analysis of their physical and functional nature.

MATERIALS AND METHODS

Bacterial Strains. *N. gonorrheae* strain MS11 (13) was used throughout these studies. Recombinant plasmids were first transformed (14) into *Escherichia coli* strain GC1 (13) and subsequently transferred to strain DHI (14) for further analysis.

DNA Manipulations. Recombinant DNA technology and high-stringency Southern blotting were as described by Maniatis *et al.* (15). Restriction enzyme digestions and BAL-31 digestions from the *Hpa* I site of pNG1100 were done under conditions recommended by the vendor (New England Biolabs). *Hin*dIII linkers (Collaborative Research) were placed at the ends of the deleted DNA by the technique described by Maniatis *et al.* (15).

DNA Sequencing. The 5′ ends of the *Hin*dIII site from the BAL-31-generated clones and from the *Sma* I and *Cla* I sites of pNG1100 were sequenced as described by Maxam and Gilbert (16).

DNA Probes. DNA probes were labeled by using T4 polynucleotide kinase (New England Biolabs) as described by Maxam and Gilbert (16).

Plasmid Isolation. Plasmids were isolated by the procedure of Kupersztock and Helinski (17) and purified by passage through a Sepharose CL-6B column (Pharmacia).

RESULTS

Cloning and Chromosomal Mapping of Pilus Gene Sequences. The large number of pilus-related sequences in the gonococcal genome (13) complicated the alignment of pilus gene loci in a linear chromosomal map when Southern hybridizations alone were used. Applying a different strategy, we obtained recombinant clones containing pilus-related sequences by subcloning, from a gonococcal chromosomal library from strain MS11 (P^+), with pBR322 as a vector and a 700-base-pair (bp) *Hpa* I/*Bgl* I fragment of pNG1100 as probe (see the legend to Fig. 1). Of 60 independent clones, we concentrated on 3—pNG1312, pNG1711, and pNG1721—because they showed a particularly strong cross-hybridization to the probe under highly stringent conditions. These plasmids, which carry *Hin*dIII or *Bcl* I inserts of 5–11 kilobases (kb) (Fig. 1 *c* and *d*), were then deleted for the pilus-related sequences, resulting in subclones pNG1704, pNG1705, and pNG1706 (Fig. 1 *c* and *d*) that contain only the flanking sequences. pNG1704, pNG1705, pNG1706, and cloned pilus sequences then were used as probes in Southern blotting experiments with chromosomal MS11 DNA that had been digested with various combinations of restriction endonucleases. The hybridizations allowed us to map the regions surrounding the pilus genes within a range of 20–30 kb in the chromosome. Certain restriction fragments hybridized to more than one probe (Fig. 1*a*). This, together with restriction maps of the inserts, allowed us to draw a physical map that links three major chromosomal pilus gene loci within a distance of ≈80 kb (Fig. 1*b*). This preliminary map was recently confirmed by analysis of larger chromosomal inserts covering this region (Fig. 1*b*).

The 80-kb mapped region represents ≈5% of the total chromosome of *N. gonorrheae*, whose size is 1600 kb (18). This mapped region could only account for three of the eight *Cla* I fragments that had been shown to hybridize strongly to pilus sequences (13). Therefore, pilus-related sequences are not localized to one region but must occur over a wide area on the gonococcal genome.

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Abbreviations: kb, kilobase(s); bp, base pair(s); P^+, piliated; P^-, nonpiliated.

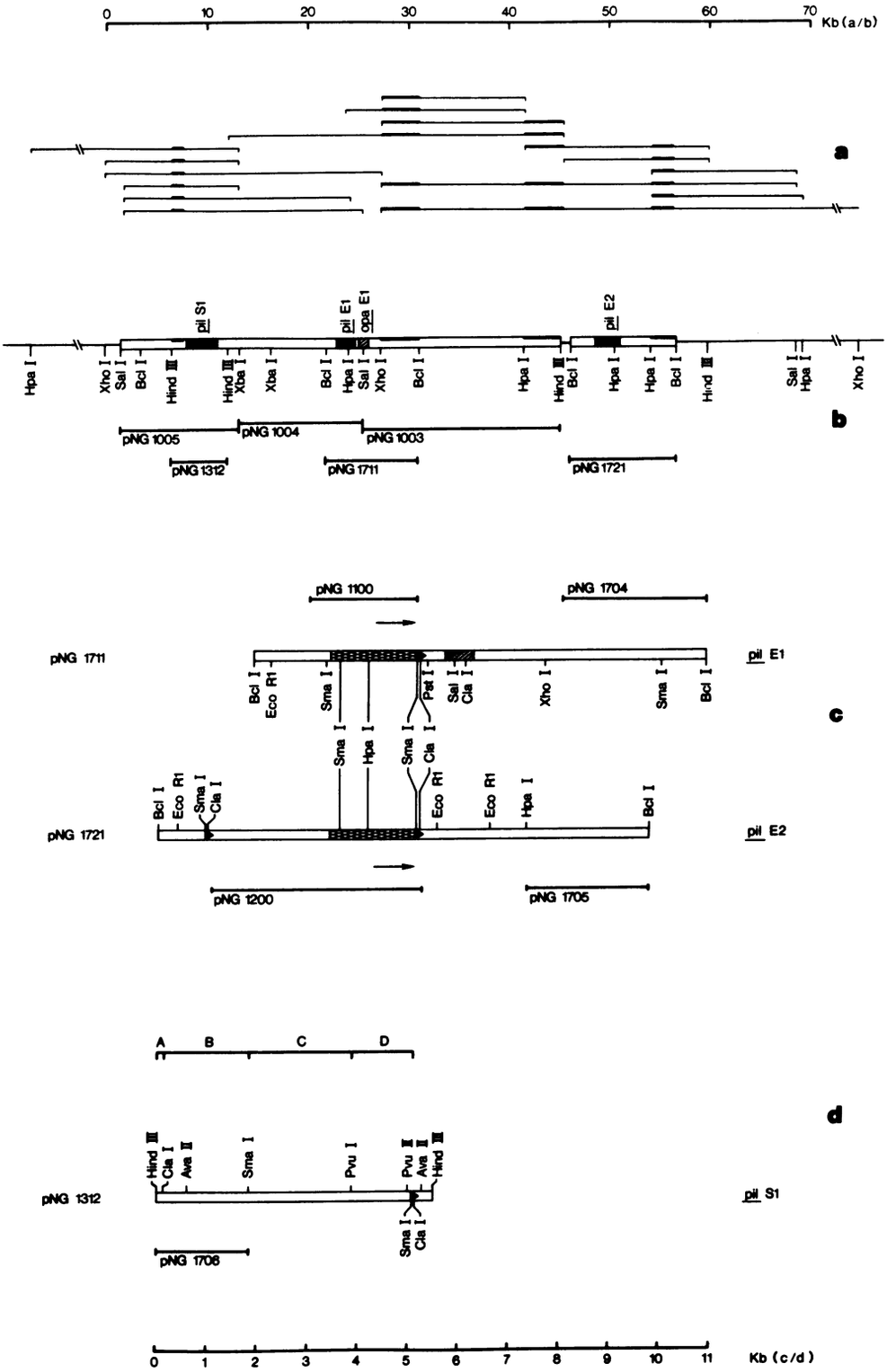

FIG. 1. Physical analysis of three major pilus gene loci and chromosome mapping of pilus gene loci. Mapping was accomplished by Southern blotting with unique gene sequences from various pilus clones (which map at the positions indicated by thickened lines at corresponding positions of *a* and *b* as hybridization probes of chromosomal DNA digested with a combination of restriction enzymes. Fragments that cross-hybridize can be aligned in a fashion as shown (*a*) and provide the basis for the restriction map (*b*). Some of the indicated fragments hybridize to more than one probe. (*b*) Chromosomal map of three major pilus gene loci (*pilE1*, *pilE2*, and *pilS1*) and of an opacity gene locus (*opa1*; ref. 18). The relative positions of these loci in the genome as determined by Southern hybridization experiments were confirmed by restriction analysis of cloned fragments from this region (indicated by lines beneath the map). ■, Areas that cross-hybridize to pilus gene probes; ▨, areas that hybridize to opacity genes. (*c*) Analysis of two expression loci. The *Bcl* I inserts of plasmids pNG1711 and pNG1721 representing *pilE1* and *pilE2*, respectively, share a region of complete homology (▤). This region contains the gene for pilin (arrow). Plasmid pNG1711 also harbors the *opaE1* locus (▨). The original pilus gene clones pNG1100 and pNG1200 (13) as well as subclones pNG1704 and pNG1705 of pNG1711 and pNG1721, respectively, which carry unique genome sequences, are aligned at the corresponding positions. ▶, Positions of 65-bp *Sma* I/*Cla* I fragments, which appear to be a characteristic component of pilus gene loci. (*d*) Analysis of a silent pilus gene locus. Plasmid pNG1312 carries a *Hin*dIII fragment containing the *pilS1* locus. Besides a 65-bp *Sma* I/*Cla* I fragment (▶), no apparent homology between this plasmid and the expression loci (*c*) is revealed by coarse restriction analysis. Subclone pNG1706 carries unique genome sequences.

Two Pilus Expression Sites Constitute the P$^+$ State of MS11 Gonococci. A detailed restriction analysis of plasmids pNG1711 and pNG1721 (Fig. 1*c*) identified their inserts as larger equivalents of the original clones expressing pilus gene, pNG1100 and pNG1200 (13). pNG1711 and pNG1721 also produced pilin in *E. coli* (data not shown). The two inserts, 9.9 and 10.2 kb, share a central homology of 1.7 kb within which is the 965-bp *Cla* I/*Hpa* I fragment previously shown to encode the actively expressing pilus gene (13). Although many genomic *Cla* I fragments hybridize to the pilus gene probes, only the 4.0-kb and 4.1-kb *Cla* I fragments show evidence of rearrangement during the P$^+$-to-P$^-$ switch (13). Subsequent mapping by Southern hybridizations showed that only these 4.0-kb and 4.1-kb *Cla* I fragments hybridized to the pilus-gene-flanking sequences of pNG1711 and pNG1721 (data not shown). In addition, only these two *Cla* I fragments were cleaved by *Hpa* I to generate 965-bp *Cla* I/*Hpa* I fragments homologous to the pilus gene probe (Fig. 2). Thus, there are two regions on the MS11 genome that actively express pilin. To distinguish these from regions that contain nonexpressed pilin-related sequences, we designate them expression locus 1 (*pilE1*) and expression locus 2

(*pilE2*), with *pilE2* being the leftmost on the map (Fig. 1*b*).

Our conclusion that pNG1711 and pNG1721 contain pilus-related expression loci is based on two assumptions. First, we assume that gonococcal genes are expressed equally well in *E. coli*. Since *N. gonorrheae* and *E. coli* are both Gram-negative bacteria, it is not unreasonable to expect gonococcal sequences to be recognized efficiently by the *E. coli* transcriptional and translational machinery. Indeed, in addition to the pilin gene, three other gonococcal genes, encoding the IgA protease (19), the opacity (PII) protein (20), and the proline biosynthetic pathway (21), also have been cloned and expressed in *E. coli*. Second, we assume that the P^+-to-P^- rearrangement affects actively expressing pilus genes. Blot-hybridization experiments using the 965-bp *Cla* I/*Hpa* I fragment as a probe against P^+ and P^- gonococcal RNA showed that transcription of the pilus-specific mRNA is abolished during the switch (unpublished data).

Whether *pilE1* and *pilE2* are or can be used simultaneously is not known at the moment. The inserts covering each of these two regions were obtained from the same chromosomal DNA preparation purified from cells derived from a single colony with the P^+ phenotype. Although it is very likely that *pilE1* and *pilE2* are both used concurrently, the possibility exists that the cells from which the DNA preparations were derived are mixtures that alternately express one or the other but not both.

Analysis of a Silent Pilus Locus. The third pilus gene locus that was cloned in plasmid pNG1312 (Fig. 1*d*) hybridized strongly with pilus structural gene sequences and much less with flanking sequences (data not shown). *E. coli* containing pNG1312 do not produce pilin, in contrast to those containing pNG1711 and pNG1721 (data not shown). By using subclones of the pNG1312 insert as probes in Southern hybridization experiments with gonococcal chromosome, this third locus was mapped to a 5-kb *Cla* I fragment (ref. 13; Fig. 1*d*). Unlike *pilE1* and *pilE2*, which mapped to 4.0-kb and 4.1-kb *Cla* I fragments, respectively (Fig. 1*b*), this third locus did not undergo rearrangement during pilus phase variation (13). A comparison of the restriction map of this third locus with the maps of *pilE1* and *pilE2* (Fig. 1 *c* and *d*) suggests that this locus does not contain an intact pilus structural gene. These data lead us to believe that this site is a silent locus, and we have designated it *pilS1*. A 73-bp *Sma* I/*Cla* I fragment occurs in the same orientation in *pilS1*, *pilE1*, and *pilE2*. In *pilE1* and *pilE2* the *Sma* I/*Cla* I sites occur 65 bp downstream from the translated region of the pilus gene (see Fig. 4). Whether the sequences in this region play a part in pilus expression remains to be seen.

Restriction fragments from a series of BAL-31-generated deletion subclones of the *Hpa* I/*Cla* I region of pNG1100 (see Fig. 4 and legend) were used as probes in Southern hybridization analysis of the *pilS1* region in pNG1312. *Hin*dIII/*Mst* III fragments from subclones BH2 and BH4, which are 170 and 240 bp in size, respectively, and contain pilus gene promoter sequences but not the ribosomal binding site (see Fig. 4), did not hybridize to the silent-region clone (Fig. 3, lanes c and d). A 250-bp *Hin*dIII/*Bgl* I fragment from BH5 (see Fig. 4) containing 5′ pilus coding sequences hybridized to the 2-kb *Sma* I/*Pvu* I C fragment and the 1.3-kb *Pvu* I/*Sma* I D fragment (Fig. 1*d*) within this silent-region insert (Fig. 3). A 350-bp *Bgl* I/*Sma* I fragment containing 3′ pilus coding sequences (see Fig. 4) also hybridized to the C and D fragment of pNG1312 (Fig. 3, lane a). DNA sequences of several variant-expressing pilin genes indicate that variability occurred in the region between the *Bgl* I site and the stop codon (unpublished data). Therefore, the latter two probes contain constant and variable pilus gene sequences, respectively. Thus, *pilS1* contains structural pilus gene information but not pilus promoter sequences found in *pilE1* and *pilE2*. Densitometer tracings of hybridization signals indicate that the variable region sequences occur 15–20 times more frequently than constant region sequences, suggesting that in some regions of pNG1312 the constant and variable region sequences are not colinear.

The variable region sequences detected in these experiments may not be exactly homologous to our 3′ pilin gene probe. Although our hybridization conditions were stringent, recent findings (unpublished data) have indicated that there are conserved as well as variable regions within the 3′ ends of expressed genes encoding variant pilin. Thus, there may be many copies of different variable regions within pNG1312.

Our previous work (13) has indicated that large sections of the gonococcal chromosome contain pilus-related sequences. Since *pilE1*, *pilE2*, and *pilS1* account for only a portion of this, many more pilus-related regions remain to be

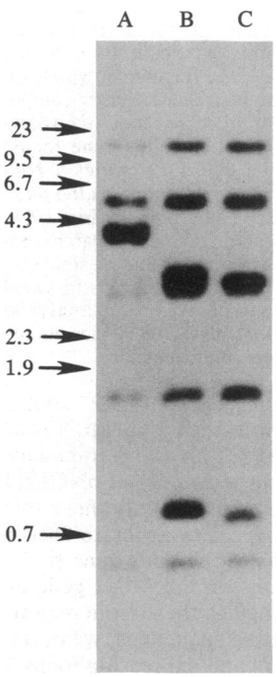

FIG. 2. Location of actively expressed pilus genes by Southern analysis. Lanes: A, P^+ chromosomal DNA digested with *Cla* I; B, P^+ chromosomal DNA digested with *Cla* I/*Hpa* I; C, P^- chromosomal DNA digested with *Cla* I. The probe is an insert from pNG1100 containing the entire pilus gene. *Hin*dIII fragments of DNA served as molecular size markers indicated in kb (arrows). Of all the *Cla* I genomic fragments that hybridized to pilus gene sequences, only the 4-kb *pilE1* and 4.1-kb *pilE2* fragments were digested by *Hpa* I to generate 3-kb and 1-kb fragments.

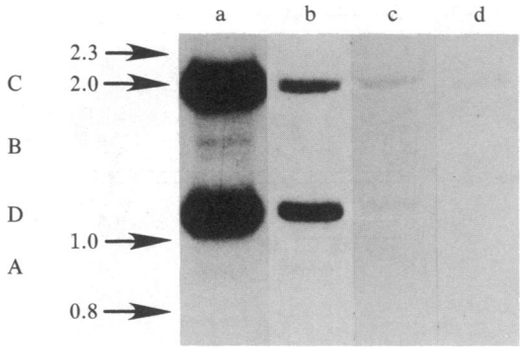

FIG. 3. Presence of pilus gene sequences in *pilS1*. pNG1312 DNA was cleaved with *Cla* I/*Sma* I/*Pvu* I/*Hin*dIII and blotted onto nitrocellulose filters for Southern analysis with probes containing 3′ pilus gene sequences within a *Sma* I/*Bgl* I fragment of pNG1100 (lane a), 5′ pilus gene sequences within a *Hin*dIII/*Bgl* I fragment of BH5 (see Fig. 4) (lane b), pilus promoter region sequences within a 240-bp *Hin*dIII/*Mst* II fragment of BH2 (see Fig. 4) (lane c), and pilus promoter region sequences within a 120-bp *Hin*dIII/*Mst* II fragment of BH4 (see Fig. 4) (lane d). Location of restriction fragments A–D within the pNG1312 insert is given above the map of this clone in Fig. 1*d*. The upper two size markers (in kb) are *Hin*dIII fragments; the lower two markers are ϕX174 *Hin*cII fragments.

examined. These uncharacterized regions also do not undergo rearrangement during the pilus phase switch and may contain other silent loci. Of 60 recombinant clones, those containing *pilE1*, *pilE2*, and *pilS1* were chosen for study because they showed the strongest hybridization signals to our pilus gene probe. Since highly stringent hybridization conditions were followed in our experiments, the uncharacterized recombinant clones giving weak signals most probably contain fewer pilin gene sequences. Whether they contain promoter sequences is not known at present.

Nucleotide Sequence of the Pilus Structural Gene. pNG1100 and pNG1200 inserts were sequenced by using the technique of Maxam and Gilbert (16). Because of a lack of good restriction sites for sequencing, BAL-31 deletions were made from the single *Hpa* I site of both plasmids. *Hin*dIII linkers were inserted at the ends of the deleted DNAs before religation. Deletion endpoints (Fig. 3) within the *Cla* I–*Hpa* I region as well as the *Cla* I and *Sma* I ends were labeled for sequencing.

Fig. 4 contains the DNA sequence of the region between the *Hpa* I and *Cla* I sites of pNG1100. In this area is a single open-reading frame, 498 bp in length, starting at position 335 and ending at position 833 near the *Cla* I site. A strong ribosome binding site A-G-G-A-G occurs seven bases upstream of the start codon. Primer extension studies using a synthetic oligonucleotide complementary to the 3′ untranslated region of pilus-specific mRNA placed the start of transcription at the guanosine at position 245 (unpublished data); five bases upstream of this putative transcription initiation site is a strong −10 sequence (T-A-T-A-A-T), although a classical −35 sequence is missing.

We also have determined a portion of the sequence of the pilin gene within the pNG1200 insert, covering the 3′ end of the coding sequence (variable region). From the *Sma* I site to the *Bgl* I site within the gene, the sequences in pNG1100 and pNG1200 are identical. pNG1100 and pNG1200 also show exact homology within a 200-bp region extending from the *Hpa* I site towards the pilin gene. Therefore, it is more than likely that both expression loci are expressing the same structural gene. This has been confirmed by sequence analysis of pilus-specific mRNA (unpublished results).

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  •BH1              16                  31                  46                  61
TTA ACG CGT AAA TTC AAA AAT CTC AAA TTC CGA CCC AAT CAA CAC ACC CGA TAC CCC ATG

•BH2              76                  91                  106                 121
CCA ATA AAA AAG TAA CGA AAA TCG GCA CTA AAA CTG ACA ATT TTC GAC ACT GCC GCC CCC

                  136                 151                 166          BH4 •  181
CTA CTT CCG CAA ACC ACA CCC ACC TAA AAG AAA ATA CAA AAT AAA AAC AAT TAT ATA GAG

                  196                 211                 226                 241
ATA AAC GCA TAA AAT TTC ACC TCA AAA CAT AAA ATC GGC ACG AAT CTT GCT TIA TAA IAC

  ⬇               256                 271                 286                 301
GCA GTT GTC GCA ACA AAA AAC CGA TGG TTA AAT ACA TTG CAT GAT GCC GAT GGC AAG CCC

Mst II  •BH5      316                 331                 346                 361
TGA GGC TTT CCC CTT TCA ATT AGG AGT AAT TTT ATG AAT ACC CTT CAA AAA GGC TTT ACC
                                                Met Asn Thr Leu Gln Lys Gly Phe Thr

                  376                 391                 406 •BH6            421
CTT ATC GAG CTG ATG ATT GTG ATC GCT ATC GTC GGC ATT TTG GCG GCA GTC GCC CTT CCC
Leu Ile Glu Leu Met Ile Val Ile Ala Ile Val Gly Ile Leu Ala Ala Val Ala Leu Pro

                  436                 451                 466                 481
GCC TAC CAA GAC TAC ACC GCC CGC GCG CAA GTT TCC GAA GCC ATC CTT TTG GCC GAA GGT
Ala Tyr Gln Asp Tyr Thr Ala Arg Ala Gln Val Ser Glu Ala Ile Leu Leu Ala Glu Gly

                  496                 511                 526                 541
CAA AAA TCA GCC GTC ACC GAG TAT TAC CTG AAT CAC GGC AAA TGG CCG GAA AAC AAC ACT
Gln Lys Ser Ala Val Thr Glu Tyr Tyr Leu Asn His Gly Lys Trp Pro Glu Asn Asn Thr

          Bgl I   556                 571                 586                 601
TCT GCC GGC GTG GCA TCC CCC CCC TCC GAC ATC AAA GGC AAA TAT GTT AAA GAG GTT GAA
Ser Ala Gly Val Ala Ser Pro Pro Ser Asp Ile Lys Gly Lys Tyr Val Lys Glu Val Glu

                  616                 631                 646                 661
GTT AAA AAC GGC GTC GTT ACC GCC ACA ATG CTT TCA AGC GGC GTA AAC AAT GAA ATC AAA
Val Lys Asn Gly Val Val Thr Ala Thr Met Leu Ser Ser Gly Val Asn Asn Glu Ile Lys

                  676                 691                 706                 721
GGC AAA AAA CTC TCC CTG TGG GCC AGG CGT GAA AAC GGT TCG GTA AAA TGG TTC TGC GGA
Gly Lys Lys Leu Ser Leu Trp Ala Arg Arg Glu Asn Gly Ser Val Lys Trp Phe Cys Gly

                  736                 751                 766                 781
CAG CCG GTT ACG CGC ACC GAC GAC GAC ACC GTT GCC GAC GCC AAA GAC GGC AAA GAA ATC
Gln Pro Val Thr Arg Thr Asp Asp Asp Thr Val Ala Asp Ala Lys Asp Gly Lys Glu Ile

                  796                 811                 826                 841
GAC ACC AAG CAC CTG CCG TCA ACC TGC CGC GAT AAG GCA TCT GAT GCC AAA TGA GGC AAA
Asp Thr Lys His Leu Pro Ser Thr Cys Arg Asp Lys Ala Ser Asp Ala Lys

                  856                 871                 886                 901
TTA GGC CTT AAA TTT TAA ATA AAT CAA GCG GTA AGT GAT TTT CCA CCC GCC CGG ATC AAC

Sma I             916                 931                 946                 961
CCG GGC GGC TTG TCT TTT AAG GGT TTG CAA GGC GGG CGG GGT CGT CCG TTC CGG TGG AAA

          Cla I
TAA TAT ATC GAT
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FIG. 4. DNA sequence of the *Hpa* I/*Cla* I fragment of pNG1100. The deduced amino acid sequence of MS11 pilin appears below the coding sequence starting at position 335. The −10 sequence and the ribosomal binding site are underlined. The arrow at position 244 indicates the start of pilus mRNA synthesis. Key restriction sites used for obtaining fragments for Southern hybridization probes are boxed. Dots above the various bases indicate the endpoint of BAL-31 deletions, where *Hin*dIII linkers were inserted. The designation for each of these deletion-generated subclones appears next to each endpoint. The boxed amino acids indicate residues predicted by the DNA sequence not present in the reported NH_2-terminal end of MS11 pilin.

The amino acid sequence of MS11 pilin, predicted from the DNA sequence, is shown in Fig. 4. Comparison of this sequence with that of purified pilin from the same strain (22) reveals that the primary translational product in MS11 contains an additional seven amino acid residues at the amino-terminal end (Fig. 4). These extra amino acid residues are likely to be the leader peptide needed for transport of the subunit across the bacterial membrane. All bacterial leader peptide sequences studied so far are at least 20 amino acids long and are distinctly hydrophobic (23). In contrast, the seven amino acids of the gonococcal "prepilin" contain a mixture of uncharged and nonpolar side chains. Since this is the first report of a gonococcal prepilin sequence, it is not clear whether this sequence is characteristic of all gonococcal leader sequences or whether it has a function specific for pilin export and assembly. Aside from these seven amino acids, the two sequences are identical.

DISCUSSION

We mapped two chromosomal loci in *N. gonorrheae* strain MS11, *pilE1* and *pilE2*, that are involved in pilin expression. Within these loci is the sequence information needed for pilin production. In MS11, at least the pilin gene is the same in both loci, and the direction of transcription is also the same. Both *pilE1* and *pilE2* undergo rearrangement during pilus phase variation (13). This rearrangement in many cases involves deletion of pilus sequences (unpublished data). A third region, a silent locus (*pilS1*), contains pilin structural gene sequences but not promoter sequences. *pilS1* maps to a 5-kb chromosomal *Cla* I fragment that does not undergo rearrangement during the phase switch. *pilE1*, *pilE2*, and *pilS1* are located within a 40-kb region of the gonococcal chromosome. In this context, note that we have recently mapped a region (*opa1*) close to *pilE1* that is important in the expression of the gonococcal opacity (Op or PII) protein (Fig. 3, lane b; ref. 21). Op, an outer-membrane protein with virulence properties, also undergoes phase and antigenic variation (24–27). The proximity of *opa1* to *pilE1* may not be entirely fortuitous, although there is as yet no indication that the Op and pilus variation systems are related.

pilE1 and *pilE2* also share some homology both upstream and downstream of the pilin gene sequence. The downstream homology is limited to a 65-bp sequence located between *Cla* I and *Sma* I sites. Southern-blot analysis and restriction-site distribution within *pilS1* give no indication of an intact structural pilin gene in this locus. However a 65-bp *Cla* I/*Sma* I fragment does occur in the extreme right-hand portion of this locus, in the same orientation as the *Cla* I/*Sma* I fragments in the *pilE1* and *pilE2* loci (Fig. 1 *c* and *d*). The role that this 65-bp region plays in pilin gene expression is not known at present. However, examination of the *pilE1* and *pilE2* loci in P^- cells shows that, in many cases, a deletion of pilin gene sequences occurs close to this 65-bp sequence (unpublished data). Nor do we know at present whether the majority of variant pilin gene sequences are derived from the *pilS1* locus.

This arrangement of expression and silent loci for pilus expression is reminiscent of the yeast mating-type interconversion system, where the *MAT* locus functions in the expression of the *a* or α gene, while silent copies of *a* and α are located elsewhere (28). However, unlike the silent *a* and α genes, the *pilS1* locus does not express pilin because it lacks pilin promoter sequences and not because of the presence of a repressor. Moreover, there appear to be more copies of the variable region pilus sequences than constant region sequences in *pilS1*. In this, *pilS1* is more analogous to the arrangement of immunoglobulin structural gene sequences, where tandem copies of variable regions of the Ig gene are separated from copies of the constant region sequences. To generate an intact, Ig-expressing gene with a particular specificity, intervening sequences in many cases are irreversibly deleted during B-cell maturation (29). We believe that no such irreversible deletion occurs during pilus phase and antigenic variation, since a single gonococcal cell expressing one pilus idiotype can express a different idiotype and subsequently switch back to express the original pilus idiotype (unpublished data).

The exact nature of the gonococcal pilus phase switch and antigenic variation remains to be determined. Both phenomena occur in a variety of prokaryotic and eukaryotic systems (30, 31). Antigenic variation is thought to comprise a serious challenge to the immune surveillance of the host. Since *N. gonorrheae* is amenable to genetic studies, it offers an exciting model system for studying the molecular basis of these two phenomena.

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