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Genetics

Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments

(recombination/phase variation/constant region/hypervariable region)

ELLYN SEGAL, PER HAGBLOM, H. STEVEN SEIFERT, AND MAGDALENE SO

Department of Molecular Biology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037

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ABSTRACT **The pilus is a major outer-membrane protein of *Neisseria gonorrhoeae* that undergoes phase and antigenic variation. In strain MS11 pilus expression is regulated at two expression loci on the chromosome, *pilE1* and *pilE2*, although many other regions contain silent pilin information. A comparison of variant pilin sequences has revealed that the gene can be divided into a constant, a semivariable, and a hypervariable region. We report here that complete pilin genes are found only at the expression loci. Silent constant and variable region pilin gene segments are located on separate and distinct restriction fragments, and the generation of a complete pilin gene within the expression loci is the result of multiple recombination events. Conserved sequences within and flanking the pilin gene are proposed to act as recombination sites during the gene conversion events needed to produce a functional pilin gene.**

The pilus of *Neisseria gonorrhoeae* is a fimbriate surface structure thought to mediate attachment of the bacterium to host (human) epithelial cells (1–3). It is composed of identical ≈18-kDa subunits (pilins) (4, 5). The pilus undergoes phase variation: at high frequencies, piliated (P^+) cells produce nonpiliated (P^-) variants and vice versa (6, 7). Pilus antigenic variation has been observed in two gonococcal strains in the laboratory and *in vivo* (8–10), and many different pilus serotypes have been identified in clinical isolates.

In strain MS11 two regions of the chromosome function as pilin expression sites (11–13), each similar in function to the *MAT* locus in yeast (14). These sites, termed *pilE1* and *pilE2*, are separated from each other by ≈20 kilobases (kb) of DNA. *pilE1* is located within a *Cla* I fragment of 4.0 kb, while *pilE2* is within a *Cla* I fragment of 4.1 kb. The expression sites are defined as regions that contain complete structural pilin genes and promoter sequences. Although both sites can be used for pilin expression, only one is needed. When the cell is in the P^+ state, either one or both expression sites contain an intact pilin gene (13). In the majority of cases, a P^+-to-P^- switch results in the deletion of pilin information from either one or both of these expression sites. The deletions vary in size and occur between any two of several directly repeated sequences present in the expression locus (13). Numerous other regions of the gonococcal genome contain pilin-related sequences. One of these silent regions, *pilS1*, maps 15 kb away from *pilE1* and contains pilin sequences but not an entire pilin gene (12). Pilin-related sequences are also present immediately upstream of the expression sites (12).

We have derived lines of variants of strain MS11 that differ only in pilus expression (refs. 10 and 13; see Fig. 1 *Upper*). We have used the technique of primer extension sequencing of pilin transcripts to determine the sequences of pilin genes actively expressed by P^+ variants within these lines of cells. Our data have brought to light several unique features of gonococcal pilus antigenic variation. (*i*) The MS11 progenitor can give rise to many piliated derivatives expressing pilins that differ in sequence and that contain new epitopes. (*ii*) Certain pilin genes are preferentially expressed by strain MS11. (*iii*) The pilin gene can be divided into constant, semivariable, and hypervariable regions. The semivariable region is characterized by transitions and transversions causing single amino acid changes. The hypervariable region contains in-frame insertions and deletions of up to four amino acids, along with single amino acid changes. (*iv*) There can be mixing and matching of semivariable and hypervariable gene segments in an expressed pilin gene. (*v*) Pilin sequence, once removed from the expression loci during the P^+-to-P^- switch, can be reexpressed in a subsequent piliated variant.

The availability of sequence data for a large number of actively expressed variant pilin genes allows us to examine the organization of the silent variant and constant pilin gene copies and the events that lead to pilin antigenic variation. We have chosen to study a hypervariable pilin sequence expressed by several members of one well-defined line of MS11 variants. Our results indicate that copies of a complete pilin gene are present only at the two expression sites. Silent loci contain either constant or variable region pilin sequences, unlike silent variable surface glycoprotein (VSG) genes in *Trypanosoma brucei*, which are complete (15). In addition, the pilin signal sequence is present in its entirety only in the expression sites. This arrangement of constant and variable pilin sequences is reminiscent of that of immunoglobulin gene segments in higher eukaryotes. However, while a complete immunoglobulin gene is generated by an often irreversible deletion of intervening sequences, a complete pilin gene is assembled by gene conversion-like events. To our knowledge, this complex assembly of genetic information has not been observed in prokaryotes until now.

MATERIALS AND METHODS

Strains and Media. *Neisseria gonorrhoeae* strain MS11 was used throughout the study and cultured as described (13). Phenotypes were as described (13).

DNA Isolation and Restriction Digestion. Gonococcal DNA isolation was done as described (13). Restriction digestion was as recommended by the manufacturers (New England Biolabs, Boehringer Mannheim).

Southern Hybridization. Agarose gels (0.67%) in Tris acetate buffer were used throughout the study. Preparation for transfer was as described (13), and DNA was transferred to aminobenzyloxymethyl paper overnight as recommended by the manufacturer (Schleicher & Schuell). Filters were washed in 0.5 M NaOH for 30 min and prehybridized for 30 min in 6× NET buffer (1× NET = 0.15 M NaCl/0.03 M

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Abbreviations: P^+, piliated; P^-, nonpiliated; kb, kilobase(s); C, constant; SV, semivariable; HV, hypervariable; bp, base pair(s); VSG, variable surface glycoprotein.

Tris·HCl, pH 8/1 mM EDTA), 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin, 0.5% Nonidet P-40, and 50 μg of salmon sperm DNA (Sigma) per ml. Kinase-treated oligonucleotide probes were added to the filter and incubated overnight. Probes SP6, ES1a, and ES12 were hybridized at 23°C; probe SP3, at 37°C; probes 4, ES10, ES11, and 1, at 42°C; and probe ES13, at 65°C. Filters were washed at hybridization temperature in 0.3 M NaCl/0.03 sodium citrate, pH 7/0.1% $NaDodSO_4$ for low stringency and 0.015 M NaCl/0.0015 M sodium citrate, pH 7/0.1% $NaDodSO_4$ for high stringency. Films (Kodak XAR-5) were exposed at −70°C with intensifying screens. Removal of probes from filters was done as suggested by manufacturers.

Synthesis of Oligonucleotides. Oligonucleotides were synthesized on an Applied Biosystems 380-A DNA synthesizer using solid-phase/phosphoamidite chemistry.

RESULTS

Analysis of Silent Pilin Gene Segments. Lines of phase variants of the original MS11 P^+ isolate have been described in detail elsewhere (13). Nomenclature for these variants is described in Fig. 1. Each line is given a number; the original P^+ is designated A. Its P^- derivative is B; the first generation P^+ revertant is C. We have synthesized oligonucleotides specific for several regions of the pilin gene expressed by the original MS11 isolate and also for genes expressed by its laboratory-derived variants (Fig. 1). These regions include the signal sequence, the constant, the semivariable, the hypervariable, and the conserved 3′ untranslated region of the pilin gene. The locations within the pilin gene of the synthetic sequences are indicated in Fig. 2. Sequences of the oligonucleotides are listed in Table 1. These probes were used in Southern hybridizations under stringent conditions against total genomic DNA from members of two lines of pilus phase variants. A single filter was used for all oligonucleotide probes. After each hybridization the probe was removed and the filter was rehybridized with a new probe. A summary of the silent loci examined below appears in Table 2. The boundaries of each locus are defined in terms of its location within a *Cla* I fragment and, as such, the genetically defined loci may differ.

Constant Region. DNA from all variants was digested with *Cla* I. Fig. 3 *a–c* shows DNA from variants of line 3; Fig. 3 *e–g* shows DNA from variants of line 2. *Cla* I fragments containing *pilE1* and *pilE2* are indicated in the margin. We have shown (10) that the first 140 base pairs (bp) of the pilin structural gene are constant. Probe 4 is specific for an area well within this region (Fig. 2) and hybridized to 1.2-kb (*pilS7*) and 3.1-kb (*pilS4*) *Cla* I fragments in all of the variants (Fig.

	A	B	C	D	E	F	G	H	I
Piliation	+ →	- →	+ →	- →	+ →	- →	+ →	- →	+
Line 3 Expression of									
ES11	-	-	+	-	-	-	+	-	+
ES12	-	-	-	-	+	-	-	-	-
ES10	-	-	+	-	+	-	-	-	-

FIG. 1. Specificity of oligonucleotide probes homologous to pilin expressed by members of line 3 variants. The state of piliation of each member is indicated by a "+" if cells are piliated and by a "−" if they are nonpiliated. (*Upper*) Letters of the alphabet are used to designate each variant. (*Lower*) Designation of oligonucleotides used for hybridization studies appear in the left-hand column; "+" indicates that the variant appearing above is expressing a pilin gene with sequences homologous to the oligonucleotide. The pilin genes of line 3 members were determined previously (10). The location of the ES10, ES11, and ES12 sequences within the pilin gene is shown in Fig. 2; the sequence of each oligonucleotide is listed in Table 1.

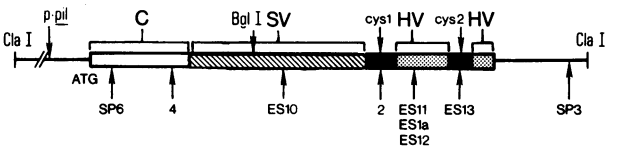

FIG. 2. Schematic diagram of the pilin gene and location of the oligonucleotide sequences within the gene. The constant region (C) is represented by a clear box; the semivariable region (SV), by a hatched box; and the hypervariable region (HV), by a stippled box. Solid black boxes denote the *cys1* and *cys2* conserved regions. *pil* indicates the location of the pilin promoter.

3 *a* and *e*). It also hybridized to the undeleted expression sites.

We have reported (13) that a P^+-to-P^- phase switch often results from a deletion event that can remove the entire pilin structural gene from *pilE1*, *pilE2*, or both. The deletions vary in size. In P^- variants 2B and 2D (members of line 2), the deletion is ≈1 kb. The 4.0- and 4.1-kb *Cla* I fragments containing *pilE1* and *pilE2* are now 3.0 and 3.1 kb, respectively. Although the entire structural gene has been removed from *pilE1* and *pilE2* in these variants, the 3.0- and 3.1-kb *Cla* I fragments still hybridized with the complete pilin gene probe (13). This residual homology is due in part to the presence of silent pilin constant region sequences. A comparison of the blotting patterns of line 2 P^+ variants A and C with P^- variants B and D shows that constant region probe 4 hybridized to the residual 3.0-kb *Cla* I fragment in variants B and D. In addition, the 3.1-kb *Cla* I fragment in these P^- variants increased in intensity, indicating that the deleted 4.1-kb *Cla* I fragment previously containing *pilE2* cross-reacted with the constant region probe.

Semivariable Region. We have reported (10) the sequences of pilin genes expressed by laboratory-derived P^+ variants. Fig. 3*b* illustrates a Southern blot involving chromosomal DNA from line 3 variants and a probe (ES10) specific for a semivariable region expressed only by line 3 variants 3C and 3E (see Fig. 1) (10). ES10 hybridized to all *Cla* I fragments recognized by a complete pilin gene probe except the 3.1-kb fragment encoding the silent constant region and the 6.4-kb fragment containing silent hypervariable sequences (see below). Additional copies of this semivariable sequence are present upstream of the expression sites (12); therefore semivariable probe ES10 hybridizes to expression loci in all variants, whether that sequence is expressed or not.

Hypervariable Region. Hypervariable region probe ES11 contains sequences specific to the pilin gene expressed by variants 3C, 3G, and 3I (10) (Fig. 2). In Southern hybridizations with *Cla* I-digested chromosomal DNA from line 3 variants, probe ES11 hybridized to a 1.8-kb *Cla* I fragment (*pilS6*) in all variants (Fig. 3*c*). It also recognized the *Cla* I fragment containing the expression site in the appropriate variants. Hypervariable probe ES12, specific for the 3E variant, hybridized to 1.8- (*pilS6*) and 6.4-kb (*pilS3*) *Cla* I fragments in all variants (Fig. 3*g*). As discussed above, silent constant region sequences are only found on *Cla* I fragments of 1.2 and 3.1 kb (*pilS7* and *pilS4*, respectively) and upstream of the expression sites. These data indicate that the silent constant and silent hypervariable pilin gene segments are separated and distinct from each other (Table 2).

Within an intact expression site pilin gene, the restriction enzyme *Bgl* I separates the 5′ constant region from the 3′ variable region (Fig. 2). Hybridization of *Cla* I- and *Bgl* I-digested genomic DNA with a hypervariable-specific probe would provide information specifically concerning the arrangement of the hypervariable segment in the expression and silent loci. We chose to use probe ES11, the hypervariable sequence specifically expressed by line 3 variants 3C, 3G, and 3I. Fig. 3*d* shows that ES11 hybridized to a 2-kb *Cla*

Table 1. Oligonucleotide probes for hybridization studies

Probe	Specificity	Sequence	Sequence of original pili
SP6	Signal sequence	5' GCC TTT TTG AAG GGT ATT CAT 3'	
4	Constant	5' GCC CGC GCG CAA GTT 3'	
ES10	Semivariable	5' TGC GAC CGT AAC GCT 3'	5' TTT AAC TTC AAC CTC 3'
2	*cys1*	5' TGG TTC TGC GGA CAG 3'	
ES11	Hypervariable 3C, 3G, 3I	5' GAC GGC TTT GTC GCG 3'	5' GTC GTC GTC GGT GCG 3'
ES12	Hypervariable 3E	5' TTT GCC GGT GGT 3'	5' TTT GGC GTC GGC 3'
ES1a	Hypervariable 2G	5' CGT CGA AGT CAC C 3'	5' CGT CGT CGG TGC G 3'
ES13	*cys2*	5' TAT CGC GGC AGG TTG ACG GCA GGT GCT TGG TGT CGA TTT 3'	
SP3	*Sma* repeat	5' CCG GAA CGG ACG ACC C 3'	

The name, specificity, and sequence of each probe appear in the first three columns. Sequences from the original MS11 pilin gene are in the right-hand column. Differences in sequence between a variant oligonucleotide and the original MS11 pilin gene are indicated by a dot over the appropriate nucleotide. A line in this column indicates complete homology between the probe and the original MS11 pilin sequence.

I/*Bgl* I fragment from the expression site. However, sequence data of the expression site pilin genes of 3C, 3G, and 3I has shown that each variant contains the ES11 sequence within a 600-bp *Cla* I/*Bgl* I fragment (10). This dichotomy is explained by the presence in *N. gonorrhoeae* of the methylase *Ngo* IV, which methylates the sequence 5' GC^{m}CGGC 3' (16). The *Bgl* I sequence within the pilin gene in the expression site is 5' GCCGGCGTGGC 3'. This sequence is methylated when present in the gonococcus, and the *Bgl* I restriction enzyme cannot cut at the site.

The ES11 hypervariable region probe recognized a single *Cla* I silent locus of 1.8 kb (*pilS6*) in all line 3 variants (Fig. 3*c*). Results obtained from the above experiment show that *pilS6* in variants 3A and 3B, which do not express the ES11 sequence, appears to contain multiple copies of the ES11 sequence (Fig. 3*d*, lanes A and B). In contrast, there appears to be only one copy of ES11 in the *pilS6* locus in variant 3C, which actively expressed ES11 (Fig. 3*d*, lane C). The change in hybridization pattern observed among variants of line 3 could be the result of two mechanisms: a change in copy number of the ES11 sequence within the silent locus or differential methylation by *Ngo* IV because of the degeneracy of the recognition sequence of *Bgl* I (5' GCCNNNNNGGC 3').

To investigate the mechanism(s) involved, *pilS6* from variants 3B and 3C were cloned into the polylinker region of pHSS6 (17) and transformed into *Escherichia coli* HB101. Kanamycin-resistant transformants were screened with probe ES11, and positives were isolated. Clones pB3-12 (from variant 3B) and pC3-18.7 (from variant 3C) were digested with *Cla* I/*Bgl* I to release the insert and probed with ES11 in a Southern blot. Results (Fig. 4) show that only one DNA fragment of ≈550 bp from each insert contains ES11-specific sequences. However, hybridization of probe ES11 to *Cla* I/*Bgl* I-digested genomic DNA of variant 3B identified more than one fragment containing the ES11 sequence within *pilS6* (Fig. 3*d*, lane B). Variant 3C, which expresses hypervariable sequence ES11 and thus contains a copy of the ES11 sequence in its expression site pilin gene, has within its *pilS6* loci a single *Cla* I/*Bgl* I fragment of 1 kb, which hybridized to prove ES11 (Fig. 3*d*, lane C). Therefore, among members of line 3, the change in hybridization patterns observed for prove ES11 is due to differential methylation by *Ngo* IV and not a change in copy number of the ES11 sequence in the silent locus. Furthermore, there was no decrease in the number of copies of the ES11 hypervariable sequence in the silent loci when a copy of ES11 appeared in an expression site pilin gene. Our results suggest that differential methylation by *Ngo* IV may also play a role in the process of pilin antigenic variation.

Table 2. Location of silent pilin gene segments within the gonococcal genome

Pilin loci	*Cla* I fragment, kb	Presence of pilin gene segments					
		C	SV	HV	*cys1*	*cys2*	*Sma* repeat
pilS2	6.8	−	+	+	+	+	+
pilS3	6.4	−	−	+	+	+	+
pilS1	5.0	−	+	−	+	+	+
pilE1	4.1	+	+	−	+	+	+
pilE2	4.0	+	+	−	+	+	+
pilS4	3.1	+	−	−	+	+	+
pilS5	3.0	−	+	−	+	+	+
pilS6	1.8	−	+	++	+	+	+
pilS7	1.2	+	+	−	+	+	+

The left-hand column lists the silent loci and the two expression loci. The size of each locus, as defined by its *Cla* I borders, is shown in column two. Columns 3–8 identify segments of the pilin gene present within the various loci, indicated by a "+". In the column marked HV, each plus represents a unique hypervariable segment (see text). As this table represents only silent gene copies, the segments listed for *pilE1* and *pilE2* do not include the expression site pilin genes. C, constant, SV, semivariable.

Signal Sequence. A complete copy of the pilin signal sequence is present only in the expression loci. Fig. 5 *a* and *b* show the results of hybridizing a pilin signal sequence probe (SP6) (12) to total genomic DNA from line 3 variants under high- and low-stringency conditions. Probe SP6 contains the entire sequence for the seven-amino acid signal (see Table 1). Under high-stringent conditions (Fig. 5*a*), SP6 hybridized only to the expression loci in either P^+ or P^- variants. Under low-stringency conditions (Fig. 5*b*), SP6 recognized additional *Cla* I fragments of 5.0, 8.4, and 15 kb in the DNA from all variants. When SP6 was used to probe an *E. coli* clone of *pilS6* (a 5.0-kb *Cla* I fragment), the presence of partial homology to the signal sequence within that locus was detected (data not shown). These results suggest that the coding region for the signal sequence may be split in the silent loci, although the possibility that the weak hybridization signals represent partial homology between the probe and genomic DNA cannot be excluded. Our data show that gonococcal signal sequences and constant region sequences are not present together in the silent loci of the gonococcal genome.

Conserved Regions. In addition to the constant 5' end of the pilin structural gene sequence, we have identified two other highly conserved regions within the gene and one downstream of the pilin stop codon (10, 12). The latter sequence is located 110 bp downstream of the pilin stop codon and spans the recognition site for the *Sma* I enzyme (probe SP3, Fig. 2). Southern hybridizations using SP3 (Fig. 3*f*) show this sequence is present in the genome where other pilin sequences are found (the ES12 hypervariable-specific 6.4-kb *Cla* I

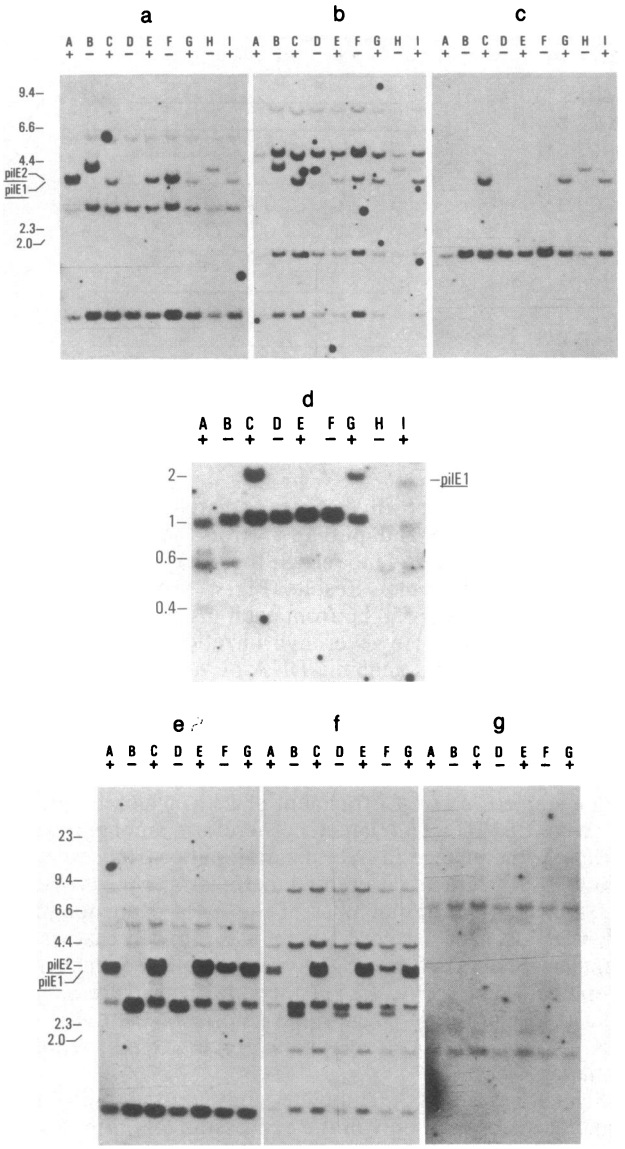

FIG. 3. Southern blot analysis of silent pilin sequences within the chromosome of line 3 (*a–c*) and line 2 (*e–g*) phase variants. Chromosomal DNA was digested with *Cla* I. Oligonucleotides used as probes are: constant region, probe 4 (*a*); semivariable region, probe ES10 (*b*); hypervariable region, probe ES11 (*c*); constant region, probe 4 (*e*); *Sma* repeat, probe SP3 (*f*); hypervariable region, probe ES12 (*g*). The locations of *pilE1* and *pilE2* are noted. *d* shows a *Cla* I/*Bgl* I digest of line 3 variants blotted with hypervariable probe ES11. All hybridizations were done under high-stringency conditions. Sizes are shown in kb.

fragment is visible with a longer exposure of the filter). The SP3 sequence, located at the 3′ end of the expression loci, is present in silent loci that harbor the 5′ segment of a pilin gene

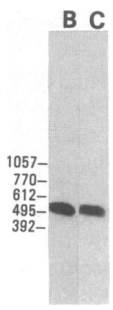

FIG. 4. Southern blot analysis of silent hypervariable locus clones. Clones pB3-12 and pC3-18.7 containing the ES11-specific 1.8-kb silent locus from variants 3B (lane B) and 3C (lane C), respectively, were subjected to restriction enzyme digests to remove their inserts from the vectors and subsequently digested with *Bgl* I. The DNAs were separated in a 1.4% agarose gel, transferred to a nitrocellulose filter, and probed with ES11. Lane B contains pB3-12 DNA; lane C contains pC3-18.7 DNA. Size markers used are ϕX174 replicative form II DNA digested with *Hinc*II.

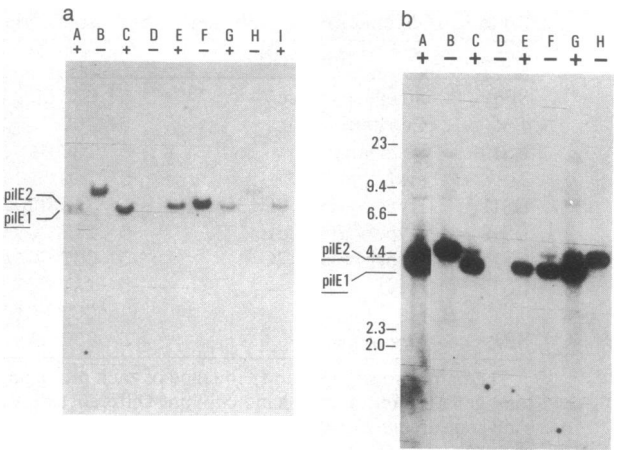

FIG. 5. Southern blot analysis of line 3 phase variants using a pilin signal sequence-specific probe. Chromosomal DNA from line 3 variants was digested with *Cla* I and probed with SP6. (*Left*) Probe SP6 was hybridized to the filter at 42°C and the filter was washed at 42°C under high-stringency conditions. (*Right*) Hybridization was at room temperature, and the filter was washed at the same temperature under low-stringency conditions. See Fig. 2 for the location of the sequence in the pilin gene and Table 1 for its sequence.

but do not contain 3′ variable sequences (Table 2).

Two internal conserved regions, termed *cys1* and *cys2* (10), are located in the variable portion of the coding sequence (Fig. 2). These DNA sequences encode a series of conserved amino acids within the pilin that probably play crucial structural and/or functional roles. Oligonucleotide probes containing the *cys1* and *cys2* sequences, 2 and ES13, respectively (Table 1), hybridized to all *Cla* I fragments that contain pilin sequences, producing a Southern blotting pattern identical to that produced by the *Sma* I repeat probe SP3 (Fig. 3*f*; also see above). Thus, silent loci containing constant, semivariable, or hypervariable pilin sequences also contain the *cys1*, *cys2*, and SP3-specific sequences (see Table 2). These conserved regions could provide homology for pairing of silent loci DNA involved in the recombination events that lead to pilin antigenic variation.

DISCUSSION

The pilin of *Neisseria gonorrhoeae* undergoes antigenic variation. A comparison of expressed variant pilin genes shows that the gene can be divided into a 5′ constant region, a middle semivariable region, and a 3′ hypervariable region. We have observed that certain P^+ variants of the MS11 strain express pilin genes with unique hypervariable region sequences. In experiments presented here, we have used synthetic oligonucleotides containing such unique sequences as well as oligonucleotides containing other pilin gene sequences in Southern hybridizations to examine the chromosomal arrangement of silent pilin loci. Our results, summarized in Table 2, show that a complete copy of the pilin gene occurs only at the expression sites *pilE1* and *pilE2*. Other regions of the chromosome, the silent loci, contain only portions of the pilin gene. *pilS4* and *pilS7* contain 5′ constant pilin gene sequences but not 3′ hypervariable sequences. *pilS3* and *pilS6* contain two different hypervariable pilin sequences but not 5′ constant pilin sequences. Experiments using a hypervariable-specific probe whose sequence is expressed by some members of line 2 (Table 1, probe ES1a) identified a third silent hypervariable locus within the gonococcal genome (*pilS2*).

All silent pilin loci contain *cys1*, *cys2*, and *Sma* repeat sequences—three regions of the pilin gene 3′ of the constant

region highly conserved among the variants. The sequence encoding a complete pilin signal sequence is present in its entirety only in the expression site. Our data indicate that silent pilin loci in the gonococcal chromosome contain truncated pilin gene segments and that silent constant and silent hypervariable pilin sequences are located on separate and distinct restriction fragments.

Gonococcal pilin expression undergoes phase variation. A P^+-to-P^- switch is often characterized by deletion of the entire pilin gene from *pilE1*, *pilE2*, or both (13). In variant 2B, the pilin gene in both *pilE1* and *pilE2* has been deleted. 2C, the P^+ revertant of 2B, has regained the pilin gene in both expression sites (13) (Fig. 3*e*, lanes B and C). However, neither the silent constant nor the silent hypervariable loci show any evidence of loss or gain of sequence during the P^--to-P^+ switch in any of the variants examined. The reestablishment of pilin gene sequences in these deleted expression sites can only occur by a gene conversion event in which pilin information is copied from the silent loci and transferred into the deleted expression site. The formation of a complete pilin gene in a deleted expression site during a P^--to-P^+ switch would require multiple recombination steps involving several silent loci and the expression locus. Providing the homology needed for initial base pairing between any two loci are the *cys1*, *cys2*, and *Sma* repeat sequences, which are present in all of the silent constant and hypervariable loci examined so far. In addition, the *Sma* repeat is present in the expression site loci of all P^+ and P^- variants studied, whether they are deleted of pilin structural gene sequences or not (13).

Some P^- variants have retained an intact expression site (13). A P^--to-P^+ switch in several of these variants does not restore the deleted expression site. Instead, the existing expression site is reactivated and used for producing pilin. Such P^+ derivatives express pilin antigenically different from that produced by its progenitor (10). The placement of a variant pilin gene sequence from the silent locus into an intact expression site could occur by a simple double crossover event, with exchange of pilin information between the two loci. Our data do not argue against this mechanism. However, in at least one case, we have observed that the placement of a variant pilin gene sequence into an expression site has occurred by gene conversion. Our studies of variants of line 3 show that the ES11 hypervariable sequence is expressed by variant 3C and not by its progenitors 3A (P+) or 3B (P−) (Fig. 3*d*). The P^- (3B)-to-P^+ (3C) switch places the ES11 variant sequence into the expression site. However *pilS6*, the silent locus containing the ES11 sequence, contains only one copy of the ES11 sequence in variants 3B and 3C. Variant 3C contains an additional copy of ES11 in the expression site. The appearance of an extra copy of ES11 in the expression site could only have resulted from a copy/insertion mechanism. The fate of the pilin gene being replaced by the ES11 sequence is not known.

Antigenic variation of the VSG gene in African trypanosomes has been postulated to occur by a combination of gene conversion and crossover mechanisms (15, 19). Gene conversion has been documented to occur for the mating-type switch in *S. cerevisiae* (14), allowing an *a* or α gene to be placed into the expression locus, *MAT*. While this process may be similar to what we have observed in the gonococcus, the silent mating type genes in yeast and certain VSG genes in *T. brucei* are present as complete copies in their respective silent loci. In *N. gonorrhoeae*, silent pilin loci contain truncated pilin sequences. This arrangement of silent constant and hypervariable pilin sequences is reminiscent of the arrangement of immunoglobulin constant and variable gene segments in lymphoid cells (18) and of the arrangement of certain silent VSG genes in *Trypanosoma equiperdum* (unpublished data).

The number of silent pilin variant sequences in the gonococcal genome is not known. However, recent sequence data on one silent locus (20) shows that within a 2-kb segment of DNA are five variant pilin gene sequences, devoid of constant region sequences, arranged in directly repeated fashion. These data support our observation that the constant and hypervariable gene segments are located in different regions of the chromosome. It is theoretically possible for recombination to occur any time between any two silent variable pilin sequences in the chromosome, resulting in the generation of new pilin sequences and ultimately new pilin epitopes. Thus, the capacity of the gonococcus to undergo pilus antigenic variation may be quite extensive.

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