Genetic Analysis of Variant Pilin Genes from *Neisseria gonorrhoeae* P9 Cloned in *Escherichia coli*: Physical and Immunological Properties of Encoded Pilins

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A series of genomic DNA fragments that encode gonococcal pilins from four well-characterized pilus variants of *Neisseria gonorrhoeae* strain P9 have been cloned in *Escherichia coli* K12. At least nine classes of cloned P9 pilin genes have been identified on the basis of restriction mapping of cloned pilin-encoding DNA and physical and immunological analysis of expressed pilin proteins. Each antigenic variant of strain P9 possesses many genomic segments of pilin gene information, although our results suggest that strain P9 contains only a single pilin-expressing (*pilE*) locus.

INTRODUCTION

The pilus of *Neisseria gonorrhoeae* is a major adhesin and pathogenicity determinant that can vary antigenically within and between strains (Pearce & Buchanan, 1978; Swanson, 1973). Pilus antigenic variation occurs both *in vitro* and *in vivo* (Lambden *et al.*, 1980, 1981; Zak *et al.*, 1984). Such variation in a major surface antigen may play a role both in the evasion of host defence mechanisms and in the sequential colonization of different tissue types during the course of an infection (Heckels, 1986).

The expression of pili by *N. gonorrhoeae* is subject to modulation by two distinct, but possibly related, genetic mechanisms: a phase variation in which pilated (P⁺) cells can become non-piliated (P⁻) and vice versa, and antigenic variation, which results in alteration of the biochemical and immunological properties of pilin, the homopolymeric protein subunit of pili (Meyer *et al.*, 1982; Saunders, 1986; Segal *et al.*, 1985; Swanson *et al.*, 1985). Production of pili by *N. gonorrhoeae* is dependent on the presence of at least one intact pilin-expressing (*pilE*) locus within the gonococcal genome (Meyer *et al.*, 1982, 1984; Swanson *et al.*, 1985). *N. gonorrhoeae* MS11, the strain most frequently utilized for genetic studies on piliation, has given rise to several distinct evolutionary variants, including strain MS11_{ms}, whose genome contains two *pilE* copies (Meyer *et al.*, 1984) and strain MS11_{mk}, whose genome contains only a single *pilE* locus (Swanson *et al.*, 1985). Phase variation from P⁺ to P⁻ may be effected by chromosomal rearrangements that give rise to deletions of all or part of a *pilE* locus (Segal *et al.*, 1985; Swanson *et al.*, 1985). In strains containing a single *pilE* gene, deletion of this locus will lead to an irreversible P⁺ to P⁻ switch and the generation of non-reverting P⁻ bacteria (Bergstrom *et al.*, 1986; Swanson *et al.*, 1985). However, in strains containing two *pilE* loci, the deleted gene can be repaired by recombination with the additional intact *pilE* copy (Segal *et al.*, 1985). Further mechanisms, not requiring deletion of *pilE* and producing gonococci with reverting P⁻ phenotypes, are also involved in piliation control (Bergstrom *et al.*, 1986; Swanson *et al.*, 1985).

Antigenic variation in gonococcal pili arises by alteration of the pilin-coding sequences at the *pilE* locus. Silent pilin sequences, representing fragments of the intact pilin gene, are widely

distributed throughout the chromosome of *N. gonorrhoeae* MS11_{ms} (Haas & Meyer, 1986; Meyer *et al.*, 1984). Data obtained from sequencing variant pilin-specific mRNA species support a model of pilus antigenic variation that operates by intragenic recombination at *pilE* loci, involving previously unexpressed pilin sequences (Hagblom *et al.*, 1985).

Studies so far on the genetic mechanisms of antigenic variation in gonococcal pili have concentrated on *N. gonorrhoeae* strain MS11. However, the biochemical and immunological properties of variant pili produced by this strain have been poorly characterized. In contrast, four well-characterized isogenic variants of *N. gonorrhoeae* P9 (designated P9-2, P9-20, P9-35 and P9-37) have been isolated, each of which produces pili that are immunologically distinct from, but related to, those of the others (Lambden *et al.*, 1981). In this paper we report the cloning of pilin-coding sequences from these variants of strain P9 and describe the physical and immunological properties of the pilins they encode.

METHODS

Bacterial strains and plasmids. The strains of *N. gonorrhoeae* used in this study have been described in detail elsewhere (Lambden *et al.*, 1981). Preliminary analysis of cloned DNA was in *Escherichia coli* GC1 (Meyer *et al.*, 1982). Subsequent plasmid analysis was performed using *E. coli* DH1 (F⁻ *recA1 hsdR17* [*r_k*⁻, *m_k*⁻] *endA1 gyrA96 thi-1 supE44 λ*⁻) (Hanahan & Meselson, 1980) as host. Plasmid pBR322 (Bolivar *et al.*, 1977) was used as a cloning vector for the study of gonococcal genes in *E. coli*.

Reagents, enzymes and isotopes. All reagents were supplied by BDH unless stated otherwise. Ampicillin, tetracycline hydrochloride and bovine serum albumin (BSA) were supplied by Sigma. Tris was obtained from Boehringer Mannheim. Restriction endonucleases were obtained from Boehringer Mannheim, except for *Bam*HI, *Eco*RI and *Hind*III, which were from P&S Biochemicals, Liverpool, UK. Other enzymes used were nuclease BAL-31 (P&S Biochemicals), ribonuclease A (Sigma), and T4 DNA ligase, DNA polymerase I and calf intestinal alkaline phosphatase (Boehringer Mannheim). Reaction conditions for each enzyme were essentially as described by Maniatis *et al.* (1982). [α -³²P]dATP (3000 Ci mmol⁻¹; 110 TBq mmol⁻¹) and ¹²⁵I-labelled *Staphylococcus aureus* protein A (>30 mCi mg⁻¹; >1.1 GBq mg⁻¹) were supplied by Amersham.

Media and culture conditions. *E. coli* was grown at 37 °C in 2.5% (w/v) nutrient broth (Lab M no. 2, London Analytical & Bacteriological Media) solidified where necessary by the addition of agar to 1.2% (w/v). Where appropriate, liquid and solid media contained ampicillin (100 µg ml⁻¹) or tetracycline hydrochloride (12.5 µg ml⁻¹).

N. gonorrhoeae P9 was grown for 18 h at 37 °C in the presence of 7% (v/v) CO₂ on CTA medium, comprising 1.125% (w/v) Proteose Peptone no. 3 (Difco); 1% (w/v) nutrient agar (Lab M no. 2); 0.1% (w/v) KH₂PO₄; 0.4% (w/v) K₂HPO₄; 0.5% (w/v) NaCl; 0.1% (w/v) soluble starch; and 1% (v/v) of a solution containing 40% (w/v) glucose, 0.5% (w/v) L-glutamine, 0.05% (w/v) Fe(NO₃)₃ and 0.002% (w/v) thiamin pyrophosphate.

DNA isolation and cloning procedures. Large-scale plasmid isolation was from chloramphenicol-amplified cultures of *E. coli* by the method of Humphreys *et al.* (1975). Small-scale plasmid isolation was by the method of Close & Rodriguez (1982). *N. gonorrhoeae* P9 total DNA was isolated by the method of Stern *et al.* (1984) from bacterial lawns derived from single colonies. The predominance of piliated (P⁺) P9 cells in lawns was confirmed by electron microscopy.

Cloning procedures were essentially as described by Maniatis *et al.* (1982). To construct representative gene libraries, total DNA from each P9 variant was partially digested with combinations of either *Eco*RI and *Sau*3AI, or *Eco*RI and *Cla*I. Approximately 2 µg of the resulting DNA fragments were then ligated to 1 µg of the purified large fragment of pBR322 possessing suitable acceptor sites. In addition, a gene library of P9-35 was constructed by the ligation of 2 µg P9-35 total DNA, cleaved to completion by *Cla*I, with 1 µg of *Cla*I-linearized pBR322 DNA that had subsequently been treated with alkaline phosphatase (100–300 ng). Each ligation mix was used to transform *E. coli* GC1 to ampicillin resistance by the method of Brown *et al.* (1979) prior to immunological screening. Subsequent plasmid analyses and subcloning procedures were carried out using *E. coli* DH1 as host.

Monoclonal antibodies. The production of monoclonal antibodies reacting with gonococcal pili has been described previously (Virji & Heckels, 1983; Virji *et al.*, 1983). Antibody SM1 recognizes a conserved epitope on all pili from all strains tested to date; antibodies SM3, SM5, SM6 and SM13 recognize epitopes responsible for antigenic differences between pili from variants of strain P9.

Immunological screening. At least 10000 ampicillin-resistant colonies from each transformation were replica-plated on to nitrocellulose filters (Schleicher & Schull BA85) at a density of 500–1000 colonies per 85 mm plate. Replica colonies were then screened for the production of SM1-reactive pilin, essentially by the method of Helfman *et al.* (1984). Monoclonal antibody SM1 was diluted 1:2000 in TSA buffer (0.02 M-Tris/HCl, pH 7.6; 0.87% NaCl; 0.05% Na₂N₃; 5 mg BSA ml⁻¹). ¹²⁵I-labelled protein A was diluted in TSA to a specific activity of 10 nCi ml⁻¹.

Dot blotting. Bacterial suspensions ($2\ \mu\text{l}$ of $>10^8$ cells ml^{-1}) were applied to sheets of nitrocellulose, allowed to dry and lysed by exposure to CHCl_3 vapour. Excess protein binding was blocked with BSA and the sheets were reacted with monoclonal antibody, washed and reacted with ^{125}I -labelled protein A as previously described (Virji & Heckels, 1983). Immunological reactivity was detected after overnight autoradiography.

Immunoblotting. Western blotting was performed after subjecting $25\ \mu\text{g}$ total cell protein per track to electrophoresis on a 12.5–17.5% (w/v) linear gradient polyacrylamide gel. Proteins were transferred from the slab gel onto a nitrocellulose filter by electroblotting (Towbin *et al.*, 1979). After incubation in BSA binding solution (3%, w/v, BSA; 150 mM-NaCl; 50 mM-Tris/HCl, pH 7.5) to block non-specific binding sites, filters were soaked for 18 h in monoclonal antibody SM1 or rabbit anti-P9 pilus polyclonal antibody, diluted in TSA 1:2000 and 1:200 respectively. The filters were then washed five times with Tris/saline solution (150 mM-NaCl; 50 mM-Tris/HCl, pH 7.5) and incubated for 18 h in ^{125}I -labelled protein A ($10\ \text{nCi}\ \text{ml}^{-1}$ in TSA). Each filter was then washed five times with Tris/saline solution prior to autoradiography.

Southern hybridizations. After electrophoresis, DNA was transferred to nitrocellulose filters by the method of Southern (1975). DNA–DNA hybridizations were performed under conditions of high stringency at 42°C in the presence of 50% (v/v) formamide as described by Maniatis *et al.* (1982). Probes were labelled to a specific activity of approximately 5×10^8 d.p.m. μg^{-1} using the Polymeraid-RH random hexanucleotide priming system (P&S Biochemicals).

RESULTS

Genetic analysis of cloned pilin genes

Plasmid gene libraries constructed using DNA isolated from each of four isogenic variants of *N. gonorrhoeae* P9 yielded a total of 85 SM1-reactive clones. Plasmid DNA preparations from clones eliciting SM1-reactive pilin were mapped using restriction enzymes (Fig. 1). All the pilin-encoding plasmids possessed a unique *Xba*I site, located at one terminus of a 1.6 kb *Eco*RI–*Xba*I fragment of gonococcal origin (Fig. 1). Restriction maps of some clones can be completely matched with those of others to produce classes of totally alignable restriction maps. On this basis, six different classes of restriction map have been found. All clones generated by the ligation of *Eco*RI–*Cla*I fragments of gonococcal DNA possess at least one small *Sma*I–*Cla*I segment. The absence of similar segments in some clones containing an *Eco*RI–*Sau*3AI gonococcal insert probably reflects the cloning strategy employed.

Subclones were constructed from pLV260 in order to localize the coding region for the structural pilin gene (Fig. 2). Further structural analysis by BAL-31 deletion about the unique *Xba*I site in pLV260 indicated that the recombinant possesses a single expressed pilin gene, the boundary of which lies approximately 0.5 kb to one side of the *Xba*I site (data not shown). Approximately 1.1 kb of the gonococcal insert of pLV260, containing all but 1 bp of the C-terminal sequence of the pilin gene (data not shown) and capable of eliciting SM1-reactive pilin, was used to probe total DNA derived from P9 variants that had been digested to completion with *Cla*I endonuclease (Fig. 3). P9 total DNA that had been subjected to Southern analysis in this way possessed one of three distinguishable hybridization patterns comprising at least seven bands. With the exception of a faintly hybridizing 2.3 kb *Cla*I fragment that was generated on cleavage of P9-37 DNA, total DNA preparations from P9-2, P9-35, and P9-37 yielded apparently identical hybridization patterns. In each case, a 3.8 kb *Cla*I fragment was present that disappeared on cleavage with *Xba*I (Fig. 3). A fragment of similar size was also represented in the P9-20 hybridization pattern. This fragment presumably harbours all, or part of, the intact pilin coding sequence of each strain, since:

- (i) All pilin-producing clones possess all, or presumably part, of a 3.8 kb *Cla*I fragment.
- (ii) All pilin-producing clones possess a single *Xba*I site, and digestion with *Cla*I and *Xba*I of plasmid DNA from clones possessing a 3.8 kb *Cla*I fragment results in the replacement of this fragment by two fragments, of 2.5 and 1.3 kb. Fragments of similar size are represented in hybridization patterns of all P9 total DNA preparations that have been digested with *Cla*I and *Xba*I.
- (iii) No other *Cla*I fragment represented is conserved in all hybridization patterns that is also digested with *Xba*I.
- (iv) P9-35 total DNA that had been digested to completion with *Cla*I was used to construct a gene library that yielded 16 pilin-producing clones. Eight of these were selected at random for

Fig. 1. Restriction analysis of DNA inserts derived from antigenic variants of *N. gonorrhoeae* strain P9 in recombinant pilin-producing plasmids. Restriction maps are aligned about a shared *EcoRI*-*XbaI* fragment of 1.6 kb. Numbers refer to classes of totally alignable restriction maps (see also Table 2). The position of the pilin gene of pLV260 is represented by a thickened bar (see text). Restriction sites: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; P, *Pvu*II; Ps, *Pst*I; S, *Sma*I; Sa, *Sau*3AI; X, *Xba*I.

restriction mapping, and all were found to contain a single gonococcal *Cla*I DNA fragment of 3.8 kb (Fig. 1 and data not shown).

(v) The hybridization pattern of a P⁻ isogenic variant of P9-2 whose total DNA had been completely digested with *Cla*I lacked a band corresponding to a fragment of 3.8 kb (Fig. 3).

The respective hybridization patterns obtained for P9-20 DNA digested to completion with *Eco*RI, *Pst*I, and *Pvu*II (data not shown) and *Cla*I were markedly different from those obtained for P9-2, P9-35 and P9-37 DNAs (Fig. 3). However, using pilin gene sequences as probes, substantial differences have also been revealed between the Southern blotting patterns of sibling pilus variants of MS11, another strain of *N. gonorrhoeae* (Swanson *et al.*, 1985).

Fig. 2. Restriction maps of gonococcal inserts in subclones of pLV260, a recombinant, pilin-producing plasmid derived from P9-2. Sub-clones encoding SM1-reactive pilin are marked P⁺. The position of the pilin gene of pLV260 is represented by a thickened bar (see text). Restriction sites are abbreviated as in Fig. 1.

Fig. 3. Southern hybridization analysis of total DNA from antigenic variants of *N. gonorrhoeae* strain P9, using the 1.1 kb *Xba*I-*Pvu*II fragment of pLV260 as probe. Lanes: 1, pLV260 DNA digested with *Cla*I; 2, pLV260 DNA digested with *Cla*I + *Xba*I; 3, P9-2 (P⁻ derivative) DNA digested with *Cla*I; 4, P9-2 (P⁻ derivative) DNA digested with *Cla*I + *Xba*I; 5, P9-2 DNA digested with *Cla*I; 6, P9-2 DNA digested with *Cla*I + *Xba*I; 7, SPP1 DNA digested with *Eco*RI; 8, λ DNA digested with *Hind*III; 9, P9-20 DNA digested with *Cla*I; 10, P9-20 DNA digested with *Cla*I + *Xba*I; 11, P9-35 DNA digested with *Cla*I; 12, P9-35 DNA digested with *Cla*I + *Xba*I; 13, P9-37 DNA digested with *Cla*I; 14, P9-37 DNA digested with *Cla*I + *Xba*I.

Fig. 4. Western blot analysis, using polyclonal antibody raised against whole P9 pili, of total protein in recombinant *E. coli* clones expressing gonococcal pilin(s). Clones representative of each size class of pilin derived from the four P9 variants are shown. Lanes: 1, DH1(pLV260); 2, DH1(pLV270); 3, DH1(pLV300); 4, DH1(pLV310); 5, DH1(pLV390); 6, DH1(pLV360); 7, DH1(pLV370); 8, DH1(pLV480).

Table 1. Reactivities of parental *N. gonorrhoeae* P9 variants with anti-pilus monoclonal antibodies (MAbs)

<i>N. gonorrhoeae</i> P9 variant	MAb reactivities				
	SM1	SM3	SM5	SM6	SM13
P9-2	+	+	+	—	+
P9-20	+	—	—	—	—
P9-35	+	—	—	+	+
P9-37	+	—	—	+	—

The same patterns of hybridizing DNA fragments were observed when total DNA from P9 variants was digested to completion with *Cla*I and probed with either the 1.1 kb *Xba*I–*Pvu*II or the 1 kb *Eco*RI fragment of pLV260 (data not shown). Southern analysis of restriction fragments of pLV260 using pLV270 as probe suggests that pLV270 shares homology with sequences distributed along the gonococcal insert of pLV260 (data not shown). These findings indicate that DNA sequences conserved within and between pLV260 and pLV270 are represented throughout the gonococcal genome.

Immunological and physical analysis of cloned pilins and relationship to restriction maps

Analysis by Western blotting of all clones revealed that each gene library (excluding that derived from P9-20) possessed recombinants capable of eliciting one of at least two size classes of SM1-reactive pilin (Fig. 4, Table 2). The size of cloned pilin differs from that of its P9 originator by up to 1.5 kDa. The immunological profile of each cloned pilin was determined using a panel of five monoclonal antibodies. Cloned pilin genes from P9-20 and P9-37 encoded pilins with immunoprofiles that were similar, or identical, to those obtained with pili from their respective P9 progenitors (Tables 1 and 2). However, the relative non-reactivity of both types of gonococcal pili limits immunological discrimination between them and cloned pilins of shared origin. Cloned pilin genes derived from P9-2 encode polypeptides of one of two sizes. Pilins in one size class possess an immunoprofile distinct from those in the other, reacting either with monoclonal antibody SM3 or SM5, both of which are regarded as specific for pili from P9-2 (Tables 1 and 2; Virji & Heckels, 1983). Superimposition of both immunoprofiles results in a spectrum of antibody reactivity indistinguishable from that produced by pili from *N. gonorrhoeae* P9-2 (Table 1). Generally pilins encoded in *E. coli* by cloned gonococcal genes were therefore apparently identical to pilins expressed by the source strain of *N. gonorrhoeae* P9.

Table 2. *Physical and immunological properties of cloned pilins and relationship to restriction maps*

Class of restriction map	Recombinant plasmid(s)	<i>N. gonorrhoeae</i> P9 originator	Apparent mol. mass of pilin (kDa)	MAb reactivities				
				SM1	SM3	SM5	SM6	SM13
1a	pLV250, pLV260	P9-2	19.0	+	+	—	—	+
1a	pLV370, pLV380 pLV493	P9-35	20.5	+	—	—	+	+
1b	pLV400	P9-35	20.5	+	—	—	+	+
1b	pLV460	P9-35	20.5	+	—	—	+	+
1c	pLV320	P9-2	20.0	+	—	+	—	+
1c	pLV430, pLV440	P9-35	20.5	+	—	—	+	+
1c	pLV350, pLV450	P9-35	20.5	+	—	+	—	+
1c	pLV340	P9-35	20.5	+	—	—	+	+
1d	pLV270	P9-2	20.0	+	—	+	—	+
2a	pLV410, pLV420 pLV490, pLV491	P9-35	20.5	+	—	—	—	+
2b	pLV360	P9-35	19.5	+	—	—	—	+
3	pLV300	P9-37	19.0	+	—	—	—	—
4a	pLV290	P9-37	18.5	+	—	—	—	—
4b	pLV310	P9-37	18.5	+	—	—	—	—
4c	pLV280, pLV281	P9-37	18.5	+	—	—	—	—
5	pLV390, pLV492	P9-35	19.0	+	—	—	—	—
6a	pLV480, pLV483 pLV486, pLV487	P9-20	19.5	+	—	—	—	—
6b	pLV481, pLV484 pLV485	P9-20	19.5	+	—	—	—	—

However, several cloned pilins possessed immunological profiles distinct from those of the P9 strain from which they were obtained and/or from those of any other clones (Table 2).

Restriction data on pilin-producing recombinant plasmids can be combined with physical and immunological characteristics of the pilin(s) elaborated by each, to identify at least nine classes of cloned gonococcal pilin (Table 2, Fig. 4). All recombinant plasmids that possess a 0.6 kb *EcoRI*-*XbaI* fragment encode SM13-reactive pilin(s). The *EcoRI* site at the terminus of the analogous fragment of pLV260 bisects the pilin gene in that recombinant, and may therefore define the coding region for the SM13-reactive epitope.

DISCUSSION

We have cloned in *E. coli* a series of pilin genes from four well-characterized pilus antigenic variants of *N. gonorrhoeae* P9. Restriction maps of the gonococcal sequences in variant pilin-producing recombinant plasmids can all be aligned on one side of the unique *XbaI* site present in each, whilst exhibiting differences on the other side that render them non-alignable. Since the pilin-coding sequence of pLV260 lies on the side of its *XbaI* site that is non-alignable, differences among clones between restriction maps of analogous segments may, therefore, reflect changes at the DNA level that are ultimately responsible for antigenic variation. Similarities between the restriction maps of recombinants, in conjunction with Southern hybridization analyses of genomic DNA from P9 variants using pilin gene sequences as probes, indicate that each variant possesses only a single expressed copy of the intact pilin gene. In this respect, strain P9 resembles MS11_{mk}, which has been shown to possess one *pilE* locus (Swanson *et al.*, 1985), but is unlike strain MS11_{ms}, which possesses two such loci (Meyer *et al.*, 1984).

The generation by *N. gonorrhoeae* of pilus variants with antigenically distinct pili has been explained by a cassette model of gene conversion (Segal *et al.*, 1986). According to this model, novel pilin genes are assembled at a *pilE* locus by intragenic recombination between segments of hitherto unexpressed pilin DNA sequences (Haas & Meyer, 1986). Such recombination may be

mediated by sequences associated with the 73 bp *SmaI*–*ClaI* repeat that is located about 65 bp downstream of the complete pilin-coding region and is distributed throughout the chromosome of *N. gonorrhoeae* MS11_{ms} (Meyer *et al.*, 1984). An identical *SmaI*–*ClaI* repeat is found approximately 65 bp downstream of the *pilE* locus of P9-2. A similar repeat is represented in all pilin clones we have isolated from *N. gonorrhoeae* P9, except where the cloning strategy employed has resulted in the deletion of all, or part, of this region. The *SmaI*–*ClaI* repeat is also present at two other positions within 2–3 kb of the P9-2 genome, upstream of its *pilE* locus (Fig. 1) and is represented more than once in several recombinant pilin-elaborating plasmids. The conservation of this sequence close to pilin-related DNA sequences in at least two different gonococcal strains suggests that it plays a crucial role in the mechanism that generates pilus antigenic variation.

Analysis of cloned pilins and their respective gene products indicates that *N. gonorrhoeae* P9 is capable of producing many antigenically distinct pilus types. Pilin clones from strain P9-2 fall into two classes, the monoclonal antibody-binding spectra of which, when superimposed, give the profile obtained with purified pilus preparations from this strain. Since there is apparently only one *pilE* site in strain P9, the parental P9-2 population used to obtain the clones presumably contained a mixture of cells expressing one or other of only two variant pilin sequences. A similar situation may exist for P9-35 and P9-37, each of which has also given rise to more than one class of cloned pilin gene.

Some reverting P[−] (P[−]rp⁺) derivatives of *N. gonorrhoeae* MS11_{mk} synthesize pilins that are not assembled into mature pili (Bergstrom *et al.*, 1986; Swanson *et al.*, 1985). Such pilins are either markedly truncated, or are of similar size to pilin from the P⁺ progenitor but contain multiple amino acid substitutions, mainly in the disulphide loop region of the protein (Bergstrom *et al.*, 1986). However, cloned pilins from *N. gonorrhoeae* P9 are all in the range 18–21 kDa, none varying in size from the pilin molecules found in their gonococcal progenitor by more than 1.5 kDa. Furthermore, several of the P9 pilins expressed in *E. coli*, most notably those derived from P9-2, contain epitopes that lie within the hypervariable region of the pilin polypeptide (Virji & Heckels, 1985). We therefore believe that most, if not all, cloned pilin genes have been derived from P⁺ P9 cells. DNA sequencing studies on these variant pilin genes have been carried out to determine the location of the epitopes recognized by the panel of anti-pilus monoclonal antibodies (Nicolson *et al.*, 1987). None of the variants possess premature termination (stop) codons; however, some variants do have TAA stop codons rather than TGA. All of the variant pilin genes with TAA translational stop sequences express pilins that do not react with type-specific monoclonal antibodies that recognize epitopes located in the hypervariable region. It is possible that this subset of pilins could be derived from P[−] rp⁺ cells that express defective pilins that are not assembled into pili (Bergstrom *et al.*, 1986).

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