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Genetics

# Genetics of protein I of *Neisseria gonorrhoeae:* Construction of hybrid porins

(shuttle mutagenesis/epitope mapping/virulence/vaccine)

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**ABSTRACT** **Protein I (PI), the major outer membrane protein of *Neisseria gonorrhoeae,* is a porin and occurs in two major immunochemical classes, A and B. By using shuttle mutagenesis to insert a selectable marker close to the PI structural gene, evidence was obtained from transformation experiments to demonstrate that the PI structural gene is equivalent to the defined locus *nmp* and that the genes for PI class A and PI class B are alleles of the same locus. The PI class B gene of strain MS11 was cloned and sequenced, and comparison of this sequence with the gene sequence of PI class A of FA19 revealed a number of regions of significant divergence. By selection for the closely linked marker in transformations between the two strains, a series of strains with a hybrid PI was obtained. Analysis of these strains with monoclonal antibodies and oligonucleotides specific to PI class A or PI class B elucidated the nature and location of some of the surface-exposed epitopes, a thorough characterization of which is a prerequisite for understanding the role of PI in gonococcal pathogenesis and its possible use as a component of a vaccine.**

Protein I (PI) of *Neisseria gonorrhoeae,* the major outer membrane protein, has a number of properties that indicate its potential as a component of a vaccine. Apart from its well-documented role as an anion-selective porin (1–3), PI is expressed on the surface of all gonococci, is highly antigenic *in vivo* (4), and has the interesting ability to insert into cell and artificial membranes directly from gonococcal cells (5, 6). Compared to certain other highly variable surface-exposed components of the gonococcus—notably, pilin (7), protein II (8), and LOS (lipooligosaccharide) (9)—PI is antigenically stable *in vitro*. It occurs in two major immunochemical classes, A and B (PIA and PIB), each composed of multiple serovars (10). The two classes of PI differ in their apparent molecular weight, their susceptibility to proteolysis (11), and their reactivity with polyclonal and monoclonal antibodies (12). Clinical isolates always contain either PIA or PIB, but never both (10). Although PIA/B hybrids can be constructed in the laboratory (13), they virtually never occur in nature (10). Since systemic gonococcal infections are usually caused by PIA strains and PIB strains are more commonly associated with local infections (14), PIA and PIB may play different roles in the pathogenicity of gonococci.

A major advance in the understanding of gonococcal PI was achieved by the cloning of the structural gene for both PIB (15) and PIA (16). The nucleotide and amino acid sequences of the two contained a large amount of homology interspersed with regions of significant diversity. Hybridization studies indicated that there is only a single PI structural gene in the gonococcal genome (15, 16), suggesting that the genes for PIA and PIB may be alleles of the same locus.

In this paper we report the use of a closely linked selectable marker introduced by shuttle mutagenesis (17) to confirm the allelic nature of the PI structural genes, to demonstrate their equivalence to the defined locus *nmp* (18), and to construct PIA/B hybrid genes by transformation. Analysis of the hybrid proteins encoded by these genes elucidated the approximate locations of epitopes for various PI-specific monoclonal antibodies.

## MATERIALS AND METHODS

**Bacterial Strains and Plasmids.** *N. gonorrhoeae* strains used in this study were FA19 (PIA) (19), MS11 (PIB) (20), and FA6149 (PIA/B) (13). *Escherichia coli* strains HB101 (21) and Y1090 (22) have been described. *E. coli* strains used for shuttle mutagenesis were RDP146 (pTCA), W3110*polA* (pOX38::mT*n*3Cm-3), and NS2114Sm (17). Plasmids used were pHSS6 (17) and pGEM-3, obtained from Promega Biotec (Madison, WI).

**Medium and Growth Conditions.** *N. gonorrhoeae* strains were grown on GC-base medium (Difco) containing Kellogg's supplements I and II (23) in a 5% $CO_2$/95% air atmosphere. The following antibiotics were used when necessary: chloramphenicol (1 $\mu$g/ml for FA19 and 10 $\mu$g/ml for MS11), spectinomycin (100 $\mu$g/ml), and streptomycin (100 $\mu$g/ml). *E. coli* strains were grown on Luria medium (21) with the following additions when appropriate: ampicillin (50 $\mu$g/ml), chloramphenicol (30 $\mu$g/ml), tetracycline (10 $\mu$g/ml), kanamycin (40 $\mu$g/ml), and streptomycin (100 $\mu$g/ml).

**Transformation of *N. gonorrhoeae*.** *N. gonorrhoeae* strains were transformed by the method of Biswas *et al.* (24). Cells were incubated for phenotypic expression of antibiotic resistance for 5 hr either in GC-base broth before plating or on GC-base agar before addition of a soft agar overlay containing the antibiotic. When using FA19 as the recipient and selecting for chloramphenicol resistance ($Cm^r$) the antibiotic at 1 $\mu$g/ml was used, whereas for MS11 the antibiotic at 10 $\mu$g/ml was used.

**Colony Immunoblotting.** Bacterial strains were assayed for binding of monoclonal antibodies by a modification of the method of Cannon *et al.* (25). A concentrated suspension of cells in GC-base broth was transferred to nitrocellulose by using a filtration manifold and lysed in chloroform vapor. The filter was soaked in TBS (10 mM Tris·HCl, pH 7.5/150 mM

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Abbreviations: PI, protein I; PIA and PIB, PI classes A and B, respectively; CAT, chloramphenicol acetyltransferase; r, resistance; s, sensitivity; Cm, chloramphenicol.

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NaCl) containing 5% (wt/vol) nonfat dried milk, incubated in TBS containing the appropriate antibody, and then washed in TBS. After incubation in alkaline phosphatase-conjugated anti-mouse IgG as secondary antibody (Sigma) and further washing, the filter was developed by using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Bethesda Research Laboratories) according to the directions of the manufacturer. The monoclonal antibodies used were 4A12, 4G5, 2F12, 6D9, 5G9, 5D1 (10), 1D3 (26), and SM101 (27), all of which are PIA-specific, and 1F5, 3C8, 2D4, and 2H1 (10), which are PIB-specific. All antibodies except SM101 were kindly supplied in ascites fluid by M. Tam (Genetic Systems) and were used at dilutions of 1:2000 to 1:10,000. SM101 was kindly provided by J. Heckels (University of Southampton).

**DNA Techniques.** Gonococcal genomic DNA was prepared by the method of Stern *et al.* (28). Preparation of plasmid DNA and transformation into *E. coli* were performed as described by Maniatis *et al.* (21). Restriction endonucleases, DNA polymerase I Klenow fragment, T4 DNA ligase, and polynucleotide kinase were obtained from either Bethesda Research Laboratories or New England Biolabs and were used according to the instructions of the manufacturer. Oligonucleotides were synthesized and used for hybridization as described (16). For λgt11 cloning, 400- to 700-base-pair (bp) *Hin*fI-digested fragments of MS11 DNA were gel-purified, end-filled by using Klenow fragment, and ligated with *Eco*RI linkers (Bethesda Research Laboratories). These fragments were then ligated with *Eco*RI-digested λgt11 and the ligation mixture was packaged and used to infect *E. coli* Y1090. Plaques were transferred to nitrocellulose and probed by hybridization by the methods of Maniatis *et al.* (21). For sequencing, fragments were subcloned into M13mp18 (29) and sequencing was performed with the sequencing kit supplied by United States Biochemical (Cleveland, OH) according to the instructions of the manufacturer.

**Shuttle Mutagenesis.** Shuttle mutagenesis was performed by a modification of the method of Seifert *et al.* (17, 30). The target DNA was subcloned into pHSS6 and introduced into RDP146 (pTCA) by transformation, and then pOX38::mT*n*3Cm-3 was introduced by conjugation. Transconjugants were grown overnight at 30°C to allow transposition to occur and then grown several hours at 37°C. A "pool" of transconjugants (≈1000 colonies) was resuspended and used as the donor in conjugation with NS2114Sm, in which plasmid cointegrates, formed as an intermediate in transposition events into the target plasmid, are resolved (17). The transposon thus inserted is stable since the transposase function, which was provided in trans on plasmid pTCA, is no longer present. The mini-transposon used in this case was mT*n3*Cm-3, in which the *bla* gene of T*n3* is replaced by the chloramphenicol acetyltransferase (CAT) gene. A full description of the development of this system for gonococci will be published separately (H.S.S., R. Ajioka, M.V. Ho, F. Heffron, M.S., unpublished work). Use of CAT for experiments in *N. gonorrhoeae* was approved by the local biosafety committees at the University of North Carolina at Chapel Hill and The Research Institute of Scripps Clinic.

## RESULTS

**Insertion of a Selectable Marker Adjacent to the PI Gene in FA19.** The 900-bp *Sau*3AI fragment of pUNC3 (16), containing the first 45 codons of the PI gene of FA19 and upstream sequences, was ligated with *Bam*HI-digested pHSS6 to form the plasmid pNCS2 (Fig. 1). This plasmid was then subjected to shuttle mutagenesis. After mapping the mT*n3*Cm-3 insertion sites within the 900-bp *Sau*3AI fragment of pNCS2, five such constructs with mT*n3*Cm-3 at various sites were digested with *Not* I, which releases the total insert as a linear fragment, and used to transform FA19. Only one digested

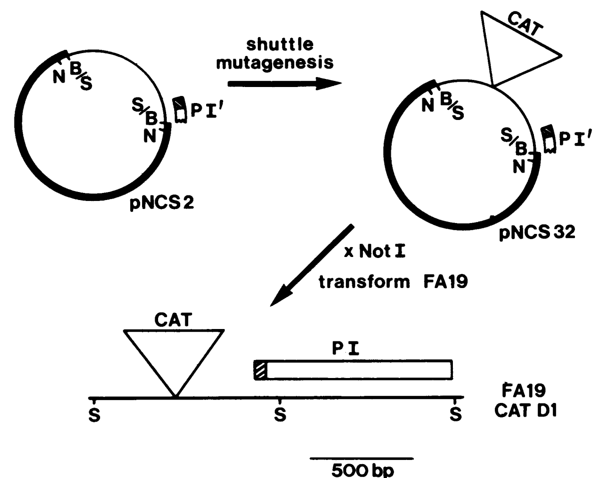


FIG. 1. Insertion of mT*n3*-CAT (CAT above an open inverted triangle) adjacent to the PI structural gene of FA19. The thick line represents the vector pHSS6 DNA. mT*n3*-CAT is 1.66 kilobases long and is not drawn to scale. The position of the PI gene on the genome of FA19 CAT D1 is indicated by the bar above the line, with the hatched box denoting the signal sequence. PI', portion of the PI gene present in the cloned constructs; N, *Not* I; B, *Bam*HI; S, *Sau*3AI.

plasmid, pNCS32 (Fig. 1), transformed FA19 to Cm$^r$, at a frequency of $10^{-7}$. The site of insertion of mT*n3*Cm-3 in pNCS32 was ≈300 bp upstream of the PIA gene promoter sequences, whereas the insertion sites in the other four plasmids were all closer to, but not within, the PI gene. The location of mT*n3*Cm-3 in the genome of the transformant FA19 CAT D1 (Fig. 1) was confirmed by Southern hybridization (data not shown).

**Evidence That the *nmp* Locus Is the PI Structural Gene and That the PIA and PIB Genes Are Alleles.** Studies (18, 31) have shown that the *nmp* locus, which affects the molecular weight and antigenicity of PI, is closely linked on the gonococcal genome to the antibiotic resistance markers *str* and *spc*, in the order *str–spc–nmp*, with cotransformation frequencies of 5% for *str* and *nmp*, and ≈23% for *spc* and *nmp*. To determine whether the CAT marker in FA19 CAT D1, adjacent to the PI structural gene, showed the same linkage pattern as the *nmp* locus, reciprocal crosses were performed between the strains FA130 (Str$^r$ Spc$^r$ Cm$^s$) (32) and FA19 CAT D1 (Str$^s$ Spc$^s$ Cm$^r$), where Str is streptomycin, Spc is spectinomycin and s is sensitivity. When FA130 DNA was used to transform FA19 CAT D1, selecting for either Str$^r$ or Spc$^r$, cotransformation frequencies were 26% (99/383) for *spc* and CAT and 7.5% (40/537) for *str* and CAT. In the reciprocal cross, selecting for Cm$^r$, the cotransformation frequencies were 10% (11/111) for *spc* and CAT and 1% (1/111) for *str* and CAT. Analysis of crossover classes confirmed that the gene order was *str* . . . *spc* . . . CAT. These data demonstrated that the CAT gene was linked to the *str* and *spc* markers in the same way as the *nmp* locus, providing strong evidence that the *nmp* locus is the structural gene for PI.

In another cross, FA19 CAT D1 DNA was used to transform the PIB strain MS11, and the transformants were selected for Cm$^r$. Among 45 transformants, 24 acquired the donor PIA, 13 retained the recipient PIB, and 8 expressed hybrid PIA/B proteins, as detected by colony immunoblotting. The replacement of the recipient PIB by the PIA of the donor when selecting for the closely linked CAT gene confirmed the allelic nature of these genes, and the frequent occurrence of hybrid proteins suggested that the sequence homology between the two alleles allowed intragenic recombination to occur. Detailed analysis of these PI hybrid strains depended on knowledge of the PIB sequence of MS11, which was cloned and sequenced.

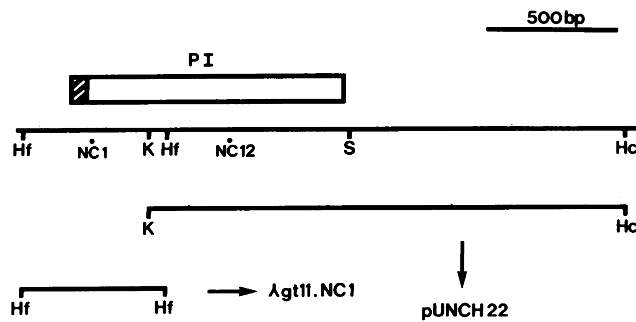


FIG. 2. Fragments of MS11 DNA cloned for the sequencing of the PI gene. The position of the PI gene is indicated by the bar above the line, with the shaded box denoting the signal sequence. The locations of the sequences of oligonucleotides NC1 and NC12 are shown. The larger fragment was cloned into the vector plasmid pGEM-3 and the smaller fragment into λgt11 to give constructs with the indicated designations. Hf, *Hin*fI; K, *Kpn* I; S, *Sau*3AI; Hc, *Hin*cII.

**Cloning and Sequencing of the PI Gene of MS11.** Since intact gonococcal PI genes apparently cannot be cloned in *E. coli* (15, 16), the strategy (15, 16) of cloning fragments that together contain the complete gene sequence was adopted for the MS11 PI gene. A DNA fragment from within the PI gene of the strain FA6149, characterized as having a hybrid PI (13), was cloned and sequenced, and the sequence revealed the presence of a *Kpn* I site in the PIB portion of the sequence, ≈300 bp from the start of the gene. Oligonucleotides NC12 (Fig. 2), derived from the PIB gene sequence downstream of the *Kpn* I site, and NC1 (Fig. 2), derived from the gene sequence corresponding to the N terminus of the protein (16), were used as probes in Southern hybridization to confirm the presence of the *Kpn* I site in the MS11 PI gene (data not shown). HB101 colonies containing *Kpn* I/*Hin*cII-digested MS11 DNA cloned into the vector plasmid pGEM-3 were probed with these oligonucleotides, and colonies hybridizing with NC12 were detected and found to contain a plasmid with a 1.8-kilobase *Kpn* I–*Hin*cII fragment that hybridized with NC12. This plasmid was designated pUNCH22 (Fig. 2). However, despite repeated attempts, no colonies hybridizing with NC1 were detected. Since NC1 hybridized with a 750-bp *Kpn* I–*Hin*cII fragment of MS11 genomic DNA in Southern hybridization (data not shown), the problem apparently was not one of size, but more likely the expression of the PI gene portion on this fragment that, in a high-copy-number vector, was lethal for HB101. For this reason the remaining portion of the MS11 PI gene was isolated as a 540-bp *Hin*fI fragment in λgt11, by using oligonucleotide NC1 as a probe. This clone was designated λgt11.NC1 (Fig. 2). The 540-bp *Hin*fI fragment of λgt11.NC1 and the 750-bp *Kpn* I–*Sau*3AI fragment of pUNCH22 were digested further, subcloned, and sequenced. The sequence is shown in Fig. 3 and predicts a protein of 350 amino acids, of which the first 19 are the putative signal peptide. The sequence is closely homologous to the PI gene sequence of R10 (15), with only 17 differences in the predicted amino acid sequences of the two proteins. A comparison of the PI gene sequences of FA19 (16) and MS11 reveals 80% nucleotide sequence similarity. A comparison of the predicted amino acid sequences of PIA (FA19) and PIB (MS11) is presented in Fig. 4 and shows a number of regions of significant diversity interspersed with long regions of homology. Some of the regions of diversity presumably correspond to surface-exposed antigenic portions of the protein, since PI-specific monoclonal antibodies raised against whole gonococcal cells are never cross-reactive between PIA and PIB (10).

**Construction and Analysis of PIA/B Hybrid Strains.** Further PIA/B hybrids were constructed by transforming MS11 with FA19 CAT D1 DNA, selecting $Cm^r$, and scoring PI by

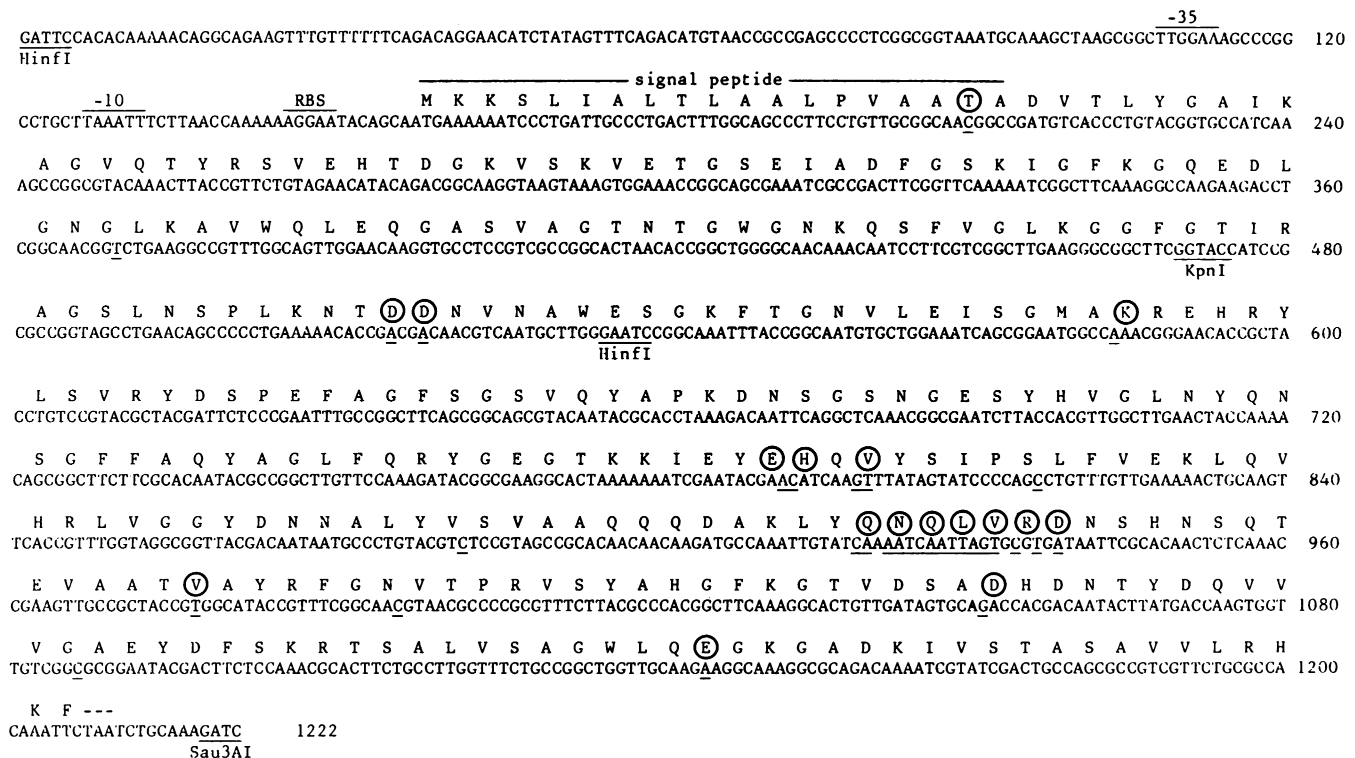


FIG. 3. DNA sequence of the PIB gene of MS11. The predicted amino acid sequence is shown above the DNA sequence, with the signal peptide indicated. The putative promoter sequences (−35 and −10) and the ribosome binding site (RBS) are shown, as are the relevant restriction enzyme sites. The last base number of each line is shown on the right. To denote differences from the PIB sequence of R10 (15), bases (other than those making up a restriction enzyme site) are underlined and amino acids are circled. The single-letter amino acid code is used.

FIG. 4. Comparison of the amino acid sequences of FA19 PIA and MS11 PIB and the structure of hybrid PI genes. PIA gene sequence is denoted by the open bar and PIB by the solid bar. Above and below the PI genes are the comparisons of amino acid sequence, a vertical line corresponding to a single difference and a solid block corresponding to a contiguous stretch of differences. The number of amino acid residues is shown on the upper scale and the base-pair number is shown on the lower scale. The oligonucleotides used to analyze the hybrids are shown between the genes, with the dotted line indicating the equivalent position on the other gene. The hybrid gene structures (classes 1–9) are shown, and regions between oligonucleotide sequences where a crossover occurred are depicted by the slanted locations. The PI serovars of the various hybrid classes are shown in Table 1.

immunoblotting. Among 100 transformants, 10 had a different PI from either parent and so were presumed to have a hybrid PI. These included the 8 strains with a hybrid PI referred to earlier in *Results*. In a reciprocal cross, DNA from a $Cm^r$ transformant of MS11 that had retained its PIB was used to transform FA19, selecting for $Cm^r$, and scoring PI as before. Among 300 transformants, 3 had a hybrid PI. Among the 13 hybrid strains, five different hybrid PI serovars were identified (Table 1). The hybrid strains were then analyzed by colony hybridization by using a number of PIA- and PIB-specific oligonucleotides (Table 2 and Fig. 4), to assess which portions of the hybrid PI genes were PIA sequences and which were PIB sequences, and nine different classes were detected by this method (Fig. 4). By analyzing the serovar of the hybrid PI genes of known structure, the approximate location of the epitopes for these monoclonal antibodies could then be determined.

Table 1. Reactivities (serovars) of hybrid PI strains with PI-specific monoclonal antibodies

| Monoclonal antibody | Hybrid class 1–4 | 5 | 6 | 7–8 | 9 | Strain FA19 | MS11 |
|---|---|---|---|---|---|---|---|
| 4G5 | + | + | + | − | + | + | − |
| 2F12 | + | + | + | − | + | + | − |
| SM101 | + | − | − | − | + | + | − |
| 6D9 | − | − | + | + | + | + | − |
| 4A12 | − | − | − | − | + | + | − |
| 5G9 | − | − | − | − | + | + | − |
| 5D1 | − | − | − | − | + | + | − |
| 1D3 | − | − | − | − | + | + | − |
| 1F5 | − | − | − | + | − | − | + |
| 3C8 | + | + | − | − | + | − | + |
| 2D4 | + | + | − | − | − | − | + |
| 2H1 | + | + | − | − | − | − | + |

+, Reaction with the monoclonal antibody; −, no reaction with the monoclonal antibody.

The epitopes for the PIA-specific monoclonal antibodies 4G5, 2F12, and SM101, which react with the class 1 hybrid, must be located near the N terminus of the protein, within the first 60 residues. Comparison of hybrid classes 1, 5, and 6 suggests that the epitope for SM101 lies at least partially in the region between residues 34 and 60. The N-terminal 60 residues contain significant diversity between PIA and PIB (Fig. 4) that could account for the specificity of these antibodies. The epitope for 6D9 (PIA-specific), which reacts with hybrid classes 6–9, lies in the region of the protein between residues 187 and 250, which also includes regions of significant diversity between PIA and PIB. The epitopes for 4A12, 5G9, 5D1, and 1D3 (all PIA-specific) were detected only in the hybrid protein of class 9 and, therefore, presumably are complex epitopes involving both N-terminal and C-terminal portions of the protein. This is supported by the observation

Table 2. Oligonucleotides used for analysis of PI hybrids

| Oligonucleotide | Sequence (5′ → 3′) | PI specificity | Location |
|---|---|---|---|
| NC8 | GCGTTAAAACCGCTACC | A | 27–32 |
| NC9 | TCGAACCCAAATCAGCG | A | 34–40 |
| NC11 | CGGTGTCGGTCTGCGCC | A | 299–305 |
| NC12 | GATACGGCGAAGGCACT | B | 182–187 |
| NC13 | CAAGGTGCCTCCGTCGC | B | 61–66 |
| NC14 | AAGTGCGCGTCGGCCGT | A | 87–92 |
| NC15 | GACTTGGCGCAACGATAA | A | 213–219 |
| NC16 | GCAGCGTACAATACGCAC | B | 144–150 |
| NC18 | GCAACATTGCCCAACCC | A | 116–121 |
| NC19 | AGGCACTGTTGATAGTGC | B | 273–279 |

Location refers to the amino acid residue numbers (where position 1 is the first residue of the mature protein) encoded in whole or in part by the sequence of the oligonucleotide.

that these antibodies do not react well with PIA in an immunoblot, where the protein is relatively denatured (data not shown).

Among the four antibodies that react with PIB of MS11, the epitope for 1F5, which reacts with hybrid classes 7 and 8, is located within the N-terminal 60 residues, and the epitopes for 3C8, 2D4, and 2H1 apparently lie within a central part of the protein, between residues 150 and 270, which includes a long stretch of divergent sequence. The epitope for 3C8 is located slightly upstream of those for 2D4 and 2H1, since the class 9 hybrid protein reacts with 3C8 only. However, the epitopes for 2D4 and 2H1 may be relatively complex since these antibodies do not react with PIB in an immunoblot (data not shown).

## DISCUSSION

PI is an abundant, ever-present, and relatively invariant structure of the gonococcal outer membrane and, accordingly, the genetics of PI seems to be relatively uncomplicated. In this study, we utilized a reporter gene inserted close to the PI structural gene to demonstrate that PIA and PIB structural genes are alleles of the same locus, which was previously identified as *nmp* (18). The presence of a single PI gene on the gonococcal genome is consistent with the observation that naturally occurring strains possess either a PIA or a PIB protein, but never both, and that their PI serotype is stably maintained. A comparison of the PIA and PIB gene and deduced amino acid sequences revealed a high degree of homology, but regions of significant sequence divergence were identified. Although PI hybrids do not occur naturally, the PI hybrids that we constructed survived stably *in vitro*. However, the relatively infrequent formation of a hybrid PI with a PIB N terminus and the surprisingly frequent occurrence of multiple crossovers within the PI gene suggests that certain classes of hybrid PI may be favored by gonococci growing *in vitro*.

The construction and analysis of PI hybrids allowed us to determine the approximate location of some of the surface-exposed portions of the proteins and provided some insight into their possible secondary and tertiary structures. The N-terminal regions of both PIA and PIB apparently are surface-exposed, along with at least one other region in the central part of each protein. The folding of PIA in the outer membrane may be such that N-terminal and C-terminal parts are closely associated on the surface, since epitopes for a number of PIA-specific monoclonal antibodies were identified only when both of these portions were present in a hybrid PI. These models for the conformation of PI in the outer membrane agree with some aspects of previous models based on proteolytic cleavage of PI in intact gonococci (11, 33), but differ from previous suggestions that only the N terminus of PIA and a central portion of PIB are surface-exposed (11, 34). Clearly further data are required to elucidate which portions of PI are surface-exposed, and how they are associated in trimeric form in the outer membrane. Identification and characterization of all surface-exposed regions of PI will be an important step toward the development of a vaccine based on this protein.

To the best of our knowledge, this is the first description of shuttle mutagenesis for the introduction of a reporter gene in the gonococcus. Insertion of mTn*3*Cm-3 provided a readily selectable $Cm^r$ phenotype, which was much easier to use than related constructions containing *bla* (data not shown). We attempted to make mutant strains lacking a porin by transforming with other constructions containing mTn*3*Cm-3 within the PIA structural gene, but were unsuccessful, presumably because a PI-mutant is not viable (data not shown). However, this powerful system should be useful for the mutagenesis of nonessential genes.

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1. Douglas, J. T., Lee, M. D. & Nikaido, H. (1981) *FEMS Microbiol. Lett.* **12**, 305–309.
2. Young, J. D. E., Blake, M., Mauro, A. & Cohn, Z. A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3831–3835.
3. Lynch, E. C., Blake, M., Gotschlich, E. & Mauro, A. (1983) *Biophys. J.* **41**, 62a.
4. Zak, K., Diaz, J. L., Jackson, D. & Heckels, J. E. (1984) *J. Infect. Dis.* **149**, 166–174.
5. Lynch, E. C., Blake, M. S., Gotschlich, E. C. & Mauro, A. (1984) *Biophys. J.* **45**, 104–107.
6. Blake, M. S. (1985) in *The Pathogenesis of Bacterial Infections*, eds. Jackson, G. G. & Thomas H. (Springer, Berlin), pp. 51–66.
7. Lambden, P. R., Robertson, J. N. & Watt, D. J. (1980) *J. Bacteriol.* **141**, 393–396.
8. Schwalbe, R. S., Sparling, P. F. & Cannon, J. G. (1985) *Infect. Immun.* **49**, 250–252.
9. Schneider, H., Hale, T. L., Zollinger, W. D., Seid, R. C., Jr., Hammack, C. A. & Griffiss, J. M. (1984) *Infect. Immun.* **45**, 544–549.
10. Knapp, J. S., Tam, M. R., Nowinski, R. C., Holmes, K. K. & Sandstrom, E. G. (1984) *J. Infect. Dis.* **150**, 44–48.
11. Blake, M. S., Gotschlich, E. C. & Swanson, J. (1981) *Infect. Immun.* **33**, 212–222.
12. Sandstrom, E. G., Chen, K. C. S. & Buchanan, T. M. (1982) *Infect. Immun.* **38**, 462–470.
13. Danielsson, D., Faruki, H., Dyer, D. & Sparling, P. F. (1986) *Infect. Immun.* **52**, 529–533.
14. Buchanan, T. M. & Hildebrandt, J. F. (1981) *Infect. Immun.* **32**, 985–994.
15. Gotschlich, E. C., Seiff, M. E., Blake, M. S. & Koomey, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8135–8139.
16. Carbonetti, N. H. & Sparling, P. F. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9084–9088.
17. Seifert, H. S., So, M. & Heffron, F. (1986) in *Genetic Engineering, Principles and Methods*, eds. Setlow, J. K. & Hollaender, A. (Plenum, New York), Vol. 8, pp. 123–134.
18. Cannon, J. G., Klapper, D. G., Blackman, E. Y. & Sparling, P. F. (1980) *J. Bacteriol.* **143**, 847–851.
19. Maness, M. J. & Sparling, P. F. (1973) *J. Infect. Dis.* **128**, 321–330.
20. Meyer, T. F., Mlawer, N. & So, M. (1982) *Cell* **30**, 45–52.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
22. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
23. Kellogg, D. S., Jr., Peacock, W. L., Jr., Deacon, W. E., Brown, L. & Pirkle, C. I. (1963) *J. Bacteriol.* **85**, 1274–1279.
24. Biswas, G. D., Sox, T., Blackman, E. & Sparling, P. F. (1977) *J. Bacteriol.* **129**, 983–992.
25. Cannon, J. G., Black, W. J., Nachamkin, I. & Stewart, P. W. (1984) *Infect. Immun.* **43**, 994–999.
26. Joiner, K. A., Warren, K. A., Tam, M. R. & Frank, M. M. (1985) *J. Immunol.* **134**, 3411–3419.
27. Virji, M., Fletcher, J. N., Zak, K. & Heckels, J. E. (1987) *J. Gen. Microbiol.* **133**, 2639–2646.
28. Stern, A., Nickel, P., Meyer, T. F. & So, M. (1984) *Cell* **37**, 447–456.
29. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26**, 101–106.
30. Seifert, H. S., Chen, E. Y., So, M. & Heffron, F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 735–739.
31. Cannon, J. G., Lee, T. J., Guymon, L. F. & Sparling, P. F. (1981) *Infect. Immun.* **32**, 547–552.
32. Sarubbi, F. A., Jr., Blackman, E. Y. & Sparling, P. F. (1974) *J. Bacteriol.* **120**, 1284–1292.
33. Judd, R. C. (1986) *Infect. Immun.* **54**, 408–414.
34. Teerlink, T., Versantvoort, H. & Beuvery, E. C. (1987) *J. Exp. Med.* **166**, 63–76.