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Cloning and Characterization of the *ponA* Gene Encoding Penicillin-Binding Protein 1 from *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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The *ponA* gene encoding penicillin-binding protein 1 (PBP 1) from *Neisseria gonorrhoeae* was cloned by a reverse genetic approach. PBP 1 was purified from solubilized membranes of penicillin-susceptible strain FA19 by covalent ampicillin affinity chromatography and used to obtain an NH_2-terminal amino acid sequence. A degenerate oligonucleotide based on this protein sequence and a highly degenerate oligonucleotide based on a conserved amino acid motif found in all class A high-molecular-mass PBPs were used to isolate the PBP 1 gene (*ponA*). The *ponA* gene encodes a protein containing all of the conserved sequence motifs found in class A PBPs, and expression of the gene in *Escherichia coli* resulted in the appearance of a new PBP that comigrated with PBP 1 purified from *N. gonorrhoeae*. A comparison of the gonococcal *ponA* gene to its homolog isolated from *Neisseria meningitidis* revealed a high degree of identity between the two gene products, with the greatest variability found at the carboxy terminus of the two deduced PBP 1 protein sequences.

Penicillin and other β-lactam antibiotics, by virtue of their structural similarity to the D-Ala-D-Ala carboxy terminus of nascent peptidoglycan, exert their lethal action by covalently binding to and inactivating enzymes involved in bacterial cell wall biosynthesis (26). Inhibition of these enzymes, known as penicillin-binding proteins or PBPs, prevents the normal cross-linking of the peptide chains of peptidoglycan and results in cell death. The pathogenic gram-negative organism *Neisseria gonorrhoeae* has three PBPs, denoted PBPs 1, 2, and 3 (1). PBPs 1 and 2 are the major antibiotic killing targets for *N. gonorrhoeae*, whereas PBP 3 is not essential for cell viability (1). Because PBP 2 has an approximately 10-fold higher affinity for penicillin G than PBP 1, penicillin kills *N. gonorrhoeae* at its MIC by inactivation of PBP 2 (1).

Early isolates of pathogenic *N. gonorrhoeae* were extremely sensitive to penicillin. However, penicillin-resistant strains have now emerged which have greatly reduced the efficacy of this antibiotic in the treatment of gonococcal infections. Penicillin resistance in *N. gonorrhoeae* has arisen by two major mechanisms: the plasmid-mediated production of a TEM-1 β-lactamase and by chromosomally mediated expression of multiple-resistance genes (3). In chromosomally mediated resistance, alterations of both membrane permeability and essential PBPs have resulted in a 1,000-fold increase in the MIC for penicillin. However, the genetic and biochemical events underlying penicillin resistance in these strains have yet to be fully elucidated (7, 10). Since high-level penicillin resistance (MIC $\geq$ 2 μg/ml) is always correlated with expression of an altered PBP 1 with a decreased affinity for penicillin (6, 7), it is highly likely that the PBP 1 gene is involved in mediating high-level penicillin resistance.

In order to delineate the role of PBP 1 in chromosomally mediated, high-level penicillin resistance, we have cloned the gene encoding PBP 1 (*ponA*) from *N. gonorrhoeae* FA19, a penicillin-susceptible laboratory strain. The *ponA* sequence shows significant homology to PBPs from other bacteria and contains all of the conserved sequence motifs found in high-molecular-mass PBPs. In addition, we report the sequence of the *ponA* gene from *Neisseria meningitidis.*

Purification and NH_2-terminal sequencing of PBP 1. Due to the lack of a genetic selection for PBP 1, a reverse genetic approach was used to clone the PBP 1 gene. Large quantities of strain FA19 were grown in liquid culture, and membranes were prepared from these cells. PBP 1 was extracted from the membrane preparation and purified by covalent ampicillin affinity chromatography (25). Figure 1 shows an aliquot of purified PBP 1 separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A major staining band at a molecular mass of ~90 kDa was visible among multiple lightly stained bands throughout the lane (Fig. 1A, lane 3). When purified PBP 1 was incubated with ^{125}I-penicillin V prior to electrophoresis and autoradiographed, it was covalently labeled with the radioactive antibiotic and comigrated with ^{125}I-penicillin V-labeled PBP 1 from FA19 membranes (Fig. 1B, lanes 1 and 2). In addition to PBP 1, several other bands of lower intensity were observed (Fig. 1B). The two minor bands directly below PBP 1 represent proteins that comigrated with PBPs 2 and 3, which likely copurified with PBP 1. These PBPs are only minor contaminants in the PBP 1 preparation since PBP 2 is not released efficiently from the antibiotic resin with hydroxylamine and PBP 3 is not readily extracted from the membranes by 1% Triton X-100–1 M NaCl (23a). The band above PBP 1 represents a PBP 1 dimer, since this band was recognized by polyclonal antibodies raised against PBP 1 (data not shown).

Aliquots of PBP 1 were separated by SDS-PAGE, electroblotted onto polyvinylidene difluoride membranes, and subjected to NH_2-terminal amino acid sequencing. A 25-amino-acid sequence, IKKILTT(?)FGLFFGF(?)VFGVGLVAI (the question marks stand for unknown amino acids), was obtained from the blotted protein. This amino acid sequence showed no significant homology to any other high-molecular-mass PBPs cloned to date or to any other known bacterial proteins.

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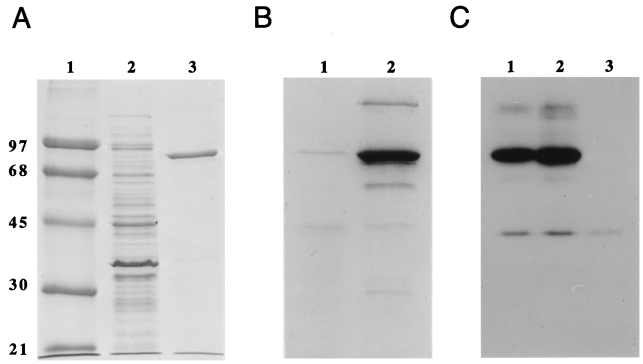

FIG. 1. Analysis of purified PBP 1 from FA19 gonococcal membranes and overexpression in *E. coli*. PBP 1 was purified from supernatants of Triton X-100-solubilized FA19 membranes as described previously (25) and subjected to SDS-PAGE. (A) Coomassie brilliant blue staining. Lane 1, 1.5 μg each of protein standards; lane 2, 20 μg of FA19 membranes; lane 3, 2 μg of purified PBP 1. (B) Autoradiography of an identical gel in which the protein samples were incubated with ^{125}I-penicillin V prior to SDS-PAGE. Lane 1, FA19 membranes; lane 2, purified PBP 1. (C) Expression in *E. coli*. The *ponA* gene was cloned into the expression plasmid pET15b and expressed in *E. coli* BL21(DE3). Membranes were prepared from cells, and aliquots were incubated with ^{125}I-penicillin V prior to SDS-PAGE and autoradiography. Lane 1, membranes prepared from bacteria transformed with the expression construct containing the gonococcal *ponA* gene; lane 2, membranes prepared from bacteria transformed with the expression construct containing the meningococcal *ponA* gene; lane 3, membranes prepared from bacteria transformed with the pET15b vector alone. Molecular mass standards are in kilodaltons.

Cloning of the *ponA* gene encoding PBP 1. Two degenerate oligonucleotides were used to clone the PBP 1 gene. The first oligonucleotide (GC2) was based on a region of the PBP 1 NH_2-terminal peptide sequence, FGLFFGF, whereas the second oligonucleotide (GC5) corresponded to a highly conserved amino acid sequence (the QGAST box [22]) found in all class A high-molecular-mass PBPs cloned to date. Duplicate filter lifts of an FA19 *Dra*I λZapII phage library (4) were probed separately with GC2 and GC5, and three clones that hybridized to both probes were isolated and plaque purified.

Sequence analysis of the 2.6-kb *Dra*I fragment in the three clones revealed a long open reading frame (ORF) spanning 2,397 bp and encoding a protein 798 amino acids in length (Fig. 2). Although two in-frame methionine codons 21 bp apart were identified at the start of the ORF, the sequence predicted from the second translation start site was identical to the NH_2-terminal sequence derived from the purified protein. Thus, the second methionine codon appears to be the preferred start site. Neither ATG codon is preceded by an optimal ribosomal binding site sequence. The stop codon of the ORF is contained within the *Dra*I restriction site (TTT **T**↓**AA** AGA; the *Dra*I site is underlined, the cleavage site is indicated by the vertical arrows, and the stop codon is in boldface). The protein product deduced from this ORF had a calculated molecular mass of 88,425 Da, very close to the size of PBP 1 derived from SDS-PAGE. The transcriptional start site was determined by primer extension to be 65 bp upstream of the ATG start codon (Fig. 2; also data not shown). While there are no strong matches to −35 and −10 consensus promoter sequences upstream of the transcriptional start site, the best matches to the −35 (TTGA CA) and −10 (TATAAT) consensus sequences are TGAAAG and TACAGT, respectively, which are spaced 15 bp apart (Fig. 2). Three copies of the 10-bp gonococcal uptake sequence GCCGTCTGAA (9) were identified within the sequenced region of the *ponA* gene. A single copy, found within the ORF of the *ponA* gene, was identified on the noncoding strand at nucleotides 1672 to 1681 (Fig. 2). Two additional copies are present within the inverted repeats immediately downstream of the *ponA* stop codon. The occurrence of uptake sequences within the inverted repeats has been observed previously in the gonococcal *uvrB* gene (2). We have named this gene *ponA* in accordance with the nomenclature used for *Escherichia coli* (24).

Analysis of the deduced amino acid sequence of *ponA* revealed all of the features characteristic of class A high-molecular-mass PBPs. PBP 1 contains a 25-amino-acid region of high hydrophobicity very near the NH_2 terminus that likely functions as both a noncleavable signal sequence and a transmembrane anchor. Following this region is the putative transglycosylase domain that catalyzes polymerization of the glycan chains of peptidoglycan. The regions comprising residues 88 to 97, 117 to 127, and 286 to 293 correspond to boxes 1, 2, and 4, respectively, described by Ghuysen and Dive (13) and display a significant conservation of amino acids when compared to other class A PBPs. These conserved regions may play a role in forming the active site of the putative transglycosylase domain of these multifunctional proteins. Within the carboxy-terminal transpeptidase domain of PBP 1 three regions can be found that are highly conserved in all members of the penicilloyl serine transferase superfamily. These regions include the SXXK tetrad containing the active-site serine at residues 461 to 464, the SXN triad at residues 521 to 523, and the KT(S)G tetrad at residues 651 to 654 (Fig. 2). On the basis of the three-dimensional structures of several β-lactamases (5, 15, 19), the *Streptomyces* R61DD-peptidase (16), and PBP 2x from *Streptococcus pneumoniae* (20), it has been determined that these conserved motifs are brought close together in the folded protein to form the active-site pocket that interacts with β-lactam antibiotics.

Comparison of the predicted amino acid sequence of PBP 1 with other class A high-molecular-mass PBPs with the GAP alignment program of the Genetics Computer Group indicated that *N. gonorrhoeae* PBP 1 displays the highest sequence identity to *E. coli* PBP 1A (41.7%), followed by *Haemophilus influenzae* PBP 1 (38.6%), *S. pneumoniae* PBP 1 (31%), *Streptococcus oralis* PBP 1 (31%), *Bacillus subtilis* PBP 1 (31%), and *E. coli* PBP 1B (31%).

Comparison of the *ponA* sequences from FA19, FA1090, and *N. meningitidis* Z2491. With primers derived from the FA19 *ponA* gene sequence, the *ponA* genes from FA1090 and *N. meningitidis* Z2491 (group A serotype) were amplified by PCR and subcloned into pUC19K. Individual clones were then sequenced. The *ponA* gene sequence from FA1090 was 100% identical to that from FA19. The *ponA* gene from *N. meningitidis* contained 62 nucleotide changes compared to the FA19 sequence, which corresponds to an overall nucleotide identity of 97.6%. However, these changes were not distributed equally throughout the gene. Instead, the differences in the nucleotide sequence of the meningococcal gene were clustered at the very 3′ end of the coding sequence (Fig. 2). There were 29 nucleotide changes within the last 124 bp of the gene (76.6% identity), compared to 33 nucleotide changes throughout the first 2,270 bp of the gene (98.5% identity). Consequently, a similar pattern was seen at the amino acid level; the proteins were 98.8% identical within the first 758 amino acid residues (749/758) versus 70% identical (28/40) within the carboxy-terminal 40 amino acids.

Expression of PBP 1 in *E. coli*. To confirm that the *ponA* gene encodes PBP 1, both the gonococcal and the meningococcal genes were subcloned into the expression vector pET15b (Novagen, Madison, Wis.) and transformed into *E. coli* BL21(DE3) cells. Membranes were prepared from over-

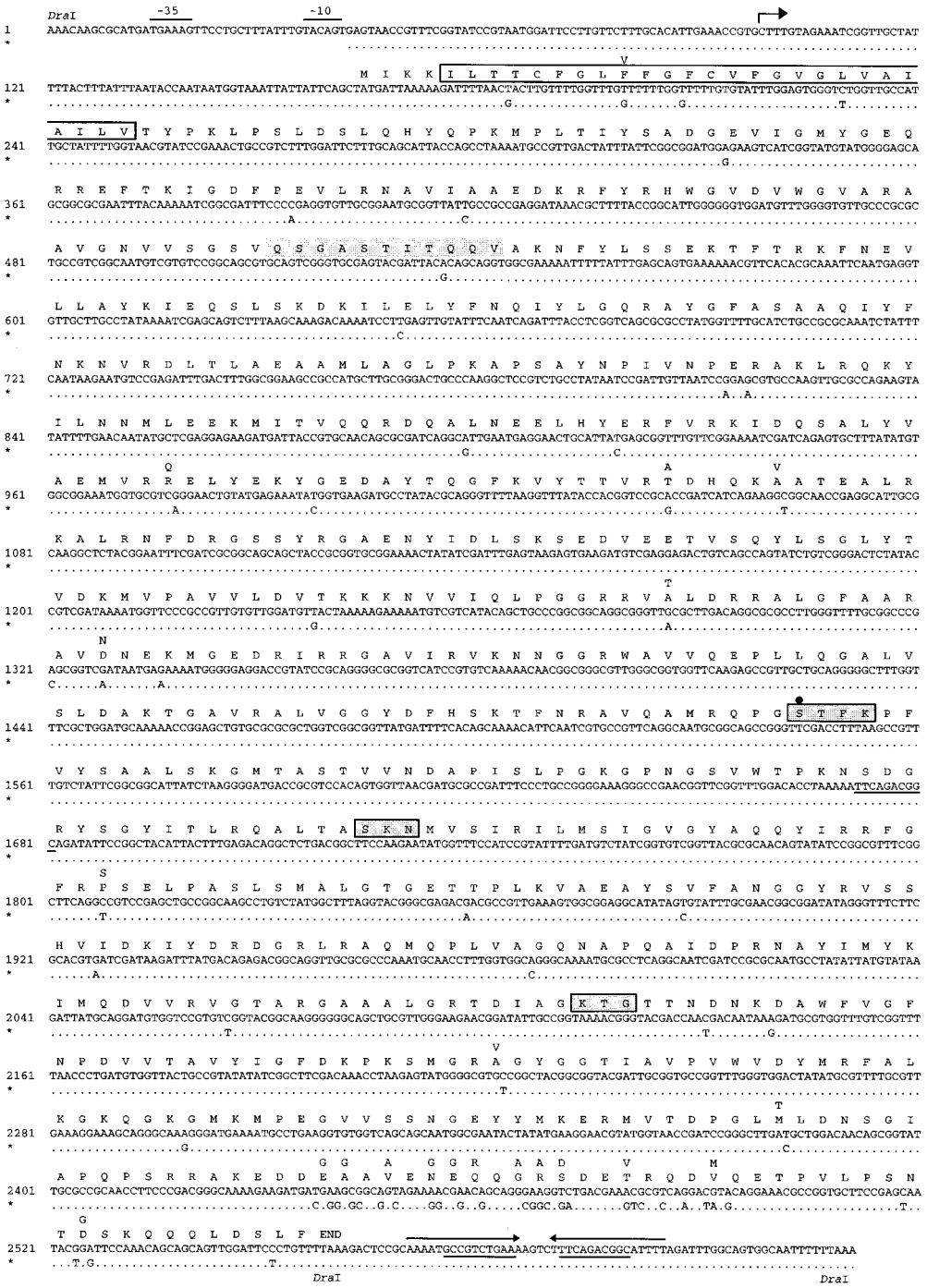

FIG. 2. Nucleotide sequences of the *ponA* genes encoding PBP 1 from both *N. gonorrhoeae* and *N. meningitidis* and deduced amino acid sequences. Duplicate filters of a λZapII library (kindly provided by Fred Sparling) containing *Eco*RI-linkered fragments from *Dra*I-digested FA19 DNA were hybridized separately with ^{32}P-labeled oligonucleotides GC2 (5′-ATIGCIACIARICCIAC-3′ where R = A or G and I = inosine) or GC5 (5′-SARGGIGSIWSIRCIHTIACICARCARYT-3′ where H = A, C, or T; S = C or G; W = A or T; and Y = C or T). Three clones hybridizing to both probes were plaque purified, and plasmid DNA was excised by using the ExAssist/Solr system protocol (Stratagene, La Jolla, Calif.). The clones were then sequenced using Sequenase version 2.0 (Amersham, Arlington, Heights, Ill.). The gonococcal PBP 1 amino acid sequence from FA19 is shown above the nucleotide sequence. The nucleotide sequence of the meningococcal PBP 1 gene is on the lines indicated by asterisks, and identical nucleotides are denoted by a dot. Differences in the amino acid sequences of the species homologs are shown on the lines marked #. Inverted repeat sequences capable of functioning as transcription terminators are indicated by the horizontally opposed arrows at the 3′ end of the gene. The putative transmembrane domain is denoted by the unshaded box at the NH_2 terminus of the translated protein, whereas the shaded boxed regions highlight the amino acid sequences conserved in the active-site regions of all penicillin-binding proteins. The active-site serine is indicated by the solid bullet. The conserved class A high-molecular-mass PBP consensus sequence used in the cloning of the PBP 1 gene is indicated by the shaded, unboxed region. The transcriptional start site of the *ponA* gene is indicated by the arrow, and putative promoter elements are overlined. Three 10-bp gonococcal DNA uptake sequences are underlined.

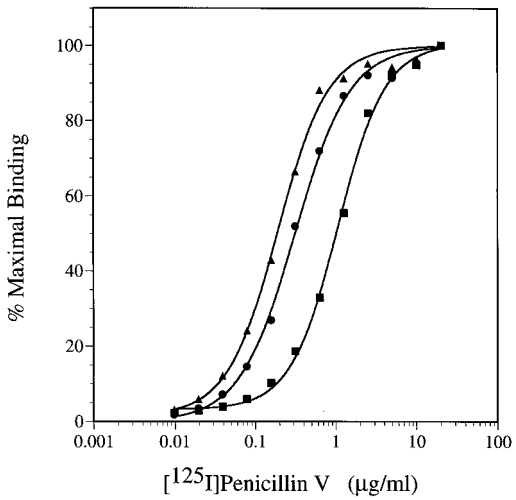

FIG. 3. Affinities of native and recombinant PBP 1 for ^{125}I-penicillin V. Aliquots of Triton X-100-solubilized membranes were incubated with increasing concentrations of ^{125}I-penicillin V for 20 min at 37°C. The samples were subjected to SDS-PAGE on 10% gels, and the amount of radioactivity in the PBP 1 bands was quantitated with a Molecular Dynamics Storm 840 PhosphorImager. ■, FA19 membranes; ●, gonococcal PBP 1 expressed in *E. coli*; ▲, meningococcal PBP 1 expressed in *E. coli.*

night cultures, incubated with ^{125}I-penicillin V, and subjected to SDS-PAGE. Autoradiography of the gel revealed the expression of a new PBP in cells containing the *ponA* gene from both neisserial species; the new PBP comigrated with PBP 1 purified from *N. gonorrhoeae* FA19 (Fig. 1C). These data indicate that the cloned *ponA* genes from both *N. gonorrhoeae* and *N. meningitidis* encode PBP 1.

Measurement of the affinities of ^{125}I-penicillin V for native and *E. coli*-expressed PBP 1. The affinities of PBP 1 isolated from FA19 membranes and of both gonococcal and meningococcal PBP 1 overexpressed in bacteria for ^{125}I-penicillin V were measured by a modified version of the protocol described by Barbour (1). The affinity of a PBP is defined as the concentration of antibiotic that results in the covalent labeling of 50% of that PBP under defined conditions of time of incubation and temperature (14). Aliquots of Triton X-100-solubilized membranes were incubated with serial dilutions of ^{125}I-penicillin V for 20 min at 37°C and were subjected to SDS-PAGE. Radiolabeled PBP 1 proteins were then quantitated with a Storm 840 PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.). The data in Fig. 3 show that the overexpressed gonococcal PBP 1 and meningococcal PBP 1 (affinities, 0.3 and 0.2 μg/ml, respectively) have affinities 3.5- to 5-fold higher than that of native PBP 1 from FA19 membranes (affinity, 1.1 μg/ml). The reason for the small differences between the affinities of PBP 1 from FA19 and PBP 1 overexpressed in *E. coli* for ^{125}I-penicillin V is not known but may involve differences in the amount of PBP 1 protein assayed or the presence of another protein in gonococcal membranes that alters the affinity of PBP 1 for the antibiotic. Barbour (1) reported an affinity of PBP 1 in FA19 membranes for [^{3}H]penicillin G of 0.2 nmol/ml (~0.095 μg/ml), which is approximately 2-fold higher than the affinity of the *E. coli*-expressed gonococcal PBP 1 for ^{125}I-penicillin V and 10-fold higher than the affinity of FA19 PBP 1 for ^{125}I-penicillin V. These differences most likely are a result of the structural differences between the two antibiotics.

Organization of the *ponA* gene region in *N. gonorrhoeae* and deletion analysis of the *ponA* gene. Biochemical experiments have indicated that PBP 1 is an essential protein in cell wall synthesis. To confirm these experiments, we disrupted the *ponA* gene by insertional inactivation and attempted to transform the disrupted gene back into FA19. In order to obtain sufficient upstream and downstream flanking sequences of the *ponA* gene for the transformation experiments, we constructed an *Eco*RI-restricted FA19 library in λDashII and screened the library with a probe derived from the *ponA* gene. Several positive clones that contained an approximately 12-kb *Eco*RI insert were isolated, and sequence analysis of the DNA flanking the *ponA* gene from one of these clones (λ5-3; Fig. 4A) revealed four ORFs upstream and oriented in the opposite direction to the *ponA* gene and one ORF downstream of the *ponA* gene, also oriented in the opposite direction. The four ORFs upstream of the *ponA* gene were homologous to pilus biosynthesis genes *pilM*, *pilN*, *pilO*, and *pilP* previously characterized in *Pseudomonas aeruginosa* and *Pseudomonas syringae* (17, 23). The FA19 *pilO* gene product is nearly identical to the gonococcal strain MS11 *pilO* gene product, differing only by a single amino acid residue. Similarly, the *pilP* gene, which spans the *Eco*RI site of the clone and is therefore incomplete at its 3′

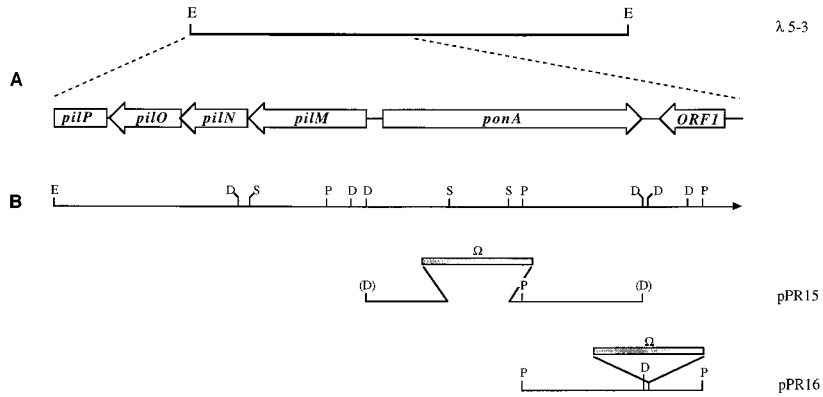

FIG. 4. Genomic organization of the *ponA* gene and flanking regions. (A) Genomic organization of 6.4 kb of λ DASHII clone 5-3 containing the *ponA* gene and flanking ORFs. Arrows indicate the six ORFs identified by sequence analysis within this 6.4-kb fragment. The gene designation of each ORF is indicated. The direction of transcription is indicated by the direction of the arrow. (B) Restriction map of the 6.4-kb fragment. Shown are the sites used to construct plasmids containing the Ω fragment, as indicated below the restriction map. The sites are as follows: E, *Eco*RI; D, *Dra*I; S, *Sty*I; and P, *Pst*I. Plasmids harboring the Ω fragment encoding streptomycin/spectinomycin resistance within either the *ponA* coding region (pPR15) or the 3′ noncoding region of the *ponA* gene (pPR16) were used in transformation experiments as described in the text.

end, is nearly identical (the gene products differ by two amino acids) to the published *pilP* sequence of MS11 (8). The organization of the pilus biosynthesis genes relative to the *ponA* gene in *N. gonorrhoeae* is identical to that of both *P. aeruginosa* (17) and *P. syringae* (23). This organization was also consistent with genomic mapping experiments (4a) that localized the *ponA* gene of *N. gonorrhoeae* near the *omc* gene recently shown to encode PilQ (8). The ORF downstream of the *ponA* gene (ORF1; Fig. 4A) encodes a polypeptide which shows significant identity (46%) to a hypothetical GTP-binding protein of unknown function in *E. coli* (21), *H. influenzae* (11), and *Mycoplasma genitalium* (12). Thus, ORF1 likely encodes the gonococcal homolog of this hypothetical GTPase.

The plasmid pPR15 was constructed to replace an internal 525-bp *Sty*I fragment of the *ponA* gene with the streptomycin/spectinomycin resistance gene (the Ω fragment; Fig. 4B), resulting in the insertional inactivation of the *ponA* gene. As a control for transformation, the Ω fragment also was inserted downstream of the *ponA* gene (pPR16; Fig. 4B). Transformation of competent FA19 with pPR15 resulted in no growth on selective medium, even after 72 h. In contrast, transformation of FA19 with pPR16 resulted in growth of streptomycin-resistant colonies after 24 h of incubation. These results strongly suggest that disruption of the *ponA* gene results in the loss of viability of the gonococci and are consistent with previous experiments indicating that PBP 1 is an essential protein involved in cell wall synthesis in *N. gonorrhoeae.*

Chromosomally mediated penicillin resistance in *N. gonorrhoeae* has given rise to an altered PBP 1 with a reduced affinity for penicillin G (6). The cloning of the *ponA* gene from *N. gonorrhoeae* will allow us to address the role of PBP 1 in the acquisition of high-level penicillin resistance in *N. gonorrhoeae*. Cloning and sequencing of the *ponA* genes from several strains resistant to high levels of penicillin may identify amino acid mutations that result in a lower affinity of PBP 1 for penicillin G. Similar studies have been carried out with the *penA* gene encoding PBP 2 from multiple clinical isolates of penicillin-resistant *N. gonorrhoeae* (18). These studies have shown that penicillin-resistant isolates of *N. gonorrhoeae* and *N. meningitidis* contain *penA* genes with mosaic structures that presumably have arisen by horizontal transfer of *penA* genes from a commensal species via transformation and homologous recombination. A similar scenario may have occurred within the *ponA* genes of gonococcal isolates with high levels of penicillin resistance.

Finally, it is apparent that *N. gonorrhoeae* has a relatively simple PBP pattern, with one low-molecular-mass PBP (PBP 3), one high-molecular-mass class B PBP (PBP 2), and one high-molecular-mass class A PBP (PBP 1). This property makes *N. gonorrhoeae* an excellent model system to study in order to better understand the roles of these PBPs in cell morphogenesis, since there appears to be no redundancy in its PBP complement.

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been submitted to the GenBank database under the accession numbers U72876 and U80933.

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REFERENCES

1. **Barbour, A. G.** 1981. Properties of penicillin-binding proteins in *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **19:**316–322.
2. **Black, C. G., J. A. Fyfe, and J. K. Davies.** 1995. A promoter associated with the neisserial repeat can be used to transcribe the *uvrB* gene from *Neisseria gonorrhoeae*. J. Bacteriol. **177:**1952–1958.
3. **Cannon, J. G., and P. F. Sparling.** 1984. The genetics of the gonococcus. Annu. Rev. Genet. **38:**111–133.
4. **Cornelissen, C. N., G. D. Biswas, J. Tsai, D. K. Paruchuri, S. A. Thompson, and P. F. Sparling.** 1992. Gonococcal transferrin-binding protein 1 is required for transferrin utilization and is homologous to TonB-dependent outer membrane receptors. J. Bacteriol. **174:**5788–5797.

4a. **Dempsey, J., and J. Cannon.** Unpublished data.

5. **Dideberg, O., P. Charlier, J.-P. Wery, P. Dehottay, J. Dusart, T. Erpicum, J.-M. Frere, and J.-M. Ghuysen.** 1987. The crystal structure of the β-lactamase of *Streptomyces albus* G at 0.3 nm resolution. Biochem. J. **245:**911–913.
6. **Dougherty, T. J.** 1986. Genetic analysis and penicillin-binding protein alterations in *Neisseria gonorrhoeae* with chromosomally mediated resistance. Antimicrob. Agents Chemother. **30:**649–652.
7. **Dougherty, T. J., A. E. Koller, and A. Tomasz.** 1980. Penicillin-binding proteins of penicillin-susceptible and intrinsically resistant *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **18:**730–737.
8. **Drake, S. L., and M. Koomey.** 1995. The product of the *pilQ* gene is essential for the biogenesis of type IV pili in *Neisseria gonorrhoeae*. Mol. Microbiol. **18:**975–986.
9. **Elkins, C., C. E. Thomas, H. S. Seifert, and P. F. Sparling.** 1991. Species-specific uptake of DNA by gonococci is mediated by a 10-base-pair sequence. J. Bacteriol. **173:**3911–3913.
10. **Faruki, H., and P. F. Sparling.** 1986. Genetics of resistance in a non-β-lactamase-producing gonococcus with relatively high-level penicillin resistance. Antimicrob. Agents Chemother. **30:**856–860.
11. **Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J. F. Tomb, B. A. Dougherty, J. M. Merrick, et al.** 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269:**496–512.
12. **Fraser, C. M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, et al.** 1995. The minimal gene complement of *Mycoplasma genitalium*. Science **270:**397–403.
13. **Ghuysen, J.-M., and G. Dive.** 1994. Biochemistry of the penicilloyl serine transferases, p. 103–129. *In* J.-M. Ghuysen and R. Hakenbeck (ed.), Bacterial cell walls. Elsevier Science B.V., Amsterdam, The Netherlands.
14. **Ghuysen, J.-M., J.-M. Frére, M. Leyh-Bouille, M. Nguyen-Disteche, and J. Coyette.** 1986. Active-site-serine D-alanyl-D-alanine-cleaving-peptidase-catalysed acyl-transfer reactions. Biochem. J. **235:**159–165.
15. **Herzberg, O., and J. Moult.** 1987. Bacterial resistance to β-lactam antibiotics: crystal structure of β-lactamase from *Staphylococcus aureus* PC1 at 2.5 Å resolution. Science **236:**694–701.
16. **Kelly, J. A., J. R. Knox, P. C. Moews, G. J. Hite, J. B. Bartolone, H. Zhao, B. Joris, J. M. Fretre, and J. M. Ghuysen.** 1985. 2.8-A structure of penicillin-sensitive D-alanyl carboxypeptidase-transpeptidase from *Streptomyces* R-61 and complexes with β-lactams. J. Biol. Chem. **260:**6449–6458.
17. **Martin, P. R., A. A. Watson, T. F. McCaul, and J. S. Mattick.** 1995. Characterization of a five-gene cluster required for the biogenesis of type 4 fimbrae in *Pseudomonas aeruginosa*. Mol. Microbiol. **16:**497–508.
18. **Matsuhashi, M., I. N. Maruyama, Y. Takagaki, S. Tamaki, Y. Nishimura, and Y. Hirota.** 1978. Isolation of a mutant of *Escherichia coli* lacking penicillin-sensitive D-alanine carboxypeptidase 1A. Proc. Natl. Acad. Sci. USA **75:**2631–2635.
19. **Moews, P. C., J. R. Knox, O. Dideberg, P. Charlier, and J.-M. Frére.** 1990. β-lactamase of *Bacillus licheniformis* 749/C at 2Å resolution. Proteins **7:**156–171.
20. **Pares, S., N. Mouz, Y. Petillot, R. Hakenbeck, and O. Dideberg.** 1996. X-ray structure of *Streptococcus pneumoniae* PBP 2×, a primary penicillin target enzyme. Nat. Struct. Biol. **3:**284–289.
21. **Plunkett, G., III, V. Burland, D. L. Daniels, and F. R. Blattner.** 1993. Analysis of the *Escherichia coli* genome. III. DNA sequence of the region from 87.2 to 89.2 minutes. Nucleic Acids Res. **21:**3391–3398.
22. **Popham, D. L., and P. Setlow.** 1994. Cloning, nucleotide sequence, mutagenesis, and mapping of the *Bacillus subtilis pbpD* gene, which codes for penicillin-binding protein 4. J. Bacteriol. **176:**7197–7205.
23. **Roine, E., D. N. Nunn, L. Paulin, and M. Romantschuk.** 1996. Characterization of genes required for pilus expression in *Pseudomonas syringae* pathovar phaseolicola. J. Bacteriol. **178:**410–417.

23a. **Ropp, P. A., D. E. Schultz, and R. A. Nicholas.** Unpublished data.

24. **Suzuki, H., Y. Nishimura, and Y. Hirota.** 1978. On the process of cellular division in *Escherichia coli*: a series of mutants of *E. coli* altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. USA **75:**664–668.
25. **Tamura, T., H. Suzuki, Y. Nishimura, J. Mizoguchi, and Y. Hirota.** 1980. On the process of cellular division in *Escherichia coli*: isolation and characterization of penicillin-binding proteins 1a, 1b, and 3. Proc. Natl. Acad. Sci. USA **77:**4499–4503.
26. **Tipper, D. J., and J. L. Strominger.** 1965. Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proc. Natl. Acad. Sci. USA **54:**1133–1141.