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Variable Expression of Class 1 Outer Membrane Protein in *Neisseria meningitidis* Is Caused by Variation in the Spacing between the −10 and −35 Regions of the Promoter

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The class 1 outer membrane protein encoded by the *porA* gene of *Neisseria meningitidis* is a candidate for a vaccine against meningococcal infection. The expression of class 1 outer membrane protein displays phase variation between three expression levels. Northern (RNA) blot and primer extension analysis revealed that this phase variation is regulated at the transcriptional level. The start site for transcription is located 59 bp upstream of the translational initiation codon. Sequence analysis of the promoter region of the *porA* gene of a variant without class 1 protein expression revealed nine contiguous guanidine residues between the −10 and −35 domains. Comparison of promoter sequences of different phase variants indicated that the length of the polyguanidine stretch correlated with the expression level of the class 1 outer membrane protein; the presence of 11, 10, or 9 contiguous guanidine residues results in high levels, medium levels, or no expression of class 1 mRNA, respectively. These results suggest that the variable *porA* expression levels seen in different isolates are modulated by guanidine residue insertion and/or deletion due to slipped-strand mispairing on the polyguanidine stretch within the intervening sequence of the −35 and −10 regions of the promoter. The phase variation of class 1 outer membrane protein may provide a molecular mechanism to evade the host immune defense. Therefore, the protective efficacy of a vaccine based on class 1 outer membrane protein may be questioned.

The major outer membrane proteins of *Neisseria meningitidis* are of interest, since they are responsible for serological differentiation of strains (5), and are under investigation as components of experimental vaccines against meningococcal infection (7, 33). Five distinct classes of outer membrane proteins can be recognized in meningococcal outer membranes (32). The class 5 proteins are hypervariable, undergoing antigenic shift during infection and phase changes between and within strains, a property which enables meningococci to evade the host immune response (29, 30). The class 4 protein is antigenically stable and can induce antibodies which block the bactericidal effect of antibodies directed against the other surface antigens (18). All meningococci express either a single class 2 or class 3 protein as the predominant protein of the outer membrane. Expression of either class is mutually exclusive, and the proteins are therefore proposed to be alleles of a single gene, *porB* (10). Class 2 and class 3 as well as class 1 proteins show antigenic variability. These variabilities of the class 2 and/or 3 and class 1 proteins of different isolates form the basis of serological differentiation into serotypes and subtypes, respectively.

Class 1 protein is a pore-forming protein with cationic selectivity (31) and is the product of the *porA* gene locus (1). Despite the antigenic variation displayed by the class 1 protein, this protein is a potential component of *N. meningitidis* vaccines. Immunization of mice with outer membrane complexes results in bactericidal antibodies directed mainly against class 1 outer membrane protein. Its value as a vaccine candidate is derived from experiments in which monoclonal antibodies directed against subtype-specific epitopes on class 1 protein were effective in bactericidal assays and conferred protection in an animal model (15, 16, 25, 26). Class 1 protein is expressed by most of the clinical isolates, but with variation in the levels of expression (11, 21).

Recently, we described clinical isolates lacking class 1 outer membrane protein. Moreover, variability of class 1 protein expression levels within a clinical isolate was observed (11). Since stable expression of this protein is a prerequisite for an effective vaccine approach, we investigated the genetic mechanism behind the variable expression of class 1 protein in more detail. First, the presence of the *porA* gene in the class 1-deficient variants was determined. The results show that all class 1 expression variants have an intact *porA* gene. Primer extension analysis revealed that the class 1-positive variants have *porA* transcripts starting 59 nucleotides upstream of the open reading frame. The class 1-negative phase variants had no *porA* transcript. Sequence analysis of the promoter region showed a poly(G) stretch flanked by −35 and −10 domains. The length of the poly(G) stretch varies and is associated with the expression level of the class 1 outer membrane protein. These results suggest that transcription of the *porA* gene can be regulated by changes in the poly(G) stretch length and thus by the spacing of the −35 and −10 regions of its promoter. Presumably, the poly(G) stretch length changes arise from slipped-strand mispairing of this region during replication. Phase variation of class 1 outer membrane protein is a possible mechanism to evade the host immune defense. Therefore, the protective efficacy of a vaccine based on class 1 outer membrane protein may be questioned.

MATERIALS AND METHODS

Strains, culture conditions, and chromosomal DNA isolation. *N. meningitidis* strains 2996 (B:2b:P1.2,5), 2257 (B:4:P1.4), and 732 (C:2b:P1.10) were clinical

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TABLE 1. Primers used in this study

Primer	Sequence[a]	Position (nt)[b]
P1-1	GGT CAA TTG CGC CTG GAT GTT CCT G	166–190
P1-2	CGC TGA TTT TCG TCC TGA TGC GGC	252–275
PorA3	CCA AAT CCT CGC TCC CCT TAA AGC C	292–317
PorA5	CCG AGA CTG CAT CCG GGC	
Random primer 10	CCT TTC TAG ATC AGG TTA AAA ACT TTC C	
P21	CTG TAC GGC GAA ATC AAA GCC GGC CGT	134–160
P22	TTA GAA TTT GTG GCG CAA ACC GAC	1226–1249
PorA6	CAG GAA ACA GCT ATG ACC GCG GAC AAT ACG AGG GCG G	84–102
PorA7	TGT AAA ACG ACG GCC AGT GCA CGA GGT CTG CGC	

[a] The underlined sequences are the −21M13 and M21Rev complementary sequences in PorA6 and PorA7, respectively.
[b] The nucleotide (nt) position is determined according to the sequence published by Barlow et al. (1).

isolates from cerebrospinal fluid collected by the Reference Laboratory for Bacterial Meningitis, University of Amsterdam and National Institute of Public Health and Environmental Protection, in the years 1974, 1989, and 1992, respectively. Bacteria were grown on a GC agar base (Difco Laboratories, Inc., Detroit, Mich.) containing 1% Vitox supplement (Oxoid Laboratories Ltd., Basingstoke, United Kingdom) at 37°C in a humified atmosphere of 5% CO_2 in air. Chromosomal DNA was prepared as described previously (14).

Northern (RNA) hybridization. Total meningococcal RNA was isolated from four isogenic variants of clinical isolate 2257 with strong, moderate, or no expression of class 1 protein as described previously (34). Equal amounts of the RNA were electrophoresed on a 1.6% agarose gel and transferred to a nylon membrane (Zeta Probe; Biorad, Upplands Vasby, Sweden) (23). The blots were probed with a PCR product made with primers P21 and P22, containing the *porA* gene. The probe was labeled with digoxigenin (DIG) (Boehringer Mannheim GmbH, Mannheim, Germany) according to the instructions supplied by the manufacturer. After hybridization, the probes were detected with anti-DIG antibodies conjugated with alkaline phosphatase and by staining according to the instructions supplied by Boehringer Mannheim GmbH. The bands on the blot were quantified by densitometry.

Oligonucleotide synthesis. The primers used in this study are described in Table 1. Primers P21 and P22 were the kind gift of M. Maiden, Hertfordshire, United Kingdom. All other primers were synthesized by Perkin-Elmer Nederland B.V., Gouda, The Netherlands.

Detection of the *porA* gene by PCR. The presence of the *porA* gene was analyzed by PCR using primers P21 and P22 as described previously (15). The PCR products were analyzed on agarose (1%) gels by the Tris-acetate-EDTA buffer system (23).

Primer extension analysis. Total meningococcal RNA was isolated as described previously (34). Primer extension analysis was performed on total meningococcal RNA from isogenic variants of isolate 2257 as described before (2) with minor modifications, using primers P1-1 and PorA6. A reaction mixture that contained 15 μg of RNA, 15 ng of primer, and 0.3 μl of [α-^{32}P]dUTP (10 mCi/ml) was used instead of an end-labeled primer.

Isolation of DNA fragments upstream of the *porA* coding sequence by targeted genome walking. DNA fragments upstream of the coding region of the *porA* gene were obtained by targeted genome walking as described by Parker et al. (19), with modifications. In separate PCRs, primer PorA3 was combined with chromosomal DNA of the class 1 protein-deficient variant of isolate 2257 and 1 of 11 random primers. The reaction mixtures were incubated in a programmable heat block (Biorad) for 5 min at 95°C prior to 30 cycles of 1 min at 95°C and 1 min at 37°C, with a subsequent increase in the temperature to 72°C at a rate of 0.25°C/s and a final period of 1 min at 72°C. The reactions were completed by a further incubation for 5 min at 72°C. After purification by phenol-chloroform extraction, the PCR products were used as templates in a second PCR (5 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 60°C, and 1.5 min at 72°C; and completed by the last cycle of 5 min at 72°C) with the random primer used in the first PCR and primer P1-2 (located upstream of primer PorA3) (Table 1; Fig. 1). After separation on a 0.7% agarose gel, the PCR products were transferred onto a nylon membrane (Zeta Probe) (23) and identified by hybridization with primer P1-2 used in the second PCR as well as with P1-1, a primer located upstream of primer P1-2 (Fig. 1). The probes were labeled with DIG (Boehringer Mannheim GmbH) at their 3′ ends according to the instructions supplied by the manufacturer. After hybridization, the probes were detected with anti-DIG antibodies conjugated with alkaline phosphatase and by staining according to the instructions supplied by Boehringer Mannheim GmbH. With random primer 10 and PorA3, a product of about 1.5 kb, which was amplified in the second PCR, was observed. Both PCR products hybridized with P1-2 as well as with P1-1. Positive bands were excised from a duplicate gel, and the DNA was purified by QIAEX according to the instructions supplied by QIAGEN Inc., Chatsworth, Calif.

Fluorescence-based sequencing and analysis. PCR products obtained by targeted genome walking were subjected to a PCR-based sequence reaction with fluorescent dye-labeled dideoxynucleotide terminators using *Taq* polymerase (Perkin-Elmer), random primer 10, and primers P1-2 and P1-1 according to the instructions supplied by Applied Biosystems Incorporated (Foster City, Calif.). The sequences were confirmed by PCR-based sequencing (according to the instructions supplied by Applied Biosystems Incorporated with fluorescence dye-labeled universal primers −21M13 and M13 reverse) of a PCR product obtained with primers PorA6 and PorA7 (Table 1; Fig. 1) and were analyzed on an automatic sequenator (model 370A; Applied Biosystems Incorporated). Sequences were analyzed by using computer programs included in the 1991 PC/GENE (intelliGenetics, Inc.) program package. Alignment of sequences was accomplished by using the CLUSTAL program and the method developed by Higgins and Sharp (9).

RESULTS

Characterization of class 1 outer membrane protein-negative isolates. It was previously observed that a number of clinical isolates of *N. meningitidis* showed variation in the expression of class 1 outer membrane protein (11, 21). Detailed analysis by colony blotting and detection of the class 1 outer membrane with a monoclonal antibody revealed that these variants are variable as well as reversible in expression of the class 1 outer membrane protein. Variants with strong and moderate (30 to 40% of the strong expression level) expression and without detectable expression can be isolated at a frequency of about 10^{-3}, indicating that these are phase variants with altered levels of class 1 protein expression (11).

The presence of the *porA* gene in these class 1 protein variants, isolated from three different clinical isolates (2996, 2257, and 732) with either strong, moderate, or no class 1 protein expression was determined by PCR using *porA*-specific primers P21 and P22 (15). Two PCR products of 1,100 and 900

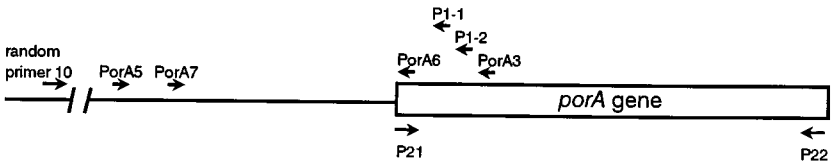

FIG. 1. Schematic representation of the locations of the different primers on the *porA* gene and its upstream region. The sequences of the primers and their positions on the sequence map of *porA* (1) are displayed in Table 1. The arrows indicate the direction of synthesis.

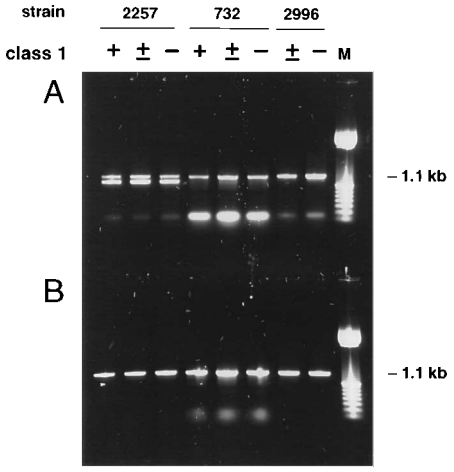

FIG. 2. Detection of the *porA* gene in clinical isolates 2996, 2257, and 732 of *N. meningitidis* by PCR using P21 and P22 as primers (15). The expression levels of class 1 outer membrane protein are indicated above the lanes. +, strong expression; ±, moderate expression; −, no expression. M, 100-bp ladder. Annealing temperatures of 60°C (A) and 70°C (B) were used in the PCRs. The position of the PCR product is marked on the right.

bp are observed after analysis by agarose gel electrophoresis using isolate 2257. Because of the homology of the *porB* gene (coding for the class 3 outer membrane protein) to *porA*, *porB* is also amplified (15), but amplification is not observed when the annealing temperature is increased to 70°C (Fig. 2B). Isolates 732 and 2996, having a class 2 outer membrane protein instead of a class 3 protein, do not exhibit the PCR product of 900 bp. The results show that variants without class 1 protein expression among colonies from three clinical isolates carry an intact *porA* gene.

Transcription of *porA* in class 1 outer membrane protein phase variants. To determine whether the phase variation of the class 1 outer membrane protein is regulated at the transcriptional level, the amount of *porA*-specific RNA in class 1 protein expression variants of isolates 2257 was estimated by Northern hybridization using the PCR product of primers P21 and P22 as a *porA*-specific probe. The *porA*-specific mRNA runs slightly faster on the agarose gel than 16S rRNA, which is about 1,600 nucleotides long (Fig. 3). The amount of *porA*-specific RNA correlated with the expression level of class 1 protein (Fig. 3). *porA*-specific transcripts were not detectable in the variant without class 1 protein expression. From these results, we conclude that phase variation of class 1 protein expression occurs at the level of transcription.

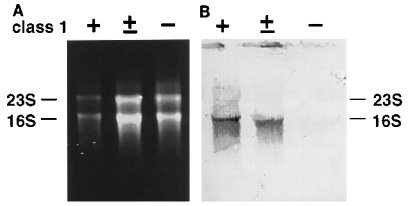

FIG. 3. Northern hybridization analysis of total RNA of class 1 outer membrane protein expression variants of *N. meningitidis* isolate 2257. (A) Ethidium bromide-stained gel. The 16S and 23S ribosomal RNA species are indicated. (B) Hybridization with the DIG-labeled PCR product of primers P21 and P22. From a duplicate gel, the RNA was transferred to a nylon membrane and *porA*-specific RNA was detected by hybridization. Strong (+) or moderate (±) expression or the absence of (−) expression of the class 1 protein is indicated.

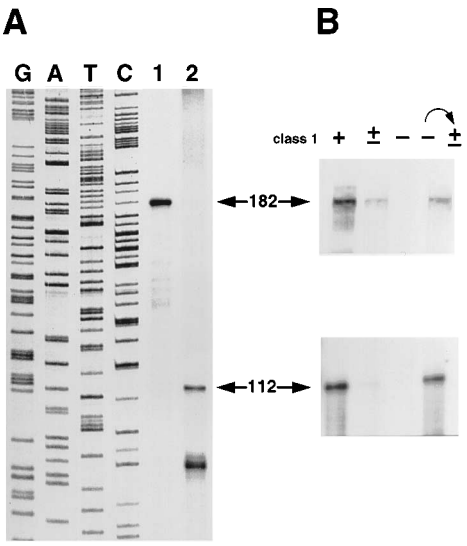

FIG. 4. (A) Primer extension analysis of total RNA from a variant of isolate 2257 expressing large amounts of class 1 protein by the use of primers P1-1 and PorA6. The positions (arrows) and sizes of the full-length cDNAs are indicated. The smaller cDNA species represent prematurely terminated cDNAs whose intensity varied from experiment to experiment. Lanes: G, A, T, and C, DNA sequencing ladder; 1, P1-1; 2, PorA6. (B) Primer extension analysis of total RNA from three class 1 protein expression variants (with strong [+], moderate [±], or no [−] class 1 protein expression) and a revertant from class 1 protein deficiency to moderate levels of class 1 protein expression (−→±) of isolate 2257.

The start point of transcription of the *porA* gene was determined by primer extension analysis. Total RNA of a variant of isolate 2257 expressing class 1 protein in large amounts was subjected to primer extension analysis using primer P1-1 and PorA6 (Table 1). The lengths of the primer extension products were 182 and 112 nucleotides, respectively (Fig. 4A). The cDNA lengths correspond to a transcriptional start point 59 nucleotides upstream of the start of the coding region, which is position 9 in the previously published sequence of the structural gene of class 1 protein of strain MC50 (1). From these results and the position of the putative termination site starting at position 1383 (1), the total length of the porA transcript can be calculated to be 1,374 nucleotides long. This value is in concordance with the observed mobility of *porA* mRNA on agarose gels (Fig. 3).

Analysis of other isogenic variants of isolate 2257 with different class 1 expression by primer extension showed that only the class 1-positive variants appeared to have *porA*-specific transcripts in an amount correlating with the level of class 1 protein expression (Fig. 4B). These results confirm the results obtained with Northern hybridization and support the conclusion that the phase variation of the levels of class 1 protein in meningococci occurs at the level of transcription.

Sequence of the promoter region of the *porA* gene. To determine the mechanism of the transcriptional phase variation of class 1 outer membrane protein, the nucleotide sequence of the region upstream of the start codon of *porA* containing the promoter of *porA* was analyzed. A DNA fragment of a class 1 protein-deficient variant of isolate 2257 containing the upstream region of *porA* was isolated by means of a genome walking technique (19). The PCR products obtained by this method, both of which hybridized with the *porA*-specific probe, were further analyzed by DNA sequencing.

The complete sequence of the region upstream of the coding region of *porA* is presented in Fig. 5. In this sequence, the

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1   GAGGTCTGCG CTTGAATTGT GTTGTAGAAA CACAACGTTT TTGAAAAAAT AAGCTATTGT
61  TTTATATCAA AATATAATCA TTTTTAAAAT AAAGGTTGCG GCATTTATCA GATATTTGGT
          -35                        -10          +1
121 CTGAAAAATG GTTTTTTGCG GGGGGGGGGG TATAATTGAA GACGTATCGG GTGTTTGCCC
181 GATGTTTTTA GGTTTTTATC AAATTTACAA AAGGAAGCCG ATATGCGAAA AAAACTTACC
241 GCCCTCGTAT TG
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FIG. 5. Sequence of the *porA* upstream region of isolate 2257 (class 1 protein negative). The transcriptional start point (T) is located at position 9 in the previously determined sequence (1). The −10, −35, and +1 transcription signals; the Shine-Dalgarno sequence; and the translational start codon are displayed in boldface type. The three oligo(T) stretches are underlined.

transcriptional start is located at position 164 and the AUG initiation codon was determined to be at position 223 (positions 9 and 63, respectively, of the previously published sequence [1]) (Fig. 5). Close examination of the sequence revealed a putative Pribnow box 7 bp upstream of the transcriptional start, with a perfect match to the consensus sequence of the −10 region of *Escherichia coli* promoters (8) (Fig. 5). This −10 region is flanked on the upstream side by a stretch of nine G nucleotides. The putative −35 sequence of the *porA* promoter is difficult to recognize. The best two candidates are ATGGTT and TGGTTT, with equal homology (two of six residues) to the consensus −35 sequence of *E. coli* (TTGACA) and located 16 and 17 bp upstream of the −10 sequence, respectively.

Phase variation of *porA* gene occurs by variation of the poly(G) stretch between the −10 and −35 regions of its promoter. The DNA sequences of both strands of the *porA* promoter regions of all variants from three different clinical isolates (732, 2257, and 2996) were determined by PCR-based sequencing using primers PorA3 and PorA5 and fluorescence dye-labeled dideoxynucleotides terminators. The sequences were confirmed by PCR-based sequencing using the primers PorA6 and PorA7 and the universal fluorescence dye-labeled primers −21M13 and M13rev.

Comparison and alignment of the sequences revealed that the poly(G) stretch spacing of the −10 and −35 regions of the *porA* promoter varied with the expression level of class 1 outer membrane protein (Fig. 6). Optimal expression of *porA* was correlated with an 11-nucleotide poly(G) stretch, whereas moderate expression and no expression occurred with poly(G) stretches 1 and 2 nucleotides shorter, respectively. Notably, the poly(G) stretch of a revertant of isolate 2257 (which reverted from class 1 protein deficiency to moderate levels of class 1 protein expression) includes one more G than its class 1-negative counterpart and exhibits moderate levels of class 1 protein expression (Fig. 6).

From these data, we conclude that the phase variation of class 1 outer membrane proteins is regulated by the variation of the G-rich spacer between the −10 and −35 regions of the *porA* promoter.

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Isolate nr.                                          Class 1 expression level

Isolate 2257
TCTGAAAAATGGTTTTTTGCGGGGGGGGGGGTATAATTGAAGACGTATCG          +
TCTGAAAAATGGTTTTTTGCGGGGGGGGGG-TATAATTGAAGACGTATCG          ±
TCTGAAAAATGGTTTTTTGCGGGGGGGGG--TATAATTGAAGACGTATCG          -

revertant 2257 - -> ±
TCTGAAAAATGGTTTTTTGCGGGGGGGGG-TATAATTGAAGACATATCG           ±

Isolate 732
TCTGAAAAATGGTTTTTTGCGGGGGGGGGGGTATAATTGATGACATATCG          +
TCTGAAAAATGGTTTTTTGCGGGGGGGGGG-TATAATTGATGACATATCG          ±
TCTGAAAAATGGTTTTTTGCGGGGGGGGG--TATAATTGATGACATATCG          -

Isolate 2296
TCTGAAAAATGGTTTTTTGCGGGGGGGGGGGTATAATTGATGACATATCG          +
TCTGAAAAATGGTTTTTTGCGGGGGGGGGG-TATAATTGATGACATATCG          ±
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FIG. 6. Sequences of the promoter region of *porA* of three clinical isolates, 2257, 732, and 2996, with variable class 1 outer membrane expression. The −10 and +1 transcription signals are displayed in boldface type. Expression levels are indicated as described for Fig. 4.

DISCUSSION

Previously, we demonstrated that the class 1 outer membrane protein of *N. meningitidis* displays phase variation between three different expression levels (11). In this study, we show by Northern hybridization and primer extension analysis that class 1 outer membrane protein phase variation occurs at the transcriptional level. Sequence analysis of class 1 protein phase variants revealed that the initiation of transcription of the *porA* gene is controlled by the variation in the spacer between the −10 and the −35 domains of the *porA* promoter due to the variation of a poly(G) stretch within this spacer region.

A number of other proteins (for a review see reference 17) of *Neisseria* species (gonococci as well as meningococci) display phase variation. Different molecular mechanisms of phase variation of protein expression have been described for *Neisseria* species, mostly through investigation of the related *Neisseria gonorrhoeae*. First, in pilin biosynthesis incorrect recombination between *pilS* and *pilE* leads to frameshifts or deletions in a process closely associated with pilin antigenic variation (6, 22, 28). Second, revertible nonpiliated phase variants are also formed when propilin is cleaved at amino acid 40 rather than at position 1. The resulting pilin is incompatible with pilus assembly and is secreted into the medium (22). A third mechanism is variation, probably due to slipped-strand mispairing, in the length of short repeated sequences within the coding region of a protein as occurs with Opa proteins and PilC. There are several other mechanisms of phase variation displayed in a variety of microorganisms, i.e., variation in repetitive domains other than the above-mentioned, site-specific recombinations (inversions) and even DNA modification (22).

Here, we describe phase variation in *N. meningitidis* controlled at the transcriptional level by length variation in the intervening sequence of the −10 and −35 regions of a promoter. Recently, it was reported that the length of a poly(C) stretch making part of the −35 domain of the *opc* promoter also varies with the expression level of Opc outer membrane protein in meningococci (24). Variations in the length of different oligonucleotide stretches between the −10 and the −35 domains have also been reported for *hifA* and *hifB* genes in *Haemophilus influenzae* [oligo(AT) stretch (34)], *bvgS* genes in *Bordetella pertussis* [oligo(C) (35)] and *vlp* genes in *Mycoplasma hyorhinis* [oligo(A) (37)]. The length variation of the oligonucleotide stretch is most probably generated during DNA replication by the slipped-strand mispairing mechanism (17).

The spacing of the −10 and −35 domains in promoters is constrained to 17 ± 1 bp, which is consistent with the notion that RNA polymerase interacts with one side of the double helix (27). With the *porA* promoter, optimal transcription is observed when the poly(G) stretch is 11 nucleotides long. Transcription is decreased to moderate levels when one G is removed, whereas no transcription is observed when two G's are deleted. Since the position of the −35 region of the *porA* promoter is uncertain, it is also unclear whether the optimal spacing between −10 and −35 is 16 or 17 (corresponding to ATGGTT or TGGTTT, respectively, as the putative −35 domain) bp. Both sequences are poorly homologous to the consensus −35 region (8).

The region upstream of the −35 domain of the *porA* promoter contains three oligo(T) (minimally 4-bp-long) stretches with interspacing of 20 and 21 bp being two turns of DNA helix at an assumed 10.5 bp per turn. These oligo(T) tracts are known to cause static bends in DNA molecules (13) and are found in upstream activator regions (4, 20). In *E. coli*, analogous regions have been shown to be responsible for 3- to 10-fold stimulation of transcription levels (12). The significance of upstream activator regions in *N. meningitidis* and in particular that of the *porA* promoter has yet to be demonstrated.

Variation between levels of class 1 outer membrane protein expression of different clinical isolates from carriers as well as from patients with meningitis was described earlier (3, 21, 36). Quantitative differences between levels of class 1 outer membrane protein expression in sequential nasopharyngeal isolates were observed. These changes in class 1 protein expression could reflect either quantitative changes in the level of expression by all the organisms in a bacterial population or heterogeneity in expression within the population colonizing the nasopharynx at any given time (36). The humoral immune response then selects for organisms with certain levels of class 1 protein expression, resulting in a population dominated by one phenotype and/or genotype. It is striking that isolates from the nasopharynx show a considerable variation not only in class 1 outer membrane expression but also and even more markedly in class 5 outer membrane proteins and pili. Possibly, the microenvironment of the nasopharynx, the natural habitat of *N. meningitidis*, or other unidentified host factors increase the frequency of changes, which gives the organism the ability to adapt to the host defense mechanisms.

The phase variation of class 1 protein has consequences for the development of a vaccine based on class 1 protein. Diminished expression of the class 1 protein results in decreased susceptibility of the bacterium to bactericidal antibodies, as was demonstrated for isolates from the nasopharynx as well as for isolates from systemic infections (3). Our results with clinical isolates show that *N. meningitidis* has a mechanism capable of inducing high-frequency phase variation of class 1 protein, although it is not known whether this holds true for replication in the host. For the development of a vaccine, for which class 1 outer membrane protein is a candidate, it is therefore important to investigate the in vivo conditions involved in the selection of organisms deficient in class 1 outer membrane protein as well as the conditions by which phase variation is increased.

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