INFECTION AND IMMUNITY, Dec. 2003, p. 6712–6720 Vol. 71, No. 12
0019-9567/03/$08.00+0 DOI: 10.1128/IAI.71.12.6712–6720.2003

Genetic Basis for Biosynthesis of the (α1→4)-Linked *N*-Acetyl-D-Glucosamine 1-Phosphate Capsule of *Neisseria meningitidis* Serogroup X

Yih-Ling Tzeng,[1,2]* Corie Noble,[2] and David S. Stephens[1,2,3,4]

Department of Medicine[1] and Department of Microbiology and Immunology,[3] Emory University School of Medicine, and Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention,[4] Atlanta, Georgia, and Department of Veterans Affairs Medical Center, Decatur, Georgia[2]

Received 12 May 2003/Returned for modification 8 August 2003/Accepted 3 September 2003

The genetic basis for biosynthesis of the (α1→4)-linked *N*-acetyl-D-glucosamine 1-phosphate capsule of *Neisseria meningitidis* serogroup X was defined. The biosynthesis gene cassette was a ~4.2-kb region located between *ctrA* of the capsule transport operon and *galE*, which encodes the UDP-glucose-4-epimerase. This location was identical to the locations of the biosynthesis cassettes in other meningococcal serogroups. Three open reading frames unique to meningococcus serogroup X were identified. Deletion-insertion mutation and colony immunoblotting confirmed that these three genes were essential for serogroup X capsule expression, and the genes were designated *xcbA*, *xcbB*, and *xcbC* (serogroup X capsule biosynthesis). Reverse transcriptase PCR indicated that the *xcbABC* genes form an operon and are cotranscribed divergently from *ctrA*. XcbA exhibited 52% amino acid similarity to SacB, the putative capsule polymerase of meningococcus serogroup A, suggesting that it plays a role as the serogroup X capsule polymerase. An IS*1016* element was found within the intergenic region separating *ctrA* and *xcbA* in multiple strains, and this element did not interfere with capsule expression.

Neisseria meningitidis is the cause of epidemic bacterial meningitis. Capsular polysaccharide is a major virulence determinant of *N. meningitidis* (23, 35). Among the 13 meningococcal serogroups classified based on capsular polysaccharide structure, serogroups A, B, C, Y, and W135 are associated with the majority of cases of meningococcal disease. In the African meningitis belt most large epidemics have been caused by serogroup A meningococci, whereas sporadic disease and outbreaks in developed countries are usually caused by serogroup B and C meningococci (1). Serogroup Y meningococci emerged as an important cause of sporadic disease and outbreaks in the United States in the late 1990s (30, 33), and in 2000 serogroup W135 meningococci caused worldwide disease in association with the Hajj pilgrimage (6, 7, 42) and large outbreaks in sub-Saharan Africa (43).

Sporadic cases of meningococcal disease caused by serogroup X meningococci have been reported in both industrialized countries (15, 18, 28, 34) and African countries (11, 31). However, recently, large serogroup X meningitis outbreaks in Niger (5, 10) and Ghana (13) have been reported. A genetic diversity study of *N. meningitidis* serogroup X isolates in which multilocus sequence typing and pulsed-field gel electrophoresis were used showed that most carrier and disease isolates recovered in the last 30 years in the African meningitis belt belonged to the same clonal group (12), while most European and American isolates were highly diverse. In a longitudinal carriage study designed to investigate the dynamics of meningococcal carriage during an interepidemic period in Ghana, the disappearance of the epidemic serogroup A strain was accompanied by a sharp increase in nasopharyngeal carriage of serogroup X meningococci (13). The carriage rate reached 18% of the population sampled, and this coincided with an outbreak of serogroup X disease. Serogroup X meningococci have also been reported to be very efficient in colonizing military recruits in the United Kingdom (21).

The capsular polysaccharides of serogroup B, C, Y, and W135 meningococci are composed of sialic acid derivatives. Serogroup B and C meningococci express (α2→8)- and (α2→9)-linked polysialic acid, respectively (3, 26), while alternating sequences of D-glucose or D-galactose and sialic acid are expressed by serogroup Y and W135 *N. meningitidis*. In contrast, the capsule of serogroup A meningococci is composed of (α1→6)-linked *N*-acetylmannosamine 6-phosphate (27), while *N. meningitidis* serogroup X synthesizes capsular polymers of (α1→4)-linked *N*-acetylglucosamine 1-phosphate (4). In order to better understand the evolution of the meningococcal capsule and its role in pathogenesis, the genetic basis of meningococcal capsule expression in serogroups A, B, C, Y, and W135 has been defined previously (37–39). Here we describe the first characterization of a capsule biosynthesis locus in *N. meningitidis* serogroup X.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The meningococcal strains, plasmids, and primers used in this study are listed in Table 1. Most meningococci were grown on gonococcal (GC) base agar (Difco Laboratories, Detroit, Mich.) or in GC broth at 37°C in the presence of 3.5% CO_2; the only exception was the meningococcal mutant grown with kanamycin, which was propagated on brain heart infusion base (Becton Dickinson and Co., Cockeysville, Md.) containing 1.25% fetal bovine serum (GIBCO BRL, Gaithersburg, Md.) at 37°C in the

* Corresponding author. Mailing address: Department of Veterans Affairs Medical Center, Research 151, Room 5A183, 1670 Clairmont Rd., Decatur, GA 30033. Phone: (404) 321-6111, ext. 6168. Fax: (404) 329-2210. E-mail: ytzeng@emory.edu.

TABLE 1. Strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Description or sequence	Reference or source[a]
N. meningitidis strains		
NMB	B:2b:P1.2,5:L2 (CDC8201085)	36
M7	NMB *synA*::Tn*916*, unencapsulated	41
F8229	Serogroup A strain	38
M328	Serogroup X reference strain	CDC
M2526	Serogroup X blood isolate from Florida, 1996	CDC[b]
M4222	Serogroup X sputum isolate from Florida, 1997	CDC[b]
M4370	Serogroup X blood isolate from Connecticut, 1997	CDC[b]
M7575	Serogroup X blood isolate from Maryland, 2000	CDC
M8210	Serogroup X blood isolate from North Carolina, 2001	CDC
M328::302	M328 with insertion-deletion mutation in *xcbABC*	This study
M2526::302	M2526 with insertion-deletion mutation in *xcbABC*	This study
Plasmids		
pCR2.1	TA cloning vector, Kan^r Amp^r	Stratagene
pUC18	Cloning vector, Amp^r	49
pHP45	Ω(Sp^r) Amp^r	29
pTA7575	TA cloning of LJ8-ga1E1 PCR product into pCR2.1	This study
pUC7575	*EcoR*I fragment of pTA7575 subcloned into *EcoR*I site of pUC18	This study
pYT302	2.447 bp of *NcoI-Eco*RV fragment in pUC7575 replaced with Ω(Sp)	This study
Primers		
CN1	5′-GGCGTTATAATGCTGGTAATTGGATTC-3′	This study
CN2	5′-CAAGCACATCTGAGACTCTACAAGG-3′	This study
CN4	5′-CGGATCATCATCGGAACATTC-3′	This study
CN5	5′-GCGAATACAGCCCACATTCTATCTG-3′	This study
CN6	5′-TTGAATTTCTGTGCACTAGATGCG-3′	This study
CN7	5′-GCGCATCTAGTGCACAGAAATTC-3′	This study
CN8	5′-CCACCCAAGAAGCCGACAAAG-3′	This study
CN9	5′-GTACCATCCGGAGCGACTGAAG-3′	This study
CN10	5′-AGTTTTGCTAATCCGCTGCTTG-3′	This study
CN12	5′-CAGATAGAATGTGGGCTGTATTCGC-3′	This study
GalE1	5′-CGTGGCAGGATATTGATGCTGG-3′	This study
LJ8	5′-CCACCACCAAACAATACTGCC-3′	This study
RN7	5′-CCAGCCGAAGCATAACCATCGC-3′	This study

[a] CDC, Centers for Disease Control and Prevention.
[b] See reference 12.

presence of 3.5% CO_2. *Escherichia coli* strains were grown in Luria-Bertani media. Antibiotics (Sigma Chemical Co., St. Louis, Mo.) were used for selection at the following concentrations: 50 μg of kanamycin per ml, 100 μg of spectinomycin per ml, and 100 μg of ampicillin per ml for *E. coli*; and 80 μg of kanamycin per ml and 60 μg of spectinomycin per ml for *N. meningitidis*. *E. coli* TOP10F′ (Invitrogen, San Diego, Calif.) and DH5α were used as the host strains for cloned PCR products and recombinant plasmids created during this study.

Transformation. Meningococcal strains were transformed by using the procedure described by Janik et al. (19). Plasmids digested with *Sca*I were used directly for meningococcal transformation. Transformants were screened by colony PCR by using a cassette-specific primer and a chromosome-specific anchoring primer. *E. coli* strains were transformed by electroporation by using a Gene-Pulser (Bio-Rad, Hercules, Calif.).

Nucleic acid purification. Chromosomal DNA was isolated from *N. meningitidis* by the following procedure. Bacteria were scraped from one confluent overnight growth plate and resuspended in 10 ml of DNA extraction buffer (10 mM NaCl, 20 mM Tris-HCl [pH 8.0], 1 mM EDTA). Proteinase K (Fisher Scientific, Pittsburgh, Pa.) was added to a final concentration of 100 μg/ml, and the suspension was incubated for at least 6 h at 50°C. An equal volume of phenol-chloroform (1:1) was then added, and the solution was mixed on a rocker for 10 min at room temperature; this was followed by 20 min of centrifugation at 10,000 × *g*. The upper aqueous layer was poured into a clean tube, and centrifugation was repeated. Chromosomal DNA was spooled out of the aqueous layer after addition of 0.1 volume of 3 M sodium acetate and 2 volumes of ethanol. The DNA was rinsed with 70% ethanol, air dried briefly, and then suspended in 2 ml of 10 mM Tris [pH 8.0]–1 mM EDTA. Total RNA was prepared from bacteria grown in GC broth to the mid-log phase by using an RNeasy mini kit (Qiagen, Valencia, Calif.) according to the manufacturer's protocol. Purified total RNA was further treated with RNase-free DNase to remove contaminating chromosomal DNA. PCRs performed with the total RNAs obtained before and after DNase treatment as templates confirmed that the RNA preparation was free of DNA contamination.

PCR, RT-PCR, and colony PCR. PCRs were performed as previously described (39). A reverse transcriptase PCR (RT-PCR) assay was performed by using a GeneAmp RT-PCR kit (Applied Biosystems, Roche) and the protocol recommended by the manufacturer. A single colony from a plated culture was collected with a sterile toothpick and suspended in sterile water, and 2 μl of the suspension was used as a template for colony PCR performed by using the standard conditions (40).

Construction of pYT302. The PCR product generated with primers LJ8 and galE1 by using chromosomal DNA from meningococcal serogroup X strain M7575 was cloned into the pCR2.1 vector with a TOPO-TA cloning kit (Invitrogen) to obtain pTA7575. This fragment was subsequently released by *Eco*RI digestion and subcloned into the *Eco*RI site of pUC18 to obtain pUC7575. After double digestion of pUC7575 with *Nco*I and *Eco*RV to remove approximately 2.4 kb, the plasmid was gel purified, blunted with the Klenow fragment, and ligated to the Ω cassette obtained from *Sma*I digestion of pHP45Ω (29). Transformants were selected with spectinomycin, and insert-containing clones were identified by colony PCR. PCR and direct sequencing analysis of the resulting plasmid, pYT302, confirmed correct deletion and insertion of the Ω cassette.

Southern blotting. PCR products were used as templates to generate random primed digoxigenin-labeled probes with the Genius nonradioactive DNA labeling and detection system (Boehringer Mannheim, Indianapolis, Ind.). DNA

hybridization was performed by following the manufacturer's suggested procedure (Boehringer Mannheim).

Whole-bacterium immunoblotting. A detailed immunoblot procedure in which whole cells are used has been described previously (22). Briefly, *N. meningitidis* cells from plate-grown overnight cultures were suspended in GC broth, and the optical density at 550 nm was determined. Sequential dilutions were made to obtain the required numbers of organisms in 50-μl aliquots. The cell suspensions were applied to a prewetted nitrocellulose membrane by using a BioDot apparatus (Bio-Rad). The membrane was subsequently processed by using the previously described procedure (22). Before the membrane was probed, polyclonal antiserum to *N. meningitidis* serogroup X (Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention) was preabsorbed with a suspension of strain M328::302 meningococci to remove nonspecific antibodies that recognize other meningococcal surface antigens. The antiserum was used at a 1:250 dilution. Alkaline phosphatase-conjugated anti-rabbit immunoglobulin G/M monoclonal antibody (ICN/CAPPEL, West Chester, Pa.) was used at a 1:2,500 dilution.

Serum bactericidal assay. A microdilution serum bactericidal assay was performed by using the procedure described by Kahler et al. (23). Pooled normal human serum was used at a 10% (vol/vol) dilution. The percent survival ($\log_{10}$) was calculated by dividing the number of CFU per milliliter obtained after incubation in serum (at 15 min) by the number of CFU per milliliter at time zero. A Student's *t* test with a two-tailed hypothesis was used to determine the significance ($P \leq 0.05$) for two variables.

Nucleotide sequence accession numbers. The nucleotide and predicted amino acid sequences of the capsule biosynthesis genes derived from strain M7575 have been deposited in the GenBank database under accession number AY289931. The GenBank accession number for the intergenic region sequence from strain M0328 is AY289932.

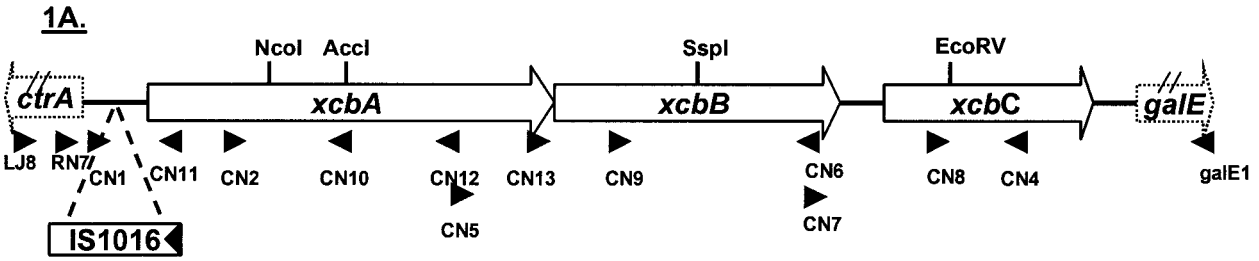
RESULTS

Characterization of the nucleotide sequence between *ctrA* and *galE* in *N. meningitidis* serogroup X. In all meningococcal serogroups characterized thus far, the capsule biosynthesis genetic cassette is flanked by *ctrA*, the first gene in the capsule transport operon, and *galE*, a gene involved in lipooligosaccharide biosynthesis (2, 44). To confirm that the biosynthesis genes of serogroup X are located in this region, primers that annealed to *ctrA* or *galE* were used to PCR amplify the region from chromosomal DNA prepared from six serogroup X isolates. A ~4.2-kb DNA fragment was obtained from strain M7575, while the other five strains yielded ~5-kb PCR products. Two independent PCR products from M7575 (RN7-galE1 and CN1-galE1) were cloned into the pCR2.1 vector, and the resulting plasmids were used as templates to obtain a nucleotide sequence by primer walking (20). The entire sequence was confirmed by 2× coverage sequencing of the two independent PCR clones. In addition, overlapping PCR amplification was performed to determine the location of the additional ~800 bp present in the five serogroup X strains that produced larger PCR products than M7575 produced. A larger PCR product was obtained when primers CN1 and CN11 were used for amplification (Fig. 1A). Nucleotide sequencing of the larger CN1-CN11 PCR product revealed the presence of an intact IS*1016* element (8). The IS*1016* open reading frame (ORF) was predicted to encode a 217-amino-acid protein homologous to the IS*1016C2* transposase and was oriented in the same direction as *ctrA* (Fig. 1A). There was no difference in the level of capsule expression between strains with and without IS*1016* when they were examined by colony immunoblotting (data not shown).

Nucleotide sequence analysis of the putative capsule biosynthesis nucleotide sequence of strain M7575 indicated that there were three putative ORFs, which were designated *xcbA*, *xcbB*, and *xcbC*. The ORFs were transcribed divergently from *ctrA*, a common feature in all meningococcal serogroups (Fig. 1A). The IS*1016* element was located in the region between the *ctrA* and *xcbA* genes. *xcbA* was 1,458 bp long and was predicted to encode a protein containing 486 residues. *xcbA* in strain M7575 was separated from *ctrA* by a 266-bp intergenic region, which exhibited no sequence similarity to the serogroup A and serogroup B intergenic regions (39). *xcbB*, which overlapped *xcbA* by 35 bp, was 1,050 bp long and was predicted to encode a 350-amino-acid protein. *xcbC*, which was separated from *xcbB* by 140 bp, was 768 nucleotides long and was predicted to encode a putative protein containing 256 amino acids. *xcbABC* had a G+C content of 35 to 39%.

Both nucleotide and predicted protein sequences were used to search the GenBank database. XcbA exhibited significant homology to the following three meningococcal proteins closely associated with meningococcal capsule loci: a hypothetical protein (*P* value, 2e-68; 40% identity and 58% similarity) encoded by a gene located between *rfbD* and *lipA* in the capsule locus of serogroup B strain B1940 (16), LcbA (*P* value, 2e-66; 38% identity and 53% similarity), and SacB (*P* value, 2e-46; 31% identity and 52% similarity) (38). LcbA, a 366-residue protein, is encoded by the first gene of a gene cluster similarly flanked by *ctrA* and *galE* in serogroup L (GenBank accession number AF112478). It has been proposed that LcbA is involved in capsule biosynthesis; however, its function and role in capsule expression have not been confirmed. SacB (545 residues) is the putative capsular polymerase encoded by the serogroup A capsule biosynthesis gene cluster (38), and a *sacB* mutant is nonencapsulated (38). An alignment of the XcbA, LcbA, and SacB sequences is shown in Fig. 2. The homology is spread throughout the protein sequences; no known domain or motif was identified. In addition, four conserved hypothetical proteins in *Streptomyces coelicolor* A3, a putative capsular polysaccharide synthesis protein in *Aeromonas hydrophila*, and a capsular polysaccharide synthesis protein (Cps1A) in *Actinobacillus pleuropneumoniae* also exhibited significant protein sequence similarity to XcbA (*P* value range, 2e-59 to 2e-41). However, the functions of these proteins have not been demonstrated.

***ctrABCD* and *lipAB* are conserved in *N. meningitidis* serogroup X.** The capsule transport genes, *ctrABCD* and *lipAB*, are highly conserved in the major disease-causing serogroups, serogroups A, B, C, Y, and W135 (2, 44). Nucleotide probes for *ctrABCD* and *lipAB* were amplified by PCR from the chromosomal DNA of a serogroup B strain, NMB, by using primers designed from the MC58 serogroup B sequence (44). Southern blotting performed under high-stringency conditions with the six serogroup X isolates confirmed that each isolate contained *ctrABCD* and *lipAB*. PCR amplification of the *ctrABCD* and *lipAB* coding sequences, as well as the linkage between these genes, gave product sizes identical to the sizes of the products amplified from serogroup B strain NMB (data not shown). Furthermore, PCR amplification with *gltS*-specific primers, located at the 3′ end of *lipB* (Fig. 3), and *lipB*-specific primers gave products for serogroup X identical to those of serogroup B strains. Thus, *ctrABCD* and *lipAB* are conserved in *N. meningitidis* serogroup X and have a genetic organization similar to that in other well-characterized serogroups (Fig. 3).



1B.

```
GAATTTGCAT TTAATTTGCA TAACCATTCC AAACCTCTAA TTATCTATCC CCTATTGTAC 60
ACAATTTAAA AGCAATTTTT CATCATTATA TAAATTTGAC ATAGATAAGC GGTAACAAAT 120
                      -10                        -35    -35
AACGACAATA GTGTTCTAAA TACCCTATTT GGTGTTATAA TGCTGGTAAT TGGATTCGGA 180
               -10
AAACATCTTA AACAAAGATA AAATATTCCC TATGTTAACA CCACAAAACA AACAAATCAC 240
TATTAAATTT TACACTCAGA AATATTTACT TAAATTATAA TTTAAGTTAT TATATTTTGG 300
GGTTTAGGAA GCAAAAATAT TAAATTGGGT TATTTCGAAG CATCTTCATT AATATTATGC 360
CGGTGGTTAA ACCTAAACTC CGTTTCTTTG AGATGCAGAT AAAACATCTC TTTGGAAATA 420
CCATAAAATT TAGCCAAACG CCCCTTAGCA TAACTCCAAA AAGATTCAAT ACCATTGATA 480
TGTTGCTTTC CTCGAGCAAA CTCATTGGAA CCATGATGGA CACGGTAATG CTTCTCATAG 540
CCCATATCGA CAAGACCGTC ATATGCTTTC CAGCCGTCAG TATTGATTTC ACTGTCAGCA 600
GATATGTGAC CACGTATGAC CTTGATTAGT GAGGCTTTCG AAGCGTCGGC AACGATTTCC 660
GTATAGACCA CGCCATTGCG TTTCAGTATG CCAAATACGA TGGTTTTACC TGACGCTCCG 720
CGACCGCGCT TACCCCTGAT ACGTCGCCCC CCAAAGTAAG ATTCATCTAA TTCGACCACA 780
CCGGACAGTG GTGAGCTTTG TTCGCACAGA GCAGCAATCC TGCGTCTGAT TTTCAGGAAA 840
ATAGGATTGA TACTGCGTAC ACTAATGCCG GTCATTTTAG CAGTATCGGA AGCGGTCAAA 900
TCAAGAGCGA AACAGCGGAT TATTTCTCTA AACTTTTGCT CTGAAATTTT GCTGAACTTT 960
                                                    -35
TGATACTTAT TTTTTAGTTT CATATTAGGA GCTTATCACG TTTTTTGATG ATTTTGCTTC 1020
                -10
CTAAGCCCCA TATTTTTTAT TAAATTTAAA TATTGAATTT AAATCAAAAC ATATTCTGGA 1080
AGGCCTTCTT ATGATTATGA GCAAAATTAG CAAATTGGTA                       1120
```

FIG. 1. (A) Schematic diagram of the capsule biosynthesis gene cassette in *N. meningitidis* serogroup X. Three ORFs, *xcbA*, *xcbB*, and *xcbC*, were identified and were transcribed divergently from the first gene of the capsule transport operon, *ctrA*. An IS*1016* element (indicated by an open box with the arrow showing the transcriptional direction of the transposase) was found in some strains. The locations of nucleotide primers used in this study are indicated by arrowheads. (B) Nucleotide sequence of the intergenic region between *ctrA* and *xcbA* of strain M0328 (accession number AY289932). The translational start codons of *ctrA* and *xcbA* are indicated by boldface type and underlining. The sequence of the IS*1016* element is indicated by lightface type. A 10-bp duplication associated with the IS*1016* insertion is enclosed in a box. The arrows indicate an imperfect 18-bp inverted repeat flanking the IS*1016* element. Probable promoters (−10 and −35) for *xcbA* in the strains containing IS*1016* in this region are indicted by dotted lines above the sequence, while the putative *xcbA* promoter in the strain without IS*1016* is indicated by the dashed line above the sequence. The putative *ctrA* promoter sequences are indicated by underlining.

```
XcbA   ---MIMSKISKLVTHPNLFFR----------------------------------------
LcbA   -----MKRLKKLTREPGVFFR----------------------------------------
SacB   MFILNNRKWRKLKRDPSAFFRDSKFNFLRYFSAKKFAKNFKNSSHIHKTNISKAQSNISS
              :  **  .*. ***

XcbA   ------------------------------------------------------------
LcbA   ------------------------------------------------------------
SacB   TLKENRKQDMLIPINFFNFEYIVKKLNNQNAIGVYILPSNLTLKPALCILESHKEDFLNK

XcbA   ------------------------------------------------------------
LcbA   ------------------------------------------------------------
SacB   FLLTISSENLKLQYKFNGQIKNPKSVNEIWTDLFSIAHVDMKLSTDRTLSSSISQFWFRL

XcbA   -------DYFLKKAPLNYGENIKPLPIETSSHSKKNTAHKTPVSSDQPIEDPYPVTFPID
LcbA   -------DYFNKKYPVRNIEQRINEIEEPAIIAN--SLHLAAVESAIHLSP-----FKID
SacB   EFCKEDKDFILFSTANRYSRKLWKHSIKNNQLFKEGIRNYSEISSLPYEEDHN---FDID
              *::  . . .  .:      :     :    : : :.*    .      * **

XcbA   VVYTWVDSDDEKFNEERLKFQNSSTSETLQGKAESTDIARFQSRDELKYSIRSLMKYAPW
LcbA   VVFTWVDNSDTQWQQRHQQYCHAASPNNLY----SNDETRFANHNELYYSLHSVRSFLPW
SacB   LVFTWVNSEDKNWQELYKKYKPDFNS-------DATSTSRFLSRDELKFALRSWEMSGSF
       :*:***:..* ::::   ::    ..        :.. :** .::** ::::*     .:

XcbA   VNHIYIVTNGQIPKWLDTNNT-KVTIIPHSTIIDSQFLPTFNSHVIESSLYKIPGLSEHY
LcbA   VNHIYIITDSQTPKWFKSAEYPNVSIIDHSEIIDKQYLPTFNSHVIEAHLHNIPNLSEHF
SacB   IRKIFIVSNCAPPAWLDLNNP-KIQWVYHEEIMPQSALPTFSSHAIETSLHHIPGISNYF
       :.:*:*:::   * *:.  :  ::  : *. *: .. ****.**.**: *::**.:*:::

XcbA   IYFNDDVMLARDLSPSYFFTSSGLAKLFITNSRLPNGYKNVKDTPTQWASKNSRELLHAE
LcbA   IYFNDDVFVARPLHREHFFHANGIASLFIADKSLQKMATKGTITPTLSASQNCIRLLNQR
SacB   IYSNDDFLLTKPLNKDNFFYSNGIAKLRLEAWGNVNGECTEGEPDYLNGARNANTLLEKE
       ** ***.:::: *  . ** :.*:*.* :      :   .   .    .::*.  **. .

XcbA   TGFWAEAMFAHTFHPQRKSVHESIEHLWHEQLNVCRQNRFRDISDINMATFLHHHFAILT
LcbA   YDCNLDHPLVHTYVPLRKSGFQTAWQYYREEIKAFLPNKFRTNQDLNLATFLVPWLMYLD
SacB   FKKFTTKLHTHSPQSMRTDILFEMEKKYPEEFNRTLHNKFRSLDDIAVTGYLYHHYALLS
             .*:  . *..      : : *:::    *:**  .*: :: :*      *

XcbA   GQALAT--RTKCIYFNIRSPQAAQHYKTLLARKGSEYSPHSICLNDHTSSNKNILSNYEA
LcbA   GKSIPN--NDICYYFNIRSNKAPTQYLKLLQKNEDNQQPHSFCANDFHSEQQ--LYDYHA
SacB   GRALQSSDKTELVQQNHDFKKKLNNVVTLTKERNFDKLPLSVCINDGADSHLN--EEWNV
       *::: .  .      *    :   :  .*  ..  :  * *.* **  ..:     ::..

XcbA   KLQSFLETYYPDVSEAEILLPTKSEVAELVKHKDYLTVYTKLLPIINKQLVNKYNKPYSY
LcbA   KLIAMLKDYFKI----------------------------------------------------
SacB   QVIKFLETLFPLPSSFEK-------------------------------------------------
       ::  :*:  :

XcbA   LFYYLGLSARFLFEETQQEHYRETAEENLQIFCGLNPKHTLALKYLADVTLTSQPXGQ
LcbA   ------------------------------------------------------------
SacB   ------------------------------------------------------------
```

FIG. 2. Alignment of the amino acid sequences of XcbA, LcbA, and SacB generated by the Clustal W method (45).

***xcbA*, *xcbB*, and *xcbC* are transcribed as an operon.** RT-PCR was performed to determine whether *xcbA*, *xcbB*, and *xcbC* are linked as an operon. The CN4 primer located in *xcbC* (Fig. 1A) was utilized as the reverse transcription primer to generate cDNA from total RNA isolated from strain M7575. The RT reaction mixture was subsequently used as the template for PCR amplification of an internal fragment of each gene. PCR products were obtained not only for *xcbC* but also for *xcbB* and *xcbA*, indicating that these three genes are transcribed as a single transcript (Fig. 4).

***xcbABC* gene products are required for capsule expression.** To determine whether the *xcbABC* gene cassette is required

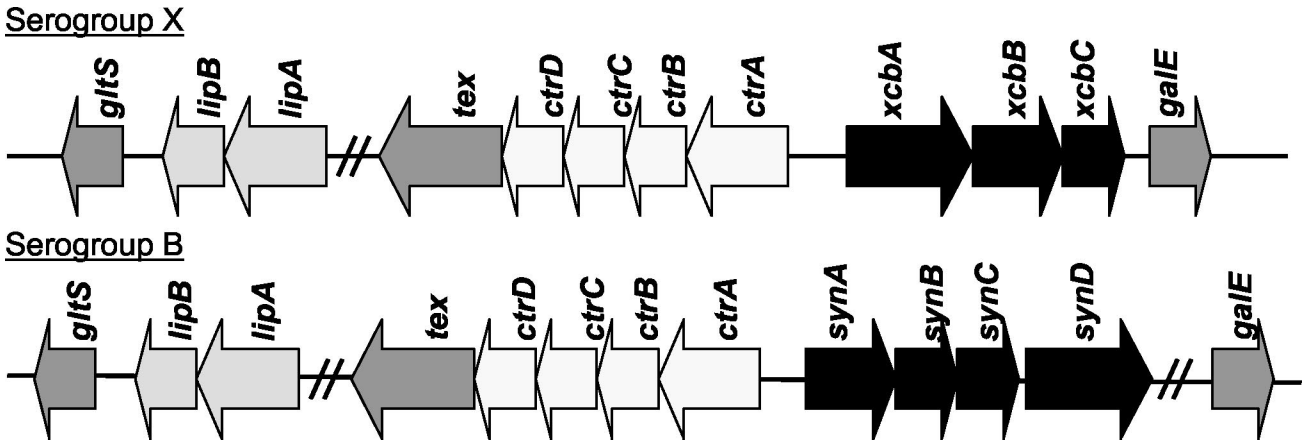


FIG. 3. Schematic diagram of the capsule genetic loci of *N. meningitidis* serogroup X and serogroup B, showing the similarity of the two serogroups.

for expression of the serogroup X capsule, a deletion-insertion mutation was introduced into the *xcbABC* gene cassette. A fragment between the *Nco*I and *Eco*RV (Fig. 1A) restriction sites was removed from plasmid pUC7575 containing the 4.2-kb *ctrA-galE* region of strain M7575 and was replaced with an Ω(Sp) cassette. This mutation resulted in 3′ truncation of *xcbA*, 5′ deletion of *xcbC*, and complete deletion of *xcbB*. The resulting plasmid construct, pYT302, was used to transform the six serogroup X meningococcal isolates. Transformants selected with spectinomycin were successfully generated in strains M328 and M2526, and deletion and insertion of the Ω cassette were confirmed by colony PCR performed with primers CN2 and CN4 and by Southern blot analyses (data not shown). Because pYT302 contained the 4.2-kb *xcbABC* DNA region without IS*1016* from strain M7575, two classes of transformants were obtained in strains carrying IS*1016*; in one of these classes IS*1016* was lost during recombination. Transformants M328::302 and M2526::302, carrying IS*1016* in the *ctrA-*

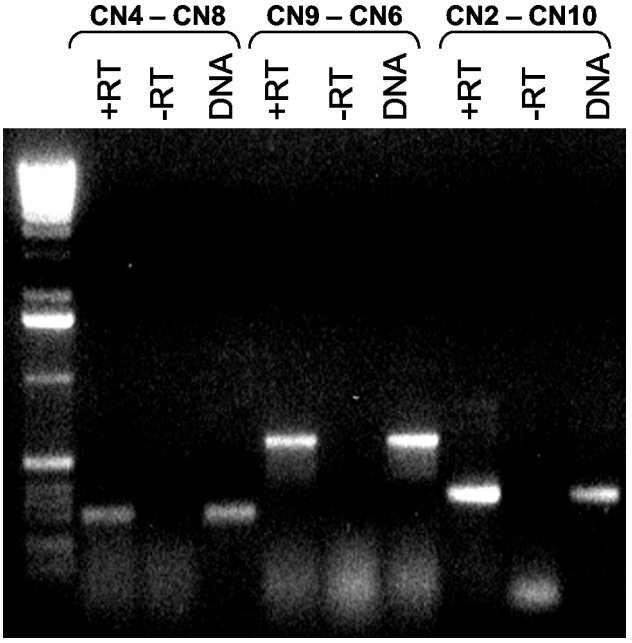


FIG. 4. RT-PCR demonstrating that *xcbA*, *xcbB*, and *xcbC* constitute an operon. An *xcbC* internal primer, CN4, was used to generate cDNA from total RNA isolated from strain M7575. Equivalent RT reactions without RT added (−RT) were also performed to check for possible chromosomal DNA contamination. The reverse transcription reaction mixtures were subsequently used as templates in PCR amplifications with primers internal to *xcbA* (primers CN2 and CN10), *xcbB* (primers CN9 and CN6), and *xcbC* (primers CN4 and CN8). PCR products generated with chromosomal DNA were used to assess the expected product size.

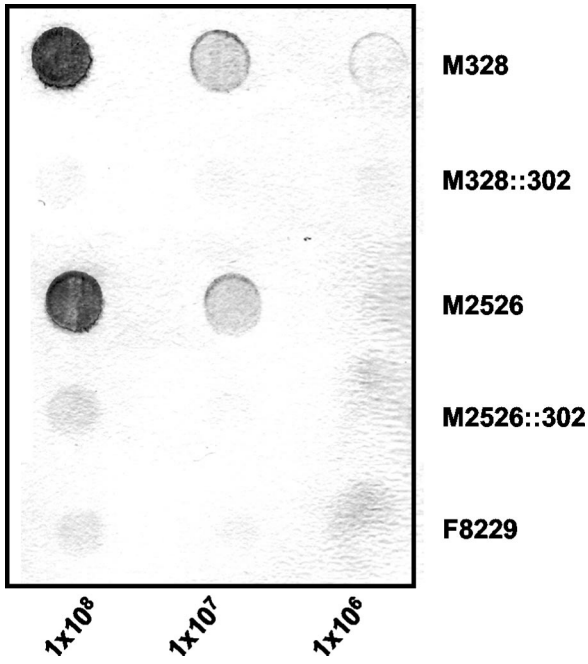


FIG. 5. Whole-bacterium immunoblot for *N. meningitidis* serogroup X. Aliquots containing 1×10^8, 1×10^7, and 1×10^6 cells of serogroup X meningococcal strains M328 and M2526 and the capsule biosynthesis insertion-deletion mutants of these strains, M328::302 and M2526::302, were dried on a nitrocellulose membrane and probed with serogroup X capsule-specific polyclonal antiserum. Serogroup A strain F8229 was included as a negative control.

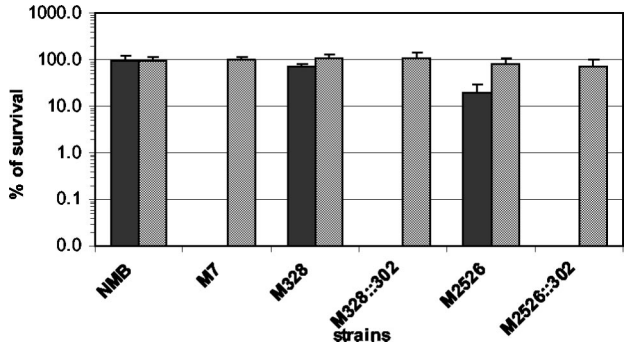


FIG. 6. Human serum bactericidal assays. *N. meningitidis* serogroup X strains M328 and M2526 and the capsule biosynthesis insertion-deletion mutants of these strains were exposed to 10% pooled normal human serum (solid bars) or heat-inactivated (56°C, 30 min) human serum (gray bars). The percentage of survival (*y* axis) is indicated by using a log scale ($n \geq 2$ for each variable). The bars indicate means, and the error bars indicate standard deviations.

xcbA intergenic region, were tested for capsule expression by whole-bacterium immunoblotting by using serogroup X capsule-specific polyclonal antiserum (Fig. 5). Neither M328::302 nor M2526::302 expressed capsular polysaccharides, further indicating that the *xcbABC* gene cluster is essential for capsule expression.

Mutations in *xcbABC* confer sensitivity to normal human serum. The importance of capsule in conferring resistance to killing by human sera in meningococci has been well documented (23). The capsule-deficient phenotype of *xcbABC* mutants shown by colony immunoblotting suggested that the mutants should have a serum-sensitive phenotype. Serum bactericidal assays were performed to confirm this prediction. As shown in Fig. 6, the *xcbABC* mutants were rapidly killed in the presence of 10% normal human serum, whereas the encapsulated parent strain survived. These data further demonstrated that no capsule is present in the *xcbABC* mutants and that capsule is important for the resistance of *N. meningitidis* serogroup X to killing by normal human serum. Meningococcal lipooligosaccharide profiles have also been shown to influence serum sensitivity (9, 16, 23, 47). However, no differences in lipooligosaccharide were noted between the parent and capsule-deficient strains when proteinase K digests of whole-cell lysates were analyzed by Tricine polyacrylamide gel electrophoresis (22) (data not shown).

DISCUSSION

The capsular polysaccharides of *N. meningitidis* and other bacterial capsules (*Haemophilus influenzae*, *E. coli* K1), designated group II capsules, have similar genetic organization and chemical properties (32, 48). Group II capsule loci are usually composed of a unique biosynthesis gene cassette flanked by conserved genes involved in translocation of the capsular polysaccharides (32, 48). Recently, *N. meningitidis* serogroup X has emerged as a serogroup that has caused large outbreaks of disease in sub-Saharan Africa (10, 13). The capsule of serogroup X, (α1→4)-linked *N*-acetylglucosamine 1-phosphate, is biochemically similar to the serogroup A capsule, (α1→6)-linked *N*-acetylmannosamine 1-phosphate. The overall organization of the capsule transport and biosynthesis genes of serogroup X meningococci, as defined in this study, showed similarity to the organization in other meningococcal serogroups characterized to date (2, 44). The capsule locus is located near *gltS*, as it is in other meningococci. In addition, *xcbABC*, *sacABCD* of *N. meningitidis* serogroup A, and *synABCD* of *N. meningitidis* serogroup B have much lower G+C contents (35 to 39, 24 to 35, and 28 to 41%, respectively) than the whole meningococcal genome (52%) (44). The difference in G+C contents suggests that horizontal gene transfer occurred.

The intergenic regions separating the divergently transcribed transport and biosynthesis genes in serogroups A, B, C, Y, and W135 have been characterized in detail and have similar organizations of transcriptional control (37, 38, 46). The sialic acid-containing serogroups (serogroups B, C, Y, and W135) utilize identical 134-bp intergenic regions to initiate transcription (37, 46), while a completely different 218-bp sequence separates the transport and biosynthesis gene clusters in *N. meningitidis* serogroup A (38). The intergenic regions of these serogroups contain overlapping promoters for controlling the divergent operons. The nucleotide sequence of the 266-bp intergenic region separating the divergently transcribed *ctrA* and *xcbA* genes differs from that found in serogroup A or serogroup B. Interestingly, expression of the serogroup X capsule was not affected by the presence of an IS*1016* element in the intergenic region (Fig. 1). IS*1016* may have inserted into the intergenic region after acquisition of the capsule locus in strain M328. Alternatively, IS*1016* may mediate acquisition of the capsule genes and may have been lost in some strains. IS*1016* was originally described as flanking the capsule locus of *H. influenzae*, and it was proposed that this element mobilizes the ~17-kb capsule gene cluster as a compound transposon in the *H. influenzae* chromosome (24, 25). Many virulent *H. influenzae* serotype b strains carry a duplicated capsule locus, and flanking IS*1016* elements may facilitate reversible gene amplification through unequal homologous recombination events (25). It has been noted that the presence of outwardly directed promoters in an insertion element or the formation of hybrid promoters between an insertion element and host DNA (14) may provide and/or enhance expression of certain genes, thus conferring a certain survival advantage. In the case of *N. meningitidis* serogroup X, predicted promoters resembling a σ70 consensus sequence can be identified in the intergenic region both within and outside the IS*1016* that could initiate transcription of *xcbA* (Fig. 1B), without interference with the putative *ctrA* promoter. There was no difference in the level of serogroup X capsule expression between strains with IS *1016* and strains without IS*1016*, suggesting that IS*1016* does not affect capsule expression. Another insertion element, IS*1301*, has been shown to mediate on-off switching of capsule expression through reversible insertion and excision within the coding sequence of the first biosynthesis gene, *synA*, in a serogroup B strain (17).

The serogroup X capsule is a polymer of (α1→4)-linked *N*-acetylglucosamine 1-phosphate. *N*-Acetylglucosamine is a common precursor of important bacterial components, such as peptidoglycan [(α1→4)-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid]. The coupling of a C-4 hydroxyl group in *N*-acetylmuramic acid and the C-1 carbon in UDP-

GlcNAc, along with the release of UDP, generates the disaccharide precursor of the peptidoglycan. Analogously, coupling of two UDP-GlcNAc molecules between the C-4 hydroxyl of one UDP-GlcNAc and the C-1 phosphate of the other UDP-GlcNAc through the energy provided by the hydrolysis of UMP is predicted to produce the (1→4) phosphodiester linkage of the serogroup X capsule. A similar sequence of reactions has also been proposed for capsule expression in serogroup A meningococci, whose capsular structure is (α1→6)-linked *N*-acetylmannosamine (ManNAc) 1-phosphate (38). SacB is believed to be responsible for the polymerization of UDP-ManNAc (38), creating the phosphodiester bond between positions 1 and 6 of individual UDP-ManNAc molecules through the release of UMP. XcbA is probably the capsular polymerase for serogroup X meningococci, considering its homology to SacB. XcbA also showed sequence similarity to the putative biosynthesis protein, LcbA, of *N. meningitidis* serogroup L, which expresses a capsule composed of (α1-P→3)-linked trisaccharide of GlcNAc.

In summary, the genetic basis of capsule expression in serogroup X meningococci was defined. Like the capsules of other meningococcal serogroups (23), the serogroup X capsule is critical for resistance to normal human serum. With the identification of the unique serogroup X biosynthesis sequence, molecular tools for diagnosis and monitoring the epidemiology and emergence of serogroup X disease can be developed. In addition, this study provided additional information on the evolution of the capsule biosynthesis region of group II encapsulated bacterial pathogens.

ACKNOWLEDGMENTS

We thank Lane Pucko for administrative assistance and Larry Martin for technical assistance. We thank Gloria Ajello, Leonard Mayer, and Nancy Rosenstein for their help.

This work was supported by National Institute of Allergy and Infectious Diseases grant AI33517 to D.S.S. and by the Meningitis and Special Pathogens Branch of the Centers for Disease Control and Prevention.

REFERENCES

1. **Achtman, M.** 1995. Global epidemiology of meningococcal disease, p. 159–175. *In* K. Cartwright (ed.), Meningococcal disease. John Wiley and Sons, Chichester, United Kingdom.
2. **Achtman, M., K. D. James, S. D. Bentley, C. Churcher, S. R. Klee, G. Morelli, D. Basham, D. Brown, T. Chillingworth, R. M. Davies, P. Davis, K. Devlin, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, S. Leather, S. Moule, K. Mungall, M. A. Quail, M.-A. Rajandream, K. M. Rutherford, M. Simmonds, J. Skelton, S. Whitehead, B. G. Spratt, B. G. Barrell, and J. Parkhill.** 2000. Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. Nature **404:**502–506.
3. **Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin, and I. C. Smith.** 1976. Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135, and BO1. Can. J. Biochem. **54:**1–8.
4. **Bundle, D. R., H. J. Jennings, and C. P. Kenny.** 1974. Studies on the group-specific polysaccharide of *Neisseria meningitidis* serogroup X and an improved procedure for its isolation. J. Biol. Chem. **249:**4797–4801.
5. **Campagne, G., A. Schuchat, S. Djibo, A. Ousseini, L. Cisse, and J. P. Chippaux.** 1999. Epidemiology of bacterial meningitis in Niamey, Niger, 1981–96. Bull. W. H. O. **77:**499–508.
6. **Centers for Disease Control and Prevention.** 2001. Risk for meningococcal disease associated with the Hajj 2001. Morb. Mortal. Wkly. Rep. **50:**97–98.
7. **Centers for Disease Control and Prevention.** 2000. Serogroup W-135 meningococcal disease among travelers returning from Saudi Arabia—United States, 2000. Morb. Mortal. Wkly. Rep. **49:**345–346.
8. **Dobson, S. R., J. S. Kroll, and E. R. Moxon.** 1992. Insertion sequence IS*1016* and absence of *Haemophilus* capsulation genes in the Brazilian purpuric fever clone of *Haemophilus influenzae* biogroup aegyptius. Infect. Immun. **60:**618–622.
9. **Estabrook, M. M., J. M. Griffiss, and G. A. Jarvis.** 1997. Sialylation of *Neisseria meningitidis* lipooligosaccharide inhibits serum bactericidal activity by masking lacto-*N*-neotetraose. Infect. Immun. **65:**4436–4444.
10. **Etienne, J., G. Sperber, A. Adamou, and J. J. Picq.** 1990. Epidemiological notes: meningococcal meningitis of serogroup X in Niamey (Niger). Med. Trop. **50:**227–229.
11. **Gagneux, S., A. Hodgson, I. Ehrhard, G. Morelli, B. Genton, T. Smith, M. Tanner, F. Binka, M. Achtman, and G. Pluschke.** 2000. Microheterogeneity of serogroup A (subgroup III) *Neisseria meningitidis* during an outbreak in northern Ghana. Trop. Med. Int. Health **5:**280–287.
12. **Gagneux, S., T. Wirth, A. Hodgson, I. Ehrhard, G. Morelli, P. Kriz, B. Genton, T. Smith, F. Binka, G. Pluschke, and M. Achtman.** 2002. Clonal groupings in serogroup X *Neisseria meningitidis.* Emerg. Infect. Dis. **8:**462–466.
13. **Gagneux, S. P., A. Hodgson, T. A. Smith, T. Wirth, I. Ehrhard, G. Morelli, B. Genton, F. N. Binka, M. Achtman, and G. Pluschke.** 2002. Prospective study of a serogroup X *Neisseria meningitidis* outbreak in northern Ghana. J. Infect. Dis. **185:**618–626.
14. **Galas, D. J., and M. Chandler.** 1989. Bacterial insertion sequences, p. 109–162. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
15. **Grahlow, W. D., H. W. Ocklitz, and H. Mochmann.** 1986. Meningococcal infections in the German Democratic Republic 1971–1984. Infection **14:**286–288.
16. **Hammerschmidt, S., C. Birkholz, U. Zahringer, B. D. Robertson, J. van Putten, O. Ebeling, and M. Frosch.** 1994. Contribution of genes from the capsule gene complex (*cps*) to lipooligosaccharide biosynthesis and serum resistance in *Neisseria meningitidis.* Mol. Microbiol. **11:**885–896.
17. **Hammerschmidt, S., R. Hilse, J. P. van Putten, R. Gerardy-Schahn, A. Unkmeir, and M. Frosch.** 1996. Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. EMBO J. **15:**192–198.
18. **Hansman, D.** 1983. Meningococcal disease in South Australia: incidence and serogroup distribution 1971–1980. J. Hyg. **90:**49–54.
19. **Janik, A., E. Juni, and G. A. Heym.** 1976. Genetic transformation as a tool for detection of *Neisseria gonorrhoeae.* J. Clin. Microbiol. **4:**71–81.
20. **Jones, D. H., and S. C. Winistorfer.** 1993. Genome walking with 2- to 4-kb steps using panhandle PCR. PCR Methods Applic. **2:**197–203.
21. **Jones, G. R., M. Christodoulides, J. L. Brooks, A. R. Miller, K. A. Cartwright, and J. E. Heckels.** 1998. Dynamics of carriage of *Neisseria meningitidis* in a group of military recruits: subtype stability and specificity of the immune response following colonization. J. Infect. Dis. **178:**451–459.
22. **Kahler, C. M., R. W. Carlson, M. M. Rahman, L. E. Martin, and D. S. Stephens.** 1996. Inner core biosynthesis of lipooligosaccharide (LOS) in *Neisseria meningitidis* serogroup B: identification and role in LOS assembly of the alpha1,2 *N*-acetylglucosamine transferase (RfaK). J. Bacteriol. **178:**1265–1273.
23. **Kahler, C. M., L. E. Martin, G. C. Shih, M. M. Rahman, R. W. Carlson, and D. S. Stephens.** 1998. The (α2→8)-linked polysialic acid capsule and lipooligosaccharide structure both contribute to the ability of serogroup B *Neisseria meningitidis* to resist the bactericidal activity of normal human serum. Infect. Immun. **66:**5939–5947.
24. **Kroll, J. S.** 1992. The genetics of encapsulation in *Haemophilus influenzae.* J. Infect. Dis. **165**(Suppl. 1)**:**S93–S96.
25. **Kroll, J. S., B. M. Loynds, and E. R. Moxon.** 1991. The *Haemophilus influenzae* capsulation gene cluster: a compound transposon. Mol. Microbiol. **5:**1549–1560.
26. **Liu, T. Y., E. C. Gotschlich, F. T. Dunne, and E. K. Jonssen.** 1971. Studies on the meningococcal polysaccharides. II. Composition and chemical properties of the group B and group C polysaccharide. J. Biol. Chem. **246:**4703–4712.
27. **Liu, T. Y., E. C. Gotschlich, E. K. Jonssen, and J. R. Wysocki.** 1971. Studies on the meningococcal polysaccharides. I. Composition and chemical properties of the group A polysaccharide. J. Biol. Chem. **246:**2849–2858.
28. **Pastor, J. M., A. Fe, M. Gomis, and D. Gil.** 1985. Meningococcal meningitis caused by *Neisseria meningitidis* of the X serogroup. Med. Clin. (Barcelona) **85:**208–209.
29. **Prentki, P., and H. M. Krisch.** 1984. *In vitro* insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
30. **Racoosin, J. A., C. G. Whitney, C. S. Conover, and P. S. Diaz.** 1998. Serogroup Y meningococcal disease in Chicago, 1991–1997. JAMA **280:**2094–2098.
31. **Riou, J. Y., S. Djibo, L. Sangare, J. P. Lombart, P. Fagot, J. P. Chippaux, and M. Guibourdenche.** 1996. A predictable comeback: the second pandemic of infections caused by *Neisseria meningitidis* serogroup A subgroup III in Africa, 1995. Bull. W. H. O. **74:**181–187.
32. **Roberts, I. S.** 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu. Rev. Microbiol. **50:**285–315.
33. **Rosenstein, N. E., B. A. Perkins, D. S. Stephens, L. Lefkowitz, M. L. Cartter, R. Danila, P. Cieslak, K. A. Shutt, T. Popovic, A. Schuchat, L. H. Harrison, and A. L. Reingold.** 1999. The changing epidemiology of meningococcal disease in the United States, 1992–1996. J. Infect. Dis. **180:**1894–1901.

34. **Ryan, N. J., and G. R. Hogan.** 1980. Severe meningococcal disease caused by serogroups X and Z. Am. J. Dis. Child. **134:**1173.
35. **Stephens, D. S., P. A. Spellman, and J. S. Swartley.** 1993. Effect of the (α 2→8)-linked polysialic acid capsule on adherence of *Neisseria meningitidis* to human mucosal cells. J. Infect. Dis. **167:**475–479.
36. **Stephens, D. S., J. S. Swartley, S. Kathariou, and S. A. Morse.** 1991. Insertion of Tn*916* in *Neisseria meningitidis* resulting in loss of group B capsular polysaccharide. Infect. Immun. **59:**4097–4102.
37. **Swartley, J. S., J. H. Ahn, L. J. Liu, C. M. Kahler, and D. S. Stephens.** 1996. Expression of sialic acid and polysialic acid in serogroup B *Neisseria meningitidis*: divergent transcription of biosynthesis and transport operons through a common promoter region. J. Bacteriol. **178:**4052–4059.
38. **Swartley, J. S., L. J. Liu, Y. K. Miller, L. E. Martin, S. Edupuganti, and D. S. Stephens.** 1998. Characterization of the gene cassette required for biosynthesis of the (α1→6)-linked *N*-acetyl-D-mannosamine-1-phosphate capsule of serogroup A *Neisseria meningitidis.* J. Bacteriol. **180:**1533–1539.
39. **Swartley, J. S., A. A. Marfin, S. Edupuganti, L. J. Liu, P. Cieslak, B. Perkins, J. D. Wenger, and D. S. Stephens.** 1997. Capsule switching of *Neisseria meningitidis.* Proc. Natl. Acad. Sci. USA **94:**271–276.
40. **Swartley, J. S., C. F. McAllister, R. A. Hajjeh, D. W. Heinrich, and D. S. Stephens.** 1993. Deletions of Tn916-like transposons are implicated in tetM-mediated resistance in pathogenic *Neisseria.* Mol. Microbiol. **10:**299–310.
41. **Swartley, J. S., and D. S. Stephens.** 1994. Identification of a genetic locus involved in the biosynthesis of *N*-acetyl-D-mannosamine, a precursor of the (α 2→8)-linked polysialic acid capsule of serogroup B *Neisseria meningitidis.* J. Bacteriol. **176:**1530–1534.
42. **Taha, M. K., M. Achtman, J. M. Alonso, B. Greenwood, M. Ramsay, A. Fox, S. Gray, and E. Kaczmarski.** 2000. Serogroup W135 meningococcal disease in Hajj pilgrims. Lancet **356:**2159.
43. **Taha, M. K., I. Parent Du Chatelet, M. Schlumberger, I. Sanou, S. Djibo, F. de Chabalier, and J. M. Alonso.** 2002. *Neisseria meningitidis* serogroups W135 and A were equally prevalent among meningitis cases occurring at the end of the 2001 epidemics in Burkina Faso and Niger. J. Clin. Microbiol. **40:**1083–1084.
44. **Tettelin, H., N. J. Saunders, J. Heidelberg, A. C. Jeffries, K. E. Nelson, J. A. Eisen, K. A. Ketchum, D. W. Hood, J. F. Peden, R. J. Dodson, W. C. Nelson, M. L. Gwinn, R. DeBoy, J. D. Peterson, E. K. Hickey, D. H. Haft, S. L. Salzberg, O. White, R. D. Fleischmann, B. A. Dougherty, T. Mason, A. Ciecko, D. S. Parksey, E. Blair, H. Cittone, E. B. Clark, M. D. Cotton, T. R. Utterback, H. Khouri, H. Qin, J. Vamathevan, J. Gill, V. Scarlato, V. Masignani, M. Pizza, G. Grandi, L. Sun, H. O. Smith, C. M. Fraser, E. R. Moxon, R. Rappuoli, and J. C. Venter.** 2000. Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. Science **287:**1809–1815.
45. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
46. **Tzeng, Y. L., J. S. Swartley, Y. K. Miller, R. E. Nisbet, L. J. Liu, J. H. Ahn, and D. S. Stephens.** 2001. Transcriptional regulation of divergent capsule biosynthesis and transport operon promoters in serogroup B *Neisseria meningitidis.* Infect. Immun. **69:**2502–2511.
47. **Vogel, U., H. Claus, G. Heinze, and M. Frosch.** 1997. Functional characterization of an isogenic meningococcal α-2,3-sialyltransferase mutant: the role of lipooligosaccharide sialylation for serum resistance in serogroup B meningococci. Med. Microbiol. Immunol. **186:**159–166.
48. **Whitfield, C., and I. S. Roberts.** 1999. Structure, assembly and regulation of expression of capsules in *Escherichia coli.* Mol. Microbiol. **31:**1307–1319.
49. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.

Editor: J. N. Weiser