JOURNAL OF BACTERIOLOGY, Feb. 2004, p. 1038–1049 Vol. 186, No. 4
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.4.1038–1049.2004

The *lgtABCDE* Gene Cluster, Involved in Lipooligosaccharide Biosynthesis in *Neisseria gonorrhoeae*, Contains Multiple Promoter Sequences

Derek C. Braun† and Daniel C. Stein*

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742

Received 1 July 2003/Accepted 6 October 2003

Biosynthesis of the variable core domain of lipooligosaccharide (LOS) in *Neisseria gonorrhoeae* is mediated by glycosyl transferases encoded by *lgtABCDE*. Changes within homopolymeric runs within *lgtA*, *lgtC*, and *lgtD* affect the expression state of these genes, with the nature of the LOS expressed determined by the functionality of these genes. However, the mechanism for modulating the amount of multiple LOS chemotypes expressed in a single cell is not understood. Using mutants containing polar disruptions within the *lgtABCDE* locus, we determined that the expression of this locus is mediated by multiple promoters and that disruption of transcription from these promoters alters the relative levels of simultaneously expressed LOS chemotypes. Expression of the *lgtABCDE* locus was quantified by using *xylE* transcriptional fusions, and the data indicate that this locus is transcribed in trace amounts and that subtle changes in transcription result in phenotypic changes. By using rapid amplification of 5′ cDNA ends, transcriptional start sites and promoter sequences were identified within *lgtABCDE*. Most of these promoters possessed 50 to 67% homology with the consensus gearbox promoter sequence of *Escherichia coli*.

Neisseria gonorrhoeae is an obligate human pathogen that causes diseases of mucosal surfaces (see reference 32 for a review). Because the gonococcus is capable of proliferating in different physiological milieus, it has developed a variety of mechanisms for adapting to these environments. Lipooligosaccharide (LOS) is indispensable for disease pathogenesis. It is immunogenic, highly pyrogenic, and responsible for the localized inflammation and scarring characteristic of gonococcal infections and for the septic shock that results from disseminated disease (21–23, 31, 39, 45; H. M. Harper, A. L. Padmore, W. D. Smith, M. K. Taylor, and R. Demarco de Hormaeche, unpublished results [presented at the Tenth International Pathogenic *Neisseria* Conference, Baltimore, Md.]). In addition, specific chemotypes of LOS confer complement resistance (34) and facilitate intracellular invasion (47). LOS molecules are heterogeneous, with a single cell often simultaneously expressing two or more chemotypes in various proportions (2, 3, 11, 46). LOS also undergoes phase variation, and distinct LOS chemotypes are favored for survival of the gonococcus within different regions of the human body and at various points during the pathogenic process (15, 32, 49, 53, 55). We believe that the expression of the correct LOS chemotype(s) by the gonococcus at the correct time during infection is essential for establishing and maintaining infection.

Our understanding of the regulatory mechanisms that affect LOS biosynthesis and expression remains incomplete. Biosynthesis of the variable oligosaccharide portion of LOS is mediated by seven LOS glycosyl transferase (*lgt*) genes (7, 13, 20). Strand slippage in homopolymeric tracts within the coding sequence of *lgtA*, *lgtC*, *lgtD*, and *lgtG* during DNA replication leads to reading frameshifts in these genes. These changes result in the production of inactivate glycosyl transferases, thus altering the LOS chemotype(s) that is expressed by a given cell (7, 11, 13, 56). Gonococcal LOS expression can be further modified by specific environmental stimuli, suggesting that regulated gene expression occurs. Gonococci grown under anaerobic conditions or in the presence of lactate, which is normally present in the female genital tract, produce an altered LOS profile (16, 30). Gonococci grown under acidic and alkaline conditions also express different LOS profiles (35). Finally, gonococci grown at different growth rates express different LOS epitopes and possess different serum sensitivities (33). The present study investigates the transcriptional organization of the *lgtABCDE* genes.

MATERIALS AND METHODS

Bacterial strains, plasmids, oligonucleotides, and culture conditions. All bacterial strains, plasmids, and oligonucleotide primers used in the present study are listed in Tables 1, 2, and 3. *N. gonorrhoeae* strains were grown in phosphate-buffered gonococcal medium (Difco) supplemented with 20 mM D-glucose and growth supplements (54) either in broth with the addition of 0.042% sodium bicarbonate or on agar where they were incubated in a 37° CO_2 incubator. All *Escherichia coli* strains were grown in Luria-Bertani medium (43). Kanamycin was used in growth media at a concentration of 30 μg/ml, ampicillin was used at 60 μg/ml, spectinomycin was used at 50 μg/ml, and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was used at 35 μg/ml. The optical densities of gonococcal and *E. coli* cultures were determined by using a Klett-Summerson colorimeter fitted with a green filter. One Klett unit corresponds to a culture density of ca. 10^7 CFU/ml. We used the DNA sequence numbering for the F62 *lgtABCDE* region, as described in the National Center for Biotechnology Information database under accession number U14554 to orient our constructs, promoter mapping, etc., to the literature.

* Corresponding author. Mailing address: Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742. Phone: (301) 405-5448. Fax: (301) 314-9489. E-mail: dcstein@umd.edu.

† Present address: Department of Biology, Gallaudet University, Washington, DC 20002.

TABLE 1. Plasmids used in this study

Plasmid	Relevant phenotype, genotype, or description	Source or reference
pGEM7Zf(−)	Cloning vector	Promega[a]
pK18	Cloning vector	38
pHP45Ω	Source of the Ω interposon	37
pXYLE20	pK18 containing the *xylE* gene from *Pseudomonas putida*	48
pCLB2	pGEM2 with a *Pst*I-to-*Age*I chromosomal DNA fragment containing *lgtABCDE* from *N. gonorrhoeae* FA19	11
pCLB2Δ1	The 5′ region between *Pst*I and *Bsm*I in pCLB2 is deleted	This study
pCLB2Δ1 *lgtDfix*#4	pCLB2D1 containing an engineered *Nru*I site within the polyguanine run of *lgtD*; primers LgtDfix-F and LgtDfix-R were used with a pCLB2Δ1 template in a PCR; the PCR amplicon was purified, digested with *Nru*I, and self-ligated	This study
pCLB2Δ1 *lgtDfix*#4Ω*Nru*I	The Ω interposon was liberated from pHP45Ω by *Sma*I digestion and inserted into the *Nru*I site of pCLB2Δ1	This study
pCLB2Δ1 *lgtDfix*#4Ω*Eco*RV	The Ω interposon was liberated from pHP45Ω by *Sma*I digestion and inserted into the *Eco*RV site of pCLB2Δ1 *lgtDfix*#4	This study
pLgtDE(*Eco*RI)	Primers IgtDE-F and IgtDE-R were used to introduce an *Eco*RI site into pCLB2D1 *lgtDfix*#4; the PCR amplicon was purified, digested with *Eco*RI, and self-ligated	This study
pLgtDEΩ*Eco*RI	pCLB2Δ1 *lgtDfix*#4 was digested with *Eco*RI and mixed with Ω interposon that had been liberated from pHP45Ω by *Eco*RI digestion and subsequently purified from an agarose gel; the mixture was ligated	This study
pDCB3	A 5,243-bp amplicon containing the *lgtABCDE* locus was generated by Expand Long-Template PCR (Roche) with primers JL-50 (contains a 5′ *Eco*RI site) and JL-51 (contains a 5′ *Bam*HI site) and was cloned into the *Eco*RI and *Bam*HI sites of pK18	This study
pDCB7	The 5,243-bp fragment of pDCB3 containing the *lgtABCDE* region was cloned into the *Eco*RI and *Bam*HI sites of pGEM7Zf(−)	This study
pDCB7Ω*Cla*I	Primer ABC was used to amplify the Ω interposon (adds *Asc*I, *Bsr*GI, and *Cla*I sites onto the ends of the Ω interposon), and this fragment was cloned into the *Cla*I site of pDCB7	This study
pDCB7Ω*Asc*I	Primer ABC was used to amplify the Ω interposon (adds *Asc*I, *Bsr*GI, and *Cla*I sites onto the ends of the Ω interposon), and this fragment was cloned into the *Asc*I site of pDCB7	This study
pDCB9	A 1,487-bp amplicon corresponding to bp 4316 to 5803 of the *lgtABCDE* region as described earlier (20) was amplified by using primers LgtDE-F (containing a 5′ *Eco*RI) and Got 5803-R (containing a 5′ *Bam*HI) and cloned into the *Eco*RI and *Bam*HI sites of pGEM7Zf(−)	This study
pDCB10	Primers PostLgtE-F (containing a 5′ *Kpn*I and *Hin*dIII sequence) and PostLgtE-R (containing a 5′ *Kpn*I sequence) were used with a pDCB9 template in a PCR to introduce a *Kpn*I site in pDCB9	This study
pDCB10*xylE*	Primers XylE-F and XylE-R were used to amplify the *xylE* gene from pXYLE20 and cloned into the *Kpn*I and *Hin*dIII sites of pDCB10	This study
pDCB17	Primers DCB17b-F (containing a 5′ *Kpn*I-*Hin*dIII sequence) and DCB17b-R (containing a 5′ *Kpn*I sequence) were used to introduce a *Kpn*I site in pDCB7	This study
pDCB17*xylE*	Primers XylE-F and XylE-R were used to amplify the *xylE* gene from pXYLE20 and cloned into the *Kpn*I and *Hin*dIII sites of pDCB17	This study
pDCB19	A ≈4,000-bp amplicon amplicon corresponding to bp 1650 to 2364 of the *lgtABCDE* region as described earlier (20) was amplified using primers DCB19b-F (contains a 5′ *Eco*RI) and DCB19b-R (contains a 5′*Bam*HI) and cloned into the *Eco*RI and *Bam*HI sites of pGEM7Zf(−)	This study
pDCB19Ω*Bsr*GI	Primer ABC was used to amplify the Ω interposon (adds *Asc*I, *Bsr*GI, and *Cla*I sites onto the ends of the Ω interposon), and this fragment was cloned into the *Bsr*GI site of pDCB19	This study
pDCB19*xyl*E	Primers XylE-F and XylE-R were used to amplify the *xylE* gene from pXYLE20 and cloned into the *Kpn*I and *Hin*dIII sites of pDCB19Ω*Bsr*GI	This study

[a] Promega, Madison, Wis.

Chemicals, reagents, and enzymes. Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beverly, Mass.). PCR buffers and enzymes and buffers for 5′ rapid amplification of cDNA ends (5′-RACE) were purchased from Invitrogen (Carlsbad, Calif.) and Roche Molecular Biochemicals (St. Louis, Mo.). All chemicals used for the present study were reagent grade or better and purchased from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise specified. The monoclonal antibodies (MAbs) 2-1-L8 and 17-1-L1 were generously provided by Wendell Zollinger of the Walter Reed Army Institute of Research, Washington, D.C. MAb 1B2 was a gift from J. McLeod Griffiss, University of California, San Francisco.

DNA and RNA isolation procedures. Chromosomal DNA was isolated as described by Rodriguez and Tait (41). Plasmid DNA was isolated by the method of Birnboim and Doly (8). RNA was purified chromatographically from gonococci grown to a Klett reading of 100, by using the High-Pure RNA isolation kit (Roche).

PCR. The PCR was generally performed by using Platinum PCR Supermix (Invitrogen) or the Expand Long-Template PCR kit (Roche) according to the

TABLE 2. Bacterial strains used in this study

Strain	Relevant phenotype, genotype, or description	Source or reference
E. coli DH5αMCR	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) *endA1 supE44 thi-1 recA1 gyrA relA1* Δ(*lacIZYA-argF*)*U169 deoR*ϕ*80dlac*Δ(*lacZ*)*M15*	BRL[a]
N. gonorrhoeae		
F62		P. F. Sparling[b]
F62ΔlgtA	239-bp *Apo*I deletion in *lgtA*; this strain produces L8LOS	47
F62ΔlgtAΔlgtE	622-bp *Bsp*EI-to-*Age*I deletion in *lgtE* of the parent strain F62ΔlgtA	47
F62ΩBsrGI	F62 containing the Ω interposon at the *Bsr*GI site between the stop codon of *glyS*β and the start codon of *lgtA*	This study
F62ΩAscI	F62 containing the Ω interposon within the *Asc*I site of *lgtB*	This study
F62ΩClaI	F62 containing the Ω interposon within the *Cla*I site of *lgtC*	This study
F62ΩNruI	F62 containing the Ω interposon within an engineered *Nru*I site in the polyguanine region of *lgtD*	This study
F62ΩEcoRV	F62 containing the Ω interposon within the *Eco*RV site of *lgtD*	This study
F62ΩEcoRI	F62 containing the Ω interposon within an engineered *Eco*RI site between *lgtD* and *lgtE*; the termination codon of *lgtD* contains part of the *Eco*RI recognition sequence, and the last base of the recognition sequence is 18 bp from the *lgtE* start codon	This study
F62LgtA(xylE)	F62 with the *xylE* reporter gene inserted at the *Bsr*GI site 58 bases before the start codon of *lgtA*	This study
F62LgtE(xylE)	F62 with the *xylE* inserted into *lgtE*	This study
F62LgtE(xylE)ΩBsrGI	F62LgtE(xylE) containing the Ω interposon at the *Bsr*GI site 58 bases before the start codon of *lgtA*	This study
F62LgtE(xylE)ΩAscI	F62LgtE(xylE) containing the Ω interposon within the *Asc*I site of *lgtB*	This study
F62LgtE(xylE)ΩClaI	F62LgtE(xylE) containing the Ω interposon within the *Cla*I site of *lgtC*	This study
F62LgtE(xylE)ΩNruI	F62LgtE(xylE) containing the Ω interposon within an engineered *Nru*I site in the polyguanine region of *lgtD*	This study
F62LgtE(xylE)ΩEcoRV	F62LgtE(xylE) containing the Ω interposon within the *Eco*RV site of *lgtD*	This study
F62LgtE(xylE)ΩEcoRI	F62LgtE(xylE) containing the Ω interposon within an engineered *Eco*RI site between *lgtD* and *lgtE*	This study

[a] BRL, Bethesda Research Laboratories.
[b] University of North Carolina, Chapel Hill.

manufacturers' directions. Primers were purchased from Bioserve Biotechnologies (Laurel, Md.) or from Integrated DNA Technologies (Coralville, Iowa). PCRs were resolved on agarose gels containing 500 μg of ethidium bromide/ml in Tris-borate-EDTA running buffer (43).

Transformation. Competent cells of *E. coli* DH5α-MCR were prepared by the method of Inoue et al. (27). Recombinant DNA transformation of *E. coli* was done according to the standard heat shock protocol (43). Transformants were verified by digestion of recovered plasmid DNA with the appropriate restriction enzymes. Recombinant DNA transformation into *N. gonorrhoeae* F62 was done by either the tube or the spot transformation method of Gunn and Stein (24).

Purification of LOS and SDS-PAGE. Gonococcal LOS was prepared from plated cultures as described by Hitchcock and Brown (25) and diluted 1:25 in lysing buffer. The suspension was boiled for 10 min immediately before 5 μl was loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. After electrophoresis, the gel was fixed overnight in a solution of 40% ethanol–5% acetic acid and then oxidized in 0.83% periodic acid for 5 min. The gel was washed for 2 h in multiple changes of H_2O every 20 min, stained for 5 min in silver staining solution (22.5 mM NaOH, 0.42% NH_4OH, 47 mM $AgNO_3$), and rewashed for 2 h in multiple changes of H_2O every 20 min. The gel was developed (100 ml of 0.005% citric acid–0.007% formaldehyde) until the bands became sufficiently visible and then photographed.

Immunological methods. Colony blots were performed by transferring cells grown overnight on GCK agar onto nitrocellulose filters. The filters were air dried for 10 min and blocked with filler solution (20 mM Tris, 150 mM NaCl, 2% nonfat dry milk, 0.2% NaN_3, 0.002% phenol red [pH 7.4]) for 30 min. Filters were blotted with Whatman 3MM paper to remove cellular debris prior to incubation in primary antibody (MAb 2-1-L8 or 17-1-L1) with gentle shaking for 2 h or longer. Filters were washed with Tris-buffered saline (TBS; pH 7.4) three times for 10 min each time. Membranes were incubated in filler solution containing goat anti-mouse immunoglobulin G conjugated with horseradish peroxidase for 2 h. Filters were washed three times for 10 min each time in TBS, and the membranes were developed by incubating them in 50 ml of 50 mM Tris-HCl–1% 4-chloro-1-naphthol–0.86% H_2O_2 (pH 8.0).

XylE assay. Qualitative screening for XylE$^+$ colonies was performed by spraying plates with 50 mM catechol, followed by incubation at 37°C for as short as 1 min to as long as 1 h to allow for color development. The presence of a yellow color indicated XylE activity. Quantitative assays were performed by inoculating cells into 20 ml of gonococcal broth plus supplements, with growth to a Klett reading of 100 (indicating an approximate density of 10^9 CFU/ml). Cells were harvested by centrifugation, washed once in 10 ml of 50 mM potassium phosphate (pH 7.5), and resuspended in 2.5 ml of 100 mM potassium phosphate–20 mM EDTA–10% (vol/vol) acetone (pH 7.2). Cells were then disintegrated with six 10-s pulses from a sonicator (Heat Systems, Inc.) set at 40% output; during this process, the tube containing the sample was kept submerged in ice water. The resulting crude lysate was clarified of cell debris with two rounds of centrifugation, first in a swinging bucket centrifuge at 4,000 × *g* for 5 min and then in a microfuge at 10,000 × *g* for 10 min. The clarified lysate was stored at −80°C. Assays were performed by diluting cell extracts in assay buffer (100 mM potassium phosphate, 0.2 mM catechol) such that a linear change in absorbance at 375 nm was seen over time. XylE activity was calculated by linear regression of the slope over six time points. One microunit of XylE activity corresponds to the formation of 1 nmol of 2-hydroxymuconic semialdehyde per min at 22°C. XylE activity was normalized against total protein concentration, as determined by the method of Bradford et al. (10), with bovine serum albumin (New England Biolabs, Beverly, Mass.) as the standard.

5′-RACE. RACE analyses were performed essentially as described by Frohman et al. (18). Synthesis of cDNA was performed with 2 μg of total RNA template, 12 pmol of antisense primer, and 1 U of avian myeloblastosis virus reverse transcriptase (Roche) in reaction buffer supplied by the manufacturer. The reaction was carried out for 1 h at 55°C, followed by 10 min of incubation at 65°C. cDNA was purified on a Qiagen spin column according to the manufacturer's directions, eluted in 50 μl of 10 mM Tris-Cl (pH 8.0), and polyadenylated with 0.5 U of terminal transferase (Roche)/μl using the manufacturer's reaction buffer supplemented with 1.5 mM $CoCl_2$ and 6.25 μM dATP. Three microliters of polyadenylated cDNA was used in a PCR with the d(T) forward primer (see Table 3) and a gene-specific antisense primer. The resulting products were

TABLE 3. Oligonucleotide primers used in this study

Primer	Site(s)	Source or reference
Anchor	GACCACGCGTGAATTC*EcoRI*GTCGAC	This study
CB-4	TATTGCGCGCACCGATGCCGACGA	11
CB-5	GCCGGCATCGAGGACGTGGAACCTGA	This study
d(T)	GACCACGCGTGAATTC*EcoRI*GTCGAC[T]$_{16}$V	This study
DA-1	CAATCATTGGCCGCCGTAGTGGGGCAGACTTGGCGCA	50
DA-5	GCCGTAAACTTTCTCAAGCTCCGCCT	This study
DCB13-F	GATC GGTACC*KpnI*GATC AAGCTT*HindIII*GGGAGAGTAAATTGCAGCCTT	This study
DCB13-R	TCGA GGTACC*KpnI* GATAATTTGATGCCGCCTGAAGGC	This study
DCB14-F2	GATC GAATTC*EcoRI* AGGATAATTTCCAATCCCCGC	This study
DCB15-F	GATC GAATTC*EcoRI* AGCGGCCCATCCCGATACGGA	This study
DCB15-R	TCGA GGATCC*BamHI* AGCTGATAACGTGGTTTTGCA	This study
DCB16-F	GATC GGTACC*KpnI* GATC AAGCTT*HindIII* GAACAGGATAAATCATGCAAAACC	This study
DCB16-R	TCGA GGTACC*KpnI* GGTTTCAATAGCTGCGGTATTTCC	This study
DCB17b-F	GATC GGTACC*KpnI* GATC AAGCTT*HindIII* GTATCGGAAAGGAGAAACGGATTG	This study
DCB17b-R	TCGA GGTACC*KpnI* CCGTCAATAAATCTTGCGTAAGAA	This study
DCB19b-F	GATC GAATTC*EcoRI* CTCCTTACCGAAGAACTCCCG	This study
DCB19b-R	ATGC GGATCC*BamHI* GCCGCAAATACGATGTCCATC	This study
Got (−180)	GATTCAGACGGCATTCGACA	This study
Got 310-R	AGGCGGTTCAGCAGGTTCAGGCGG	This study
Got 660-R	CGGAATTTTGAGCTTGTGCA	This study
Got 1020	ACACCGAGCGGGATTGGGCGGAAG	This study
Got 1576-R	GATC GGATCC*BamHI* GCCAAGCTGATAACGTGGTTTTGC	This study
Got 3240-R	TGCGCCATCTTTGAAGCATACA	This study
Got 3742-R	CAATGGCGGCAAGCACGCTT	This study
Got 3869-F	GTTCGATCCAGCCTATATCCAC	This study
Got 3891-R	GTGGATATAGGCTGGATCGAAC	This study
Got 3997-R	TGGAAGAAGTCTGGTCTTGA	This study
Got 4232-R	AGCAAATCGGTCAAAGAATAC	This study
Got 5803-R	AGCCCG GGATCC*BamHI* GCGAACGCTGCATCGTCC	This study
Got F	AGCT GAATTC*EcoRI* CTGCAGGCCGTCGCCGTATTCAAACAACTG	This study
JL-9	ACCACGTTATCAGCTTGGCT	This study
JL-12	AGCGGCCCATCCCGATAC	50
JL-50	CT GAATTC*EcoRI* GGCCGACATCGCGCTTTTGGGCG	47
JL-51	AT GGATCC*BamHI* GGG GCGATTTTACCTAGCAGATGAA	47
LgtDE-F	GGAAATACCGCAGCTATTGAATTC*EcoRI* CGA	50
LgtDE-R	GCATGATTTATCCTGTTCGAATTC*EcoRI* AAT	50
LgtDfix-F	GGA TCGCGA*NruI* GAACCGATGCCGACGATATTGCCT	This study
LgtDfix-R	GGTTC TCGCGA*NruI* TATATTCTCCACCTCCGCCA CCCGACTTTGCCATTCGTCCAGCCCGAT	This study
OmegaABC	TCAGAT GGCGCGCC*AscI* TGTACA*BsrGI* TCGAT*ClaI* GGTGA TTGATTGACGAAGCTTTATGC	This study
PostLgtE-F	GATC GGTACC*KpnI* AAGCTT*HindIII* CAGAAATGGACACACTGTCATTCC	This study
PostLgtE-R	TCGA GGTACC*KpnI* TGATTTTAATCCCCTATATTTTACAC	This study
XylE-BSR-F	GATC TGTACA*BsrGI* AGGAGGA*RBS* TGACGTCATGAAC	This study
XylE-BSR-R	TCGA TGTACA*BsrGI* TCAGGTCAGCACGGTCAT	This study
XylE-F	GATC GGTACC*KpnI* AGGAGGA*RBS* TGACGTCATGAAC	This study
XylE-R	TCGA AAGCTT*HindIII* TCAGGTCAGCACGGTCAT	This study

subjected to a second round of PCR with the anchor primer and a nested reverse primer and then resolved on 3% low-melting-temperature agarose gels. The transcriptional start site was determined by DNA sequence analysis of the RACE products.

RESULTS

Analysis of transcriptional linkage by interposon mutagenesis. The organization of the genes found in the *lgt* gene cluster suggested that they would be transcribed as an operon. Analysis of the DNA sequence (up to 500 bp) upstream of the putative *lgtA* start codon failed to identify any sequences with homology to known F 70-like promoters. This analysis did identify a DNA sequence that might form a rho-dependent termination sequence in the intergenic region between *glyS* stop codon and the putative *lgtA* start codon (see Fig. 1). However, if this stem-loop structure (26 bp upstream of the putative *lgtA* start codon) was functioning as a transcriptional stop signal, it was unclear what DNA sequence could be promoting the expression of the *lgt* gene cluster.

In order to determine whether the stem-loop was functioning as a transcription termination signal, we introduced a strong transcriptional stop site 6 bp upstream from the start of the stem-loop structure by inserting the Ω interposon into a *Bsr*GI site and analyzed the effect of its insertion on LOS expression. The location of the insertion event in each transformant was verified by PCR amplification of the appropriate region and restriction digestion of the PCR products. The data indicate that all transformants analyzed incorporated the Ω interposon sequence at the appropriate chromosomal location (data not shown). LOS was purified from 10 individual transformants and analyzed by SDS-PAGE, and the bands visualized by silver staining the gels. Each of the Ω interposon-derived mutants exhibited an altered LOS phenotype that possessed the same SDS-PAGE profile. A representative of

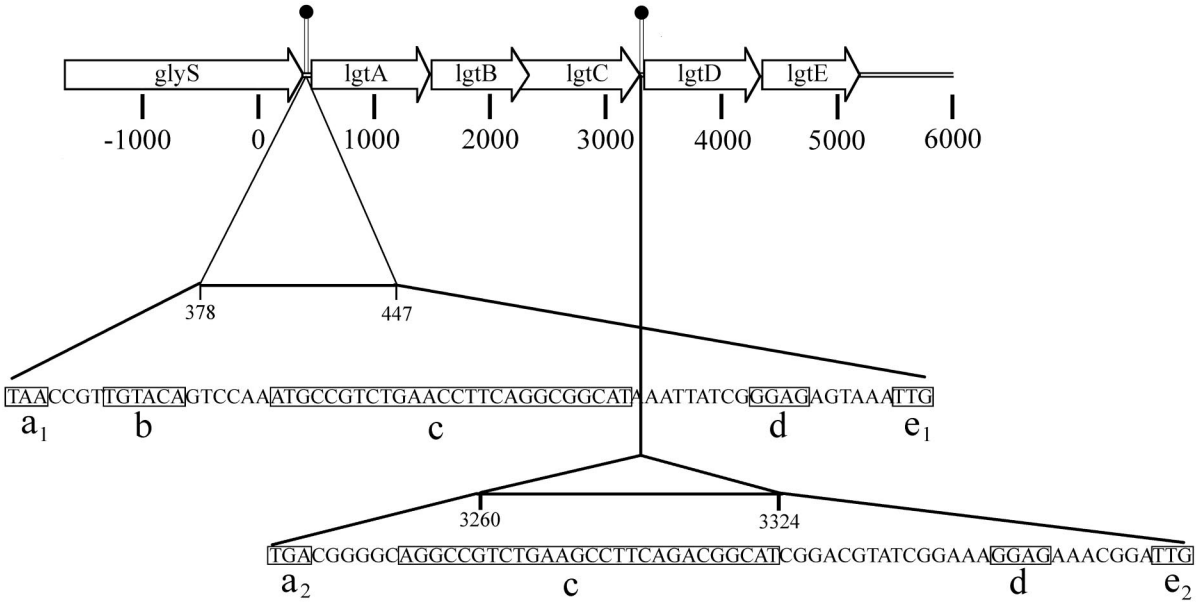

FIG. 1. Genomic organization of the *lgt* gene region. This diagram is derived from the DNA sequence of the *lgt* gene cluster as originally published by Gotschlich (20), with the NCBI accession number U14554. The sequence numbers given in the figure correspond to those described in this accession. The features identified in this figure are indicated as follows: a_1, *glyS* stop codon; a_2, *lgtC* stop codon; b, *Bsr*GI restriction site; c, potential stem-loop structure than could function as a transcriptional terminator; d, putative ribosome-binding site; e_1, putative *lgtA* start codon; and e_2, putative *lgtD* start codon.

the SDS-PAGE profile of one of these mutants (F62ΩBsrGI) is shown in Fig. 2, lane 3.

The *lgtE* gene is the most downstream gene in the *lgtABCDE* locus, and yet it mediates the first biochemical step in the assembly of a growing LOS molecule. If the *lgtABCDE* locus is an operon driven by a single promoter, then an Ω interposon insertion at the *Bsr*GI site should produce a strain whose LOS phenotype resembles the LOS of strains deleted in *lgtE* (produces LOS that will be referred to as ΔLgtE LOS). The data presented in Fig. 2 indicate that F62ΩBsrGI produces an LOS that is larger than the ΔLgtE LOS chemotype, suggesting that this strain possesses LgtE activity.

Western blot analysis of LOS expressed by F62ΩBsrGI indicated that the single band seen in Fig. 2, lane 3, reacted with MAb L1-1-17 (data not shown), an MAb that binds to an alternate LOS chemotype in neisserial LOS, one that is produced when LgtA is nonfunctional and LgtC is functional. From the data obtained in the analysis of LOS expressed by F62ΩBsrGI, we concluded that transcription of *lgtA* must initiate 5′ of the *Bsr*GI. These data also indicate that the potential rho-dependent termination sequence still allows for the transcription of sufficient message such that the amount of LgtA present in in vitro-grown cells is not limiting. Because F62ΩBsrGI was able to produce an LOS that requires transcription of *lgtC*, we concluded that a promoter must exist 3′ of the *Bsr*GI site. These data indicate that the overall transcriptional organization of the genes contained within this gene cluster must be driven from multiple promoters.

In order to localize promoter containing regions, we created a family of isogenic strains of *N. gonorrhoeae* F62 containing the Ω interposon inserted at different points within the *lgtABCDE* locus and observed the resulting LOS phenotypes. The plasmids used to make the various gonococcal constructs are described in Table 2. LOS was purified from 10 to 12 individual transformants for each construct and visualized after separation on SDS-PAGE gels (data not shown). Each of the Ω interposon mutants exhibited an altered LOS phenotype that was consistent across all of the individual transformants of each mutant that was analyzed, indicating that the change in LOS phenotype did not occur due to phase variation. We verified that the Ω interposon had inserted into the correct region by PCR amplification of the *lgtABCDE* locus of each of these mutants, with subsequent restriction digestion analysis of the amplicons. The expected restriction pattern was generated on an agarose gel from the amplicons generated from each of the mutants, indicating that the Ω interposon had been incorporated into the correct sites (data not shown).

The data presented in Fig. 2 indicate that all strains containing Ω interposon insertions within *lgtABCDE* continued to produce LOS chemotypes that require LgtE activity, except when the S interposon was inserted into an engineered *Eco*RI site immediately upstream of the *lgtE* ribosome-binding site (F62ΩEcoRI; Fig. 2, lane 9). Analysis of these results in detail allowed us to map the location of potential promoters. Strain F62ΩAscI (lane 4) produced two LOS components: one that possesses a mobility consistent with a ΔLgtB LOS (which corresponds to an LOS structure made when LgtB is nonfunctional) and one that possesses a mobility that corresponded to ΔLgtE LOS. The LOSs expressed by this strain failed to bind MAbs 1B2, 1-17-L1, and 2-1-L8. That this strain does not produce L1 LOS suggests that *lgtC* transcription has been inhibited. By combining these two results (data shown in Fig. 2, lanes 2 to 4), we could map a promoter region to between the *Bsr*GI and *Asc*I sites.

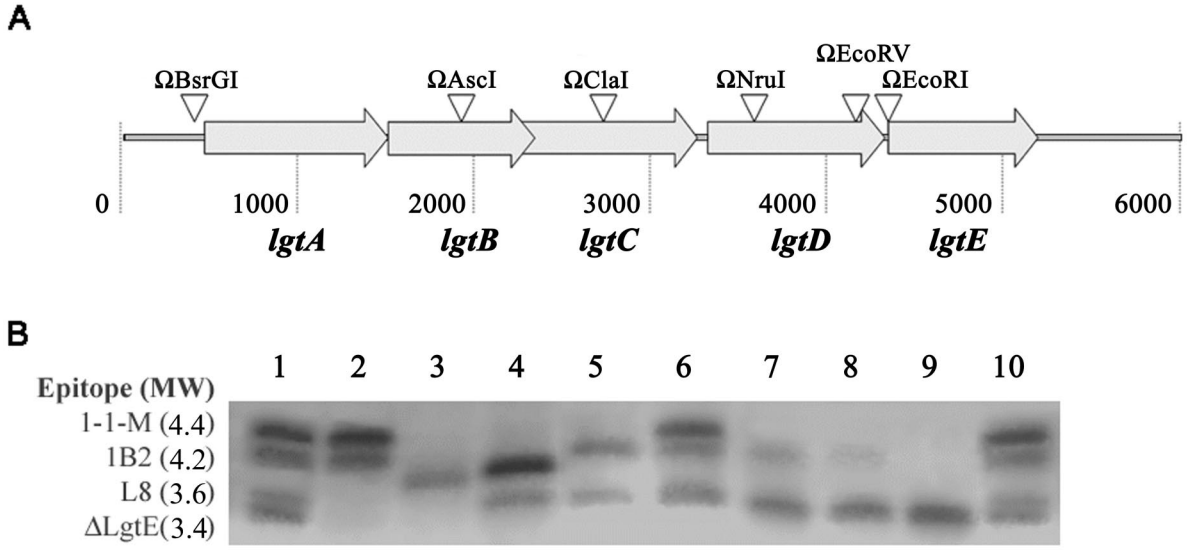

FIG. 2. Phenotypic analysis of LOS produced by Ω mutants. (A) *lgtABCDE* gene locus. Inverted triangles represent Ω insertion points. The names above the triangles refer to the names of the F62 insertion mutants. (B) Silver-stained SDS-PAGE gel of LOS isolated from various mutants. Lanes 1, 6, and 10 are an LOS ladder derived from LOS isolated from F62, F62ΔlgtA, and F62ΔlgtE. The four bands show the mobilities of the MAb 1-1-M, 1B2, and L8 reactive LOSs and the ΔLgtE LOS chemotype. The remaining lanes represent LOS species from strains F62 (lane 2), F62ΩBsrGI (lane 3), F62ΩAscI (lane 4), F62ΩClaI (lane 5), F62ΩNruI (lane 7), F62Ω*Eco*RV (lane 8), and F62ΩEcoRI (lane 9).

Simultaneous expression of at least two LOS components is seen in four mutant strains (see Fig. 2, lanes 5 and 7 to 9). In these strains, a mixture of the ΔLgtE LOS and a second LOS chemotype that binds MAb 1B2 (Western blot data are not shown) is produced. Since the Ω interposon is placed more proximal to the start codon of *lgtE*, a proportionally greater amount of ΔLgtE LOS is produced. In fact, the shift in the proportions between these two LOS components occurs in a stepwise fashion, suggesting the presence of multiple promoters.

Additional promoter regions were mapped by analyzing changes in the proportion of the two chemotypes that are produced. F62ΩClaI (Fig. 2, lane 5) produces a roughly 60:40 proportion of 1B2 LOS (LOS chemotype made when strain expresses a functional LgtA, and a nonfunctional LgtC and LgtD) and ΔLgtE LOS chemotypes, indicating inhibition of *lgtD* transcription and decreased transcription of *lgtE* versus that of the parental strain. Strain F62ΩNruI (Fig. 2, lane 7) produces a roughly 40:60 proportion of 1B2 LOS and ΔLgtE LOS. Strain F62ΩEcoRV (Fig. 2, lane 8) expresses less 1B2 LOS and more ΔLgtE LOS than strain F62ΩNruI. Strain F62ΩEcoRI mutant (Fig. 2, lane 9) only expresses ΔLgtE LOS. Altogether, the introduction of the Ω interposon in successive sites between *Cla*I and *Eco*RI results in stepwise increases in the amount of ΔLgtE LOS that is produced, suggesting a corresponding decrease in LgtE production. These data suggest the existence of promoters between each of the Ω interposon insertion sites, except between *Asc*I and *Cla*I, and support a model of multiple, weak promoters existing throughout the *lgtABCDE* locus. This further suggests that the cumulative contribution of each of these promoters defines the nature of the LOS that is expressed.

Quantitation of gene expression. In order to quantitate the level of gene expression within the *lgtABCDE* locus, we constructed three *xylE* transcriptional fusions. The *xylE* gene was inserted immediately upstream of *lgtA* [F62LgtA(xylE)], at the beginning of lgtD [F62LgtC(xylE)], and immediately downstream of *lgtE* [F62LgtE(xylE)]. Analysis of the LOS profiles of these strains by SDS-PAGE and colony blotting (data not shown) showed that the transformants expressed the expected wild-type LOS profile when the *xylE* gene was inserted before or after the *lgtABCDE* locus and the Δ*lgtD* LOS when the *xylE* gene was inserted into *lgtD* (Fig. 3).

We assayed these *xylE* fusion strains for catechol-2,3-dioxygenase (XylE) activity (Table 4). The assays showed a difference in XylE activity among the three strains. XylE activity was sevenfold higher in F62LgtA(xylE) than in F62LgtE(xylE) and F62LgtC(xylE). These data indicate that the different genes within the *lgtABCDE* gene locus can be expressed at different levels due to different mRNA concentrations. They further indicate that most transcription through this region terminates before it extends through the entire region.

Positional effects of the Ω interposon insertion on XylE activity in F62LgtE(xylE). The data presented above suggest the presence of multiple promoter sequences located within

TABLE 4. XylE activity of selected mutants

Strain	XylE activity[a] (μU)
F62	0.2
F62LgtA(xylE)	220
F62LgtC(xylE)	31.6
F62LgtE(xylE)	32.8
F62RfaF(xylE)[b]	15,400

[a] One microunit of XylE activity corresponds to the formation of 1 nmol of 2-hydroxymuconic semialdehyde per min at 22°C.

[b] This strain possesses a *xylE* insertion at the start of the gonococcal *rfaF* gene (14).

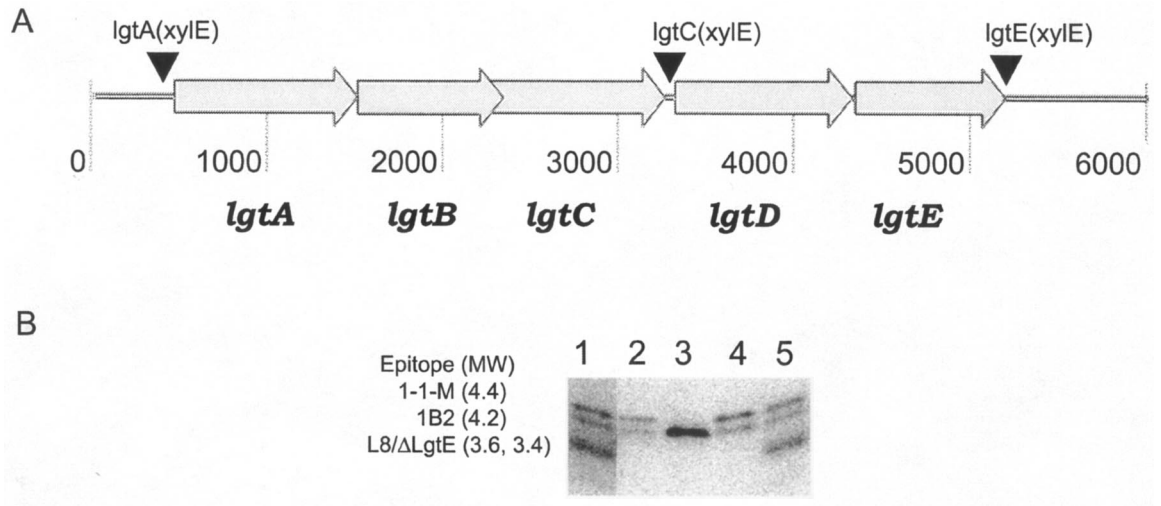

FIG. 3. (A) Phenotypic analysis of LOSs produced by *xylE* fusion strains. The dark triangles represent *xylE* insertion points within the *lgtABCDE* gene cluster. (B) Silver-stained SDS-PAGE gel of LOSs isolated from these strains. Lanes 1 and 5 represent an LOS ladder derived from LOS that has been isolated from strains F62, F62ΔlgtA, and F62ΔlgtE. The four bands show the mobility of the MAb 1-1-M, 1B2, and L8 reactive LOSs and the ΔLgtE LOS chemotype. Lane 2 represents LOS isolated from F62LgtA(xylE), lane 3 represents LOS isolated from F62 *lgtC* (*xylE*), and lane 4 represents LOS isolated from F62 *lgtE* (*xylE*).

the *lgtABCDE* locus. In order to quantify the relative contribution that these sequences might play in overall gene expression, we introduced the Ω interposon into various chromosomal locations in F62LgtE(xylE). Each transformant was analyzed by PCR amplification of the region of interest, with subsequent restriction digestion analysis to verify that the insertion had incorporated into the correct chromosomal location. In addition, the SDS-PAGE profiles of each isogenic pair [i.e., F62LgtE(xylE)::ΩBsrGI compared to F62ΩBsrGI] were identical, indicating that the interposon incorporation was exerting the same phenotypic modulation in each pair of strains. The data presented in Fig. 4 indicate that insertion of the Ω interposon at the *Bsr*GI site did not result in a change in XylE expression. This further supports the data presented above that indicated that transcription terminates within the *lgtABCDE* region. Insertions that occurred within the coding sequence reduced but did not eliminate XylE expression compared to the isogenic parent strain lacking the interposon insertion [F62LgtE(xylE)]. As the insertion site of the Ω interposon neared the *lgtE* coding sequence, the level of transcription decreased. Insertion of the Ω interposon in the engineered *Eco*RI site allowed for XylE activity, suggesting that weak promoter sequences were located within the *lgtE* coding sequence. Overall, these data suggest that multiple promoter sequences must occur within the *lgtABCDE* coding sequence.

Identification of transcriptional start sites. Initial attempts to identify the size of transcripts produced from the *lgtABCDE* region by Northern hybridization experiments were unsuccessful. However, the XylE expression data described above suggest that the reason for this failure is due to the low level of mRNA that would correspond to this region. Therefore, we used RACE, a more sensitive approach, to identify putative transcriptional start sites.

RACE products were analyzed on an agarose gel (Fig. 5). In most of the lanes, multiple DNA fragments were generated by the RACE reactions. These fragments represent transcriptional start sites (TS) within the *lgtDE* region or RNA polymerase pause sites. The sequence of the RACE products was determined, and the upstream DNA sequences were analyzed for homology to known promoter sequences. Six of the seven sites identified by RACE corresponded to upstream sequences that showed between 50 and 79% homology with the consensus gearbox promoter sequence of *E. coli* (1, 9, 29). The sequence upstream of the seventh fragment (TS-3626) showed overlapping sequences with 58 and 75% homology to the σ^{70} consensus of *Neisseria* spp. and the σ^{28} consensus of *E. coli*, respectively (Fig. 6) (4, 6). In light of the fact that promoters with less homology have frequently been shown to have high expression levels, these results indicate that the identification of these sites as promoters is realistic.

Due to the extensive homology between *lgtA* and *lgtD* and between *lgtB* and *lgtE*, we hypothesized that promoters found in *lgtDE* may have homologous counterparts in *lgtAB*. We examined the *lgtAB* sequence for homology within the identified regions. Of the seven promoter sequences identified within *lgtDE*, only TS-3626 had an identical counterpart within *lgtAB*. Two other sequences showed homology with a two-base mismatch (corresponding to TS-4267 and TS-4493). The remaining regions had counterparts in which three or more bases mismatched in a manner which gave them less homology to the consensus promoter sequence.

DISCUSSION

The *lgtABCDE* gene cluster encodes most of the genes needed to make the gonococcal α-oligosaccharide (20). Our data indicate that a complex transcriptional control mechanism is responsible for regulating the expression of *lgtABCDE*. First, we demonstrate that multiple promoter regions exist throughout this cluster. Second, we show that the *lgtABCDE* genes are transcribed at minute levels relative to the transcriptional ex-

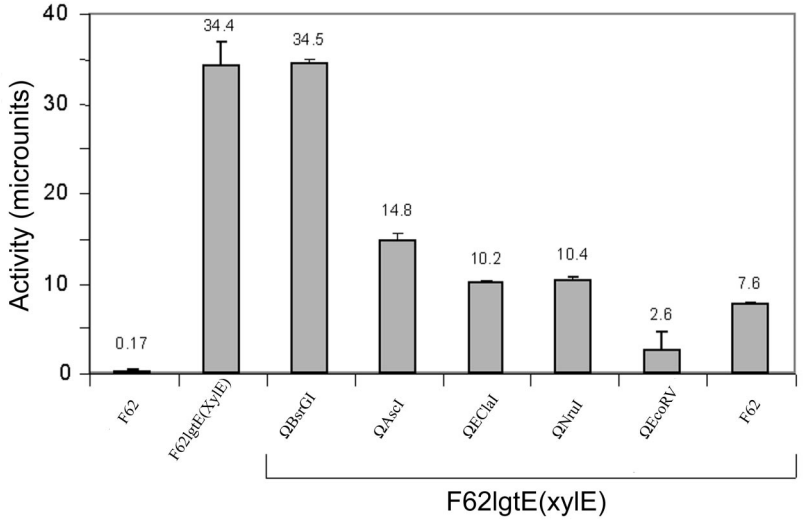

FIG. 4. Catechol-2,3-dioxygenase activity of *xylE* fusion strains. Enzymatic activity was detected biochemically by a spectrophotometric procedure that detects the appearance of 2-hydroxymuconic semialdehyde. Activity is measured in microunits (nanomoles per minute per milligram). Error bars indicate the standard error between triplicate assays.

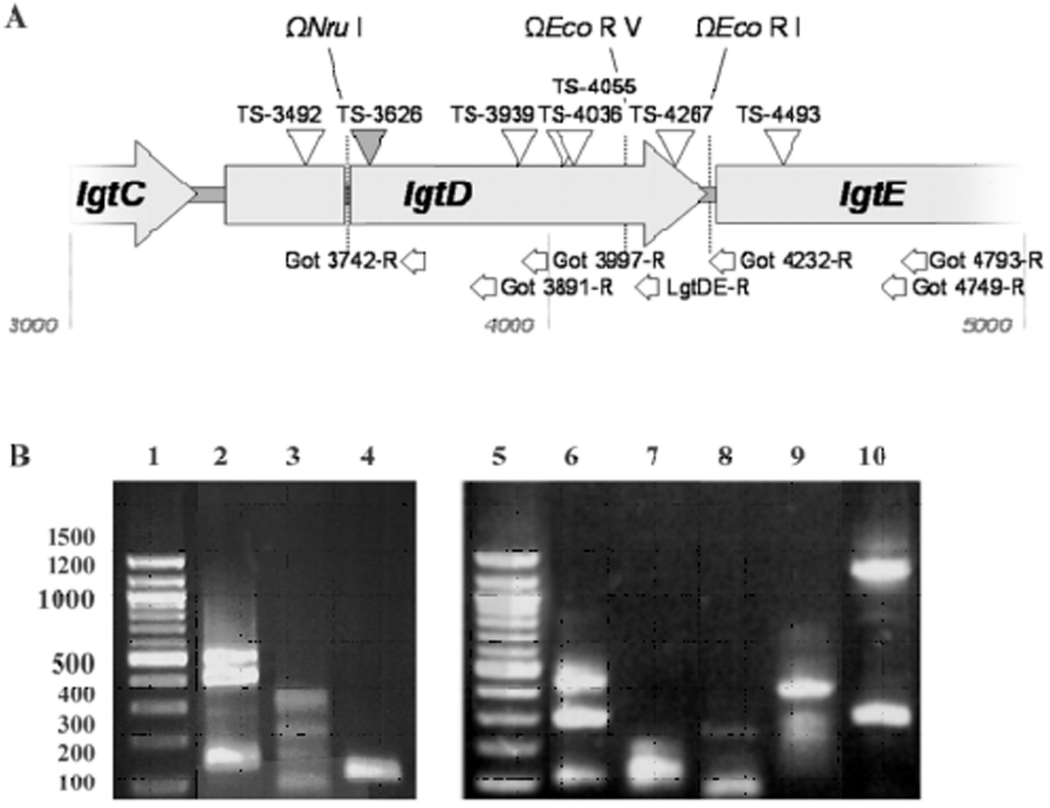

FIG. 5. RACE products and associated transcriptional start sites. (A) Diagram showing elements of the 5′-RACE experiment. Vertical arrows represent the locations of putative promoters, named according to the relative position of the transcriptional start sites. Open arrows represent putative gearbox promoters, and the closed arrow represents the single putative σ^{70} promoter. Horizontal arrows represent the location of the reverse primers which were used. (B) Agarose gel electrophoresis of RACE products. DNA was resolved on an agarose gel. Lanes 1 and 5, 100-bp ladder (New England Biolabs). Major bands, from top to bottom, represent PCR products that initiate at cDNA 5′ ends (transcriptional start sites), as follows: lanes 2 and 3, TS-4267, TS-4055, and TS-3939; lane 4, TS-3939; lane 6, TS-4267, TS-4493 and unknown; lane 7, TS-4267; lane 8, TS-4055; lane 9, TS-3626; lane 10, TS-3492 and unknown.

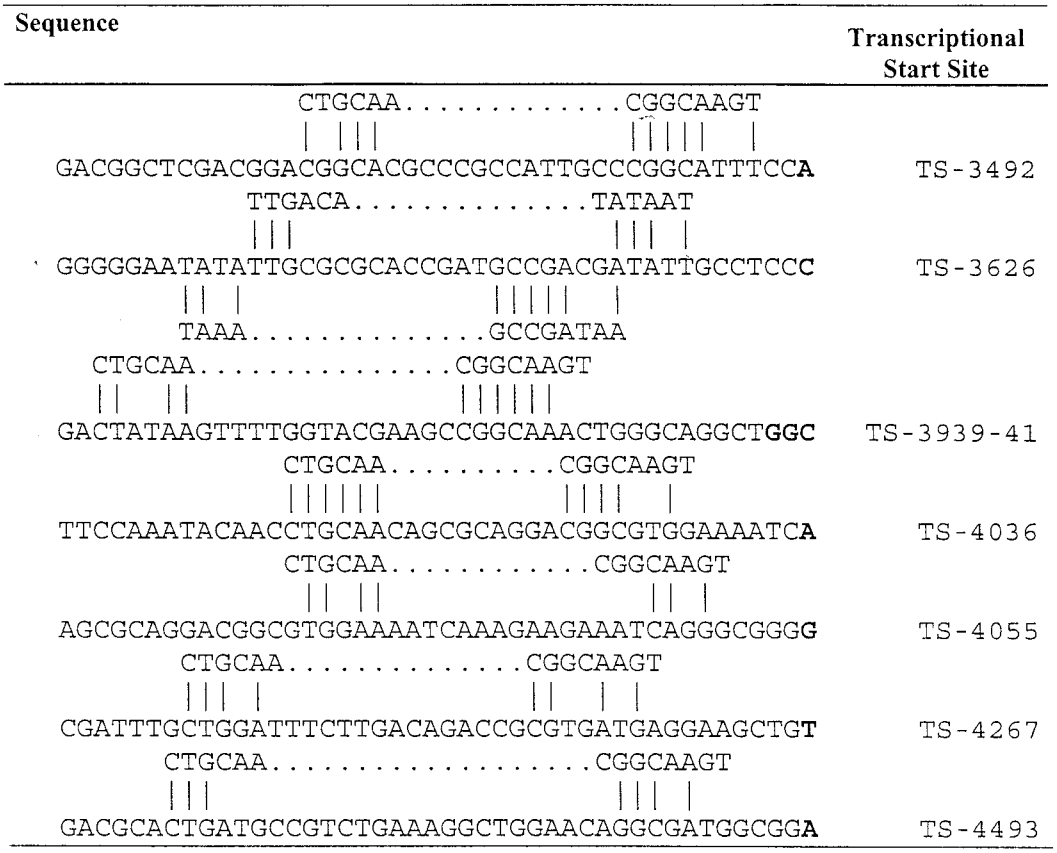

FIG. 6. Putative promoters identified by sequence homology. Putative transcriptional start sites were examined for potential homology to known consensus *E. coli* and *Neisseria* promoter sequences by calculating the percent identity between sequence upstream of TS and known consensus promoter sequences. The spacing between the −10 and −35 sequences was required to be within ±3 nucleotides of the consensus spacing; this requirement is not reflected in the homology percentages. Nucleotides in boldface represent putative TS identified by RACE.

pression of *lsi-1* (*rfaF*) glycosyl transferase (see Table 4). Subtle differences in the level of transcription appear to modulate the LOS phenotype in terms of the identity and relative amounts of each of the chemotypes that are simultaneously surface expressed.

The Ω interposon is a spectinomycin cassette (*aadA*) flanked by *rho*-independent transcriptional terminators derived from bacteriophage T4. The introduction of the Ω interposon in either orientation results in at least a 1,000-fold reduction in transcription, causing polar mutations (17, 18). This interposon cassette has been used to determine linkage relationships and to map the location of promoters in a number of organisms, including *N. gonorrhoeae* (12, 17, 37, 42). Using Ω interposon insertions within the *lgtABCDE* region, we were able to generate evidence that supports the presence of multiple promoters.

As the Ω interposon is placed more proximal to *lgtE*, a greater number of transcripts are terminated at the Ω interposon insertion site and overall transcription of *lgtE* is reduced, hence also the lower amounts of LgtE. When *lgtE* is transcribed in limiting amounts, not enough LgtE is produced to process all of the LOS precursors before they are surface expressed. Using *xylE* as a reporter gene for measuring *lgtE* transcription indicated that the overall level of transcription of this gene cluster is quite low. The level of XylE expression at all insertion points within this region is very low. This can be seen when we compared the level of expression of XylE to that seen when this gene was linked to another LOS biosynthetic gene, *rfaF* (14). Expression of this gene is almost 1,000 times greater than what is seen for the two *lgt* (*xylE*) insertions. Our data show that *lgtE* transcription (Fig. 4) and LgtE activity (Fig. 2) decreases if the Ω interposon is inserted within *lgtABCDE*. The amount of the decrease is more if the Ω interposon is inserted closer to the start codon of *lgtE*. This decrease in LgtE activity is seen as an increase in the proportion of ΔLgtE LOS between any two Ω interposon insertion mutants, which occurs between five of the six mutants examined. Therefore, promoter activity originates between each pair of adjacent Ω interposon insertion sites (see Fig. 2).

Seven potential transcriptional start sites were identified between the *Cla*I and *Eco*RI Ω interposon insertion sites by using RACE analysis. Each of these transcriptional start sites contained −10 and −35 upstream regions that possessed between 50 and 79% homology to consensus *E. coli* and *Neisseria* promoter sequences (see Fig. 5 and 6). Three of these TS had homologous promoter sequences within the *lgtAB* region. The location of these putative promoters identified by RACE to be within *lgtDE* is consistent with the phenotypes and reporter gene activities of the F62 *lgtE* (*xylE*) Ω interposon insertion mutants. The identification of a transcriptional start site within

the *lgtE* coding sequence (TS-4493) indicates a promoter which, without further rationalization, appears to serve no biological function in this strain. However, the existence of this promoter within *lgtE* is logical in terms of evolutionary descent because extensive homology exists found between *lgtB* and *lgtE*. Any existing promoters are likely to be shared between these two genes. It has been demonstrated that intergenic recombination occurs between *lgt* genes, giving rise to recombinant *lgt* loci (5, 28). Therefore, the putative promoter upstream of TS-4493 may have had biological function in the context of *lgtB* transcription.

The identification of six out of seven putative promoter regions as gearbox promoters is consistent with recent studies and with data from the present study. In *N. gonorrhoeae*, the gearbox promoter has been implicated in the expression of two virulence factors, AniA (26) and Tpc (19). Gearbox promoters generally express at a strength that is inversely proportional to growth rate (52), a finding consistent with our observations that transcription of *lgtE* (*xylE*) increases fourfold between the mid-logarithmic and early stationary phase (data not shown).

Most significantly, we demonstrated here that limiting the transcription of *lgtE* leads to simultaneous production of two LOS chemotypes in a balance that is contingent upon the level of transcription. Assays of the *xylE* fusion strains allowed us to correlate visible proportions of $LgtE^+$ and ΔLgtE LOSs with measured levels of transcriptional expression (Fig. 2 and 4). In addition, these results show that the *lgtABCDE* genes are transcribed at very low levels, demonstrating that subtle changes in transcription are likely to incur significant phenotypic changes.

The transcriptional fusion data also support an earlier study that demonstrated that a strain of *N. gonorrhoeae* FA19 with *lgtA* frameshifted to the "off" position, continued to produce trace amounts of $LgtA^+$ LOS (11). Burch et al. speculated that transcriptional and/or translational strand slippage was occurring at a frequency just high enough to allow for visible amounts of $LgtA^+$ LOS. Transcriptional strand slippage by the RNA polymerase holoenzyme would result in a small amount of in-frame *lgtA* transcript, whereas translational strand slippage of out-of-frame mRNA by the ribosomes would allow for the production of in-frame LgtA protein. Since the present study demonstrates that small changes in the amount of LgtE dramatically impact the nature of the expressed LOS, it is feasible that a low level of transcriptional or translational strand slippage of *lgtA* mRNA could result in sufficient LgtA to account for the $LgtA^+$-$LgtA^-$ mixed phenotype of these strains.

From our data, we are able to formulate a logical explanation for why *N. gonorrhoeae* F62 normally produces a mixture of the 1-1-M and 1B2 LOS chemotypes. The LgtD glycosyl transferase facilitates the addition of GalNAc to the terminal Gal of the α chain, converting the α chain from the 1B2 chemotype to the 1-1-M chemotype. Our data show that the *lgtD* gene is transcribed at a level ~1,000-fold less than that of *lsi-1* (*rfaF*) (14). The low level of *lgtD* transcriptional expression is such that a distribution of both $LgtD^+$ (1-1-M) and $LgtD^-$ (1B2) LOS chemotypes are produced. If transcriptional expression of *lgtD* were to be increased, then we would predict a phenotypic shift toward producing a greater proportion of the 1-1-M chemotype.

Control of LOS phenotype by limiting transcription is the likely mechanism in spontaneous L1-reactive phase variants of *N. gonorrhoeae* F62. These variants typically express a mixture of immunotype L1 ($LgtC^+$) and other ($LgtC^-$) LOS chemotypes (D. C. Stein, unpublished data). The data from the present study suggests that in this strain, *lgtC* is transcribed in amounts such that insufficient LgtC is being produced to catalyze all its available substrate. It is also possible that *lgtC* is transcribed and translated in strains that fail to make the L1 LOS but that the enzyme is outcompeted for substrate by LgtA.

Sequence analysis of the *lgtABCDE* region using the Neural Network algorithm (40) revealed 29 sequences that share significant homology with the σ^{70} promoter consensus of *E. coli* and *Neisseria* spp. Remarkably, the polyguanine regions of the *lgtA*, *lgtC*, and *lgtD* genes were among the highest scorers, suggesting that the polyguanine tracts may affect promoter functions. These regions all had clearly identifiable −35 and −10 regions, but the spacing between these regions was too long to suggest that these sequences were functional in these genes. There are other examples of promoter regions spanning homopolymeric runs in the pathogenic neisseriae. The *porA* and *opc* genes of *N. meningitidis*, respectively, contain a polyguanine and polycytosine run between the −35 and −10 consensus s^{70} sequence (44, 51). Frameshifting in these runs alters the spacing between the −35 and −10 sequences and leads to phase shifting between high, low, and intermediate transcriptional levels. It is feasible that this same mechanism may be occurring within the *lgtABCDE* locus and that these promoters are up- or downregulated based upon heritable changes to their sequence.

Our model is consistent with observations made by researchers who have shown that LOS expression is modulated by these environmental stimuli: growth rate, pH, aerobic versus anaerobic conditions, and carbon source (glucose versus lactate versus pyruvate) (16, 30, 33, 35). At present, the genetic mechanism that mediates this variation in expression is unknown. Most of these promoters identified by the present study are gearbox promoters, which exhibit expression at levels inversely proportional to growth rate. Since environmental changes can either increase or decrease the growth rate, changes in the environment would influence the level of expression from these promoters. Alternatively, additional epistatic factors may interact with these promoters and contribute to LOS expression in response to environmental conditions.

Our cumulative understanding of LOS phase variation in *N. gonorrhoeae* can be summarized as follows: *N. gonorrhoeae* regulates LOS biosynthesis and phase variation via a variety of disparate mechanisms. The specific LOS components produced by a particular strain are defined by on-off strand slippage of homopolymeric tracts within the *lgtA*, *lgtC*, *lgtD*, and *lgtG* genes (7, 13). Simultaneous production of multiple LOS epitopes is mediated by production of limiting amounts of the LgtA, LgtD, or LgtE. This occurs via transcriptional or translational strand slippage (11), via regulation of transcriptional expression (the present study), and via the low kinetic efficiencies of specific glycosyl transferases (36). In addition, recombination between glycosyl transferases is seen in some strains of *Neisseria* and these recombinant loci may consequently invoke one or more of the above mechanisms of phase variation (5, 36).

ACKNOWLEDGMENTS

We thank Peijun He and Anne Corriveau for excellent technical assistance.

This study was supported by a grant from the National Institutes of Health to D.C.S. (AI24452) and an F31 fellowship to D.C.B. (AI09636).

REFERENCES

1. **Aldea, M., T. Garrido, J. Pla, and M. Vicente.** 1990. Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. EMBO J. **9:**3787–3794.
2. **Apicella, M. A., M. Ketterer, F. K. Lee, D. Zhou, P. A. Rice, and M. S. Blake.** 1996. The pathogenesis of gonococcal urethritis in men: confocal and immunoelectron microscopic analysis of urethral exudates from men infected with *Neisseria gonorrhoeae.* J. Infect. Dis. **173:**636–646.
3. **Apicella, M. A., M. Shero, G. A. Jarvis, J. M. Griffiss, R. E. Mandrell, and H. Schneider.** 1987. Phenotypic variation in epitope expression of the *Neisseria gonorrhoeae* lipooligosaccharide. Infect. Immun. **55:**1755–1761.
4. **Arhin, F. F., F. Moreau, J. W. Coulton, and E. L. Mills.** 1998. Sequencing of *porA* from clinical isolates of *Neisseria meningitidis* defines a subtyping scheme and its genetic regulation. Can. J. Microbiol. **44:**56–63.
5. **Arking, D., Y. Tong, and D. C. Stein.** 2001. Analysis of lipooligosaccharide biosynthesis in the *Neisseriaceae*. J. Bacteriol. **183:**934–941.
6. **Arnosti, D. N., and M. J. Chamberlin.** 1989. Secondary sigma factor controls transcription of flagellar and chemotaxis genes in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **86:**830–834.
7. **Banerjee, A., R. Wang, S. Uljohn, P. A. Rice, E. C. Gotschlich, and D. C. Stein.** 1998. Identification of the gene (*lgtG*) encoding the lipooligosaccharide β chain synthesizing glucosyl transferase from *Neisseria gonorrhoeae.* Proc. Natl. Acad. Sci. USA **95:**10872–10877.
8. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
9. **Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter.** 1991. Stationary-phase-inducible "gearbox" promoters: differential effects of *katF* mutations and role of sigma 70. J. Bacteriol. **173:**4482–4492.
10. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
11. **Burch, C. L., R. J. Danaher, and D. C. Stein.** 1997. Antigenic variation in *Neisseria gonorrhoeae*: production of multiple lipooligosaccharides. J. Bacteriol. **179:**982–986.
12. **Carson, S. D., P. E. Klebba, S. M. Newton, and P. F. Sparling.** 1999. Ferric enterobactin binding and utilization by *Neisseria gonorrhoeae.* J. Bacteriol. **181:**2895–2901.
13. **Danaher, R. J., J. C. Levin, D. Arking, C. L. Burch, R. Sandlin, and D. C. Stein.** 1995. Genetic basis of *Neisseria gonorrhoeae* lipooligosaccharide antigenic variation. J. Bacteriol. **177:**7275–7279.
14. **Danaher, R. J., E. F. Petricoin III, and D. C. Stein.** 1994. Use of *xylE* fusions to demonstrate that *lsi-1*, a *Neisseria gonorrhoeae* lipooligosaccharide biosynthetic gene, and *lsi-3* are not transcriptionally linked. J. Bacteriol. **176:**3428–3432.
15. **de Vries, F. P., A. van Der Ende, J. P. van Putten, and J. Dankert.** 1996. Invasion of primary nasopharyngeal epithelial cells by *Neisseria meningitidis* is controlled by phase variation of multiple surface antigens. Infect. Immun. **64:**2998–3006.
16. **Frangipane, J. V., and R. F. Rest.** 1993. Anaerobic growth and cytidine 5′-monophospho-*N*-acetylneuraminic acid act synergistically to induce high-level serum resistance in *Neisseria gonorrhoeae*. Infect. Immun. **61:**1657–1666.
17. **Frey, J., and H. M. Krisch.** 1985. Omega mutagenesis in gram-negative bacteria: a selectable interposon which is strongly polar in a wide range of bacterial species. Gene **36:**143–150.
18. **Frohman, M. A.** 1994. On beyond classic RACE (rapid amplification of cDNA ends). PCR Methods Appl. **4:**S40–S58.
19. **Fussenegger, M., A. F. Kahrs, D. Facius, and T. F. Meyer.** 1996. Tetrapac (*tpc*), a novel genotype of *Neisseria gonorrhoeae* affecting epithelial cell invasion, natural transformation competence, and cell separation. Mol. Microbiol. **19:**1357–1372.
20. **Gotschlich, E. C.** 1994. Genetic locus for the biosynthesis of the variable portion of *Neisseria gonorrhoeae* lipooligosaccharide. J. Exp. Med. **180:**2181–2190.
21. **Gregg, C. R., M. A. Melly, C. G. Hellerquist, J. G. Coniglio, and Z. A. McGee.** 1981. Toxic activity of purified lipopolysaccharide of *Neisseria gonorhoeae* for human fallopian tube mucosa. J. Infect. Dis. **143:**432–439.
22. **Gregg, C. R., M. A. Melly, and Z. A. McGee.** 1980. Gonococcal lipopolysaccharide: a toxin for human fallopian tube mucosa. Am. J. Obstet. Gynecol. **138:**981–984.
23. **Griffiss, J. M.** 1995. The role of bacterial lipooligosaccharides in the pathogenesis of human disease. Trends Glycosci. Glycotechnol. **7:**461–478.
24. **Gunn, J. S., and D. C. Stein.** 1996. Use of a non-selectable transformation technique to construct a multiple restriction modification deficient mutant of *Neisseria gonorrhoeae.* Mol. Gen. Genet. **251:**509–517.
25. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver stained polyacrylamide gels. J. Bacteriol. **154:**269–277.
26. **Householder, T. C., W. A. Belli, S. Lissenden, J. A. Cole, and V. L. Clark.** 1999. *cis*- and *trans*-acting elements involved in regulation of *aniA*, the gene encoding the major anaerobically induced outer membrane protein in *Neisseria gonorrhoeae*. J. Bacteriol. **181:**541–551.
27. **Inoue, H., H. Nojima, and H. Okayama.** 1990. High efficiency transformation of *Escherichia coli* with plasmids. Gene **96:**23–28.
28. **Jennings, M. P., Y. N. Srikhanta, E. R. Moxon, M. Kramer, J. T. Poolman, B. Kuipers, and P. van der Ley.** 1999. The genetic basis of the phase variation repertoire of lipopolysaccharide immunotypes in *Neisseria meningitidis.* Microbiol. **145:**3013–3021.
29. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor sigma S. J. Bacteriol. **173:**4474–4481.
30. **McGee, D. J., and R. F. Rest.** 1996. Regulation of gonococcal sialyltransferase, lipooligosaccharide, and serum resistance by glucose, pyruvate, and lactate. Infect. Immun. **64:**4630–4637.
31. **McGee, Z. A., C. M. Clemens, R. L. Jensen, J. J. Klein, L. R. Barley, and G. L. Gorby.** 1992. Local induction of tumor necrosis factor as a molecular mechanism of mucosal damage by gonococci. Microb. Pathog. **12:**333–341.
32. **Merz, A. J., and M. So.** 2000. Interactions of pathogenic neisseriae with epithelial cell membranes. Annu. Rev. Cell Dev. Biol. **16:**423–457.
33. **Morse, S. A., C. S. Mintz, S. K. Sarafian, L. Bartenstein, M. Bertram, and M. A. Apicella.** 1983. Effect of dilution rate on lipopolysaccharide and serum resistance of *Neisseria gonorrhoeae* grown in continuous culture. Infect. Immun. **41:**74–82.
34. **Mühlecker, W., S. Gulati, D. P. McQuillen, S. Ram, P. A. Rice, and V. N. Reinhold.** 1999. An essential saccharide binding domain for the mAb 2C7 established for *Neisseria gonorrhoeae* LOS by ES-MS and MSn. Glycobiology **9:**157–171.
35. **Pettit, R. K., E. S. Martin, S. M. Wagner, and V. J. Bertolino.** 1995. Phenotypic modulation of gonococcal lipooligosaccharide in acidic and alkaline culture. Infect. Immun. **63:**2773–2775.
36. **Piekarowicz, A., and D. C. Stein.** 2002. Biochemical properties of *Neisseria gonorrhoeae* LgtE. J. Bacteriol. **184:**6410–6416.
37. **Prentki, P., and H. M. Krisch.** 1984. In vitro insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
38. **Pridmore, R. D.** 1987. New and versatile cloning vectors with kanamycin-resistance marker. Gene **56:**309–312.
39. **Ramsey, K. H., H. Schneider, R. A. Kuschner, A. F. Trofa, A. S. Cross, and C. D. Deal.** 1994. Inflammatory cytokine response to experimental human infection with *Neisseria gonorrhoeae*. Ann. N. Y. Acad. Sci. **730:**322–325.
40. **Reese, M. G., F. H. Eeckman, D. Kulp, and D. Haussler.** 1997. Improved splice site detection in Genie. J. Comput. Biol. **4:**311–323.
41. **Rodriguez, R. L., and R. C. Tait.** 1983. Isolation of chromosomal DNA recombinant DNA techniques: an introduction. Addison-Wesley Publishing Company, Reading, Mass.
42. **Ronpirin, C., A. E. Jerse, and C. N. Cornelissen.** 2001. Gonococcal genes encoding transferrin-binding proteins A and B are arranged in a bicistronic operon but are subject to differential expression. Infect. Immun. **69:**6336–6347.
43. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
44. **Sarkari, J., N. Pandit, E. R. Moxon, and M. Achtman.** 1994. Variable expression of the Opc outer membrane protein in *Neisseria meningitidis* is caused by size variation of a promoter containing poly-cytidine. Mol. Microbiol. **13:**207–217.
45. **Schneider, H., J. M. Griffiss, J. W. Boslego, P. J. Hitchcock, K. O. McJunkin, and M. A. Apicella.** 1991. Expression of paragloboside-like lipooligosaccharide may be a necessary component of gonococcal pathogenesis in men. J. Exp. Med. **174:**1601–1605.
46. **Schneider, H., T. L. Hale, W. D. Zollinger, R. C. Seid, C. A. Hammack, and J. M. Griffiss.** 1984. Heterogeneity of molecular size and antigenic expression within lipooligosaccharides of individual strains of *Neisseria gonorrhoeae* and *Neisseria meningitidis.* Infect. Immun. **45:**544–549.
47. **Song, W., L. Ma, W. Chen, and D. C. Stein.** 2000. Role of lipooligosaccharide in *opa*-independent invasion of *Neisseria gonorrhoeae* into human epithelial cells. J. Exp. Med. **191:**949–959.
48. **Stein, D. C.** 1992. Plasmids with easily excisable *xylE* cassettes. Gene **117:**157–158.
49. **Stein, D. C., J. S. Gunn, and A. Piekarowicz.** 1998. Sequence similarities between the genes encoding the S.*Ngo*I and *Hae*II restriction/modification systems. Biol. Chem. **379:**575–579.
50. **Tong, Y., B. Reinhold, V. Reinhold, B. Brandt, and D. C. Stein.** 2001. Structural and immunochemical characterization of the lipooligosaccharides expressed by *Neisseria subflava* 44. J. Bacteriol. **183:**942–950.

51. **van der Ende, A., C. T. Hopman, S. Zaat, B. B. Essink, B. Berkhout, and J. Dankert.** 1995. 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. J. Bacteriol. **177:**2475–2480.
52. **Vicente, M., S. R. Kushner, T. Garrido, and M. Aldea.** 1991. The role of the "gearbox" in the transcription of essential genes. Mol. Microbiol. **5:**2085–2091.
53. **Virji, M., K. Makepeace, I. R. Peak, D. J. Ferguson, M. P. Jennings, and E. R. Moxon.** 1995. Opc- and pilus-dependent interactions of meningococci with human endothelial cells: molecular mechanisms and modulation by surface polysaccharides. Mol. Microbiol. **18:**741–754.
54. **White, L. A., and D. S. Kellogg, Jr.** 1965. *Neisseria gonorrhoeae* identification in direct smears by a fluorescent antibody counterstain method. Appl. Microbiol. **13:**171–174.
55. **Yamasaki, R., D. E. Kerwood, H. Schneider, K. P. Quinn, J. M. Griffiss, and R. E. Mandrell.** 1994. The structure of lipooligosaccharide produced by *Neisseria gonorrhoeae*, strain 15253, isolated from a patient with disseminated infection: evidence for a new glycosylation pathway of the gonococcal lipooligosaccharide. J. Biol. Chem. **269:**30345–30351.
56. **Yang, Q. L., and E. C. Gotschlich.** 1996. Variation of gonococcal lipooligosaccharide structures is due to alterations in poly-G tracts in *lgt*genes encoding glycosyl transferases. J. Exp. Med. **183:**323–327.