CrgA Is an Inducible LysR-Type Regulator of *Neisseria meningitidis*, Acting both as a Repressor and as an Activator of Gene Transcription

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The *crgA* gene of *Neisseria meningitidis*, which codes for a LysR-type regulator, is divergently oriented with respect to the *mdaB* gene, which codes for a hypothetical NADPH-quinone oxidoreductase. Transcriptional studies of the intergenic region between *crgA* and *mdaB* showed that two overlapping and divergent promoters, *P*<sub>crgA</sub> and *P*<sub>mdaB</sub>, control transcription of these genes. Deletion of the *crgA* gene led to a strong increase in transcription from the *P*<sub>crgA</sub> promoter and a concomitant strong decrease in transcription from the *P*<sub>mdaB</sub> promoter, indicating that CrgA acts both as an autorepressor of transcription at its own promoter and as an activator of transcription at the *mdaB* promoter. Addition of α-methylene-γ-butyrolactone (MBL), an inducer of NADPH-quinone oxidoreductase, to wild-type *N. meningitidis* cells specifically resulted in further activation of transcription of the *P*<sub>mdaB</sub> promoter and more repression of transcription of the *P*<sub>crgA</sub> promoter. No such regulation was observed when MBL was added to *crgA*-deficient cells, indicating that the transcriptional response to MBL is CrgA mediated. Under the same experimental conditions, no regulation of transcription by either CrgA or MBL was detected at the pilus and capsule genes. The role of CrgA in the regulation of gene expression during the infectious cycle of *N. meningitidis* is discussed.

The human pathogen *Neisseria meningitidis* is a common colonizer of the nasopharynx, and in a small percentage of carriers, this bacterium can cross the epithelial barrier to enter the bloodstream, causing septicemia, and then further cross the blood-brain barrier, causing meningitis. The ability to interact with host cells plays a major role in the ability of *N. meningitidis* to establish a productive infection. Numerous bacterial attributes have been identified as factors that play a role in these interactions. Among these, the type IV pili play an essential role by allowing the initial adhesion of bacteria to host cells via the adhesin PilC1 (20, 22). The expression of PilC1 is upregulated during the initial interaction of the bacteria with the cells; this upregulation is required for complete adhesion of the bacterium (28). It has been proposed that regulation of PilC1 expression is controlled by a 150-bp sequence located upstream of PilC1. This 150-bp element was designated CREN, for contact regulatory element of *Neisseria* (28). Further analysis demonstrated that a 150-bp sequence very similar to that found upstream of pilC1 corresponds to a sequence repeat, designated Rep2, containing a ribosome binding site upstream of an ATG codon, which is the predicted start codon of a downstream open reading frame (ORF) (23). This full-length element is present 16 times in the *N. meningitidis* genome. Fourteen of 16 ORFs located downstream of Rep2 are upregulated during the initial contact of the bacterium with the cells in a manner similar to that of pilC1 (21), suggesting that these 14 Rep2-associated genes are coordinately upregulated in the initial interaction of *N. meningitidis* with host cells. Interestingly, one of the CREN/Rep2 elements lies upstream of a gene designated *crgA* (contact-regulated gene A) (7), which encodes a 299-amino-acid protein belonging to the LysR family of transcriptional regulators (14, 26, 32).

It has been reported that in the absence of epithelial cells, *crgA* is expressed at low levels from two transcription start sites, P1 and P2, which map upstream of and within the CREN/Rep2 element, respectively. RNA analyses led to the hypothesis that transcription starting at P2 is responsible for inducing *crgA* expression when *N. meningitidis* comes into contact with target cells (7). As a consequence, it has been proposed that the product of this gene, CrgA, represses the expression of several genes, including the pilC1, pilE, and *sia* genes involved in adhesin, pilin, and capsule biosynthesis, respectively. However, there are reasons to doubt aspects of the previous model. For example, the transcript starting at the P2 site poorly resembles the −24/−12 GG-N<sub>16</sub>GC consensus sequence characteristic of a sigma 54-dependent promoter (1). Furthermore, in *N. meningitidis* the sigma 54 factor encoded by the *rpoN* gene is inactive (17, 29), and in vitro the P2 promoter appears to be transcribed by the *Escherichia coli* RNA polymerase containing sigma 70, the housekeeping sigma factor (4). Although it has been suggested that the P1 and P2 transcriptional start sites of the *crgA* gene arise from two distinct promoters, no functional evidence has been provided yet, and the nature of the mapped 5′ ends of RNA remains unclear.

CrgA has been reported to function by binding to the pro-
motors of the crgA and pilC1 genes (both harboring a CREN/Rep2 element), as well as the pilE and sia genes (both devoid of a CREN/Rep2 element), repressing transcription upon adhesion of bacteria to target epithelial cells (5). This suggested that, independent of the CREN/Rep2 element, CrgA regulates all four promoters by a similar mechanism (3, 5–7), possibly by binding to a T-N11- A motif characteristic of other LysR regulators (9).

In this paper, we report functional identification of the crgA gene promoter and provide evidence that the CREN/Rep2 repeat element is not involved in initiation of gene transcription. We show that CrgA is a regulatory protein controlling transcription both as a repressor and as an activator of overlapping and divergent promoters. Furthermore, activation and repression of transcription controlled by CrgA are enhanced by the addition of an inducer of NADPH-quinone oxidoreductase to N. meningitidis cells. It is likely that this inducer, or a similar inducer, activates the CrgA protein to control the expression of sets of genes; however, transcription of pilus and capsule genes appeared to be unaltered irrespective of CrgA and α-methylene-γ-butyrolactone (MBL).

### MATERIALS AND METHODS

**Bacterial strains and culture conditions.** The bacterial strains used in this study are listed in Table 1. The N. meningitidis strains are MC58 derivatives (29) and were routinely cultured in GC-based (Difco) agar supplemented with Kellogg’s supplement I (15) at 37°C in a 5% CO2–95% air atmosphere at 95% humidity. Strains were stocked in 10% skim milk and stored at –80°C. For liquid cultures, N. meningitidis strains were grown overnight on solid medium, resuspended in phosphate-buffered saline (PBS) to an optical density at 600 nm of 1, and inoculated at a 1:20 dilution into GC broth supplemented with Kellogg’s supplement I, 12.5 μM Fe(NO3)3, and, when required, erythromycin, kanamycin, and/or chloramphenicol added at final concentrations of 5, 100, and 5 μg/ml,

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<tr>
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<td>Plasmid containing the ermAM erythromycin resistance gene</td>
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<td>Plasmid containing the chloramphenicol resistance cassette from Campylobacter coli</td>
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<td>pILL600</td>
<td>Plasmid containing the kanamycin resistance cassette from Campylobacter coli</td>
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<td>pMMB206</td>
<td>Plasmid containing the P Puc promoter and the lacI gene</td>
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<tr>
<td>pSL-Fla-Ery</td>
<td>Plasmid consisting of a promoterless lacZ gene and the ermAM erythromycin resistance gene flanked by upstream and downstream regions for allelic replacement at a chromosomal location between ORFs NMB1074 and NMB1075</td>
<td>This study</td>
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respectively. For transformation by naturally competent \textit{N. meningitidis}, four or
five single colonies of a freshly grown overnight culture were resuspended in 20 
μl of PBS, spotted onto GC agar plates to which 5 to 10 μg of linearized plasmid DNA was added, allowed to dry, and incubated for 6 to 8 h at 37°C. Transformants were then selected on plates containing erythromycin (5 
μg/ml), and single colonies were
restreaked on selective media for further analysis. Single colonies were resus-
pended in 50 
μl of distilled water, placed in a boiling water bath for 5 min, and
centrifuged in a bench top centrifuge for 5 min at 8,000 ×g.

**DNA techniques.** DNA manipulations were carried out routinely as described
by Sambrook et al. (25). Small-scale plasmid DNA preparation and large-scale plasmid DNA preparation were carried out with a QiAprep Spin mini kit and a
plasmid midi kit (QIAGEN, Inc.) used according to the manufacturer’s instruc-
tions. DNA fragments or PCR-amplified products were purified from agarose
gels with a QiaEX DNA purification kit (QIAGEN, Inc.). Each PCR was per-
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\( ^{a} \) Uppercase letters in Roman type indicate \textit{N. meningitidis}-derived sequences, italicized uppercase letters indicate \textit{E. coli}-derived sequences, lowercase letters indicate sequences added for cloning purposes, and underlined letters indicate recognition sites.

\( ^{b} \) Restriction enzyme sites added for cloning purposes.
Δ35 (MC-P<sub>crgA</sub>2), Δ10 (MC-P<sub>crgA</sub>5), ΔP (MC-P<sub>crgA</sub>4), and ΔR (MC-P<sub>crgA</sub>10), respectively (Table 1).

Construction of the crgA mutant of N. meningitidis and complementation. To construct a crgA deletion mutant, the crgA gene was replaced with a kanamycin cassette by double crossing over. To do this, plasmid pG3::Km was generated as follows. Upstream and downstream flanking regions of crgA were amplified from the MCS8 chromosome with primer pairs crgA-L2−crpA-R2 and crpA−L−crpA-R and cloned as 428-bp PstI/BamHI and 489-bp BamHI/EcoRI fragments into pGem3Z, respectively; a kanamycin cassette from plasmid pILL600 was cloned into the BamHI site between the two flanking regions. This plasmid was used to transform N. meningitidis NMB1856, and crgA was verified by PCR. The selected strain was named MC58.

To amplify the chloramphenicol resistance cassette was added to this construct, generating a 500-bp XmaI/NsiI fragment downstream of NMB1428 and a 430-bp BamHI/SpeI fragment upstream of NMB1429 were obtained by PCR amplification with the primer Com1 and Com4, respectively. These fragments are consecutive fragments that have overlapping ends bearing the BamHI and NsiI restriction sites for cloning purposes. These two fragments were used as templates to amplify an NADPH-quinone oxidoreductase (192 amino acids). The arrows indicate the direction of transcription. Underlined boldface letters indicate +1 promoter consensus sequences. The Rep2/CREN DNA sequence is enclosed in a box. The dotted lines indicate the CrgA binding site according to Deghmane et al. (3). SD, Shine-Dalgarno sequence.

Western blot analysis. To prepare sera against CrgA, 20 μg of recombinant CrgA protein obtained under denaturing conditions was used to immunize 6-week-old CD1 female mice (Charles River Laboratories) according the procedure described by Pizza et al. (24). One milliliter of a single culture in the exponential growth phase was harvested by centrifugation at 8,000 × g and resuspended in 100 μl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer, and 5 μl of each total protein sample was fractionated on a 10% polyacrylamide gel.
RESULTS

Intergenic region between the crgA and mdaB genes of N. meningitidis contains two overlapping and divergent promoters. The structure of the locus and the nucleotide sequence of the 238-bp intergenic region between the two divergent genes crgA and mdaB are shown in Fig. 1. This region contains a 138-bp DNA element, termed Rep2 or CREN (21, 28), directly upstream of the putative ATG start codon of the crgA gene. To define the start point of transcription of crgA and mdaB, we carried out an S1 nuclease protection assay and primer extension analysis for total RNA extracted from N. meningitidis.

Figure 2A shows the results of urea-acrylamide gel electrophoresis in an S1 nuclease protection experiment carried out by hybridizing Probe1 (Fig. 1A) to RNA of wild-type N. meningitidis. The results show a major S1-resistant band migrating at a position corresponding to 375 nucleotides, which defined the PcrgA start site of transcription (Fig. 2A, lane 2). Other faster-migrating bands may have been derived from the presence of weaker start points or from in vivo degradation of the RNA, and most of these 5′ ends of RNA mapped within the CREN/Rep2 region. Primer extension analysis (Fig. 2D, lane 5) confirmed the position of the PcrgA start site of transcription at a position 179 nucleotides from the ATG start codon of the crgA gene and minor 5′ ends of RNA mapping within the CREN/Rep2 element.

To define the start point of the mRNA encoded by the mdaB gene, we carried out primer extension of total RNA extracted from N. meningitidis. Figure 2B shows a unique extended product mapping at a position 24 nucleotides upstream of the ATG gene, indicating the position of the PmdaB start site of RNA transcription.

These results indicate that the intergenic region between the crgA and mdaB genes contains at least two promoters, which we call PcrgA and PmdaB. Analysis of the DNA sequence revealed the presence of −10-TATAAT and −35-ATGAA regions upstream of PcrgA and −10-CACAA and −35-TTA regions upstream of PmdaB. These sequences show conservation with the E. coli sigma 70 −10-TATAAT and −35-TTGACA recognized promoters and are likely to define the N. meningitidis PcrgA and PmdaB promoters (Fig. 1B). Analysis of the sequences upstream of the 5′ ends mapping within the CREN/Rep2 element revealed no sequence conservation with known promoter consensus sequences.

In order to obtain information on the nature of the 5′ ends of transcripts mapping downstream of the PcrgA promoter, we generated progressive deletions of the PcrgA promoter region fused to lacZ, introduced into the N. meningitidis MC58 genome by double recombination, and assayed for activity. As the beta-galactosidase activity values were very close to the background levels for all constructs, we decided to investigate transcription by primer extension analyses. Figure 2C shows a diagrammatic representation of the strains harboring the PcrgA deletion mutations. Total RNA was extracted from each mutant strain and used in primer extension analyses. Figure 2D shows that the PcrgA promoter was active in the strain carrying the full-length promoter construct (strain wtA) (lane 1), as well as in the strain carrying the deletion of the CREN/Rep2 region (strain ΔR) (lane 5). No extension products were detected with RNA extracted from strains Δ35, Δ10, and ΔP (lanes 2, 3, and 4), which harbored deletions from position −63 to position −15, from position −63 to position −6, and from position −63 to position 43 of PcrgA, respectively. Therefore, transcription of the region studied was abolished when deletions affected the −35 or −10 regions of the PcrgA promoter. Consequently, we concluded that the 5′ ends mapping within the CREN/Rep2 region are PcrgA dependent and that no promoters map within this region. These results were confirmed by primer extension of RNA extracted from E. coli transformed with plasmids carrying the crgA promoter mutations (data not shown). Furthermore, five independent beta-galactosidase experiments with the E. coli system gave Miller unit values of 61.5 ± 6.3, 68 ± 1.4, 9.8 ± 1.6, 5.4 ± 1.2, and 98.4 ± 3.3 for the wtA, Δ35, Δ10, ΔP, and ΔR constructs, respectively.

We concluded that the crgA gene is transcribed from the PcrgA promoter upstream of the Rep2/CREN element.

CrgA represses transcription from PcrgA and activates transcription from PmdaB. To establish the role of CrgA in transcription of the PcrgA and PmdaB promoters, we decided to assay transcription from these promoters in a crgA deletion mutant. To do this, we substituted the crgA gene with a kanamycin cassette in strain ΔR (Fig. 2C), which carried the PmdaB promoter fused to lacZ in a heterologous locus, generating strain ΔRcrgA (Table 1). Expression of CrgA in these strains was assessed by Western blot analysis of total protein extracts, whereas transcription from the PcrgA and the PmdaB promoters was assayed by primer extension analysis.

Figure 3A shows a Western blot of protein extracts from strains ΔR (CrgA+) and ΔRcrgA (CrgA−). As expected, in the wild-type background a protein band corresponding to CrgA was detected (lane 1), while this band was not detected in the crgA deletion mutant (lane 2).

Total RNA was extracted from these strains, and primer extension was carried out with primers hybridizing to RNA synthesized from the PcrgA and PmdaB promoters in the heterologous genomic location. Figure 3B shows that transcription from the PmdaB promoter was increased in the CrgA mutant (lane 2) compared to RNA extracted from the wild-type background (lane 1). Surprisingly, the amount of transcripts at the PmdaB promoter was strongly reduced and transcripts were undetectable in the CrgA mutant (lane 2) compared to the wild-type background (lane 1). As a control, primer extension was carried out with a primer complementary to the adenylyl kinase gene (adk), and this experiment showed no variation in the amount of mRNA (lanes 1 and 2). These results indicate that CrgA represses transcription from the PcrgA promoter and activates transcription from the PmdaB Promoter.

Complementation of the crgA mutation. To complement the crgA mutation and to obtain a better understanding of the regulation of transcription by CrgA, we constructed a strain expressing the crgA gene under the control of an inducible promoter to monitor accumulation of the CrgA protein and
correlate it to regulation of transcription. In this strain, ARcrgA-C (Table 1), the expression of the crgA gene was inducible by addition of IPTG, as its transcription was under the control of the P\textsubscript{tac} promoter and the LacI repressor. Cells were grown in liquid cultures in the presence of increasing amounts of IPTG to the mid-log phase, and aliquots of each sample were collected and used to prepare total protein extracts and total RNA.

The Western blot in Fig. 4A shows that the CrgA protein was detected when cells were grown in the presence of 10\,\mu M IPTG (lane 2) and that the amount increased with increasing amounts of IPTG in the culture medium (lanes 3 to 4). Primer extension analysis showed that transcription from the P\textsubscript{crgA} promoter was repressed in cells grown in the presence of 30\,\mu M IPTG or in the presence of higher concentrations of IPTG.

FIG. 2. Mapping of the promoters in the crgA-mdaB intergenic region. (A) Identification of the crgA transcripts. The 441-bp DNA of Probe1 (Fig. 1A) was end labeled at one extremity, hybridized to N. meningitidis total RNA, and used for S1 nuclease digestion to map 5' ends of transcription products synthesized by the P\textsubscript{crgA} promoter. The G+\,A lane contained a G+\,A sequence reaction mixture for the DNA probe used as a size marker (18). A control sample was processed identically but contained no RNA (lane 1). Lane 2 contained 40 \mu g RNA. The nucleotide sequence of the coding strand upstream of the transcriptional start site is shown on the right, and the −10 promoter element is indicated by a vertical bar. The P\textsubscript{crgA} promoter in strain MC58 appears to be localized 10 bp upstream of the P1 promoter mapped by Deghmane and coworkers (7) in strain CS013. The nature of this discrepancy was not investigated. (B) Identification of mdaB transcript. Total RNA (30 \mu g) from N. meningitidis was hybridized to the end-labeled mdaB-PE oligonucleotide (Table 2) and elongated with reverse transcriptase to map 5' ends of transcription products synthesized by the P\textsubscript{mdaB} promoter (lane 1). Precise mapping was performed by sequencing the cloned region in plasmid pG3cr:Km (Table 1) with the same primer (lanes G, A, T, and C). DNA regions corresponding to promoter and Rep2/CREN elements are indicated on the left. (C) Schematic representation (not to scale) of the chromosomal promoter-Rep2 mutations fused to the lacZ gene and inserted between the NMB1074 and NMB1075 ORFs of the N. meningitidis genome. The strains are referred to by their short names; the full names are given in Table 1. (D) Detection of the transcript generated by the promoter-Rep2 mutations fused to lacZ. Lanes 1 to 5 contained 30 \mu g of total RNA extracted from strains wtA, Δ35, Δ10, ΔP, and ΔR, respectively, hybridized to the end-labeled LAC oligonucleotide (Table 2), and elongated with reverse transcriptase. Lanes 1 and 5 contained RNA synthesized by the P\textsubscript{crgA} promoter; no bands were detected in lanes 2 to 4. Similar band patterns have been obtained with RNA extracted from E. coli transformed with plasmids carrying the promoter mutations shown in panel C. Precise mapping was performed by sequencing the cloned region in plasmid pSL-Perg5 (Table 1) with primer LAC (lanes G, A, T, and C).

FIG. 3. (A) Western blot analysis with anti-CrgA antisera. Total cell lysates from strains ΔR and ΔRcrgA were used to detect CrgA. The asterisk marks a cross-reactive band. (B) Regulation of transcription of the P\textsubscript{crgA} and P\textsubscript{mdaB} promoters. Total RNA from strains ΔR (lanes 1) and ΔRcrgA (lanes 2) was hybridized to primers MDER-PE1 (upper panel) and LAC (middle panel) and elongated with reverse transcriptase. As a control (lower panel), RNA from strains ΔR and ΔRcrgA was elongated with primer adk-PE for adk mRNA.

FIG. 4. (A) Detection of the transcript generated by the promoter-Rep2 mutations fused to lacZ. Lanes 1 to 5 contained 30 \mu g of total RNA extracted from strains wtA, Δ35, Δ10, ΔP, and ΔR, respectively, hybridized to the end-labeled LAC oligonucleotide (Table 2), and elongated with reverse transcriptase. Lanes 1 and 5 contained RNA synthesized by the P\textsubscript{crgA} promoter; no bands were detected in lanes 2 to 4. Similar band patterns have been obtained with RNA extracted from E. coli transformed with plasmids carrying the promoter mutations shown in panel C. Precise mapping was performed by sequencing the cloned region in plasmid pSL-Perg5 (Table 1) with primer LAC (lanes G, A, T, and C).
Fig. 4B, compare lanes 3 and 4 with lanes 1 and 2). By contrast, at the same concentrations of IPTG the Pmtdb promoter was increasingly activated (Fig. 4C, lanes 1 to 4). In a control experiment, transcription from the adk promoter showed no variation in the amount of RNA in response to IPTG (data not shown). We concluded that CrgA controls transcription from the PcrcgA and Pmtdb promoters in a dose-dependent manner.

Activity of the CrgA regulator is induced by α-methylene-γ-butyrolactone. In E. coli, the mdaB gene encodes a protein with NADPH-specific quinone oxidoreductase activity that has been shown to be induced more than 25-fold by MBL (13). To investigate whether addition of this small molecule to N. meningitidis cells influences transcription of the mdaB gene in a CrgA-dependent manner and in turn transcription of the crcgA gene, we extracted RNA from the wild-type background (ΔR), the crcgA mutant (ΔRCrgA), and the complementing (ΔRCrgA-C) strains before treatment and after 10 min of treatment with 2 mM MBL. These RNA preparations were used in primer extension experiments to monitor accumulation of RNA synthesized from the PcrcgA and Pmtdb promoters, and the results are shown in Fig. 5.

Treatment of the ΔR strain (CrgA+) with MBL clearly resulted in an increase in the amount of transcript from the Pmtdb promoter (Fig. 5A, lanes 1 and 2). Independent of MBL addition, no extended products were detected in the CrgA mutant (lanes 3 and 4); however, the pattern of RNA accumulation in response to MBL was restored in the CrgA complemented strain (CrgA-C) (lanes 5 and 6). The same RNA preparations were then used in primer extension experiments to monitor transcription from the PcrcgA promoter. Figure 5B shows that in the wild-type background, the amount of transcript from the PcrcgA promoter was decreased upon MBL treatment (lanes 1 and 2) and was not influenced in the mutant (lanes 3 and 4) and that these changes could be complemented by CrgA (lanes 5 and 6).

From these results, we concluded that addition of MBL to growing cells activates transcription of the Pmtdb promoter and represses transcription from the PcrcgA promoter. Furthermore, these modulations of transcription are CrgA dependent.

CrgA and MBL have no effect on transcription of pilus and capsule genes. The results described above prompted us to investigate whether other CrgA-regulated promoters are similarly regulated. As it has been reported that CrgA regulates transcription of the pilC1, pilE, and sia genes (5), we selected these genes to study the CrgA-mediated MBL response. To do this, we carried out primer extensions of the pilE, sia, and pilC1 mRNA using the same RNA sample that was used for the experiment whose results are shown in Fig. 5, and the results are shown in Fig. 6.

Fig. 6A shows the extended products of the pilE mRNA, with a major band mapping 90 nucleotides upstream of the ATG start codon, which identified the PpilE start site of RNA transcription. This is in agreement with previous studies (2). Surprisingly, regardless of MBL addition to the cells the intensity of this band remained unchanged in the three strains used (lanes 1 to 6). Fig. 6B shows two extended products of the sia mRNA, with a major band identifying the PpilE start site of
used for the experiment shown in Fig. 5 and with primers pilE-PE1
and PilE-PE2 (C) to assess regulation of the PilC1, PilD, and PilC promoters, respectively. Sequencing reactions carried
out with each cloned promoter fragment served as size markers (lanes
G, A, T, and C). Independent of the strain and of MBL treatment,
major bands show no appreciable variation in the amount of elongated
products. Analyses of the DNA sequence upstream of the identified
major bands show no appreciable variation in the amount of elongated
G, A, T, and C). Independent of the strain and of MBL treatment,
out with each cloned promoter fragment served as size markers (lanes
panel B).

FIG. 6. Mapping and regulation of the pilE, sia, and pilC1 promoters. Primer extension analyses were performed with the same RNA
transcription 106 nucleotides upstream of the ATG start codon. This corresponds to a previously identified promoter
(27), which showed no variation in the amount of RNA among strains or upon MBL treatment (lanes 1 to 6). Figure 6C shows
the extended products of the PilC1 mRNA. The slowly migrating
band mapped 255 nucleotides upstream of the ATG start
codon of the gene and corresponded to the PilC1 start site of
RNA transcription (28). This band and the faster-migrating
bands had similar intensities in RNA extracted from the three
strains and from cells treated or not treated with MBL (lanes
1 to 6). These results indicate that transcription from the PilC1,
pilE, and sia promoters is regulated neither by CrgA nor by
MBL addition.

It is worth noting that the 5’ ends mapping downstream of the
P PilC1 promoter are located in the CREN/Rep2 region of
the PilC1 gene; thus, analogous to results obtained for the P
PilA promoter (Fig. 2), these 5’ ends of RNA and those mapping
downstream of the PilE promoter may arise from in vivo pro-
cessing of longer mRNAs.

DISCUSSION

The CrgA protein of N. meningitidis is a LysR-type transcrip-
tional regulator (29), which is upregulated during the initial
phase of adhesion of the bacterium to the target cells, and this
protein was proposed to be a repressor controlling expression
of a set of genes during bacterial adhesion to epithelial cells
(7). The gene encoding the CrgA regulator, crgA, is preceded
by a CREN/Rep2 repetitive DNA element and maps diver-
gently with respect to another gene, the mdaB gene coding for
an NADPH-quinone reductase.

In this study, we identified a single promoter, P crgA-1, which
is responsible for transcription of the crgA gene. We carried out
deletion and 5’ end mapping analyses, which showed that the
5’ ends of transcripts mapping within the CREN/Rep2 region
depend on transcription from the upstream P PilC1 promoter (Fig.
2). Furthermore, the P mdaB promoter of the upstream
gene is divergently oriented and overlaps the P PilC1 promoter
(Fig. 1 and 2). This promoter architecture is compatible with
coordinated regulation of transcription of the crgA and mdaB
genes. Accordingly, the amounts of transcripts synthesized
from the P PilC1 and P mdaB promoters are increased and de-
creased in a crgA knockout background, respectively (Fig. 3).
Furthermore, repression of the P PilC1 promoter and activation
of the P mdaB promoter are restored in a complementing strain
(Fig. 4). In addition, the degree of complementation of the
P PilC1 and P mdaB transcriptional regulation correlates well with
the intracellular amount of CrgA (Fig. 4). Thus, the autoreg-
ulatory mechanism of gene transcription primarily controls the
intracellular concentration of CrgA, a condition used by many
regulatory proteins to modify their activities in response to
environmental changes. In addition, CrgA activates the diver-
gently oriented upstream gene, another typical feature of
LysR-type regulators. Interestingly, two CrgA binding sites
have been mapped within this region (4, 5). One CrgA binding
site spans positions −16 to 13 of the PilC1 promoter and this
region corresponds to positions −20 to −49 of the P mdaB
promoter. Another CrgA binding site spans positions 17 to 46
of the PilC1 promoter that correspond to positions −53 to −82
of the P mdaB promoter (Fig. 1B). Therefore, the two CrgA

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binding sites are located close to the transcriptional start site of the P_crgA promoter and upstream of the P_mdaB promoter, respectively. These DNA binding positions could be compatible with a mechanism of repression and activation of transcription of the P_crgA and P_mdaB promoters, respectively.

It has been proposed that a second promoter, termed P2, controls expression of the crgA gene mapping within the Rep2/CREN element (7). We have no evidence of promoters mapping within this region. The possibility that this element is responsible for posttranscriptional regulation of crgA within this region. The possibility that this element is responsible for posttranscriptional regulation of crgA is enhanced by the presence of MBL. As crgA is upregulated during the initial phase of adhesion (5), it would be interesting to understand the role of MBL or a similar inducer in the coordination of CrgA-regulated genes during infection.

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