Differential Expression of Genes That Harbor a Common Regulatory Element in *Neisseria meningitidis* upon Contact with Target Cells

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The expression of several genes in *Neisseria meningitidis* upon contact with epithelial cells was associated with the presence of the contact regulatory elements of *Neisseria*. These genes are involved in various aspects of meningococcal biology and could be coordinately regulated upon contact with target cells.

*Neisseria meningitidis* is a gram-negative bacterium of the genus *Neisseria*. It is an occasional pathogen that provokes septicemia, meningitis, and arthritis in humans. Bacterium-host interaction may require pleiotropic regulatory systems that could act through cis-regulatory promoter elements to coordinate the expression of several genes. Contact regulatory element of *Neisseria* (CREN) is a 150-bp sequence specific for pathogenic *Neisseria* species (*N. meningitidis* and *N. gonorrhoeae*). It was first described in the promoter regions of pilC1 and crgA genes immediately upstream of the ribosome-binding site (4). CREN is involved in the induction of the transcription of pilC1 and crgA genes upon contact with the target cells. This induction is necessary for optimal adhesion of *N. meningitidis* to cells (3, 9). CREN harbors a transcriptional regulator that is involved in the switch of bacterial adhesion to intimate adhesion through downregulation of capsule, pilC1, and pili (3, 4). Binding of CrgA to target promoters is independent from the presence of the CREN element. Indeed CrgA-binding sites are located upstream of the CREN element (in pilC1). CrgA also binds to promoters of the pilE and sia genes that are devoid of CREN elements (3, 4). A probe corresponding to CREN of pilC1 of the strain 8013 was able to hybridize to several chromosomal loci in several strains of *N. meningitidis* from different genetic lineages (4). Moreover, several copies of CREN homologs are present in the published complete genomes of two meningococcal strains (7, 11). We aimed in this work to explore the involvement of new CREN-like elements in the regulation of gene expression upon contact with target cells.

In silico analysis of strain MC58 (serogroup B) (http://www.tigr.org) revealed that different loci showed homology to CREN of pilC1. The sequence identity ranged between 58.5 and 97.4% (Fig. 1). Similar observations were made with the strain Z2491 (serogroup A) (http://www.sanger.ac.uk). CREN corresponds to the repeat element (Rep2) that was described on the complete genomic sequence of *N. meningitidis* (7). We selected 12 loci on the basis of the presence of an open reading frame (ORF) downstream of these CREN homologs in the strain MC58 (Table 1). All CREN elements from these loci showed a GG-N8-(A/G)C-related motif in comparison to pilC1 and crgA (Fig. 1).

Two of the 12 selected loci corresponded to two known genes in *N. meningitidis*. The first gene was pilC1 (NMB1847), and the second gene was crgA (NMB1856) (Table 1). As for the other 10 loci, 1 (NMB0776) corresponded to the gly1 gene that has been identified in *N. gonorrhoeae* (1). Another locus (NMB2132) showed homology to transferrin-binding proteins. Four other loci showed homologies to known genes in other bacterial species, and four loci showed no homology to the proteins in the sequence data bank (8, 12, 13) (Table 1).

We studied the conservation of these genes among 10 different strains of *N. meningitidis* from different genetic lineages and serogroups. Oligonucleotides were designed to amplify entirely each ORF from the MC58 strain (Table 2). Forward oligonucleotides (coding strand) start at the ATG codon, while reverse oligonucleotides (noncoding strand) start immediately upstream of the stop codon of each ORF. PCR amplification using these oligonucleotides showed that these genes were present in all tested meningococcal strains (Table 1). For one locus corresponding to NMB0607 in the MC58 strain, no amplification was obtained from strain 8013, but Southern hybridization using the entire PCR product corresponding to the NMB0607 ORF from the strain MC58 as a probe confirmed the presence of this locus in strain 8013 (data not shown; Table 1). The absence of PCR amplification may be due to the DNA polymorphisms between strains MC58 and 8013 in the sequences binding to the oligonucleotides. The same analysis revealed that the presence of these genes was variable among six strains belonging to six different species of nonpathogenic *Neisseria* (Table 1). Next, we tested whether genes from these different meningococcal strains harbored the CREN element in their promoter regions. Oligonucleotides were designed to amplify the promoter regions of genes from the MC58 strain except for NMB0607. Oligonucleotides at the 5′ side were upstream from each CREN element, whereas the oligonucleotides at the 3′ side were designed immediately downstream from the start codon of each ORF (Table 2). The expected size of PCR products corresponding to different loci was 400 bp. All tested strains revealed PCR fragments identical in size to...
the fragments from the strain MC58 for the loci corresponding to NMB1047, NMB1136, NMB1347, NMB1508, and NMB1664 as well as crgA and pilC1. DNA sequencing confirmed the presence of CREN elements in these loci in strain 8013. For the locus NMB0776, PCR-amplified fragments of a larger size than that of the strain MC58 was obtained for few strains (550 and 400 bp, respectively) (Table 1). DNA hybridization using the CREN element of pilC1 as a probe confirmed the presence of CREN homologs in these strains. Moreover, DNA sequencing of the PCR fragment obtained from strain 8013 verified the

![Conserved motif: GG doublet](image)

FIG. 1. Alignment of nucleotide sequences of CREN elements of the 12 genes identified in this work. The start codons of the ORFs and the Shine-Dalgarno sequence (SD) are indicated. The conserved GG doublet is also shown. Black background indicates conserved bases.

<table>
<thead>
<tr>
<th>Locus in MC58 (%) identity</th>
<th>Homology</th>
<th>Predicted size (amino acids) of protein</th>
<th>Presence of ORF in:</th>
<th>Presence of CREN in N. meningitidis strains</th>
<th>Induction of expression in 8013 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMB0607 58.5</td>
<td>secD protein, export membrane protein</td>
<td>618</td>
<td>+</td>
<td>+</td>
<td>Not determined</td>
</tr>
<tr>
<td>NMB0697 66</td>
<td>ksgA dimethyl adenosine transferase involved in protein synthesis</td>
<td>259</td>
<td>+</td>
<td>–</td>
<td>Not determined</td>
</tr>
<tr>
<td>NMB0776 73.1</td>
<td>gly1 of N. gonorrhoeae; effect on cytotoxicity</td>
<td>139</td>
<td>+</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;, +&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>NMB0783 60</td>
<td>No homology</td>
<td>159</td>
<td>+</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;, +&lt;sup&gt;f&lt;/sup&gt;</td>
<td>–&lt;sup&gt;S&lt;/sup&gt;</td>
</tr>
<tr>
<td>NMB1047 59.9</td>
<td>No homology</td>
<td>123</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NMB1136 61</td>
<td>No homology</td>
<td>184</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NMB1347 68.5</td>
<td>suhB extragenic suppressor protein</td>
<td>264</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NMB1508 77.1</td>
<td>No homology</td>
<td>472</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NMB1664 68.4</td>
<td>Peptidase family U32</td>
<td>451&lt;sup&gt;i&lt;/sup&gt;</td>
<td>+</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
</tr>
<tr>
<td>NMB1847 97.4</td>
<td>pilC1; adhesion and pilus assembly</td>
<td>1,028</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NMB1856 68.5</td>
<td>crgA; regulation of adhesion</td>
<td>299&lt;sup&gt;f&lt;/sup&gt;</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NMB2132 74.8</td>
<td>Related to transferrin-binding protein</td>
<td>493</td>
<td>+</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;, +&lt;sup&gt;e&lt;/sup&gt;</td>
<td>–&lt;sup&gt;S&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of identity to the CREN element of pilC1 gene from the 8013 strain.

<sup>b</sup> Strains of N. meningitidis tested were LNP10817 (serogroup A); LNP16645, LNP16646, LNP14912, and MC58 (serogroup B); LNP18012, 290/94, and 8013 (serogroup C), and LNP17617 and LNP18399 (serogroup W135).

<sup>c</sup> Nonpathogenic Neisseria strains were N. lactamica LNP411, N. denitrificans LNP412, N. animalis LNP413, N. flavescens LNP414, N. flavua LNP3264, and N. sicca LNP3265.

<sup>d</sup> ORF was absent from some strains belonging to nonpathogenic Neisseria species (N. denitrificans strain LNP412, N. animalis strain LNP413, and N. flavua strain LNP 3264).

<sup>e</sup> The CREN element was of a higher size in strains LNP18012, 290/94, LNP17617, and LNP18399.

<sup>f</sup> The CREN element was only present in strains LNP16645, LNP16646, LNP14912, and MC58 (serogroup B).

<sup>g</sup> The CREN element was only present in strains LNP14912 and MC58 (serogroup B, ET-5 clonal complex).

<sup>h</sup> Absence of CREN in 8013 strain.

<sup>i</sup> Signal sequence.
PCR fragments obtained from strain 8013 further con-
other genetic lineages showed a smaller promoter region than
However, homologs to the locus NMB2132 (but not to the
than that from the MC58 strain (250 and 400 bp, respectively).
 group B and belong to the ET-5 clonal complex) were smaller
NMB0783 and NMB2132, PCR fragments from all the strains
presence of CREN elements. For two loci corresponding to
NMB0783 and NMB2132, PCR fragments from all the strains
(except for strains LNP14912 and MC58, which are of sero-
group B and belong to the ET-5 clonal complex) were smaller
than that from the MC58 strain (250 and 400 bp, respectively).
However, homologs to the locus NMB2132 (but not to the
locus NMB0783) from other serogroup B strains belonging to
other genetic lineages showed a smaller promoter region than
that of MC58 and LNP14912 strains. DNA sequencing of the
PCR fragments obtained from strain 8013 further confirmed
the absence of CREN elements in the two loci corresponding
to NMB0783 and NMB2132 (Table 1). We next monitored the
expression of the genes identified in this work during bacterial
adhesion to target cells. Hec-1-B epithelial cells were infected
by strain 8013 or MC58 as previously described (4). Total RNA
was prepared from the same number of bacteria for both
strains from cell-associated bacteria and bacteria grown alone
in cell culture medium in the absence of Hec-1-B cells. Bacte-
ria were counted under microscopy using a Petroff-Hauser
counting chamber (Touzart & Matignon). Reverse transcrip-
tase PCR (RT-PCR) analysis was performed as previously
described (3). In order to prevent the RT-PCR from being
saturated, we first standardized the assays using the abundantly
expressed porA gene that lacks a CREN element and that is
not induced. RT-PCR was performed using 1, 2, 5, 7, and 10 μg
of total RNA. The increase of the signal was linear up to 5 μg
(data not shown). RT-PCR assays were subsequently per-
formed using 5 μg of total RNA. After 1 h of adhesion, the
expression of loci corresponding to NMB0697, NMB0776,
NMB1047, NMB1136, NMB1347, NMB1664, and NMB1508
was higher in cell-associated bacteria (Fig. 2). However, no
induction of the transcription was detected in the strain 8013
for the genes corresponding to NMB0783 and NMB2132 (de-
void of CREN element in their promoter regions in strain
8013). By contrast, these two genes were induced in the MC58
strain (Fig. 2). These results reinforce the association between
the CREN element and the induction of the transcription upon
contact with target cells.

### Table 2. Oligonucleotides used in this study

<table>
<thead>
<tr>
<th>ORF purpose and name</th>
<th>Sequence Relevant characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF amplification</td>
<td>Coding strand</td>
</tr>
<tr>
<td>0697-1</td>
<td>5'-ATGATGGACCCGTATCCCTTTATGAAAA-3' Coding strand</td>
</tr>
<tr>
<td>0697-2</td>
<td>5'-CTGAAAGAAACCAAAAGGGCGGCGG-3' Noncoding strand</td>
</tr>
<tr>
<td>0697-3</td>
<td>5'-GACGATCCCTGGCCGAGATA-3' Coding strand</td>
</tr>
<tr>
<td>0776-1</td>
<td>5'-ATGAAAAACTTTTCCATGCGCC-3' Coding strand</td>
</tr>
<tr>
<td>0776-2</td>
<td>5'-GAAAATATCGTATTTCCCGCAGGAA-3' Noncoding strand</td>
</tr>
<tr>
<td>0776-3</td>
<td>5'-TGAAAACAGCCGACCTAATTTG-3' Coding strand</td>
</tr>
<tr>
<td>0776-4</td>
<td>5'-GGTTTCAACGCCGCTGAGAA-3' Noncoding strand</td>
</tr>
<tr>
<td>2132-2</td>
<td>5'-GATTAAACACCTTGGTTTGCCGGG-3' Coding strand</td>
</tr>
<tr>
<td>2132-3</td>
<td>5'-GGTTTCAACGCCGCTGAGAA-3' Noncoding strand</td>
</tr>
<tr>
<td>porA0</td>
<td>5'-GATGTCACCGTCACCGGGAATCAA-3' Coding strand</td>
</tr>
<tr>
<td>porA101</td>
<td>5'-GCCGATAAACAGACGAAATCC-3' Noncoding strand</td>
</tr>
<tr>
<td>CREN element amplification</td>
<td>Coding strand</td>
</tr>
<tr>
<td>0697-3</td>
<td>5'-CTGCCGGCAAACCCGCGCCCGGCACGAA-3' Coding strand</td>
</tr>
<tr>
<td>0697-4</td>
<td>5'-CTGCCGGCAAACCCGCGCCCGGCACGAA-3' Noncoding strand</td>
</tr>
<tr>
<td>0776-3</td>
<td>5'-CTGCCGGCAAACCCGCGCCCGGCACGAA-3' Coding strand</td>
</tr>
<tr>
<td>0776-4</td>
<td>5'-CTGCCGGCAAACCCGCGCCCGGCACGAA-3' Noncoding strand</td>
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<tr>
<td>2132-2</td>
<td>5'-GATTAAACACCTTGGTTTGCCGGG-3' Coding strand</td>
</tr>
<tr>
<td>2132-3</td>
<td>5'-GATGGATATATGTTTGCCG-3' Noncoding strand</td>
</tr>
<tr>
<td>porA0</td>
<td>5'-GCCGATAAACAGACGAAATCC-3' Noncoding strand</td>
</tr>
</tbody>
</table>
The presence of the CREN element for the NMB2132 locus only in strains belonging to the clonal complex ET-5 needs to be confirmed in a larger collection of strains. However, it may be responsible for a physiological polymorphism caused by a differential gene expression pattern upon contact with target cells. Such a polymorphism may provoke a distinct behavior for these strains that are thought to be virulent but with low transmissibility (2). The initial events occurring upon contact of \textit{N. meningitidis} to target cells at the nasopharynx appear to be crucial in establishing effective colonization (and subsequent invasion of internal compartments) or shedding and dissemination to other hosts (10).

CREN elements seem to control various genes (contact regulon) that are involved in several aspects of the meningococcal biology (metabolism, adhesion, and transcriptional regulation). The molecular signals recognized by \textit{N. meningitidis} upon contact with target cells and the regulatory proteins involved in signaling pathways have not yet been identified. Other contact-regulated genes have been recently reported using a DNA microarray technique, suggesting that several contact-inducing mechanisms may exist (5). Their interference with CREN-associated genes remains to be analyzed. The pattern of coordinate expression of bacterial genes harboring CREN elements may permit an optimal physiological status enabling either colonization or transmission between hosts.

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REFERENCES


