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A cell-contact-regulated operon is involved in genetic variability in *Neisseria meningitidis*

Anne Jamet [a,*], Eric Frapy [b], Dominique Schneider [c,d], Xavier Nassif [a,b,e], Patricia Martin [f]

[a] *Institut National de la Santé et de la Recherche Médicale, U1002, F-75015 Paris, France*
[b] *Université Paris Descartes, Faculté de Médecine René Descartes, F-75006 Paris, France*
[c] *Institut Jean Roget, Université Joseph Fourier, F-38042 Grenoble, France*
[d] *CNRS UMR5163, F-38042 Grenoble, France*
[e] *Assistance Publique–Hôpitaux de Paris, Hôpital Necker–Enfants Malades, F-75015 Paris, France*
[f] *Institut National de la Recherche Agronomique, U1043, F-31076 Toulouse, France*

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Abstract

The ability of *Neisseria meningitidis* to establish efficient interaction with host cells is crucial for its survival. We recently demonstrated that an entire operon containing genes *NMA1802* to *NMA1806* was overexpressed during the early stage of the colonization process. In this work, we investigated whether upregulation of the expression of this operon facilitated the ability of *N. meningitidis* to adapt to growth on host cells. Using a strain displaying an inducible operon, we demonstrated that the *NMA1802-NMA1806* cell-contact-regulated operon could potentially improve the adaptability of meningococcus during growth on the cell surface through enhanced generation of variants.

Keywords: Meningococcus; Operon; Mutation

1. Introduction

Neisseria meningitidis asymptomatically colonizes the nasopharynx of 8–25% of the human population (Stephens et al., 2007) and can thus be considered part of the normal flora. Transmission of *N. meningitidis* occurs directly from person to person through aerosol inhalation. Since the bacterium cannot survive outside the human host, its ability to establish efficient interaction with host cells is crucial for its survival as a commensal organism. However, in a very small proportion of colonized individuals, via a yet unknown mechanism, *N. meningitidis* is responsible for life-threatening invasive infections. Indeed, the bacterium is able to cross the nasopharyngeal epithelium and enter the bloodstream. During bacteremia, *N. meningitidis* interacts with endothelial cells of brain microvessels and may cross the blood–brain barrier leading to meningitis (Coureuil et al., 2009). Therefore, during the process of the disease, efficient adhesion of *N. meningitidis* to host cells is also required.

Although adherence to host cells is necessary to both successfully colonize and survive within the human host, the molecular mechanisms responsible for efficient interaction between *N. meningitidis* and the host cell remain mostly unknown. To face selective pressure encountered within the host, *N. meningitidis* exhibits high genome plasticity and contains a large number of genes that undergo phase variation promoting genetic diversity (Davidsen and Tonjum, 2006). This phenotypic diversity could lead to enhanced fitness at a population level.

A group of 14 genes belonging to a regulon named “REP2” has been shown to be upregulated by contact with both endothelial and epithelial host cells (Morelle et al., 2003). The REP2 sequence, which is a repeated sequence of

* Corresponding author.
E-mail addresses: anne.jamet@inserm.fr (A. Jamet), eric.frapy@inserm.fr (E. Frapy), dominique.schneider@ujf-grenoble.fr (D. Schneider), xavier.nassif@inserm.fr (X. Nassif), p.martin-bizon@envt.fr (P. Martin).

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approximately 150 bp located upstream from the 14 genes, contains a promoter involved in cell-contact upregulation of the regulon (Taha et al., 1998). The REP2 regulon is likely to encode functions important for the adaptation of the bacterium during growth on the human cell surface. For instance, *pilC1* is essential for adhesion of *N. meningitidis* to human cells (Taha et al., 1998) and induction of *xseB*, another member of the regulon, has been shown to increase bacterial ability to repair DNA (Morelle et al., 2005).

We recently demonstrated that a whole operon containing genes *NMA1802* to *NMA1806* was part of the REP2 regulon (Jamet et al., 2010) and was induced upon contact with host cells. The operon comprises *NMA1802* encoding the A subunit of the topoisomerase IV (ParC), *NMA1803* encoding a non-functional two-component signal transduction system sensor, *NMA1805* encoding a regulatory protein and *NMA1806* encoding a protein that shares homology with S-adenosylmethionine-dependent methyltransferases.

In this work, using a meningococcal strain displaying an inducible operon, we sought to investigate whether over-expression of the entire operon, as it occurs during the colonization process, could play a role in the adaptation of *N. meningitidis* to growth on host cells. Indeed, we showed that cell-contact regulation of the expression of the operon could facilitate the adaptability of *N. meningitidis* through increased genetic variability (i.e. spontaneous mutations and phase variation).

2. Materials and methods

2.1. Bacterial strains

The meningococcal strain used in this study was Z5463. Sequenced strains Z2491 (Parkhill et al., 2000) and Z5463 were isolated during the same epidemic in The Gambia in 1983 (Achtman et al., 1988). Z5463 is a naturally transformable serogroup A strain belonging to the same sequence type as strain Z2491, i.e. ST-4, subgroup IV-1, expressing OpaA and OpaC. Contrary to strain Z2491, strain Z5463 is piliated and is therefore naturally transformable and able to adhere to cells. *Neisseria* was grown at 37 °C in 5% CO_2 on GC Medium Base (GCB) (Difco) or in GC liquid medium containing Kellog's supplement. *Escherichia coli* strain DH5α was used for DNA cloning and plasmid propagation. *E. coli* was grown on Luria–Bertani (LB) agar or broth. For antibiotic selection of *E. coli* strains, kanamycin (Km) was used at 30 μg ml^{-1} and erythromycin (Em) at 150 μg ml^{-1}. To select strains derived from *N. meningitidis* strain Z5463, the Km concentration was 200 μg ml^{-1}, spectinomycin (Sp) was used at 75 μg ml^{-1}, Em at 2 μg ml^{-1} and tetracycline (Tet) at 10 μg ml^{-1}.

2.2. Construction of strain Z5463 harboring the NMA1802-NMA1806 operon under control of an IPTG-inducible promoter

A *Not*I restriction site was inserted immediately upstream from the ribosome binding site of the *NMA1802* gene using the following oligonucleotides: NMA1801-up/NMA1801-*Not*I and NMA1802-*Not*I/NMA1802-XbaI (Table 1). The IPTG-inducible promoter was then introduced into this site. The pHSX-*ermC-lacIOP* plasmid (Seifert, 1997) was cleaved with *Not*I to release a 3.1 kb fragment containing *ermC*, *lacIq* and the tandem *lac* operator promoter sequences *tacOP* and *UV5OP*. This fragment containing the IPTG-inducible promoter was cloned into the *Not*I site engineered upstream of the *NMA1802* gene. The resulting IPTG-inducible *NMA1802-1806* operon was sequenced check the correct orientation of the promoter and introduced by transformation into strain Z5463. Transformants were selected using Em and 1 mM IPTG. It should be pointed out that the presence of IPTG in the plate was necessary to obtain transformants. Insertion of the IPTG-inducible promoter (P_{ind}) upstream of the *NMA1802* gene was confirmed by PCR using primers NMA1801-up and NMA1802-XbaI (Table 1).

2.3. Adhesion assays

Human umbilical vein endothelial cells (HUVECs obtained from PromoCell) were cultured and infected as already described (Morelle et al., 2003). Briefly, 2×10^8 bacteria in exponential growth phase were used to infect a monolayer of 5×10^5 HUVECs. Thirty min later, supernatant was removed and cells were washed with infection medium (IM), i.e. RPMI 1640 medium with Glutamax supplemented with 10% heat-inactivated fetal calf serum (PAA laboratories). Monolayers were then washed every hour and fresh medium was added. The bacteria adherent to monolayers were harvested at 1 and 4 h of adhesion.

For competition assays, HUVECs were infected with both 2×10^8 cells of strain Z5463 and 2×10^8 cells of strain Z5463P_{ind}*NMA1802-NMA1806* in the presence of two different concentrations of IPTG. Adherent bacteria were harvested after 1 and 4 h of adhesion and plated on GCB supplemented with kanamycin or erythromycin to allow quantification of strain Z5463 and Z5463P_{ind}*NMA1802-NMA1806*, respectively.

2.4. Total RNA isolation, real-time RT-PCR

Total RNA isolation and real-time RT-PCR were performed as previously described (Jamet et al., 2009) from bacteria grown in IM and harvested after 1 h and 4 h of adhesion to HUVECs. The *aphA3* gene that encodes kanamycin resistance, located in the intergenic region between *NMA1655* and *NMA1654* genes, was used as an internal reference. The level of transcription of *aphA3* and *NMA1802* genes was determined using pairs of primers RT-aphA3-3′/RT-aphA3-5′and RT-1802-Up/RT-1802-Down (Table 1).

2.5. Determination of spontaneous mutation frequencies

Bacteria from overnight culture on GCB plates or GCB plates supplemented with different concentrations of IPTG