migA, a quorum-responsive gene of *Pseudomonas aeruginosa*, is highly expressed in the cystic fibrosis lung environment and modifies low-molecular-mass lipopolysaccharide

Hongjiang Yang,¹ Mauricia Matewish,² Isabelle Loubens,³ Douglas G. Storey,³ Joseph S. Lam² and Shouguang Jin¹

Author for correspondence: Shouguang Jin. Tel: +1 352 392 8323. Fax: +1 352 392 3133. e-mail: sjin@mgm.ufl.edu

**Pseudomonas aeruginosa** is an opportunistic human pathogen which poses a major threat to patients with cystic fibrosis (CF). Excessive amounts of mucus present in the lungs of CF patients promotes the colonization of *P. aeruginosa*. The migA gene, encoding a putative glycosyltransferase, has been shown to be highly inducible by respiratory mucus derived from CF patients. In this study, it is further demonstrated by population transcript analysis that the migA gene is highly expressed in the CF lung environment. Deletion analysis of the migA promoter identified a las-box-like sequence commonly found in promoters that are responsive to quorum sensing regulation. Further analysis of migA expression in quorum-sensing-defective strains, as well as its expression in response to autoinducer molecules, demonstrated that migA is regulated by the RhlI/RhlR quorum sensing regulatory system. Functionally, as the MigA sequence homology data suggested, the migA gene indeed affects the structure of LPS in *P. aeruginosa*. Increased expression of the migA gene results in a loss of core-plus-one LPS, while having no obvious effect on the long-chain O-antigen-bearing LPS. Although the exact biological role of the core-plus-one LPS is not clear, these experimental results suggest that migA up-regulation in the CF lung environment is part of the adaptive response which confers on *P. aeruginosa* a survival advantage.

### Keywords
migA, mucus, LPS and quorum response

**INTRODUCTION**

*Pseudomonas aeruginosa* is an opportunistic pathogen which poses a major threat to immunocompromised patients, burn victims and cystic fibrosis (CF) patients (Bodey *et al*., 1983; Gilligan, 1991; Holder, 1993). More than 90% of mortality among CF patients is caused by lung infection with *P. aeruginosa*. The presence of excessive amounts of viscous mucus in the lungs of CF patients is thought to be a major contributing factor to their susceptibility to infection by *P. aeruginosa*. The mucus not only affects the host defence system by blocking the movement of alveolar macrophages and inhibiting cilia movement-associated bacterial clearance, but it also provides a unique growth environment, allowing *P. aeruginosa* to persist (Carnoy *et al*., 1993; Marshall & Carroll, 1991). The host immune response mounted against the infecting bacteria causes lung tissue damage and eventually leads to the failure of lung function (Gilligan, 1991).

As an opportunistic pathogen, *P. aeruginosa* relies on a combination of virulence factors to infect its host. Production and secretion of many of these virulence factors are increased at high cell density through a quorum sensing mechanism. Two such regulatory systems in *P. aeruginosa*, *las* and *rhl*, have been well characterized (Latifi *et al*., 1996, 1995; Pearson *et al*., 1994, 1995; Pesci *et al*., 1997; Pesci & Iglewski, 1997; Winson *et al*., 1995). Each is composed of two
components, an inducer locus, lasI or rhlI, that controls the synthesis of diffusible autoinducer molecule 3-oxo-dodecanoyl homoserine lactone (C12-HSL; PAI-1) or butanoyl homoserine lactone (C4-HSL; PAI-2), respectively, and as a response locus, lasR or rhlR, that encodes transcriptional factors which become active upon binding to the respective autoinducers (Passador et al., 1993). The PAI-1-bound form of LasR enhances the expression of genes encoding secreted proteases, siderophore, ADP-ribosylating enzymes and genes that control secretion of virulence factors (Passador et al. 1993; Seed et al., 1995; Toder et al., 1994, 1991; Winson et al., 1995). PAI-2-bound RhlR controls some genes affected by LasR and, in addition, activates the expression of loci responsible for the production of toxic chemicals, including hydrogen cyanide, rhomolipid and phenazines (Latif et al., 1996, 1995; Ochsner & Reiser, 1995; Ochsner et al., 1994, 1997).

Chronic CF lung infections by *P. aeruginosa* start from acute infections early in the life of CF patients. The chronic infection has interspersed episodes of acute lower respiratory tract symptoms. As the chronic stage of the infection develops, there is a gradual transition of the *P. aeruginosa* isolates from a predominately non-mucoid, smooth LPS, piliated and motile phenotype to a predominately mucoid, rough LPS, non-piliated and non-motile phenotype (Govan, 1988; Luzar & Montie, 1985; Mahenthiralingam et al., 1994; Hancock et al., 1983; Woods et al., 1986). These morphological changes reflect bacterial adaptation to the CF lung environment, resulting from changes in gene regulation as well as mutation and selection. The mechanism for conversion to the mucoid phenotype was shown to be due to mutations in the anti-σ factor gene, mucA, which alleviate repression of a 22 kDa σ factor encoded by algU (or algT) that is required for the expression of the alginate biosynthetic pathway genes, in combination with environmental signals that activate genes under the control of two transcriptional regulators, AlgR and AlgB (Deretic et al., 1995; Govan & Deretic, 1996; Ma et al., 1998). Alginate is the predominant exopolysaccharide moiety of the mucoid substance in CF isolates. In the case of pili and motility losses, mutation in the rpoN gene, which is required for pilus and flagellum assembly, accounts for the majority of the cases (Mahenthiralingam et al., 1994). However, the molecular mechanism for the conversion from smooth LPS to rough LPS is not as clear. *P. aeruginosa* LPS consists of distinct A and B bands; the high-molecular mass B-band determines the O-antigenic specificity of the bacterium, whereas the antigenically conserved A-band is a common antigen consisting of α-d-rhamnose trisaccharide repeating units. Wild-type *P. aeruginosa* strains express a proportion of their LPS as long-chain molecules, containing up to 50 repeating units (smooth LPS). In addition, these strains will have LPS that is devoid of O-polysaccharide (rough LPS) and LPS containing only one O chain repeating unit (core-plus-one LPS) (Rocchetta et al., 1999).

To understand the molecular mechanism of bacterial adaptation to the CF lung environment, we have previously isolated and characterized *P. aeruginosa* genes that are specifically inducible by respiratory mucus derived from CF patients (Wang et al., 1996). One of these encodes a putative glycosyltransferase which was predicted to be involved in LPS or exopolysaccharide modification. In this report, we show that the migA gene product indeed modifies bacterial LPS structure, predominantly the core-plus-one LPS. Furthermore, we demonstrate that the migA gene is regulated by the RhlR/RhlR quorum sensing regulatory system and is highly expressed in the CF lung environment. The significance of these observations is discussed.

**METHODS**

**Bacterial strains and plasmids.** Bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* and *P. aeruginosa* were grown in L-agar or L-broth at 37 °C. Minimal medium A (Min A; Davis & Mingioli, 1950) was also used for the growth of *P. aeruginosa*. Antibiotics were used at the following final concentrations (µg ml⁻¹): for *E. coli*, ampicillin, 100; spectinomycin, 50; streptomycin, 25; tetracycline, 20; for *P. aeruginosa*, carbenicillin, 150; spectinomycin, 200; streptomycin, 200; tetracycline, 100. *P. aeruginosa* isolates from soil, blood of infected individuals and CF sputa were designated as environmental, non-CF and CF isolates, respectively. Both CF and non-CF clinical isolates were obtained from the Medical Center of the University of Arkansas for Medical Sciences, USA.

To generate the migA insertional mutant, a 2 kb Ω fragment was inserted into the SalI site of the 5’ migA coding region in pMSB3A (Wang et al., 1996). The resulting plasmid, pMSB3AΩ, was linearized by *EcoR* digestion and electroporated into PAK as described previously (Jin et al. 1994). Colonies with resistance to spectinomycin/streptomycin were selected, followed by screening for sensitivity to carbenicillin. Chromosomaial double crossovers were further confirmed by Southern hybridization.

To generate migA promoter deletion constructs, either available restriction sites upstream of the migA promoter or EcoRI sites generated in desired positions by site-directed mutagenesis (Kunkel et al., 1987) were used in combination with the BamHI site in pMS18 (Wang et al., 1996). Isolated migA promoter fragments were fused to a promoterless lacZ by cloning into the EcoRI/BamHI sites of pDN19lacZ (Totten & Lory, 1990). To introduce an EcoRI site at positions —200, —89 and —38 relative to the ATG translational start codon of migA in pMS18, the following three oligonucleotides were used: migA-200, 5’-GGCTACCTGATCAATGTTCC-3’; migA-100, 5’-TACGCGGCGCCGCGATTTGGC-3’; and migA-50, 5’-GACGGGCCCCCTTGGAAATCTACCGGGGAGACG-3’.

**Sputum collection and extraction of total RNA.** Sputum samples from nine paediatric patients were utilized in this study. These patients were part of a larger cohort of patients currently being followed (Storey et al., 1992, 1997, 1998). The patients in this study all attend the Alberta Children’s Hospital Cystic Fibrosis Clinic, Canada. Voluntary consent was obtained from all patients and their guardians. Ethical approval of the study design was given by the Conjoint Research Ethics Board of the University of Calgary, Canada. All samples used in this study were collected from patients who were in the
Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Description</th>
<th>Source</th>
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<tr>
<td><strong>E. coli</strong></td>
<td></td>
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<tr>
<td>DH5α</td>
<td>F− 80lacZΔM15 endA1 recA1 bsdR17(m4134) supE44 thi-1 relA1 ΔlacZYA-argF gyrA96 deoR</td>
<td>Hanahan (1983)</td>
</tr>
<tr>
<td>CJ236</td>
<td>ung−/dut− mutant strain</td>
<td>Kunkel et al. (1987)</td>
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<tr>
<td><strong>P. aeruginosa</strong></td>
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<tr>
<td>PAK</td>
<td>Laboratory strain</td>
<td>David Bradley, Newfoundland, Canada</td>
</tr>
<tr>
<td>PAKmigA::Ω</td>
<td>migA insertion mutant of PAK</td>
<td>Belanger et al. (1999)</td>
</tr>
<tr>
<td>PAKwbpL::GmR</td>
<td>wbpl mutant strain of PAK, deficient in A-band and B-band LPS</td>
<td>This study</td>
</tr>
<tr>
<td>PAO1</td>
<td>Laboratory strain</td>
<td>Holloway et al. (1979)</td>
</tr>
<tr>
<td>PAO1algR::GmR</td>
<td>algR mutant derivative of PAO1</td>
<td>Wozniak &amp; Ohman (1994)</td>
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<tr>
<td>PDO100</td>
<td>rhl mutant of PAO1</td>
<td>Print &amp; Ohman (1995)</td>
</tr>
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<td>PAO-JP1</td>
<td>lasI mutant strain of PAO1</td>
<td>Passador et al. (1993)</td>
</tr>
<tr>
<td>PAO-JP2</td>
<td>last and rhl double mutant of PAO1</td>
<td>Pearson et al. (1997)</td>
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<td><strong>Plasmids</strong></td>
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<td>pDN18</td>
<td>Broad-host-range plasmid, IncP, Tc</td>
<td>Nunn et al. (1990)</td>
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<td>pS9610</td>
<td>migA gene clone under the control of lac promoter in pDN18</td>
<td>This study</td>
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<td>pDN19lacΩ</td>
<td>A promoterless lacZ transcriptional fusion vector; Sp+ Sm− Tc+</td>
<td>Totten et al. (1990)</td>
</tr>
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<td>pMS18</td>
<td>5′ migA clone in pTZ18R</td>
<td>Wang et al. (1996)</td>
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<td>pMSB3A</td>
<td>3 kb EcoRI fragment containing migA cloned into pTZ18R</td>
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<td>migA gene in pMSB3A disrupted by Ω insertion</td>
<td>This study</td>
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<td>pMS18lacΩ</td>
<td>migA::lacZ fusion in pDN19lacΩ</td>
<td>Wang et al. (1996)</td>
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<td>pS9717</td>
<td>migA::lacZ fusion in pDN19lacΩ</td>
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<td>pTS400</td>
<td>lasB−lacZ translational fusion construct</td>
<td>Passador et al. (1993)</td>
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<td>pIL17</td>
<td>migA gene of PA103 cloned into pUC18</td>
<td>This study</td>
</tr>
<tr>
<td>pCC27</td>
<td>algD-containing clone</td>
<td>Chitin &amp; Ohman (1996)</td>
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<tr>
<td>pPS1816</td>
<td>lasA-containing clone</td>
<td>Schad &amp; Iglewski (1988)</td>
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Moderate to severe disease categories. Also, the selected patients had acquired solely or predominantly P. aeruginosa infections.

Sputum from the CF patients was collected as described previously (Storey et al., 1998). RNA was extracted from the sputum samples, blotted onto Nylon membrane, hybridized and signal intensity was measured as described by Storey et al. (1992). A 613 bp internal HindII fragment of migA from pIL17 was used as a probe to measure migA-specific transcripts. A 700 bp internal EcoRI fragment of algD from plasmid pCC27 (Chitin & Ohman, 1990) and a 450 bp PstI fragment of lasA from pPS1816 (Schad & Iglewski, 1988) were used to examine algD-specific and lasA-specific transcripts, respectively.

**Primer extension.** Total bacterial RNA was isolated from strain PAK that was grown in minimal medium A with or without 10% respiratory mucus from CF patients. For primer extension experiments, an oligonucleotide, migA-down (5′-CTTCTTCCAGGTACTTTTCCGCGTTG-3′) was used as primer and according to the protocol described by Maniatis et al. (1982). The same primer was also used to sequence the migA promoter region to identify the transcription initiation site.

**LPS preparation, SDS-PAGE and Western blot analysis.** LPS was prepared from whole-cell lysates of P. aeruginosa by the method of Hitchcock & Brown (1983). LPS samples were run on standard discontinuous 12.5% glycine SDS-PAGE gels (Hancock & Carey, 1979) or by commercially prepared 16% Tricine SDS-PAGE gels (Novex). LPS was visualized by the silver-staining methods of Dubray & Bezdor (1982) for glycine SDS-PAGE and Tsai & Frasch (1982) for Tricine gels. For Western immunoblots, the procedure was essentially as...
Hybridized with the patients over a period of 3 years and blotted onto a isolated from 25 sputum samples obtained from nine CF.

In the samples that we selected, approximately 100- to 400-fold relative intensity (Fig. 1). In vivo the sole or the predominant pathogenic organism in the samples were collected from CF patients who had conducted population transcript analysis. Sputum lung environment of migA were measured at various cell densities.

RESULTS

migA of P. aeruginosa is highly expressed in the CF lung environment

The migA gene was identified by virtue of its inducibility in vitro by respiratory mucus derived from CF patients (Wang et al., 1996). To test if this gene is indeed expressed in vivo during infection of CF lungs, we conducted population transcript analysis. Sputum samples were collected from CF patients who had confirmed P. aeruginosa infections. Total RNA was isolated from 25 sputum samples obtained from nine CF patients over a period of 3 years and blotted onto a nitrocellulose membrane. When the membrane was hybridized with the migA probe, all 25 samples showed a hybridization signal above background levels of approximately 100- to 400-fold relative intensity (Fig. 1).

In the samples that we selected, P. aeruginosa was either the sole or the predominant pathogenic organism in the sample. Thus, hybridization of the migA probe is not likely to be due to hybridization of the probe to RNA from other species. This suggested that in the lungs of patients with CF, migA was commonly expressed. We have also probed these RNA samples with algD, encoding a key enzyme in the biosynthesis of alginate, which is known to hybridize strongly to these RNA samples (Storey et al., 1997). Our second probe, lasA, encoding an elastase enzyme, usually hybridizes at an intermediate level to these RNA samples (Storey et al., 1997, 1998). The hybridization signals with the migA probe appear to be at a level nearer to the algD probe, suggesting abundant transcript accumulation in the bacterial populations found in the sputa from CF patients (Fig. 1).

The data in Fig. 1 indicate that migA transcript levels positively correlate to that of algD. This suggests that these genes might be controlled by the same regulatory system. This possibility seems more likely when we consider the fact that both genes are possibly involved in polysaccharide biosynthesis or modification. To test this, we examined the effect of algR, a known regulator of algD, on migA expression. A migA::lacZ fusion construct, pMS19lacfl, was introduced into wild-type PAO1 and its algR mutant derivative, and the levels of β-galactosidase activity were compared. Results showed that mucus-mediated migA gene activation was not altered in the algR mutant background compared to the wild-type strain (data not shown), indicating that mucus-mediated expression of migA is independent of algR.

Deletion analysis of the migA promoter

To identify functional promoter elements of the migA gene, a series of promoter deletion constructs were generated using both existing restriction sites as well as sites generated by site-directed mutagenesis. The promoter deletion fragments were fused to the promoterless lacZ gene in pDN19lacQ and PAK cells harbouring the resulting constructs were tested for β-galactosidase activities with or without the addition of CF respiratory mucus. As shown in Fig. 2, promoter deletion up to –200 (relative to the putative ATG start codon) did not affect wild-type promoter activity but deletion to −89 totally abolished the inducibility by mucus. These results indicate that important migA promoter elements reside within the 111 bp region between −200 and −89.

The migA transcription initiation site was further determined. Based on the above promoter deletion analysis, an oligonucleotide that hybridizes downstream of the ATG start codon was used as a primer to conduct a primer extension experiment. Total RNA was prepared from the same number of PAK cells grown in MinA medium with or without 10% CF respiratory mucus. Under both growth conditions, a single transcription initiation site corresponding to the A at position −23 upstream of the ATG start codon was identified (Fig. 3). A higher intensity of signal was detected in the RNA sample prepared from cells grown in the presence of CF mucus, representing a higher transcript level. An identical size transcript was found in the lungs of patients with CF, migA was commonly expressed. We have also probed these RNA samples with algD, encoding a key enzyme in the biosynthesis of alginate, which is known to hybridize strongly to these RNA samples (Storey et al., 1997). Our second probe, lasA, encoding an elastase enzyme, usually hybridizes at an intermediate level to these RNA samples (Storey et al., 1997, 1998). The hybridization signals with the migA probe appear to be at a level nearer to the algD probe, suggesting abundant transcript accumulation in the bacterial populations found in the sputa from CF patients (Fig. 1).

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Regulation and function of migA gene

**Fig. 1.** Comparison of population transcript accumulation of migA (white bars), algD (hatched bars) and lasA (black bars) in *P. aeruginosa* cells chronically colonizing the lungs of CF patients. Total RNA was isolated from sputum samples of CF patients and blotted onto a nitrocellulose membrane. Relative intensities of radioactive signals after hybridization with individual probes are shown (see Methods).

![Graph showing comparison of population transcript accumulation of migA, algD, and lasA in P. aeruginosa cells chronically colonizing the lungs of CF patients.](image)

**Fig. 2.** Promoter deletion analysis of the migA gene. DNA fragments containing various lengths of the migA promoter region were fused with the promoterless lacZ reporter gene and introduced into the wild-type PAK strain. β-Galactosidase activities (Miller units) were measured after overnight growth in MinA with (+) or without (−) 10% CF respiratory mucus. B, BamHI; K, KpnI; R, EcoRI site introduced by site-directed mutagenesis (see Fig. 3 for sequence). A chloramphenicol acetyltransferase homologue (cat) and two exotoxin A regulatory genes (regA and regB) reside upstream of migA.
H. YANG and OTHERS (EcoRI in pSJ9729) las-box-1 las-box-2 (EcoRI in pSJ9728) (EcoRI in pSJ9727) (Translation start) (a) – AC GTG GGC CGC GGT TTT TTT TTT CTT C TTG C GT C GT C G T G A C (b)............................................................................................................................... ..................

**Fig. 3.** Determination of the transcription initiation site of the migA gene by primer extension. (a) DNA sequence of the migA promoter region. EcoRI sites introduced by site-directed mutagenesis which were used to construct plasmids pSJ9727, pSJ9728 and pSJ9729 are shown. Lower case letters represent mismatch mutations introduced to generate EcoRI sites. The transcription initiation site 'A' is shown in bold and underlined. (b) Autoradiogram of primer extension experiment. Lanes marked with – and + contain RNA samples prepared from PAK grown in the absence or presence, respectively, of 10% CF mucus. Equal amounts of the two RNA samples were used. DNA sequencing termination reactions for A, C, G and T are marked. The arrow indicates the extended primer and the DNA sequence of the anti-sense strand is shown next to it.

**Fig. 4.** RhlI/RhlR-mediated activation of migA::lacZ expression. (a) Cell-density-dependent expression of the migA gene. Strain PAK containing the migA::lacZ fusion construct pSJ9729 (■) or vector pDN19lacΩ (▲) was cultured in L-broth at 37 °C and β-galactosidase activity was plotted against cell density. (b) rhlI is required for the cell-density-dependent migA expression. Plasmid pSJ9729 was introduced into wild-type PAO1 as well as isogenic lasI, rhlI and lasI/rhlI mutant derivatives PDO100, PAO-JP1 and PAO-JP2, respectively. β-Galactosidase activities were measured at low (OD$_{600}$ 1–0; black bars) or high (OD$_{600}$ 4–5; white bars) cell density after growth in L-broth. (c) Effect of PAI-1 and PAI-2 autoinducers on the expression of migA. β-Galactosidase activities of the lasI/rhlI double mutant (PAO-JP2) harbouring migA::lacZ and lasB::lacZ fusion constructs pSJ9729 and pTS400, respectively, were grown to an OD$_{600}$ of 3.5 in L-broth in the absence (white bars) or presence of 100 nM C12-HSL (shaded bars), 100 nM C4-HSL (black bars) or 100 nM each of C12-HSL and C4-HSL (hatched bars).

migA is regulated by the Rhl/RhlR quorum sensing system

To investigate whether migA is indeed regulated by the quorum sensing system, cell-density-dependent expression of the migA::lacZ fusion was first tested in a PAK background. PAK(pSJ9729) was grown in L-broth with appropriate antibiotics at 37 °C. As shown in Fig. 4(a), β-galactosidase activity increased proportional to cell density and more than fourfold greater β-galactosidase activity was observed at high cell density than that at
Regulation and function of \( \text{migA} \) gene

![Fig. 5. Effect of \( \text{migA} \) on the production of high-molecular-mass LPS. (a) Silver-stained SDS-PAGE. (b, c) Western immunoblots reacted with the A-band-specific mAb N1F10 and B-band-specific mAb O25G3D6, respectively. The \( \text{migA} \) gene was constitutively expressed by a \( \text{lac} \) promoter in pSJ9610. Lanes: 1, wild-type PAK; 2, PAK(pSJ9610); 3, PAK(\( \text{migA} \)::\( \Omega \)).](image)

low cell density (\( \text{OD}_{600} \) 4.5 vs 1.0), demonstrating cell-density-dependent expression, a characteristic of genes under the control of quorum sensing systems. We further examined whether \( \text{migA} \) is regulated by either one or both of the two well characterized quorum sensing systems in \( \text{P. aeruginosa} \), \( \text{lasI}/R \) or \( \text{rhlI}/R \). Expression of \( \text{migA}::\text{lacZ} \) in \( \text{lasI} \) and \( \text{rhlI} \) single mutants, as well as in a \( \text{lasI}/\text{rhlI} \) double mutant background was compared to that in the wild-type PAO1 background. At high cell density (\( \text{OD}_{600} \) 4–5), \( \text{migA} \) expression levels were much lower in either the \( \text{rhlI} \) or \( \text{lasI}/\text{rhlI} \) double mutant background than in wild-type strain. However, the \( \text{migA} \) expression level in the \( \text{lasI} \) mutant background was not different from that in the wild-type PAO1 background (Fig. 4b), suggesting that \( \text{migA} \) is regulated by the RhII/R system. Furthermore, \( \text{migA} \) expression in a \( \text{lasI}/\text{rhlI} \) double mutant background is stimulated by the addition of C4-HSL (PAI-2) but not by C12-HSL (PAI-1). Interestingly, addition of both \( \text{PAI}-1 \) and \( \text{PAI}-2 \) did not induce \( \text{migA} \) expression (Fig. 4c), which is similar to other RhlI/RhlR-regulated genes described by Pesci et al. (1997). It was postulated in these cases that \( \text{PAI}-1 \) inhibits the binding of \( \text{PAI}-2 \) to RhlR. As a control, the expression of \( \text{lasB} \), which is known to be regulated by LasR, was stimulated by \( \text{PAI}-1 \) but not by \( \text{PAI}-2 \) in the same mutant background (Fig. 4c).

\( \text{migA} \) affects low-molecular-mass LPS production

The \( \text{migA} \)-encoded protein shows homology to glycosyltransferases associated with LPS biosynthesis in other Gram-negative bacteria (Wang et al., 1996), suggesting a possible role of \( \text{migA} \) in LPS core synthesis. To determine the role of \( \text{migA} \) in LPS biosynthesis, LPS banding patterns were compared among strains of wild-type PAK, a \( \text{migA}::\Omega \) mutant and PAK harbouring a plasmid that constitutively expresses the \( \text{migA} \) gene (pSJ9610). Two gel systems were used to examine the LPS banding patterns, Tricine SDS-PAGE and the standard glycine discontinuous SDS-PAGE system followed by silver-staining and Western immunoblotting with appropriate mAbs. The Tricine SDS-PAGE system allows for significantly improved resolution of low-molecular-mass LPS of the core region (Lesse et al., 1990) while standard SDS-PAGE provides good resolution of high-molecular-mass LPS, such as O antigen. High-molecular-mass LPS of wild-type \( \text{P. aeruginosa} \) strain PAK, which includes A-band and B-band LPS, was analysed by silver-stained SDS-PAGE gels and Western immunoblots using B-band-specific mAb O25G3D6 and A-band-specific mAb N1F10. No significant difference in the production of high-molecular-mass LPS was observed between the parent strain PAK and the \( \text{migA}::\Omega \) mutant (Fig. 5). Results from the Tricine gel analysis showed, however, that the expression of \( \text{migA} \) clearly affected core LPS production. Wild-type PAK exhibits two LPS bands corresponding to ‘complete-core’ (band 2) and ‘core-plus-one O antigen repeat’ (band 1) (Fig. 6, lane 1). For comparison, LPS from a PAK \( \text{wbpL} \) mutant, deficient in the initial glycosyltransferase required for both A-band and B-band LPS production, was included as a control in lane 4. As expected, only band 2 (complete core) was detected. PAK(pSJ9610) produced only band 2 (Fig. 6,
The observation that MigA shares amino acid sequence homology with glycosyltransferases, implies a potential role of this protein in LPS modification. We have shown here that migA indeed affects the modification of low-molecular-mass LPS. High-level expression of migA leads to the loss of the core-plus-one LPS band, whereas migA mutation results in an increase in this band. We hypothesize that MigA is a glycosyltransferase that transfers a sugar residue onto the core region and consequently modifies the attachment point for O antigen on the outer-core oligosaccharide. The MigA modified outer-core molecule would therefore be less able to serve as an acceptor for O antigen. A ligase enzyme, WaaL, joins the O antigen polysaccharide to the lipid A core and there is a strong evidence that the nature of the acceptor molecule is important for this ligation reaction. Evidence to support this hypothesis is needed.
as follows. (i) In *P. aeruginosa*, the structure of the outer-core region in a fully assembled O chain containing LPS differs from that of the O-chain-deficient rough LPS. The point of attachment of the O chain to the outer core is at the 1,3-linked rhamnose (Sadovskaya *et al.*, 2000). When this residue is missing, the core molecule is less able to serve as an acceptor for O antigen attachment. Genetic evidence suggests that the rhamnose residue in the outer-core region is necessary for O antigen attachment (Rahim *et al.*, 2000). (ii) A rhamnosyltransferase, required for the addition of the 1,3-linked rhamnose which serves as the attachment point for O antigen to the outer core, shares amino acid sequence homology with MigA (Rahim *et al.*, 2000). (iii) MigA is homologous to glycosyltransferases, including WaaV of *E. coli*, possessing a type R1 core (Heinrichs *et al.*, 1998). The proposed function of WaaV is that of a UDP-glucosyltransferase required for the addition of a 1,3-linked glucose, which is a side-chain substituent in the outer core and acts as the attachment site for long-chain O antigen to an R1-type core.

Recently, Ernst *et al.* (1999) have demonstrated that the lipid A portion of the *P. aeruginosa* LPS undergoes specific modification during chronic colonization of CF lungs. The CF-specific lipid A apparently contains palmitate and aminoarabinose which are associated with bacterial resistance to cationic antimicrobial peptides and increased inflammatory responses, indicating that they are likely to be involved in airway disease. Since the migA gene is highly expressed in the CF lung environment and interferes with the formation of core-plus-one LPS, it is possible that suppression of such low-molecular-mass LPS renders *P. aeruginosa* a survival advantage in the CF lung environment. If indeed this is the case, a prolonged colonization in the CF lung environment could result in enrichment of adaptive mutants in which migA gene expression becomes constitutive, similar to the enrichment of mucoid isolates of *P. aeruginosa*. Our survey of CF- and non-CF-derived *P. aeruginosa* strains indicates that a high proportion of CF isolates lost core-plus-one LPS, whereas all of the non-CF isolates retained it (unpublished results). However, it is not clear if this is due to migA up-regulation or a defect in the ligase which mediates core-plus-one LPS synthesis. Further studies are underway to understand this as well as the role of migA up-regulation in the adaptation of *P. aeruginosa* to the CF lung environment.

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**REFERENCES**


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