Characterization of MexT, the Regulator of the MexE-MexF-OprN Multidrug Efflux System of Pseudomonas aeruginosa

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We investigated the regulation of the MexEF-OprN multidrug efflux system of Pseudomonas aeruginosa, which is overexpressed in nfxC-type mutants and confers resistance to quinolones, chloramphenicol and trimethoprim. Sequencing of the DNA region upstream of the mexEF-oprN operon revealed the presence of an open reading frame (ORF) of 304 amino acids encoding a LysR-type transcriptional activator, termed MexT. By using T7-polymerase, a 34-kDa protein was expressed in Escherichia coli from a plasmid carrying the mexT gene. Expression of a mexE::lacZ fusion was 10-fold higher in nfxC-type mutants than in the wild-type strain; however, transcription of mexT as well as the mexT DNA region was unchanged. Located adjacent to mexT but transcribed in opposite direction, the beginning of an ORF termed qrh (quinone oxidoreductase homologue) was identified. Expression of a qrh::lacZ fusion was also found to be activated by MexT. Further, we present evidence for coregulation at the transcriptional and posttranscriptional level between the MexEF-OprN efflux system and the OprD porin responsible for cross-resistance of nfxC-type mutants to carbapenem antibiotics.

Pseudomonas aeruginosa is a leading cause of hospital acquired infections. Its high intrinsic antibiotic resistance and the ability to develop multidrug resistance pose serious therapeutic problems. For a long time it has been assumed that this elevated intrinsic resistance was mainly due to the low outer membrane permeability of P. aeruginosa which was correlated to the appearance of outer membrane proteins with sizes in the range of 50 kDa. These proteins (OprM, OprJ, and OprN) have now been shown to be part of multidrug efflux systems with broad specificity (13, 25, 26) which catalyze the energy-dependent extrusion of antibiotics such as β-lactams, quinolones, tetracycline, chloramphenicol, macrolides, and trimethoprim. The three efflux systems of P. aeruginosa have similar patterns of genetic organization. The first gene of each operon encodes a periplasmic fusion protein (MexA, MexC, or MexE), the second encodes a cytoplasmic membrane protein (MexB, MexD, or MexF) thought to be the actual efflux pump, and the third gene encodes an outer membrane protein (OprM, OprJ, or OprN). The three proteins are believed to form a channel across the inner and outer membranes. The mexAB-oprM operon is expressed constitutively and contributes to the intrinsic resistance of P. aeruginosa to a variety of toxic substances (14, 15). Transcription of the mexAB-oprM operon is increased in nalB-type mutants (30) due to mutations in the repressor protein MexR (27, 43). The mexCD-oprJ operon (25) is not expressed constitutively but is overexpressed in mutants displaying mutations in nfxB, the gene coding for the transcriptional repressor of this efflux system (24, 35).

The third efflux operon, mexEF-oprN, which confers resistance to quinolones, chloramphenicol, and trimethoprim, is overexpressed in nfxC-type mutants of P. aeruginosa (13). NfxC-type mutants (7) are also cross-resistant to the carbapenem imipenem (3, 8, 18), since they show decreased expression of OprD, an outer membrane protein facilitating the diffusion of basic amino acids, small peptides (40), and several carbapenem antibiotics (39). The mexEF-oprN efflux operon differs from the other efflux systems in that it is positively regulated by a protein belonging to the LysR family of transcriptional activators (13). In the present study, we characterize this activator, called MexT, and show that it is required for the expression of the MexEF-OprN efflux pump. We also demonstrate the involvement of MexT in the regulation of an open reading frame (ORF) adjacent to mexT and present evidence for coregulation at the transcriptional and posttranscriptional levels between the OprD porin and the MexEF-OprN efflux system.

(The MexT sequence and the regulation of the mexEF-oprN operon were presented at the Pseudomonas meeting in Madrid, Spain, in September 1997.)

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. Escherichia coli DH10B was used for cloning experiments and plasmid propagation. Plasmids pUC119 and pC20H were used for subcloning and sequencing. Plasmids were conjugated from E. coli S17-1 (36) or by triparental mating with the helper plasmid pRK2013. Transconjugants were selected on M9 minimal medium (16) supplemented with 40 mM citrate as a carbon source or on Luria-Bertani (LB) medium supplemented with ampicillin at 40 μg/ml to counterselect against E. coli. MICS in Mueller-Hinton broth were determined by the microdilution method (11).

DNA sequencing and protein analysis. DNA sequences were determined from double-stranded templates according to the dyeoxy chain termination method (32) with an automated sequencer (Applied Biosystems model 373A). DNA sequences were processed and analyzed with the PCGENE program (Intelligenetics Inc., Mountain View, Calif.) and the BLAST algorithm (1). Protein alignments were generated by the program CLUSTAL.

Strain and plasmid constructions. The mexT mutant was constructed by inserting a 2.2-kbp BamHI fragment of POM4 (13) carrying the entire mexT gene into the suicide vector pJQ200mp18 (28), yielding pJQ3. MexT was inactivated by inserting the Hg′ determinant as a 4.8-kbp BamHI fragment of pHP45Hg (6) into the unique BglII site of pJQ3. The resulting construct, called pJQ2C, was mobilized from strain S17-1 into PA01. Hg′ and Gm′ colonies were recovered after counterselection on sucrose-containing plates. Three independent colonies were analyzed by Southern blot analysis with a digoxigenin-labeled DNA fragment. All three showed the same banding pattern, in agreement with an integration of the Hg′ cassette into the mexT structural gene. The mexT::Hg mutation was then transduced by phase E79v2 (22) into the nfxC-type mutant PT149. The mexE::lacZ fusion plasmid pNFZ4 was constructed by cloning a 0.5-kbp BglII-EcoRI fragment of cosmID pOPN4 (13) containing the 3′ end
of MexT into the promoter probing vector pMP220 (37). To generate the gsh::
lacZ fusion plasmid pQRZ4, a 0.5-kbp DNA fragment was amplified from ge-
monic DNA of strain PAO1 with primers procxP1 (5′-CTGCTCGGGGGCCA
GGTTCTGAC-3′) by GGTGGGCTCCATGCTGCGTC-3′ from pMP220 (37). To generate the
MexT expression with T7 polymerase. An E. coli strain harboring the T7
polymerase expression plasmid pFG1-2 was transformed with the control plasmid
pWSK29 or the mexT-expressing plasmid pWSNC5. Single transformants were
grown at 30°C in LB medium supplemented with the appropriate antibiotics.
At an optical density at 600 nm of approximately 0.4, the cultures were shifted for
growth at 30°C in LB medium supplemented with the appropriate antibiotics. At
50 μg/ml.

**RESULTS**

**Nucleotide sequence of the mexT gene.** The mexE-mexF-
oprN operon was previously shown to be located on pOPN4, a
plasmid-based cosmid clone (13). The DNA region upstream of
mexE was sequenced and a single ORF of 912 nucleotides
(nt) was identified. This ORF, called mexT, is transcribed in the
same direction as the mexE-mexF-oprN operon. A Shine-Dal-
garno sequence (GAGGA) was located 6 nt upstream of the
start codon. The 304-amino-acid sequence of the putative
MexT polypeptide was compared to the entries in the Swiss-
prot and EMBL databases by using the BLASTP program (1).

**TABLE 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristic(s)</th>
<th>Reference or source</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
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<tr>
<td><em>E. coli</em></td>
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</tr>
<tr>
<td>MC1061</td>
<td>F′ araD139 Δ(ara-leu)7606galE15 galK16 Δ(lac)X74 rpsL thi pro bsdR recA4 strR RP4-2</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>S17-7-1</td>
<td>thi pro bsdR recA4 strR RP4-2</td>
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</tr>
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<td>DH10B</td>
<td>F′ araD139 Δ(ara-leu)7606galU1 galK1 Δ(lac)X74 mcrA(mrr-hsdRMS-mcrBC) deoR</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
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<td></td>
</tr>
<tr>
<td>PAO1</td>
<td>Wild-type</td>
<td>Laboratory collection</td>
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<tr>
<td>PT149</td>
<td>PAO-7H, PAO1 overproducing MexE-OpR, selected on ciprofloxacin</td>
<td>13</td>
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<tr>
<td>PT579</td>
<td>PAO1 mexT::Gm</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
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<td>pUC19</td>
<td>High-copy-number cloning vector, Ap′</td>
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<td>pSK29mp18</td>
<td>Mobilizable suicide vector, sacB, Gm′</td>
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<td>pWSK29</td>
<td>Low-copy-number T7 expression vector, Ap′</td>
<td>42</td>
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<td>pWNCS</td>
<td>pWSK29 with 1.5-kbp KpnI-EcoRI fragment expressing mexT under T7 control, Ap′</td>
<td>This study</td>
</tr>
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<td>pOPN4</td>
<td>pLAFR3-based cosmid containing 27-kbp insert harboring mexE-mexF-oprN, Tc′</td>
<td>13</td>
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<td>pME6001</td>
<td>Broad-host-range vector, Gm′</td>
<td>D. Haas</td>
</tr>
<tr>
<td>pMEXT</td>
<td>pNFCS, 1.5-kbp HindIII-EcoRI fragment of pOPN4 cloned in HindIII-EcoRI-cleaved pME6001, Gm′</td>
<td>13</td>
</tr>
<tr>
<td>pMP220</td>
<td>Promoterless lacZ fusion vector, IncP, Tc′</td>
<td>37</td>
</tr>
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<td>pNFZ4</td>
<td>mexE::lacZ fusion on pMP220, Tc′</td>
<td>This study</td>
</tr>
<tr>
<td>pTZ4</td>
<td>mexT::lacZ fusion on pMP220, Tc′</td>
<td>This study</td>
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<td>pSE50</td>
<td>oprD::phoA translational fusion on pMP220, Tc′</td>
<td>This study</td>
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</table>

*Hg, mercury; Tc, tetracycline; Gm, gentamicin; Ap, ampicillin.

**Determination of β-galactosidase and alkaline phosphatase activities.** Strains were
incubated at 30°C on nutrient agar plates supplemented with the
appropriate antibiotics and grown overnight at 37°C. Cultures were diluted 1:100 infresh LB without antibiotics. When strains carried two plasmids, antibiotics for
the marker on each plasmid were added (gentamicin at 15 μg/ml and tetracycline
at 50 μg/ml). β-Galactosidase (21) and alkaline phosphatase (4) activities were
determined in triplicate samples at various times during growth.

**Nucleotide sequence accession number.** The DNA sequence of mexT and its
upstream region have been deposited in the EMBL databank and assigned
accession number AJ007825.
respectively. The mexT gene was therefore a likely candidate to harbor a mutation responsible for overexpression of the mexEF-oprN operon. The mexT region, encompassing the structural gene and the regulator region, was amplified by PCR from strain PT149 (formerly PAO-7H) overexpressing the MexEF-OprN efflux system and from its parental strain PAO1. However, sequence analysis of these PCR fragments showed no nucleotide changes. The mexT region of two other spontaneous nfxC-type mutants was sequenced. Again, neither of them displayed any nucleotide changes, suggesting that the mutation responsible for the nfxC phenotype was not located in mexT or its regulatory region.

Cloning of the DNA region upstream of mexT. To further investigate the regulation of the mexEF-oprN efflux operon, the DNA region upstream of mexT was cloned by plasmid rescue as described in Materials and Methods. The restriction pattern of three recovered clones was analyzed and found to contain about 10 kbp of chromosomal DNA. The DNA sequence upstream of mexT was determined from one of the plasmids and found to contain the beginning of an ORF transcribed divergently from mexT and located 221 bp from the mexT start codon. The deduced amino acid sequence of the ORF was homologous to a family of quinone oxidoreductases from E. coli (38) and P. aeruginosa (EMBL accession number X85015) as well as to eucaryotic homologues. The intergenic region between mexT and this ORF, called tentatively qrh (quinone oxidoreductase homologue), contained two putative promoter sequences located on the two different DNA strands and overlapping at their –10 regions. The putative mexT (CTGACA-18 bp-GATAAT) and qrh (CTGACA-15 bp-AATAAC) promoter sequences were very similar to the E. coli σ70 consensus sequence (TTGACA-15 to 17 bp-TATAAT).

Examination of the previously sequenced mexE promoter region (13) did not reveal significant homology to the E. coli σ70 consensus sequence. However, the sequence GTATCAC TGTTCGGTGATAATACCAAATCTCGTGTTCAGATTGT was found 58 bp upstream of the mexE start codon. This sequence showed a striking similarity to the sequence of the nod box (9) (NYATCCAYNNYRGATGNNNNYNATC NAAACAATCGGTTTTTA) located upstream of genes regulated by NodD in a Rhizobium spp. MexT is therefore likely to activate mexEF-oprN transcription by binding to the nod-box-like sequence.

Analysis of the mexT gene product and phenotype of a mexT mutant. T7 polymerase-directed expression of mexT in E. coli revealed a protein band with a size of 34 kDa (Fig. 1). This size is close to the molecular mass of 34,418 deduced from the nucleotide sequence of mexT, and the labeled protein is therefore likely to correspond to the mexT gene product.

A PAO1 derivative, called PT579 and inactivated by the insertion of an ΩHg cassette in the coding region of mexT, was constructed. As expected, MICs of the mexT mutant were indistinguishable from those of the wild-type strain (data not shown). The mexT::ΩHg mutation was transferred by transformation into the multidrug-resistant nfxC-type mutant PT149. All of the tested transductants recovered the antibiotic susceptibility profile of the wild-type strain, demonstrating that a functional mexT gene is required for expression of the multidrug resistance phenotype in nfxC-type mutants.

MexT is required for selection of the nfxC phenotype. Selection of the nfxC phenotype can easily be achieved by plating wild-type PAO1 cells on LB agar containing at least 500 μg of chloramphenicol per ml. To establish the role of MexT in selection of the nfxC phenotype, PAO1 and the mexT::ΩHg mutant PT579 were plated on LB agar plates containing chloramphenicol at 600 μg/ml. While resistant colonies of PAO1 appeared at a frequency of about 10^-8, no colonies (<10^-10) were obtained with the mexT mutant. Among the 10 colonies derived from PAO1, all presented the nfxC phenotype (data not shown). These results demonstrate the absolute requirement of mexT for the selection of the nfxC antibiotic resistance phenotype. Indeed, PAO1 strains unable to yield nfxC mutants carry mutations in mexT (unpublished result).

Expression of mexE, mexT, and qrh. To further study the regulatory circuits of the qrh-mexT-mexEF-oprN DNA region, transcriptional lacZ fusions to mexE (pNFZ4), mexT (pTZ4), and qrh (pQRZ4) were constructed with the low-copy-number IncP derivative pMP220. In PAO1, β-galactosidase activities expressed from the mexE::lacZ fusion were always comparable to those of the control vector pMP220. However, in the nfxC-type mutant PT149 expression of the mexE::lacZ fusion was already higher in the lag phase and further increased during exponential and stationary growth (Fig. 2A). A similar increase in mexE::lacZ expression was found when MexT was introduced in trans on the multicopy plasmid pMEXT (data not shown). These results clearly show a correlation in the level of expression of the mexEF-oprN operon with the antibiotic resistance phenotype. They also suggest that the amount of MexT might be critical to the regulation of the mexEF-oprN operon. Therefore, the mexT::lacZ fusion plasmid pTZ4 was introduced into the wild type and the nfxC mutant PT149. In both strains, the fusion showed similar levels of elevated constitutive expression of β-galactosidase during growth in LB (data not shown), in agreement with a gene displaying a σ70 consensus promoter sequence (Fig. 2B). This result suggests that overexpression of MexEF-OprN in the nfxC-type mutant PT149 was not due to increased levels of mexT transcription.

Genes controlled by LysR-type activators are often located adjacent to the regulator and transcribed in the opposite direction. We therefore analyzed expression of the qrh::lacZ fusion plasmid pQRZ4. Like that for mexT, qrh expression was found to be constitutive; however, four- to fivefold-higher levels of β-galactosidase were measured in strains PT149 and PAO1 (pMEXT) compared to the wild type (Fig. 2B), suggesting that the qrh gene is also positively regulated by MexT.

To confirm that plasmid-encoded MexT was sufficient to activate transcription, the lacZ fusions were analyzed in E. coli. In the presence of plasmid pMEXT, expression levels of the mexE::lacZ fusion were increased about 40-fold, while those of the qrh::lacZ fusion were increased fivefold. Plasmid pMEXT

FIG. 1. Labeling of E. coli cells with [35S]Met and [35S]Cys carrying either the control T7 expression vector pWSK29 or the mexT-carrying plasmid pWNC5. The molecular masses (in kilodaltons) of standard protein markers are indicated on the right.
had no significant effect on expression of the mexT::lacZ fusion (Fig. 3). These results are in agreement with those found for P. aeruginosa, where plasmid-encoded MexT had a greater effect on mexE than on oprD transcription.

In order to verify that the observed operon expression was not a singularity of the nfxC-type strain PT149, the three lacZ fusions were introduced into four other nfxC-type strains as well as into strains overexpressing either the mexAB-oprM (nalB) or the mexCD-oprJ (nfxB) operon. All four nfxC-type strains showed increased expression of the mexE::lacZ fusion (10- to 20-fold) and of the qrh::lacZ fusion (fivefold). In the strains overexpressing either of the two other efflux operons, all three fusions were expressed at levels comparable to those of the wild type (data not shown).

**MexT coregulates oprD expression.** Several reports have clearly established that in nfxC-type mutants, overexpression of the mexEF-oprN efflux operon is linked to decreased amounts of the OprD porin in the outer membrane (18, 19). Since OprD is the port of entry of the carbapenem imipenem, nfxC-type mutants are cross-resistant to imipenem (3, 8, 18). Whether this cross-resistance is due to transcriptional repression of the oprD gene by MexT was investigated by constructing a pMP220-based lacZ fusion to the oprD gene (pSE45). Twofold-lower LacZ activity was found in the nfxC-type mutant PT149 compared to the wild type (Table 2). Since this weak effect could probably not account for the dramatic decrease in OprD expression in nfxC mutants as determined previously by Western blot analysis (13, 18, 19), we further examined the possibility of posttranscriptional regulation by MexT. A plasmid-encoded oprD::phoA translational fusion (pSE50) was constructed and introduced into PAO1 and PT149. Compared to the wild type, a fivefold decrease was observed in the nfxC-type mutant PT149 (Table 2). Furthermore, a similar fivefold decrease in alkaline phosphatase expression was found when plasmid pMEXT was introduced into PAO1 carrying the oprD::phoA fusion. These results strongly suggest that MexT downregulates oprD expression also at the posttranscriptional level.

**DISCUSSION**

Our results clearly establish MexT as the transcriptional activator of the mexEF-oprN efflux operon and demonstrate its requirement for the expression of the nfxC multidrug resistance phenotype. Furthermore, we found that when the gene is expressed from a multicopy plasmid, mexT on its own is able to activate transcription of a mexE::lacZ fusion in both P. aeruginosa and E. coli. This finding is in agreement with the previous observation that the mexT-carrying plasmid pMEXT (pNFZ8) is sufficient to confer a nfxC resistance phenotype to a susceptible strain.

**TABLE 2. Effects of nfxC mutation and mexT on transcriptional oprD::lacZ and translational oprD::phoA fusions in P. aeruginosa**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>LacZ or PhoA activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1</td>
<td>pSE45 (oprD::lacZ)</td>
<td>2.086 ± 0.373</td>
</tr>
<tr>
<td>PT149(nfxC)</td>
<td>pSE45</td>
<td>1.012 ± 0.31</td>
</tr>
<tr>
<td>PAO1</td>
<td>pSE50 (oprD::phoA)</td>
<td>10.4 ± 2.1</td>
</tr>
<tr>
<td>PT149(nfxC)</td>
<td>pSE50</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>PAO1</td>
<td>pSE50, pME6001 (control plasmid)</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>PAO1</td>
<td>pSE50, pMEXT (pME6001::mexT)</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

*Results are the means ± standard deviations of triplicate determinations. Samples were taken during the mid-exponential growth phase. Enzymatic activities are expressed in Miller units (LacZ) or alkaline phosphatase units (PhoA).*
tible PAO1 wild type strain (13). We also show that the mexT DNA region is unchanged in the nfxC-type mutant PT149 and that mexT transcription levels are comparable to those of the wild type. How can one account for the increased mexEF-oprN transcription in the nfxC mutants? The majority of the LysR-type regulators are synthesized in a nonactive form and become activated upon binding of a cognate effector molecule(s) (34). We therefore assume that in the nfxC-type mutants, the effector molecule of MexT is produced constitutively or in larger amounts than in the wild type, thereby causing permanent activation of MexT and hence overexpression of the MexEF-OprN efflux system. The fact that introduction of additional plasmid-encoded copies of MexT into a wild-type strain also causes increased mexEF-oprN expression can be explained by a shift in the equilibrium between the inactive and active forms of MexT. Such a mechanism has been suggested for the XylS regulator protein of the TOL plasmid pWW0 from P. putida (29), a member of the AraC family of transcriptional activators. Therefore, even in the absence of MexT effector molecules, plasmid-encoded MexT is able to activate mexEF-oprN transcription and to confer a nfxC multidrug resistance phenotype on the susceptible wild type strain.

The MexT homologues NahR and NodD are activated upon binding of salicylate (33) and phenolic plant-derived compounds (20), respectively. Interestingly, MexT not only has significant amino acid similarity to NodD, but the regulatory region of the mexEF-oprN operon also contains a nod box element (9) found upstream of NodD-regulated genes in Rhizobium species. However, neither NahD effector factors (flavone, trigonellin, naringenin, and vanilline, etc.) provided at a final concentration of 2 mM nor the NahR inducer salicylate at concentrations of up to 50 mM showed any significant effect on mexEF-oprN transcription (unpublished results). Furthermore, the addition of the MexEF-OprN substrate molecules chloramphenicol, norfloxacin, or trimethoprim at subinhibitory concentrations had no effect on the expression of the mexE::lacZ fusion. Supposing that effector molecules are also substrates of the efflux pump, these results are further evidence that antibiotics are not the natural substrates of the MexEF-OprN efflux pump.

We found that MexT activates transcription not only of the mexEF-oprN efflux operon but also of an adjacent ORF, which we tentatively called qrh. The protein encoded by qrh shows homology to a family of quinone oxidoreductases of eucaryotic species. However, neither NodD effectors (flavone, trigonellin, and vanilline, etc.) provided at a final concentration of 2 mM nor the NahR inducer salicylate at concentrations of up to 50 mM showed any significant effect on mexEF-oprN transcription (unpublished results). Furthermore, the addition of the MexEF-OprN substrate molecules chloramphenicol, norfloxacin, or trimethoprim at subinhibitory concentrations had no effect on the expression of the mexE::lacZ fusion. Supposing that effector molecules are also substrates of the efflux pump, these results are further evidence that antibiotics are not the natural substrates of the MexEF-OprN efflux pump.

Numerous reports have demonstrated a decrease in OprD expression in nfxC-type mutants (12, 13, 18). OprD is a porin which facilitates diffusion of basic amino acids and small peptides and is also the port entry of carbapenem antibiotics. In addition to MexEF-OprN substrates, nfxC-type strains are therefore cross-resistant to imipenem. Our results with oprD::lacZ and oprD::phoA fusion experiments suggest that the coregulation between oprD and the mexEF-oprN operon is exerted both at the transcriptional and posttranscriptional levels. A 2.5-fold decrease in the expression of a transcriptional oprD::xylE fusion in the presence of MexT has been reported recently by Ochs et al. (23). This result is in agreement with the twofold decrease in expression of our transcriptional oprD::lacZ fusion observed in a nfxC mutant. However, this modest effect seems unlikely to account solely for the almost complete absence of OprD in outer membranes of nfxC mutants (10, 13, 18). Therefore, the observed posttranscriptional effect on oprD expression offers a further explanation. A similar type of coregulation is found in E. coli mar mutants in which increased expression of the AcrAB efflux system is correlated with decreased expression of the porin OmpF (5). This effect is mediated by the antisense mcrF RNA (2). Alternatively, one can assume that transport of OprD across the cytoplasmic membrane is decreased in nfxC-type mutants by the overexpression of the MexEF-OprN efflux system.

Among the three multidrug efflux systems characterized so far, only the mexAB-oprM system (14) is constitutively expressed. It also displays the broadest substrate specificity and might therefore represent a natural defense mechanism against a variety of harmful substances. The fact that the other two efflux systems are not expressed under normal laboratory conditions and are tightly regulated by their respective regulator protein could suggest a role in more specific tasks, for example in the secretion of cellular metabolites. Identification of their substrates should help to elucidate the physiological role of these efflux pumps.

ACKNOWLEDGMENTS

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