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FptA, the Fe(III)-Pyochelin Receptor of *Pseudomonas aeruginosa*: a Phenolate Siderophore Receptor Homologous to Hydroxamate Siderophore Receptors

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The *Pseudomonas aeruginosa* siderophore pyochelin is structurally unique among siderophores and possesses neither hydroxamate- nor catecholate-chelating groups. The structural gene encoding the 75-kDa outer membrane Fe(III)-pyochelin receptor FptA has been isolated by plasmid rescue techniques and sequenced. The N-terminal amino acid sequence of the isolated FptA protein corresponded to that deduced from the nucleotide sequence of the *fptA* structural gene. The mature FptA protein has 682 amino acids and a molecular mass of 75,993 Da and has considerable overall homology with the hydroxamate siderophore receptors FpvA of *P. aeruginosa*, PupA and PupB of *Pseudomonas putida*, and FhuE of *Escherichia coli*. This observation indicates that homologies between siderophore receptors are an unreliable predictor of siderophore ligand class recognition by a given receptor. The *fptA* gene was strongly regulated by iron; *fptA* transcription was totally repressed by 30 μM $FeCl_3$, as determined by Northern (RNA) blotting. The promoter of the *fptA* gene contained the sequence 5′-ATAATGATAAGCATTATC-3′, which matches the consensus *E. coli* Fur-binding site at 17 of 18 positions. The −10 promoter region and transcriptional start site of the *fptA* gene reside within this Fur-binding site.

Pseudomonas aeruginosa, a ubiquitous gram-negative rod, is nutritionally versatile and is one of the most distinct species among the pseudomonads (54). This bacterium is considered highly pathogenic for individuals with compromised immunity; septicemic infections involving this organism have a poor prognosis despite recent advances in antimicrobial chemotherapy (43).

In response to iron deprivation, many bacteria produce siderophores, low-molecular-weight iron chelators, which mediate high-affinity iron transport via membrane receptors. *P. aeruginosa* produces two unrelated siderophores, pyoverdin and pyochelin. Pyoverdin is a small peptide containing a fluorescent dihydroxyquinoline derivative which is characteristic of the fluorescent siderophores produced by members of *Pseudomonas* rRNA homology group I (1, 21). In addition to two hydroxamate groups possessed by pyoverdin, the 6,7-dihydroxyquinoline derivative is also believed to serve as an iron-chelating group (58).

Pyochelin is a structurally unique phenolate siderophore which has neither hydroxamate nor catecholate character (16). Pyochelin has been assigned the structure 2-[2-(*o*-hydroxyphenyl)-2-thiazolin-4-yl]-3-methyl-4-thiazolidinecarboxylic acid and apparently chelates Fe(III) in a 2:1 pyochelin-Fe(III) stoichiometry (5, 17). Two other pseudomonads, *Pseudomonas cepacia* and *Pseudomonas fluorescens*, also produce pyochelin (18, 51). The unusual complement of thiazoline and thiazolidine heterocyclic rings in pyochelin has not been observed in other siderophores. Among other known siderophores, only anguibactin (34), the siderophore produced by *Vibrio anguillarum*, possesses either of these heterocyclic rings (i.e., thiazoline); however, unlike pyochelin, anguibactin has both hydroxamate- and catecholate-chelating groups.

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Studies on Fe(III)-pyochelin transport have identified at least two outer membrane proteins that are required for efficient iron transport via pyochelin. The first of these proteins to be identified is a 14-kDa outer membrane protein, designated ferripyochelin-binding protein (FBP) (52, 53); FBP mutants are slightly attenuated in Fe(III)-pyochelin transport (31), and the actual role of FBP remains obscure. Recently, a 75-kDa outer membrane protein has been identified as the Fe(III)-pyochelin receptor (4, 31). In vivo transport assays with mutants defective in this 75-kDa outer membrane protein and in vitro assays with the isolated protein demonstrated that this protein bound Fe(III)-pyochelin and was absolutely required for Fe(III)-pyochelin transport. The gene encoding the Fe(III)-pyochelin receptor (*fptA*) has now been isolated and sequenced.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. All *P. aeruginosa* strains are PAO1 derivatives.

Media and growth conditions. *Escherichia coli* strains were routinely grown in LB medium with the following antibiotics: carbenicillin (Cb), 100 μg/ml; ampicillin (Ap), 100 μg/ml; chloramphenicol (Cm), 15 μg/ml; kanamycin (Km), 40 μg/ml; and tetracycline (Tc), 20 μg/ml. Isopropylthiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were used at 100 and 50 μg/ml, respectively. *P. aeruginosa* strains lacking plasmids were also grown on LB. *P. aeruginosa* strains bearing plasmids were selected and grown on GG agar containing tetracycline (110 μg/ml). CPS medium was routinely used as an ironlimiting medium for *P. aeruginosa*, and CDS agar was used for screening pyochelin production and transport for *P. aeruginosa*. The compositions of GG (glucose-glycerol) medium, CPS (casamino acids-phosphate-sulfate) medium, and CDS (CPS-dipyridyl-salicylic acid) agar

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics[a]	Source or reference
E. coli		
DH5α	ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 endA recA1 hsdR17 deoR thi-1 supE44 gyrA96 relA1*	BRL[b]
DH10B	*mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80d*lacZ*ΔM15 Δ*lacX74 deoR recA1 endA1 araD139* Δ(*ara, leu*)*7697 galU galK rpsL nupG*	BRL
DH11S	ϕ80d*lacZ*ΔM15 *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Δ(*lac-proAB*) Δ(*recA1398*) *deoR rpsL srl thi*/F′ *proAB*$^+$ *lacI*^{q}ZΔM15	BRL
JB377	*hsdS leuB6 thr* Δ(*srlR-recA*)*306*::Tn*10*	J. A. Heinemann
GM119	*dcm-6 dam-3 metB1 thi-1 lacY1* (or *lacZ4*) *galK2 galT22 mtl-2 tonA2* (or *tonA31*) *tsx-1* (or *tsx-78*) *supE44*	K. Tilly
P. aeruginosa		
IA602	*pvd-2* Pch$^-$ Sal$^-$	4
IA613	*fptA1 pvd-2* Pch$^-$ Sal$^-$	4
Plasmids		
pHSG298	Km^r pUC derivative	57
pHSG396	Cm^r pUC derivative	57
pRK2013	IncP *tra*$^+$ ColE1 replicon	26
pRKY55	pTJS133-derived cloning vector containing *lacZ*α complementation fragment of pUC128	4
pRML200	RK2-derived *tetAR* cassette carried by pUC128	This study
pRML400	RK2-derived *tetAR* cassette carried by pHSG396	This study
pRML403	RK2-derived *tetAR* cassette carried by pHSG298	This study
pRML461	pHSG396 derivative containing a 655-bp *Cla*I-*Eco*RI fragment encoding *fptA* promoter and 5′-most 594 bp of *fptA*	This study
pSP329	pTJS75-derived IncP cloning vector	59
pUC128	Extended-polylinker pUC derivative	37
pUC21	Extended-polylinker pUC derivative	60

[a] *P. aeruginosa* phenotypes and genotypes: *fptA*, defective in the Fe(III)-pyochelin receptor, *pvd*, inability to produce pyoverdin; Pch$^-$, inability to produce pyochelin; Sal$^-$, inability to produce salicylic acid.
[b] GIBCO BRL, Gaithersburg, Md.

have been described previously (4). Long-term storage of bacterial cultures was at −80°C in 40% glycerol.

DNA manipulations and plasmid constructions. Plasmid isolation, transformation, triparental mating, use of restriction endonucleases and other DNA-modifying enzymes, agarose gel electrophoresis, cloning methods, and other in vitro DNA techniques were carried out as described previously (4). In order to specifically cut and clone DNA with *dam* methylase-sensitive sites, plasmids were passed through the *dam dcm E. coli* strain GM119. Electroporation of plasmids into *E. coli* was done with a Gene Pulser apparatus with a Pulse Controller (Bio-Rad, Richmond, Calif.) as recommended by the manufacturer. Electroporation of plasmids into *P. aeruginosa* was done similarly with the modifications reported by Diver et al. (22). Desired fragments from the recombinant pUC-type derivatives were subcloned into the mobilizable broad-host-range IncP cloning vector pRKY55 in order to allow replication in *P. aeruginosa*.

A tetracycline resistance cassette was constructed from a 2.1-kb *Stu*I-*Bgl*I fragment containing the *tetAR* gene of RK2 (63) as follows. pSP329, a mini-RK2 cloning vector, was digested to completion with *Stu*I, which cuts at the 3′ end of the *tetA* gene. This DNA was partially digested with *Bgl*I and blunt-ended with T4 DNA polymerase. A 2.1-kb fragment containing *tetAR* was cloned into *Eco*RV-digested pUC128 to yield pRML200. In order to have access to the *tetAR* cassette in the absence of the Ap^r determinant of pUC128, the *tetAR* cassette was cloned from pRML200 into the Cm^r pUC derivative pHSG396 and the Km^r pUC derivative pHSG298 to yield the Cm^r Tc^r derivative pRML400 and the Km^r Tc^r derivative pRML403, respectively.

Plasmid rescue procedure. A 3.6-kb *Eco*RI-*Bam*HI fragment including the 3′ end of the *fptA* gene was cloned into pUC21 to yield pRML231. The 2.1-kb *Eco*RI *tetAR* cassettes of pRML400 and pRML403 were cloned into *Eco*RI-digested pRML231 to yield pRML408 and pRML409, respectively. pRML408 and pRML409 were electroporated into *P. aeruginosa* IA602. The electroporated IA602 culture was resuspended in SOC broth, incubated at 37°C for 4 h, and then plated onto GG-tetracycline agar and incubated at 37°C for 48 h. Individual Tc^r transformants were picked and purified by restreaking. Genomic DNA preparations from these transformants were made by the hexadecyltrimethyl ammonium bromide (CTAB)-proteinase K method (6) and dissolved in TE (Tris-EDTA) buffer. To free the integrated pUC derivative and adjacent chromosomal sequences from the chromosome, genomic DNA from these transformants was digested to completion with *Bam*HI or *Pst*I and religated at a concentration of 5 μg/ml in order to effect intramolecular ligation events. The DNA was purified from the ligation mix, and 1 μg was electroporated into electrocompetent *E. coli* DH10B. Transformants bearing rescued plasmids were recovered after plating on LB plates with carbenicillin.

ssDNA purification. Single-stranded DNA (ssDNA) was prepared from pUC21- and pUC128-derived recombinant phagemids cloned into *E. coli* DH11S by using the helper phage VCSM13 (Stratagene, La Jolla, Calif.). M13-packaged phagemid DNA was precipitated from culture supernatants by the addition of 0.25 volume of 3.75 M ammonium acetate–20% polyethylene glycol 8000. ssDNA was purified by a modification of the CTAB-proteinase K method (6) for bacterial genomic DNA preparation. The precipitated phagemid pellet from a 20-ml culture was resuspended in 2 ml of TE buffer, and the CTAB protocol was followed, with appropriate adjustment of reagent volumes. After the $CHCl_3$-isoamyl alcohol (24:1) extraction step, the emulsion is centrifuged in Eppendorf tubes for 30 to 45 min to resolve the phases. After precipitation with isopropanol and washing with 70% ethanol, the ssDNA pellet is dissolved in 150 μl of TE buffer. This modified protocol gives a high yield of ssDNA which serves as an excellent template for DNA sequencing.

DNA sequencing. Sequence was obtained by using phagemid

ssDNA as the template. Sequencing primers were synthesized by phosphoramidite methodology on a Cyclone Plus DNA synthesizer (Millipore Corp., Burlington, Mass.). Sequencing was done with Sequenase version 2.0 (USB, Cleveland, Ohio) and [α-^{35}S]dCTP (New England Nuclear, Wilmington, Del.). Both strands of the target DNA were sequenced with dGTP and dITP mixes to resolve sequencing artifacts associated with high-G+C% DNA.

Protein sequencing. The outer membranes of CPS-grown IA602 were isolated, extracted with Triton X-100, and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (4). Electrophoretic transfer of separated outer membrane proteins to Immobilon-P polyvinylidene difluoride membranes (Millipore Corp.) for amino acid sequencing was performed in 25 mM sodium phosphate buffer (pH 7.2) for 2 h at 32 V and 1.0 A. Protein adhering to the membrane was stained with 0.1% Coomassie blue R-250 in 50% high-pressure liquid chromatography (HPLC)-grade methanol for 5 min, destained with 50% HPLC-grade methanol, and then rinsed extensively with deionized distilled water. Bands corresponding to the 75-kDa Fe(III)-pyochelin receptor were excised, and the N-terminal sequence was determined by gas-phase sequencing with an Applied Biosystems model 470A protein sequencer equipped with an on-line 120A PTH-Analyzer.

RNA purification, Northern (RNA blot) analysis, and primer extension. RNA was purified from *P. aeruginosa* by both the hot phenol extraction method (28) and the acid guanidinium thiocyanate-phenol-chloroform extraction method (13). *P. aeruginosa* IA602 cells were grown in 200 ml of 0.8% CPS–1 mM $MgSO_4$ with and without 30 μM $FeCl_3$ to an A_{600} of ~0.65 for RNA isolation. The isolated RNA was dissolved in diethylpyrocarbonate-treated water, and the concentration was determined by measuring the optical density of the solution at 260 and 280 nm. Northern blotting was carried out essentially as described previously (27, 48), with the modifications described by Jones and Jones (35). Nytran nylon membranes (Schleicher & Schuell, Keene, N.H.) were used for the blots, and radiolabeled DNA probes were generated by random primer labeling (25) with the Prime-a-Gene labeling system (Promega, Madison, Wis.) with [α-^{32}P]dCTP (New England Nuclear). Primer extension was carried out essentially as described by Gammie and Crosa (28). An oligonucleotide (5′-TCACCTTCGTCTCCGTTTTC-3′) complementary to the *fptA* mRNA was labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (New England Nuclear); this was used as the primer for primer extension. The same primer was used unlabeled to sequence *fptA* ssDNA by the sequencing methods described above. The primer extension and sequencing reactions were run in parallel to map the start site of the transcript.

DNA sequence and homology analysis. Sequence data bases at the National Center of Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health, Bethesda, Md., were searched with the BLAST algorithm (2). Sequence alignments and dot matrix presentation of data were executed with the Pustell protein and DNA matrices. Putative coding sequences were identified with the GCWIND program (50) on the sequence. The GCWIND program, similar to the FRAME program (8), predicts actual coding sequences based on the G+C content in the third position of codons in organisms with high G+C content.

Nucleotide sequence accession number. The nucleotide sequence of the *fptA* region has been deposited with the GenBank/EMBL data base under accession number U03161.

RESULTS

Isolation of the *fptA* gene. An earlier study from this laboratory reported that the gene encoding the Fe(III)-pyochelin receptor was cloned on a 3.6-kb *Eco*RI-*Bam*HI fragment by complementation of the Fe(III)-pyochelin transport defect in a mutant (Fpt$^-$) strain (4). Subclones of this fragment in the vector pRKY55 were used in further complementation analyses and demonstrated that a 1.7-kb region (an *Eco*RI-*Xho*I fragment) was sufficient for complementation of the Fpt$^-$ defect in IA613. Because a clone containing the entire gene encoding the 75-kDa Fe(III)-pyochelin receptor would have to be approximately 2.1 kb long, the entire gene was not present on this fragment. Subsequent sequence analysis of this 1.7-kb fragment (see below) demonstrated that the 3′ and 5′ ends of separate open reading frames (ORFs) were present and that only the 3′ coding region of the *fptA* gene had been cloned. Complementation of the Fpt$^-$ phenotype with the 3.6-kb *Eco*RI-*Bam*HI fragment at a frequency of 1 was observed and probably resulted from high-level recombination with the genome of the *recA*$^+$ *P. aeruginosa* strain IA613. Recombinational complementation was supported by the observation that the complementing 1.7-kb *Eco*RI-*Xho*I fragment had no recognizable promoter and gave rise to the Fpt$^+$ phenotype in either orientation from the *lac* promoter in pRKY55.

To clone the 5′ remainder of the *fptA* gene, the method of plasmid rescue was used. The plasmid rescue protocol was based on allelic exchange methods for nonenteric gram-negative bacteria (36) and is shown schematically in Fig. 1A. pUC-derived plasmids carrying the 3′ *fptA* coding region and a tetracycline resistance cassette were integrated into the genome of the *fptA*$^+$ strain IA602 by electroporation, followed by selection for Tc^r transformants. pUC-derived plasmids were used because of their inability to replicate as an extrachromosomal element in *P. aeruginosa*, and a tetracycline resistance cassette was used because the ampicillin resistance determinant on pUC plasmids is a poor selective agent in this bacterium. Genomic DNA from four independently isolated Tc^r transformants of IA602 was digested with *Bam*HI or *Pst*I (enzymes which did not cut within the cloned 3′ end of the *fptA* gene), ligated in dilute solution, and electroporated into *E. coli* DH10B, with selection for Ap^r transformants. The rescued plasmids from the various *Bam*HI digestions were identical by restriction enzyme analysis, as were those isolated from *Pst*I digestions. Internal sites in the rescued DNA were common to both *Bam*HI- and *Pst*I-rescued plasmids, as shown in Fig. 1B. The common identity of these rescued DNA fragments was confirmed by primer extension sequencing upstream of the *Eco*RI site in all plasmids.

N-terminal sequencing of the FptA protein. Outer membranes of IA602 were prepared from CPS-grown cells as described in Materials and Methods and further extracted with Triton X-100 to remove any cytoplasmic membrane contamination. The outer membrane proteins were separated by SDS-PAGE, and the proteins were electroblotted onto an Immobilon-P polyvinylidene difluoride membrane. The N terminus of the 75-kDa Fe(III)-pyochelin receptor was sequenced by gas-phase sequencing and determined to be DARK DGETELPDM. An independent N-terminal sequence analysis of the Fe(III)-pyochelin receptor by another laboratory resulted in the sequence AKDGETELPDMVIS (44a), which was very similar to our results.

Nucleotide sequence of the *fptA* region. A stretch of 4,312 bp was sequenced on both strands with dGTP and dITP and had an overall G+C content of 69.4%. The nucleotide sequence determined for the *fptA* region and the translated products of

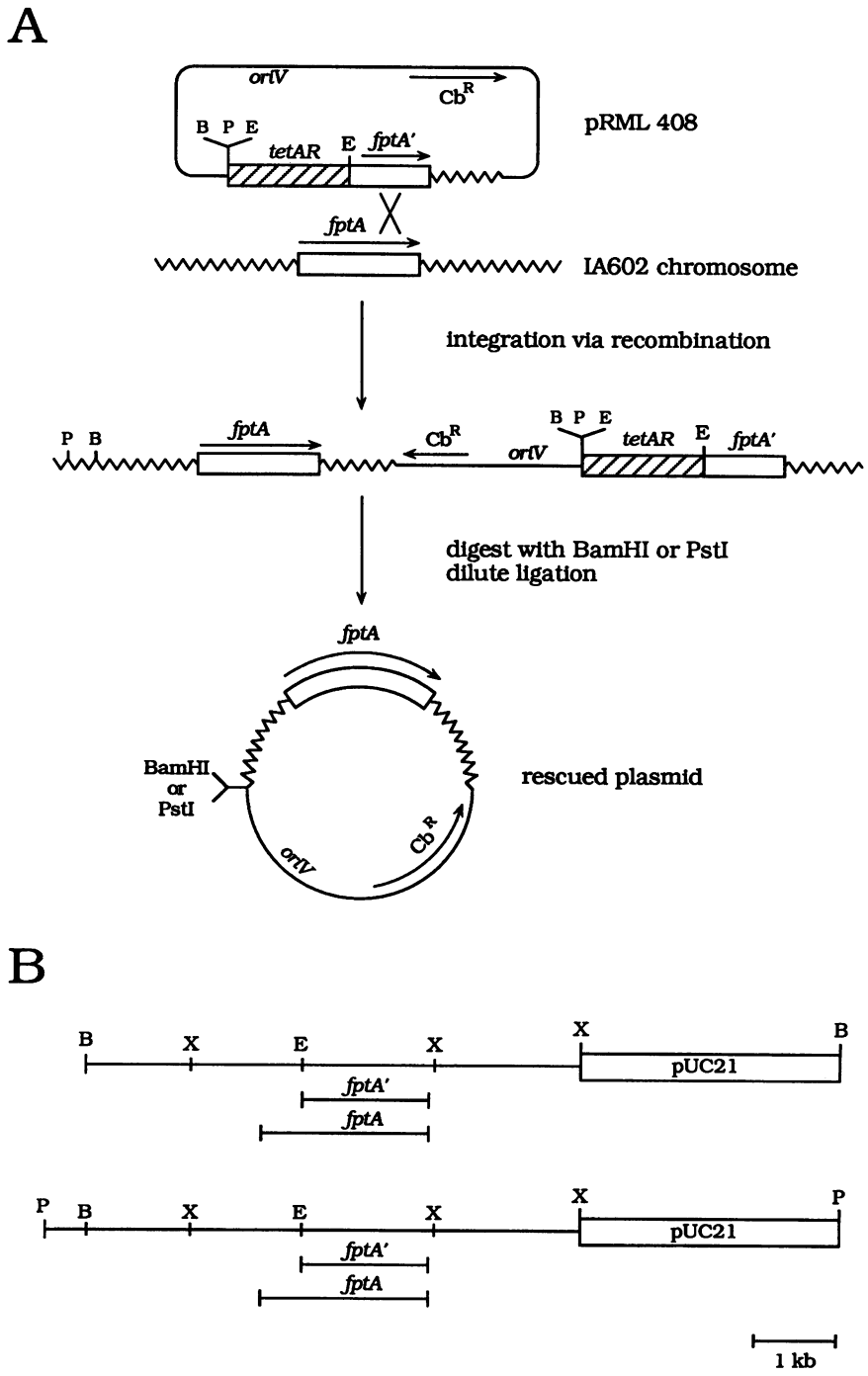

FIG. 1. Cloning of the complete *fptA* gene by plasmid rescue. (A) Plasmid rescue protocol. Plasmid pRML408 is a pUC derivative that is unable to replicate in *P. aeruginosa* and contains the 3′ end of *fptA* (*fptA′*) (see Materials and Methods for its construction). pRML408 was electroporated into the *fptA*$^+$ strain IA602, and plasmid integrants were selected as tetracycline-resistant transformants. Genomic DNA from these integrants was isolated, digested with either *Bam*HI or *Pst*I, and religated in dilute conditions. *Bam*HI and *Pst*I were chosen for two reasons: (i) neither *Bam*HI nor *Pst*I cuts within the cloned *P. aeruginosa* DNA in pRML408 (the extant *Bam*HI site had been removed by ligation with a *Bgl*II site) and sites for both *Bam*HI and *Pst*I were located 3′ of both the integrated pUC vector and the DNA targeted for rescue; (ii) *Bam*HI and *Pst*I both have G+C-rich recognition sites that cut *P. aeruginosa* DNA relatively frequently and therefore result in rescued plasmids with small inserts likely to be stable in pUC. The plasmid rescue protocol was also done with pRML409 (see Materials and Methods) with identical results. (B) Restriction map of rescued plasmids. The upper diagram represents rescued plasmids derived from *Bam*HI digestion, and the lower diagram shows those from *Pst*I digestions. The *fptA′* fragment originally cloned in pRML408 corresponds to the DNA between the *Eco*RI site and pUC21 in the rescued plasmids. Abbreviations: B, *Bam*HI; E, *Eco*RI; P, *Pst*I; X, *Xho*I; *fptA′*, 3′ end of the *fptA* gene; *tetAR*, RK2-derived tetracycline resistance cassette; Cb^R, carbenicillin and ampicillin resistance determinant.

the predicted coding sequences are shown in Fig. 2. The restriction map of the *fptA* region and the ORFs predicted by GCWIND analysis (50) to be actual coding sequences are shown in Fig. 3. GCWIND analysis predicts actual coding sequences based on the G+C content in the third position of codons in high-G+C organisms, as does the similar program FRAME (8).

ORF1 was unequivocally identified as the *fptA* gene when the translated product possessed the amino acid sequence DARKDGETELPDMVIS, which directly corresponded to the N-terminal sequence of the isolated Fe(III)-pyochelin receptor. The predicted *fptA* start codon is ATG, and a putative Shine-Dalgarno sequence, GAGG, was positioned 11 bases upstream of the methionine start codon. Although this proposed ribosome-binding site is somewhat different from the *E. coli* consensus Shine-Dalgarno sequence, many other *P. aeruginosa* genes have been reported to have similar ribosome-binding sites. Translation of the *fptA* coding region predicts a protein of 720 amino acids with a molecular mass of 79,987 Da and a pI of 5.8. The mature FptA protein lacking the 38 N-terminal amino acids has 682 amino acids with a mass of 75,993 Da and a pI of 5.2. This corresponds well with the observed size of 75 kDa on SDS-PAGE (4).

Hydrophobicity predictions by the method of Kyte and Doolittle (41) indicated that the mature protein was relatively hydrophilic, with no obvious transmembrane domains; similar observations have been made for a number of other outer membrane receptors (49). The leader peptide of FptA is 38 amino acids long and has all the characteristics of a bacterial leader peptide (62). There are seven basic amino acids in the N-terminal region, followed by an extended hydrophobic domain; a helix-breaking proline is at position −7 relative to the cleavage site, and alanine residues are present at positions −3 and −1. The mature FptA protein contained no cysteine residues and possessed a C-terminal phenylalanine, a feature common to many outer membrane proteins (56). The G+C content of the *fptA* coding region was 67%, and the codon usage demonstrated a strong bias for C and G in the third position of codons, as expected for high-G+C DNA. An AT-rich sequence located 31 bases upstream of the *fptA* start codon was virtually identical to the consensus *E. coli* Fur-binding site. This putative "iron box" in the *fptA* promoter, 5′-ATAATGATAAGCATTATC-3′, matched the consensus Fur-binding site, 5′-ATAATGATAATCATTATC-3′ (10, 20), at 17 of 18 positions.

ORF2 appears to be translationally coupled to the *fptA* gene, as the predicted start codon of ORF2 (ATG) and the termination codon of *fptA* (TGA) overlap. The nucleotide sequence of ORF2 predicts a protein of 93 amino acids with a mass of 9,569 Da. The nucleotide sequence of ORF3 predicts a protein of 394 amino acids with a mass of 42,247 Da. The predicted proteins of both ORF2 and ORF3 are very basic, with pIs of approximately 12, and both are very hydrophobic, on the basis of Kyte-Doolittle analysis. It is unknown whether either of these ORFs has any function in Fe(III)-pyochelin transport, but the translational coupling of ORF2 to *fptA* suggests that this protein may have some function in Fe(III)-pyochelin transport. However, attempts to specifically inactivate ORF2 by gene replacement or gene interruption were unsuccessful.

The *fptA* gene mapped to the *Spe*I-A fragment and the *Dpn*I-F doublet (F_1 assumed) fragments of the PAO map (23) by contour-clamped homogenous electric field pulsed-field gel electrophoresis and Southern hybridization methods, as reported earlier by Farinha et al. (24). The probe for hybridization was generated by random primer labeling of a 655-bp *Cla*I-*Eco*RI fragment (Fig. 2 and 3) containing the *fptA* promoter and 594 bp downstream of the transcriptional start site. This mapping places *fptA* at 21.7 to 24.6 min on the recalibrated genetic map and at kb 687 to 921 relative to *oriC* on the physical map.

Identification of the *fptA* transcriptional start site. To determine the transcriptional start site of the *fptA* promoter, primer extension was used to map the 5′ end of the RNA isolated from iron-starved *P. aeruginosa*. RNA was not isolated until the *P. aeruginosa* culture reached the slower rate of exponential growth associated with iron depletion and expression of siderophore biosynthesis and uptake systems, which occur at an A_{600} of ~0.65 in CPS broth (15). A radiolabeled synthetic oligonucleotide complimentary to a sequence approximately 50 to 70 bases downstream of the suspected −10 region was extended with reverse transcriptase. Comparison of the product on a DNA sequencing gel with the sequence produced with the same primer in ssDNA sequencing indicated that the cDNA transcript comigrated with an A residue (Fig. 4A). Therefore, the transcriptional start site is a T residue within the iron box of the *fptA* promoter (Fig. 4B).

Transcriptional regulation and Northern analysis of *fptA*. The presence of a highly conserved iron box in the *fptA* promoter suggested that *fptA* would be strongly regulated by iron, as Fur-regulated promoters are in *E. coli*. Therefore, the regulation of the *fptA* gene was analyzed by Northern analysis of cellular RNA. As done with the transcriptional start site determination above, RNA was not isolated until the *P. aeruginosa* culture reached the slower rate of exponential growth associated with iron depletion and expression of siderophore biosynthesis and uptake systems, which occur at an A_{600} of ~0.65 in CPS broth (15). In a parallel culture containing 30 μM $FeCl_3$, RNA was similarly isolated at an A_{600} of ~0.65. RNA samples (5 and 20 μg) from both the iron-starved and iron-replete cultures were electrophoresed in a formaldehyde-agarose gel and transferred to nylon membranes by standard Northern-blotting methods. The probe for hybridization was generated by random primer labeling of a 655-bp *Cla*I-*Eco*RI fragment (Fig. 2 and 3) containing the *fptA* promoter and 594 bp downstream of the transcriptional start site.

An autoradiogram of the Northern blot is shown in Fig. 5. The blot shows that *fptA* transcription is strongly regulated by iron, as RNA isolated from cells grown in 30 μM $FeCl_3$ (Fig. 5, lanes 1 and 2) shows no hybridization to the probe, whereas the RNA from iron-starved cells (Fig. 5, lanes 3 and 4) demonstrates strong hybridization to the probe. The autoradiogram revealed a band corresponding to a transcript of ~2.5 kb which corresponds well with *fptA* and ORF2 existing as an operon; a transcript encoding only *fptA* would have a size of ~2.2 kb.

Northern blotting with RNA isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method (13) from iron-starved cells routinely gave the smearing observed in Fig. 5. Even more extensive smearing and no bands were observed with RNA obtained by the hot phenol extraction method (28). Ethidium bromide staining of the formaldehyde-agarose gel before blotting showed intact, undegraded rRNA bands when RNA isolated by the guanidinium thiocyanate method was used, a highly valued method for the efficient isolation of RNA. Difficulties in transcript size determination and similar degradation of iron-regulated transcripts and RNA isolated from iron-starved cells have been observed in investigations of the *ent* and *fep* operons of *E. coli* (11); rapid mRNA turnover rates occurring specifically during iron starvation have been hypothesized.

Homology analysis. The FptA sequence was compared with

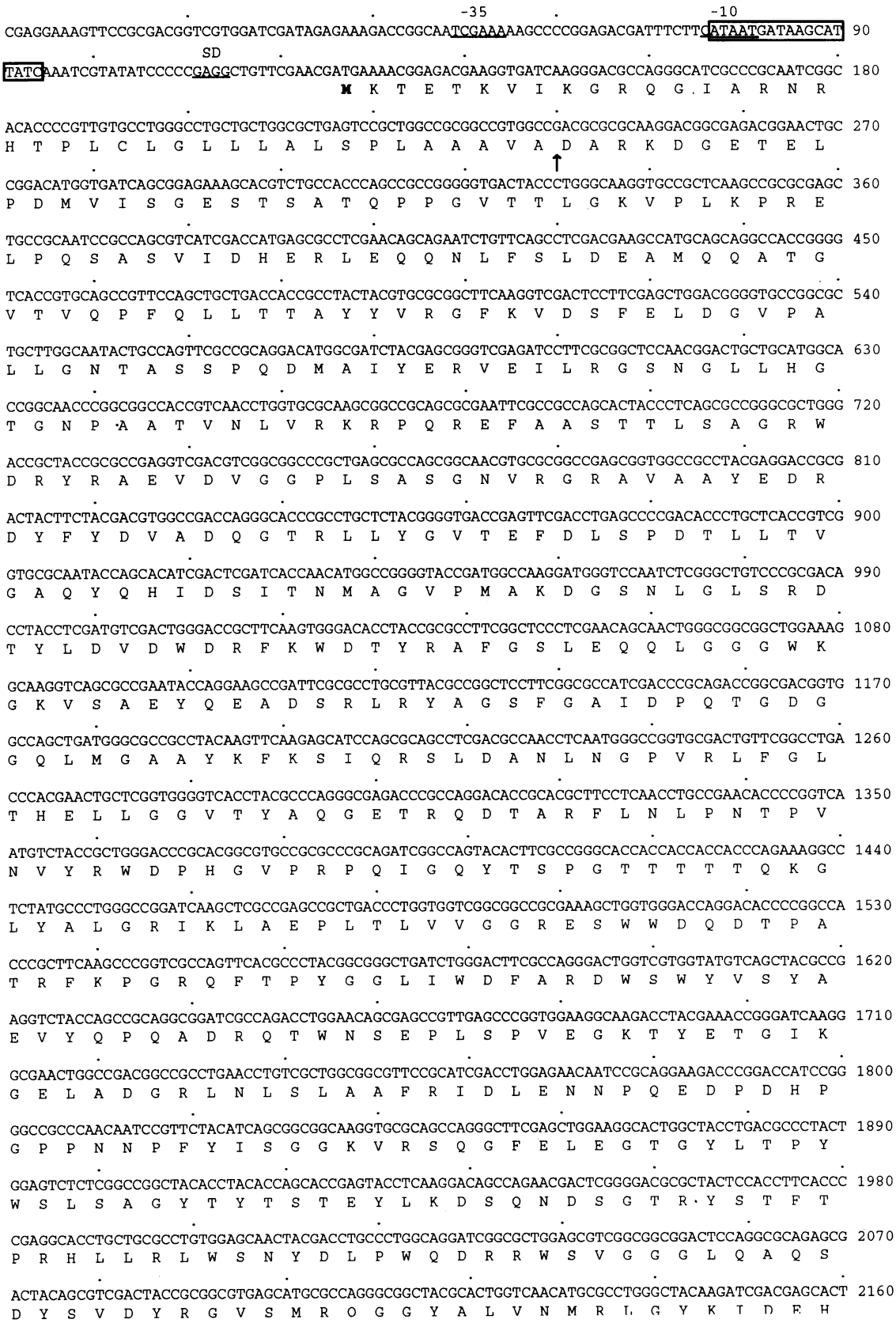

FIG. 2. Nucleotide sequence of the *fptA* region and the deduced amino acid sequence of proteins predicted by GCWIND analysis. The nucleotide sequence is marked every 20 bases for reference. The amino acid sequence is shown in the single-letter code, with the N-terminal methionines (**M**) and stop codons (*) shown in boldface type. The leader peptide cleavage site in FptA is shown as a vertical arrow near nucleotide

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GGACGGCGGCGGTCAACGTCAACAACCTGTTCGACCGGACCTACTACCAGAGCCTGTCCAACCCCAACTGGAACAACCGCTACGGCGAAC 2250
W  T  A  A  V  N  V  N  N  L  F  D  R  T  Y  Y  Q  S  L  S  N  P  N  W  N  N  R  Y  G  E

CGCGCAGCTTCAACGTCAGCCTGCGGGGCGCGTTCTGATGCCGCGCCAGTCGGGCTTCGGCTGGGCCTGGCGGGTTCCGCTGGCGCTCGC 2340
P  R  S  F  N  V  S  L  R  G  A  F  *
                                   M  P  R  Q  S  G  F  G  W  A  W  R  V  P  L  A  L  A

CGGCAGCCTGGCGGCGGCGACCGCCAGCGGCTATCTGTTGACTCGCGGGCTGCCCCTGGACGACCCGCTCGAGCGTCTCTACGCCGGGCT 2430
  G  S  L  A  A  A  T  A  S  G  Y  L  L  T  R  G  L  P  L  D  D  P  L  E  R  L  Y  A  G  L

CTTCGGCGCGCTCGGGGTGGGCTTGTTGCTGCTTGTCGGCGGGTTGCTGGCGCGTGGTCCGGGCAACTTCGCCTGGCGCCTGGGCGGTAG 2520
  F  G  A  L  G  V  G  L  L  L  L  V  G  G  L  L  A  R  G  P  G  N  F  A  W  R  L  G  G  S

CCTGCTGGTACTGGGCTTGGCGTTGTGGCTGCTGGCGGGGCGCGGTTGAAGTGGCGGCGCGTCCTCTCCTGCTCTCGCTGCACGGTGGCG 2610
  L  L  V  L  G  L  A  L  W  L  L  A  G  R  G  *

CCGGCGCGCTGTTCGGCGTGTTGCTGTTCGTGGTGCTGTTCAGTGGCGCCTGGAGTCTTGGCCACGACGACCTGCGCGAGTGGCTGCGGG 2700

CCCCGGCGCAGGCGGGAGGAGAAGCCCTTGCGCTGGAGCGCCTGCTCGAACGGGCGGGCGAGGAAGGCGTCGACATCCGCGATGCGACCC 2790

TGCTGCTGCCCGCTCCCGGCCATGCCGCCTTCAGCGTCTGCGATGCGCGCCTGGACTGCCGGCTGGACCTCGACCCGGCCAGCGGCCGGG 2880

TGCTGCCACCGATGCCGGCGCTGGACCTCCTGCTGAACCTGCACAAGAGCCTGTTCGTCGGCTTCCCCGGGCGGGTGCTGGTCAGCCTGT 2970
          M  P  A  L  D  L  L  L  N  L  H  K  S  L  F  V  G  F  P  G  R  V  L  V  S  L

TCGGCGTATCGCTGTTGCTGCTGTGCCTGGCCGGCGTGCTGCTGCACAGCCGCCGCTGGCGCGACCTGCGGCGTTGGCGGCGGGATCGCG 3060
F  G  V  S  L  L  L  L  C  L  A  G  V  L  L  H  S  R  R  W  R  D  L  R  R  W  R  R  D  R

GGCTGCGCCTGGCGCTGTTCGACCTGCATGGCCTGATCGGCATCTGGGGACTGCCCTGGCTGCTGTTGTTCGGTTTCACCGGCGCGCTCA 3150
G  L  R  L  A  L  F  D  L  H  G  L  I  G  I  W  G  L  P  W  L  L  L  F  G  F  T  G  A  L

GCGGGCTGGGCGCTCTCGGGACCCTGCTGCTGGCGCCGGTGGCCTACCCGCAGGAACCGAACCGGGTGTTCGTCGAGTTGATGGGACCGC 3240
S  G  L  G  A  L  G  T  L  L  L  A  P  V  A  Y  P  Q  E  P  N  R  V  F  V  E  L  M  G  P

CGCCGCCCGCCGCCGAGGGGCGGCCATTGGCGTCCCGCATCGATCTCGACCGCCTGCTTGCCGGCGATGCCGTGCGGGCGCCCGGCTTCG 3330
P  P  P  A  A  E  G  R  P  L  A  S  R  I  D  L  D  R  L  L  A  G  D  A  V  R  A  P  G  F

TCGCCCAGCGTTTGAGCCTCAGTCATGCCGGGGATGTCGCCGGCAGCGTGGAGATCGCCGGTATCCGGCGTGGCCTGCCGAGCACCGCGA 3420
V  A  Q  R  L  S  L  S  H  A  G  D  V  A  G  S  V  E  I  A  G  I  R  R  G  L  P  S  T  A

ACTTCGAGCGGCACCGCTATCGGCTGGCCGACGGCACCCTGCTCGGCGAGCGCAGCTCGGCGCAGCGCGGTTTCTGGCTGCGCGCCTTCA 3510
N  F  E  R  H  R  Y  R  L  A  D  G  T  L  L  G  E  R  S  S  A  Q  R  G  F  W  L  R  A  F

TCGCCGTGCAGCCGTTGCATTTCGCCCAGTACCAGTGGCTCGGGCCGGGCTGGTCCGCCGCGTTGCGCGGCCTGCACCTGGCGATGGGCC 3600
I  A  V  Q  P  L  H  F  A  Q  Y  Q  W  L  G  P  G  W  S  A  A  L  R  G  L  H  L  A  M  G

TCGGCGCCTGCCTGCTCTGCGCCAGCGGCCTGTACCTGTGGCTGCAACGACGCGCCCTCGGCGCCGGACGCCCGCGTACGGCTCTTGCAGC 3690
L  G  A  C  L  L  C  A  S  G  L  Y  L  W  L  Q  R  R  A  S  A  P  D  A  R  V  R  L  L  Q

GCCTGAGCCAGGGTTTCTGTGCCGGCCTGGTGGCGGCGGCCGCGTTGCTCCTGCTGGGGCTGCAACTCGCCCCCTCGGAGCTACTCGCCG 3780
R  L  S  Q  G  F  C  A  G  L  V  A  A  A  A  L  L  L  L  G  L  Q  L  A  P  S  E  L  L  A

GACCCTGGCCGGGGCGGCTGTTCCTTGTCCTGTGGGCCGCCGCTGGCCTGGCGGCGTTGCTGCTGCCGGGCGACTGGCCGCTGGCGCGCG 3870
G  P  W  P  G  R  L  F  L  V  L  W  A  A  A  A  G  L  A  A  L  L  L  P  G  D  W  P  L  A  R

GATTGCTCGGCGTCGCCGGGCTGGCCTGCCTGGCGGCCGCCGTCGCGCACCTGGCGCCCTGGCTCATGCGCGGCCGGCTGCCGGCCCTGG 3960
G  L  L  G  V  A  G  L  A  C  L  A  A  A  A  V  A  H  L  A  P  W  L  M  R  G  R  L  P  A  L

GCCCCGACCTCACCCTGATCCTCTGCGGCGCGCTGCTCATCCGGCACGCCTGGATGCAGGCGCGCGCCGCCGCGCCACCCGCCCACCCCC 4050
G  P  D  L  T  L  I  L  C  G  A  L  L  I  R  H  A  W  M  Q  A  R  A  A  A  P  P  A  H  P

GTGTCACCGGAGACCACCATGCTTGAGCTGTACCGCCACCGCCGCCTGGTCATCACCCTGGCGTTGCTCTACCTGTCCCAGGGCATTCCC 4140
R  V  T  G  D  H  H  A  *

ATCGGCCTGGCCATGGACGCCCTGCCCACCCTCCTGCGCCAGGATGGCGCGCCATTGCAGGCGCTGGCCTTCCTGCCCCTGGTGGGGCTG 4230

CCCTGGGTGGTCAAGTTCCTCTGGGCACCCTGGGTCGACAATCACTGGTCGCGCCGTCTCGGCCGGCGGCGTAGCTGGATCC 4312
```

250. A sequence highly homologous to the *E. coli* Fur-binding site is shown within the box at nucleotides 77 to 94. The putative −35 and −10 promoter sequences and Shine-Dalgarno sequence of the *fptA* gene are underlined at nucleotides 49 to 54, 78 to 83 (within the box), and 111 to 114, respectively.

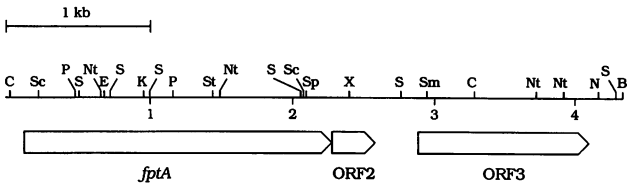

FIG. 3. *fptA* region of *P. aeruginosa*. The restriction map of the sequenced *fptA* region is shown at the top. The ORFs predicted by GCWIND analysis (50), *fptA*, ORF2, and ORF3, are shown below the restriction map, with the direction of transcription indicated. Abbreviations: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; K, *Kpn*I; N, *Nco*I; Nt, *Not*I; P, *Pvu*II; S, *Sal*I; Sc, *Sac*II; Sm, *Sma*I/*Xma*I; Sp, *Sph*I; St, *Stu*I; X, *Xho*I.

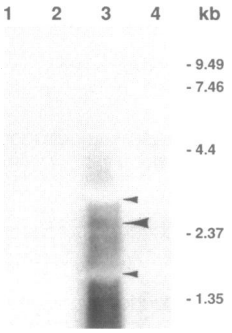

FIG. 5. Northern analysis and transcriptional regulation of *fptA*. RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method (13) from IA602 cells and subjected to Northern analysis as described in Materials and Methods. The probe included ~600 bases complementary to *fptA* mRNA. Lanes: 1, 20 μg of RNA from cells grown in 30 μM $FeCl_3$; 2, 5 μg of RNA from cells grown in 30 μM $FeCl_3$; 3, 20 μg of RNA from iron-starved cells; 4, 5 μg of RNA from iron-starved cells. Sizes of RNA standards are shown on the right (in kilobases). The two small arrowheads show the position of the rRNA species at 1.5 kb (lower) and 2.9 kb (upper), and the large arrowhead indicates the position of the *fptA* transcript.

the complete sequence data bases at NCBI by using the BLAST algorithm. The homology searches revealed that FptA had significant homology to various pyoverdin and pseudobactin receptors of the fluorescent pseudomonads (FpvA, PupA, and PupB) and the FhuE protein of *E. coli*; lower homology to the FhuA protein of *E. coli* and the FoxA protein of *Yersinia enterocolitica* was observed. All of these proteins are receptors for hydroxamate siderophores: FpvA of *P. aeruginosa* is the receptor for pyoverdin (45); PupA of *P. putida* is the receptor for pseudobactin 358 (9); PupB of *P. putida* is the receptor for pseudobactins BN7 and BN8 (39); FhuE recognizes coprogen, rhodotorulic acid, and ferrioxamine E (49); FhuA recognizes ferrichrome (14); and FoxA serves as the receptor for ferrioxamine (7). Dot matrix projection of the homology data show strong diagonals for FptA versus FpvA, PupA, PupB, and FhuE and weaker ones for FptA versus FhuA and FoxA (Fig. 6). As a separate control, an alignment of FptA with FepA, the *E. coli* receptor for enterochelin/enterobactin, was also calculated, and no similarity was observed (Fig. 6). Comparison of FptA with FcuA, the ferrichrome receptor of *Y. enterocolitica* (38), and FatA, the anguibactin receptor from *Vibrio anguillarum*, showed a similar lack of homology.

Siderophore receptors are believed to interact with the cytoplasmic membrane protein TonB to facilitate the movement of the outer membrane-bound ligand into the periplas-

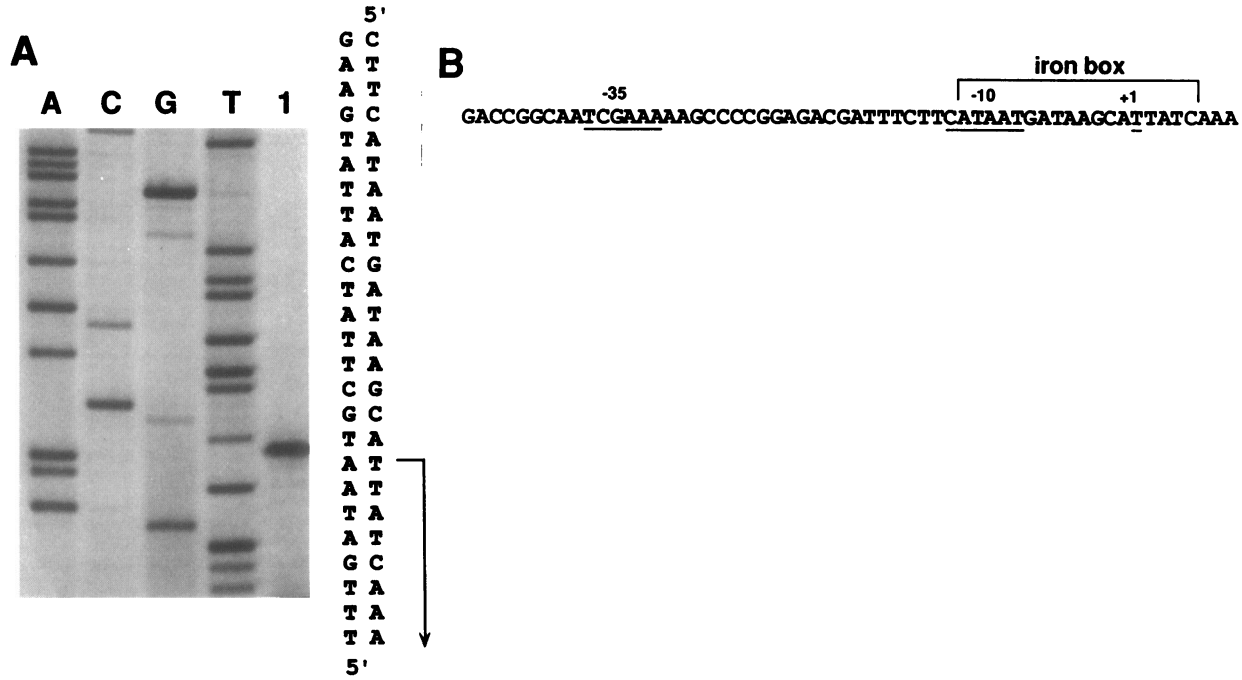

FIG. 4. Identification of the transcriptional start site of *fptA*. (A) Primer extension analysis of *fptA* mRNA. Primer extension was done as described in Materials and Methods. Lanes A, C, G, and T are sequencing ladders generated from *fptA* ssDNA, and lane 1 is the primer extension reaction. To the right of the autoradiogram is the double-stranded DNA sequence of this region, with the transcriptional start site and direction of transcription indicated by the arrow. (B) Promoter region of *fptA*. Putative −35 and −10 regions are indicated. The putative Fur-binding site is indicated (iron box), and +1 indicates the transcriptional start site.

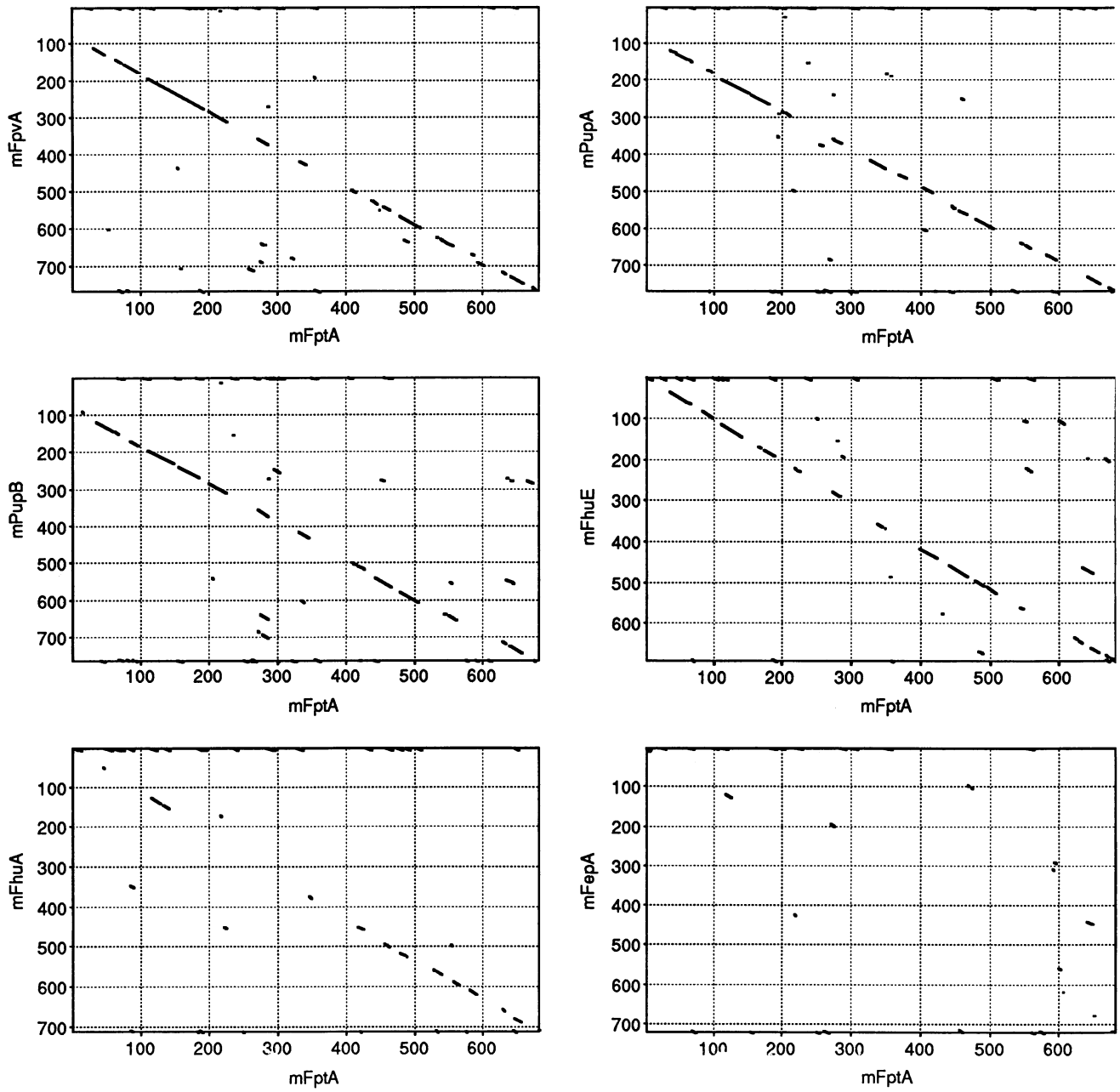

FIG. 6. Homology between FptA and other siderophore receptors. Dot matrix presentations of homology alignments of FptA versus FpvA, PupA, PupB, FhuE, FhuA, and FepA. Dot matrix analysis was done with the Pustell protein matrix with the following settings: window size, 15; minimum percent score, 40; hash value, 1; scoring matrix, pam250 matrix.

mic space. Siderophore receptor proteins possess an N-terminal sequence that is proposed to interact with TonB, which has been termed the TonB box. Although somewhat degenerate, the consensus TonB box can be represented as (D/E)TXX VXA(A/S), X is variable. Although FptA is quite similar to the TonB-dependent siderophore receptors, the FptA protein does not possess a consensus TonB box. The only other receptors lacking a consensus TonB box are the closely related FpvA and PupB.

BLAST analysis of the predicted proteins from ORF2 and ORF3 did not reveal any significant homologies within the complete sequence data bases at NCBI.

DISCUSSION

The Fe(III)-pyochelin receptor of *P. aeruginosa* has been identified biologically in studies with mutants that are unable to transport Fe(III)-pyochelin and lack this 75-kDa outer membrane protein (4, 31) and physically in experiments showing that the purified protein bound Fe(III)-pyochelin in protease protection assays (31). The current study unequivocally confirms the assignment of the 75-kDa outer membrane protein as the Fe(III)-pyochelin receptor via N-terminal sequencing of the purified protein and sequencing of the *fptA* gene.

	I		II		
BtuB	4	SPDTLVVTAN	BtuB	565	IANLFDKRY
Cir	4	DGETMVVTAS	Cir	608	VLNLFDKDL
FatA	12	ADESITVYGQ	FatA	658	VNNVTDEAY
FcuA	28	TNDTITVVGA	FcuA	687	IENVTNERY
FecA	21	SGFTLSVDAS	FecA	705	VKNIFDQDY
FepA	10	HDDTIVVTAA	FepA	674	VDNLFDKRL
FhuA	5	DEDTIVVTAA	FhuA	680	VNNLFDREY
FoxA	1	-DDTIEVTAK	FoxA	650	VNNIADKKY
IrgA	6	TDETMVVTAA	IrgA	597	VYNLFDQEV
IutA	4	DDETFVVSAN	IutA	660	IENLFDRDY
PfeA	12	GEQTVVATAQ	PfeA	678	VDNLFDKRL
TbpA	12	QLDTIQVKAK	TbpA	842	VYNLLNHRY
FhuE	4	TEETVIVEGS	FhuE	661	VNNLFDKTY
PupA	66	DGNTVTVTAS	PupA	739	VNNIFDKKY
PupB	70	ANASYSLQAS	PupB	732	LNNVFDREY
FpvA	66	QGNAITISVA	FpvA	736	VNNVFDKTY
FptA	5	DGETELPDMV	FptA	647	VNNLFDRTY
		+++ + +			+ *+ +

	III		ΔI/III
BtuB	105	QRVEYIRGPRSAVYG---SDA-IGGVVNIITTR	101
Cir	106	ERIEVVRGPMSSLYG---SDA-LGGVVNIITKK	102
FatA	124	QRIDVLKGPASLLNGMPPNGS-VGGSINLVTKR	112
FcuA	149	ERVEVFKGANAFINGISPSGSGVGGMINLEPKR	121
FecA	190	DAIDVVRGGGAVRYG---PQS-VGGVVNFVTRA	169
FepA	120	ERIEVLRGPARARYG---NGA-AGGVVNIITKK	110
FhuA	127	ERAEIMRGPVSVLYG---KSS-GPGLLNMVSKR	122
FoxA	121	ERIDVIKGPSSALYG---QSI-PGGVVMMTSKR	121
IrgA	110	ERIEVIRGPMSTLYG---SDA-IGGVINIITRK	104
IutA	103	HHIEVIFGAT-SLYG---GGS-TGGLINIVTKK	99
PfeA	122	ERIEVIRGPAAARYG---NGA-AGGVVNIITKQ	110
TbpA	125	KAVEISKGSNSVEQG---SGA-LAGSVAFQTKT	113
FhuE	120	ERVEVVRGATGLMTG---TGN-PSAAINMVRKH	116
PupA	202	DRIEIVRGATGLMTG---AGD-PSAVVNVIRKR	136
PupB	201	DRVEIVRGATGLISG---MGN-PSATINLIRKR	131
FpvA	201	DRVEVLKGATGLLTG---AGS-LGATINLIRKK	135
FptA	116	ERVEILRGSNGLLHG---TGN-PAATVNLVRKR	111
		+++++++*+ + * ++ +++ +	

FIG. 7. Alignments of conserved regions among TonB-dependent outer membrane receptors and FptA. Regions I, II, and III are those designated by Bitter et al. (9). The numerals following the receptor designations indicate the first residue of the indicated region in the mature protein. Region I is the sequence previously designated the TonB box (42). The column headed ΔI/III indicates the number of amino acids between the first residues of regions I and III. A + below the sequences indicates a conserved residue (unique or class of amino acid) in 15 of 17 of the receptor sequences, and an asterisk (*) indicates an identical residue in all receptor sequences. The sequences were obtained from references 9 and 45 except for FatA (40), FcuA (38), IrgA (29), PfeA (19), TbpA (44), and PupB (39). The receptors are listed in alphabetical order except for FhuE, PupA, PupB, FpvA, and FptA, which are grouped together because of their strong homologies.

Although quite homologous to other siderophore receptors, the FptA protein is unusual in many aspects. Pyochelin, although structurally unique, can be classed loosely with other phenolate siderophores, based on the presence of either 2,3-dihydroxybenzoic acid or salicylic acid. Siderophores such as vibriobactin, parabactin, agrobactin, and mycobactin possess 2-(*o*-hydroxyphenyl)-2-oxazoline groups (or closely related derivatives), which can be viewed as having some structural similarities with pyochelin. Since pyochelin is a phenolate siderophore, it was reasonable to expect a closer relationship between FptA and other phenolate/catecholate siderophore receptors than between FptA and hydroxamate siderophore receptors. However, the homology searches clearly show that FptA has significant overall homology to hydroxamate siderophore receptors, while no homology was observed between FptA and receptors for catecholate siderophores.

The hydroxamate siderophores recognized by FpvA (pyoverdin), PupA (pseudobactin 358), PupB (pseudobactins BN7 and BN8), and FhuE (coprogen, rhodotorulic acid, and ferrioxamine E) are relatively large extended-chain structures possessing multiple amide bonds and a polypeptide character, with the chelating groups extending from the chain. These siderophores are all hydrophilic compounds, a common character among the hydroxamates. In contrast to these structures, pyochelin is very compact and hydrophobic, lacking any recognizable similarity to the extended-chain structures discussed above. Although the primary structures of these hydroxamate siderophores and pyochelin are distinctly different, it is possible that once the Fe(III) chelates of these different siderophores are formed, the molecular surfaces presented to the bacterium may be similar.

The homology between FptA and hydroxamate siderophore receptors indicates that predictions of siderophore class (hy-

droxamate or phenolate) recognition based on the primary sequence structure of a receptor are unreliable. From the primary structure of FptA, it could be predicted that the siderophore recognized by this receptor would be a hydroxamate. The results presented in this article stress the requirement for functional assays with receptor mutants and the isolated receptor in assigning a functional identity to siderophore receptors. Since pyochelin is structurally unique, its interaction with its receptor may also be unique among siderophores. However, Koebnik et al. (38) recently reported that the ferrichrome receptor FcuA of *Y. enterocolitica* was significantly homologous to FatA, the anguibactin receptor of *V. anguillarum*. The dissimilar structures of ferrichrome and anguibactin indicate that there is no necessary relationship between receptor structure and ligand specificity among siderophores and their receptors.

The strong regulation of *fptA* gene transcription by iron and the presence of a highly conserved Fur-binding site in the *fptA* promoter provide additional evidence of the importance of the Fur protein in iron regulation in *P. aeruginosa* (46, 47). Expression of the FptA protein is iron repressible (31), and its probable regulation by Fur, as evidenced by the presence of the putative Fur-binding site, fits these observations well. The location of the transcriptional start site and −10 region within this Fur-binding site would result in promoter occlusion, preventing both promoter recognition and transcriptional initiation. Such an arrangement has been recognized as providing strong repression of the Fur-regulated *fep* and *ent* promoters in *E. coli* (12). The recent discovery of a transcriptional activator of pyochelin and FptA biosynthesis, PchR (30), indicates that both positive and negative transcriptional regulation is occurring at the *fptA* promoter. The presence of both positive (PchR) and negative (Fur) regulatory systems in *fptA* expression is unusual and will be a continuing subject of investigation.

The hierarchy of homologies between FptA and other siderophore receptors strongly support the evolutionary relationships proposed by Koebnik et al. (38). FptA probably has a common ancestor with FhuE, FpvA, PupA, and PupB and is more distantly related to FhuA and FoxA. FptA has significant homology to the family of TonB-dependent outer membrane receptors; specific regions appear to be highly conserved throughout this family (9, 42, 45) (Fig. 7). Homology analysis and alignment of conserved domains (determined by the distances between regions I and III) indicate that three of these receptors, FptA, FpvA, and PupB, lack a consensus TonB box at their N termini. The absence of a consensus TonB box in these proteins is unusual in view of its high conservation among other siderophore receptors. It remains a possibility that the alignment of FptA, FpvA, and PupB in region I (Fig. 7) represents extremely degenerate TonB boxes or that transport mediated by these receptors is TonB independent. However, it is probable that transport via these receptors is TonB dependent and that regions II and III interact with TonB. The testing of these hypotheses in *P. aeruginosa* awaits the identification of a TonB analog and corresponding mutants.

The homology between FptA and the various pyoverdin/pseudobactin receptors of the fluorescent pseudomonads (FpvA, PupA, and PupB) is of special interest because of the close evolutionary relationship between *P. putida* and *P. aeruginosa*. Although similar at the amino acid level, *fptA* and the genes for the fluorescent siderophore receptors show little similarity at the nucleotide level. The G+C contents of the coding sequences are distinct (*pupA*, 55.3%; *fpvA*, 60.9%; and *fptA*, 67%), as are the codon usages. The nucleotide content, codon bias, and chromosomal map position suggest that *fptA* has long been present in the *P. aeruginosa* genome. In contrast, the transport genes for the fluorescent siderophores (pyoverdin/pseudobactin) are likely to have been recent acquisitions in the evolution of *Pseudomonas* rRNA homology group I. The *P. aeruginosa pvd* (3, 32, 61) and *P. putida pvd* (55) loci are located within the "catabolic-rich" regions of their respective genomes, and therefore the hypothesis that the genes for fluorescent siderophores are recent acquisitions in the evolution of *Pseudomonas* rRNA homology group I is in accord with the gene accretion model of pseudomonal genome evolution proposed by Holloway and Morgan (33).

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