JOURNAL OF BACTERIOLOGY, May 1996, p. 2586–2592 Vol. 178, No. 9
0021-9193/96/$04.00+0

PchR, a Regulator of Ferripyochelin Receptor Gene (*fptA*) Expression in *Pseudomonas aeruginosa*, Functions Both as an Activator and as a Repressor

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Received 4 December 1995/Accepted 28 February 1996

The product of the *pchR* gene, an AraC-like regulatory protein, is required for production of the FptA ferric pyochelin receptor in response to iron limitation and pyochelin (D. E. Heinrichs and K. Poole, J. Bacteriol. 175:5882–5889, 1993). The influence of iron, pyochelin, PchR, and FptA on *fptA* and *pchR* gene expression was assessed with *fptA-lacZ* and *pchR-lacZ* transcriptional fusions. As was expected, the expression of *fptA* decreased dramatically following the inactivation of *pchR* by the insertion of an ΩHg cartridge, although the effect (>10-fold) was not as dramatic as that of pyochelin deficiency, which obviated *fptA* gene expression. Insertional inactivation of *pchR* in a pyochelin-deficient (Pch^-) background restored *fptA* expression to levels observed in the pyochelin-producing (Pch^+) $PchR^-$ strain, suggesting that PchR represses *fptA* expression in the absence of pyochelin. Consistent with this, the cloned gene caused a five-fold decrease in the expression of the *fptA-lacZ* fusion in *Escherichia coli*. *pchR* gene expression was inducible by iron limitation, a result in agreement with the previous identification of a Fur box upstream of the gene, although the magnitude of the induction was less than that observed for *fptA* in response to iron limitation. Expression of *pchR* was effectively absent in a pyochelin-deficient strain, and insertional inactivation of *pchR* in a Pch^+ or Pch^- background caused an increase in *pchR* gene expression. PchR, thus, negatively regulates its own expression. Two related heptameric sequences, CGAGGAA and CGTGGAT, were identified upstream of the putative −35 region of both *fptA* and *pchR* and may function as a binding site for PchR. Insertional inactivation of *fptA* caused a marked decrease in *fptA* expression in a Pch^+ background and obviated the apparent repression of *fptA* expression in a Pch^- background, reminiscent of the effect of a *pchR* mutation. The *fptA* mutant did not, however, exhibit a defect in *pchR* expression. Interestingly, *fptA* mutants were unable to grow in the presence of pyochelin, suggesting that FptA is the sole outer membrane receptor for ferric pyochelin. These data indicate that PchR functions as both an activator and a repressor in controlling the expression of *fptA* and *pchR*. The involvement of FptA in this control is unclear, although it may be important in mediating the pyochelin effect on *fptA* expression, possibly by modulating PchR activity.

Most bacteria, with the possible exception of some lactobacilli, require iron for growth and must obtain it from their environment (41). This requirement is complicated by the extremely low solubility of iron in nature (41) and, in the case of pathogenic microorganisms, by the iron-limiting nature of the host (22, 34, 47). To obtain iron in what are, thus, extremely dilute conditions, many bacteria synthesize high-affinity iron chelators termed siderophores together with cell surface receptors specific for the iron-siderophore complexes (39, 40). Given the importance of iron for growth and, therefore, infection, it is not surprising that siderophore-mediated iron uptake systems are important determinants of virulence in many bacterial pathogens (16, 17, 21, 30, 32).

Pseudomonas aeruginosa, a ubiquitous gram-negative rod associated with acute and chronic infections of humans (7), is considered highly pathogenic for individuals with compromised immunity. It produces two unrelated siderophores, pyoverdin (16, 28) and pyochelin (14), in response to iron deprivation and is capable of utilizing a number of heterologous siderophores, including enterobactin (43) and ferrioxamine B (13). Although it is the inferior iron chelator in vitro (2), pyochelin is capable of removing transferrin-bound iron (53), a significant source of in vivo iron. This fact might explain the siderophore's known contribution to enhanced in vivo growth and virulence (15), although its ability to catalyze the formation of tissue-damaging free radicals (8, 9) may also be significant.

The receptor for ferric pyochelin is a 75-kDa outer membrane protein, FptA, which is produced in response to iron limitation (26). This fact is consistent with the presence of a sequence with homology to the consensus Fur binding sequence (18) upstream of the recently cloned and sequenced *fptA* gene (3, 5). The demonstration that pyochelin-deficient strains are deficient in FptA production and that the addition of exogenous pyochelin restores receptor production (20, 26) suggests that FptA expression is pyochelin dependent. This supposition conflicts with the conclusion of another report (3) describing the pyochelin-independent expression of FptA. It is possible, however, that differences in the growth media used in these studies explain the apparent conflict and that, under certain conditions, the pyochelin dependence of FptA is abrogated. Siderophore-dependent expression of a receptor protein is uncommon in, e.g., *Escherichia coli*, in which the ferric dicitrate uptake system is the sole example of a system inducible by the cognate chelator (citrate) (58), although such expression appears to be the rule in *P. aeruginosa*. Indeed, in addition to that for FptA, expression of the receptors for ferric pyoverdin (FpvA) (20) and ferric enterobactin (PfeA) (43) as

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description[a]	Source or reference
P. aeruginosa		
CDC5	PAO1 *pvd-2*	1
DH143	CDC5 *fptA*::ΩTc (FptA$^-$)	This study
DH119	CDC5 *pchR*::ΩTc (PchR$^-$)	This study
IA614	Pyochelin-deficient derivative of CDC5	4
DH54	IA614 *fptA*::ΩTc (FptA$^-$)	This study
K649	IA614 *pchR*::ΩTc (PchR$^-$)	This study
E. coli		
JM101	Δ*pro-lac thi* F′ (*traD36 proAB*$^+$ *lacI*^{q}*ZM15*)	55
S17-1	*thi pro hsdR recA* Tra$^+$	50
Plasmids		
pUCP18	Multicopy *E. coli-P. aeruginosa* shuttle vector; MCS within α *lacZ* fragment; Ap^r Cb^r	49
pHP45Ω-Tc	pUC18 derivative carrying the Ω interposon with a Tc^r gene replacing the normally resident Sm^r and Sp^r genes; Ap^r Tc^r	19
pSUP202	pBR325 derivative carrying the Mob (mobilization) site of plasmid RP4; Ap^r Tc^r Cam^r	50
pDH6	pUCP18 derivative carrying the *pchR* gene on a 1.8-kb *Eco*RI-*Sal*I fragment; Ap^r Cb^r	25
pDH8	pUCP18 carrying the *fptA* gene on a 3.5-kb *Xho*I fragment; Ap^r Cb^r	This study
pMP190	Broad-host-range, low-copy-number *lacZ* fusion vector; Cam^r Sm^r	52
pDH9	pMP190 carrying a *fptA-lacZ* fusion; Cam^r Sm^r	This study
pDH10	pMP190 carrying a *pchR-lacZ* fusion; Cam^r Sm^r	This study

[a] *pvd*, defect in pyoverdin biosynthesis; Ap^r, ampicillin resistant; Cb^r, carbenicillin resistant; Cam^r, chloramphenicol resistant; Sm^r, streptomycin resistant; Sp^r, spectinomycin resistant; Tc^r, tetracycline resistant; MCS, multiple cloning site; *fptA*::ΩTc or *pchR*::ΩTc, insertional inactivation of chromosomal *fptA* or *pchR* with the Ω-Tc interposon.

well as of the putative ferrioxamine B receptor (13) is inducible by the corresponding siderophore.

Expression of FptA is dependent upon the product of a gene, *pchR*, which encodes a 31-kDa AraC-like regulator of *fptA* gene expression and which appears to mediate the pyochelin-dependent expression of FptA (25). A Fur box was identified upstream of *pchR*, although the influence of iron on *pchR* expression was not assessed (25). The present report highlights additional findings concerning the influence of iron, pyochelin, and PchR on *fptA* as well as *pchR* gene expression and provides data implicating FptA in the regulation of *fptA* expression.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are described in Table 1.

Media. Iron-deficient succinate medium has been described previously (43). L broth (43) was employed as the rich medium throughout, except for the β-galactosidase assays, for which brain heart infusion (BHI; Difco) broth was used. BHI broth was made iron deficient by the addition of ethylenediamine-di(*o*-hydroxyphenylacetic acid) (EDDHA) (5 to 25 μg/ml). Solid media were obtained via the addition of Bacto Agar (Difco; 1.5% [wt/vol]). Ampicillin (100 μg/ml), carbenicillin (200 μg/ml), chloramphenicol (*P. aeruginosa*, 200 μg/ml in agar and 100 μg/ml in broth; *E. coli*, 30 μg/ml), and tetracycline (*P. aeruginosa*, 100 μg/ml; *E. coli*, 10 μg/ml) were included in growth media as required.

DNA methodology. Plasmid DNA was routinely isolated by the alkaline lysis procedure (46). Restriction endonucleases and T4 DNA ligase were obtained from Gibco-BRL and used according to the manufacturer's instructions or as described by Sambrook et al. (46). The transformation of *E. coli* (46) and *P. aeruginosa* (6) with plasmid DNA has been previously described. Restriction fragments were isolated, as required, from agarose gels (0.8%, wt/vol) with the Prep-a-gene matrix (Bio-Rad, Mississauga, Ontario, Canada) according to the manufacturer's instructions.

Isolation of pyochelin. Pyochelin was prepared as previously described (25).

Growth assays. Bacterial growth was assayed by monitoring the change in A_{600} of cultures inoculated at an A_{600} of 0.05 to 0.10 with stationary-phase cells and shaken (200 rpm) at 37°C.

In vitro mutagenesis and gene replacement. Construction of the *pchR* mutant strains via in vitro mutagenesis and gene replacement has been described previously (25). The *fptA* gene was mutated in vitro by insertion of a tetracycline resistance gene (Ω-Tc), obtained on a 2.1-kb *Sma*I fragment from plasmid pHP45-Tc, into the *Not*I site in the *fptA* coding region (Fig. 1). The bulk of the *fptA* gene, including the Ω-Tc insert, was then recovered on a 3.8-kb *Eco*RI fragment which was inserted into the unique *Eco*RI site in plasmid pSUP202. pSUP202 carrying the Ω-Tc-mutated *fptA* gene was transformed into mobilizing *E. coli* S17-1, which was subsequently used in conjugations with *P. aeruginosa* as described previously (25). *P. aeruginosa* strains carrying the Ω-Tc-mutated *fptA* gene in the chromosome were selected on L agar containing streptomycin (to counterselect the donor *E. coli*) and tetracycline and were subsequently screened for carbenicillin sensitivity. The FptA deficiency of these strains was ultimately confirmed by screening isolated outer membranes on sodium dodecyl sulfate-polyacrylamide gels (25).

β-Galactosidase assays. *P. aeruginosa* strains carrying *lacZ* transcriptional fusion plasmid pMP190 and its derivatives were grown overnight at 37°C in BHI broth containing chloramphenicol with or without EDDHA (as indicated) and diluted 100-fold into the same medium. Following growth to the mid- to late-log phase, cultures were assayed for β-galactosidase activity by the method of Miller (38).

RESULTS

Regulation of *fptA* gene expression. To obtain a more quantitative measure of the influence of iron, pyochelin, and PchR

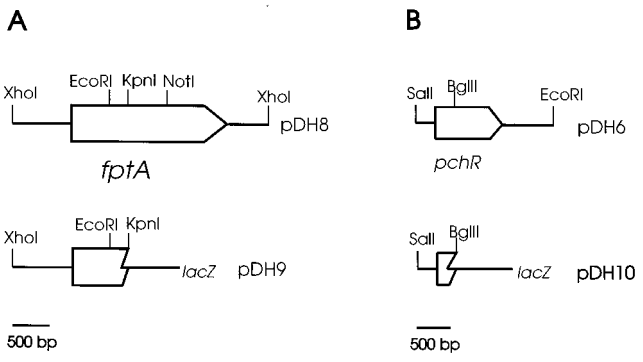

FIG. 1. Construction of *fptA-lacZ* (A) and *pchR-lacZ* (B) reporter fusions. The pUCP18 derivatives carrying *fptA* (pDH8) and *pchR* (pDH6) were used as the source of the upstream promoter regions which were excised as indicated and inserted into *lacZ* transcriptional fusion vector pMP190 to yield pDH9 and pDH10, respectively. The *Not*I site of insertion of an Ω-Tc interposon in the *fptA* coding region (used in the construction of an *fptA* knockout mutation) is shown. Vector regions are not shown.

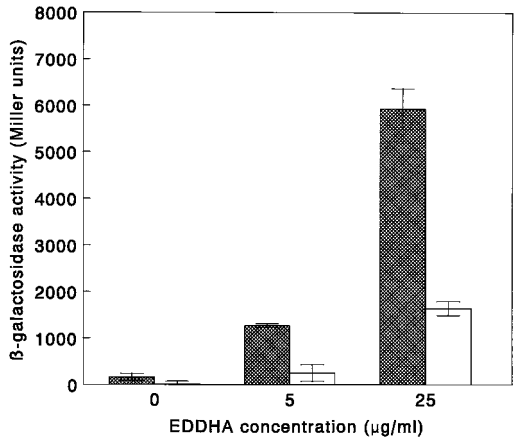

FIG. 2. Iron regulation of *fptA* and *pchR* gene expression. *P. aeruginosa* CDC5 harboring pDH9 (*fptA-lacZ*) (hatched bars) or pDH10 (*pchR-lacZ*) (open bars) was grown overnight at 37°C in chloramphenicol-containing BHI in the absence (for iron-replete growth) or presence (for iron-limited growth) of EDDHA (5 or 25 μg/ml). Following dilution (100-fold) in the same medium, the cells were cultured to an A_{600} of 0.6 to 1.0 and assayed for β-galactosidase activity. The values reported represent the means of three determinations ± the standard deviations (error bars) and have been corrected for background values obtained with *lacZ* fusion vector pMP190 without the insert.

on the expression of *fptA* and to demonstrate that this influence is mediated at the level of transcription, an *fptA-lacZ* fusion (pDH9) was constructed (Fig. 1) and introduced into a variety of *P. aeruginosa* strains. As was expected, the imposition of iron limitation via the addition of the nonmetabolizable iron chelator EDDHA markedly increased the expression of the *fptA-lacZ* fusion in *P. aeruginosa* CDC5 (Fig. 2). In addition, the elimination of *pchR* in strain CDC5 via the insertion of an Ω-Tc cartridge within the gene (to produce strain DH119) caused a marked decrease in *fptA* expression (Fig. 3) paralleling the loss of FptA observed previously in such mutants (25). Significantly, however, *fptA* was still expressed in the *pchR* null mutant, indicating that a basal level of *fptA* transcription occurs independent of PchR. In contrast, the absence of pyochelin production in CDC5 derivative IA614 correlated with an almost total lack of *fptA* expression (Fig. 3), and elimination of *pchR* in this strain (to yield K649) restored the low-level PchR-independent *fptA* expression that was observed in DH119. Consistent with the apparent ability of PchR to repress *fptA* gene expression in the absence of pyochelin, introduction of the *pchR* gene on plasmid pDH6 into *E. coli* carrying fusion vector pDH9 decreased *fptA-lacZ* expression circa five-fold, from 1,609 ± 141 Miller units of β-galactosidase activity (for *E. coli* carrying pDH9 and pUCP18) to 305 ± 2 Miller units of activity (for *E. coli* carrying pDH9 and pDH6).

Regulation of *pchR* gene expression. A sequence upstream of the *pchR* coding region which showed homology to the consensus binding sequence for the *E. coli* Fur repressor was identified (25), suggesting that *pchR* expression was likely iron regulated. In agreement with this conclusion, *P. aeruginosa* CDC5 harboring a *pchR-lacZ* fusion vector (pDH10) (Fig. 1) displayed increased β-galactosidase activity in response to iron limitation imposed via the addition of EDDHA (Fig. 2). The activities measured were, however, markedly less than those obtained for the *fptA-lacZ* fusion (Fig. 2).

To assess whether *pchR* expression was also regulated in response to pyochelin and whether PchR regulates its own expression, pDH10 was introduced into pyochelin-producing strain CDC5 and its pyochelin-deficient (IA614) and PchR-deficient (DH119) derivatives. β-Galactosidase levels were twofold higher in DH119 compared with those in CDC5 (Fig. 4), in contrast to the marked decrease in *fptA* expression seen in the same mutant. Again, however, the absence of pyochelin in IA614 correlated with a total loss of *pchR* gene expression, and the introduction of a *pchR* knockout mutation in this strain (to yield K649) restored *pchR* expression (Fig. 4).

The observed regulation of *fptA* and *pchR* by PchR, a putative transcriptional regulatory protein, suggests that a common sequence, likely to function as a binding site for PchR, should exist upstream of these genes. Examination of the upstream regions of *pchR* and *fptA* revealed, in fact, two related heptameric sequences that were separated by 14 (*fptA*) or 13 (*pchR*) bp and that were upstream of the putative −35 regions of both of these genes (Fig. 5).

Influence of FptA on *fptA* and *pchR* gene expression. Receptor-dependent receptor gene expression is a common feature of *P. aeruginosa* and the related *Pseudomonas putida*. Indeed, mutants of *P. aeruginosa* lacking the ferric pyoverdin receptor FpvA are devoid of *fpvA* gene expression (as assessed with an *fpvA-lacZ* transcriptional fusion [42]), and mutants of *P. putida* WCS358 lacking the ferric pseudobactin receptor PupB fail to activate *pupB* gene expression in response to pseudobactins BN7 and BN8 (33). To determine, therefore, whether FptA is required for *fptA* gene expression, the influence of an *fptA* knockout mutation on the expression of *fptA-lacZ* was assessed. Interestingly, strain DH143, the *fptA*-deficient derivative of CDC5, was unable to grow under iron-limiting conditions, although DH54, the FptA-deficient derivative of IA614, exhibited no such difficulty (Fig. 6). The addition, however, of exogenous pyochelin to cultures of DH54 completely inhibited growth in an iron-limited medium (Fig. 6), indicating that pyochelin produced by DH143 was likely responsible for the observed growth defect. In order to assess, then, the influence of an FptA deficiency on the expression of the *fptA* and *pchR* genes under iron-limiting conditions, it was necessary first to culture cells to the early-log phase in iron-rich conditions prior to the imposition of iron deficiency (by adding EDDHA). By

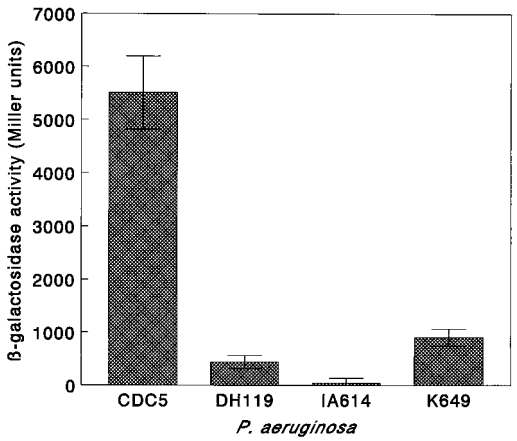

FIG. 3. Influence of pyochelin and PchR on *fptA* gene expression. *P. aeruginosa* CDC5 (Pch^+ $PchR^+$), DH119 (Pch^+ $PchR^-$), IA614 (Pch^- $PchR^+$), and K649 (Pch^- $PchR^-$) harboring pDH9 (*fptA-lacZ*) were grown overnight at 37°C in chloramphenicol-containing BHI with EDDHA (5 μg/ml), diluted 100-fold in the same medium, and grown to early- to mid-log phase. The EDDHA concentration was subsequently increased (to 25 μg/ml), and the cultures were allowed to grow for a further 2 h prior to being assayed for β-galactosidase activity. The values reported represent the means of three determinations ± the standard deviations (error bars) and have been corrected for background values obtained with *lacZ* fusion vector pMP190 without the insert.

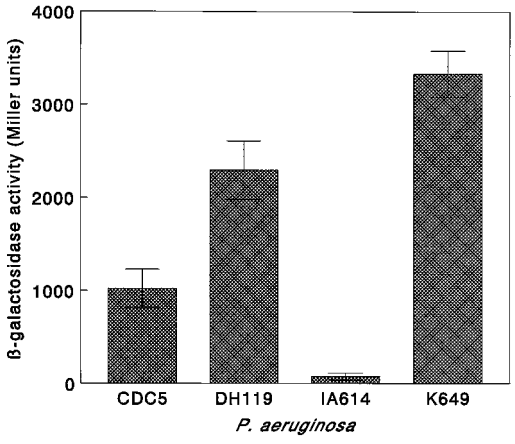

FIG. 4. Influence of pyochelin and PchR on *pchR* gene expression. *P. aeruginosa* CDC5 (Pch$^+$ PchR$^+$), DH119 (Pch$^+$ PchR$^-$), IA614 (Pch$^-$ PchR$^+$), and K649 (Pch$^-$ PchR$^-$) harboring pDH10 (*pchR-lacZ*) were cultured and assayed for β-galactosidase activity as described in the legend to Fig. 3. The values reported represent the means of three determinations ± the standard deviations (error bars) and have been corrected for background values obtained with *lacZ* fusion vector pMP190 without the insert.

this approach, FptA-deficient strain DH143 showed a threefold decrease in *fptA-lacZ* expression compared with that of the CDC5 parent strain (Fig. 7). Still, a substantial amount of *fptA* expression was occurring in the absence of FptA, and it was certainly more than what was evident in pyochelin-deficient IA614. Elimination of FptA in IA614 (yielding strain DH54) restores *fptA* expression to the level seen in DH143 (Fig. 7).

The influence of FptA deficiency on the expression of the *fptA-lacZ* transcriptional fusion was strangely reminiscent of the effect of a *pchR* mutation (compare Fig. 3 and 7). To determine whether the *fptA* mutation adversely affected *pchR* gene expression, the *pchR-lacZ* fusion was introduced into CDC5, IA614, and their FptA-deficient derivatives. As can be seen in Fig. 8, the loss of FptA in DH143 had no effect on *pchR* expression (compare CDC5 and DH143), while the loss of FptA in DH54 actually alleviated the apparent repression of *pchR* expression observed in IA614.

DISCUSSION

Knockout mutations in *pchR* previously rendered cells of *P. aeruginosa* unable to synthesize the ferric pyochelin receptor FptA in response to iron limitation and pyochelin, suggesting that PchR mediates the pyochelin-dependent expression of this receptor protein (25). The conflicting report that FptA expression occurs in the absence of pyochelin (3) is puzzling, in light of the data presented here and elsewhere (20, 26). It is possible that the use of a Casamino Acids-based medium in the Ankenbauer study (3) somehow overrides the normal pyochelin inducibility of FptA or that the pyochelin-deficient mutant described in the current study carries an additional mutation(s) (it was generated by nitrosoguanidine mutagenesis) which uncouples the pyochelin dependence of *fptA* expression. In support of medium influences on FptA expression, we have noted previously that FptA and pyochelin production are markedly curtailed in pyoverdin-producing wild-type strains grown in the phosphate-succinate medium used in this study (24, 26), while this curtailment has not been reported by other researchers using a Casamino-Acids-based medium (14, 51).

The observation, here, that PchR$^-$ strains exhibit a marked decrease in *fptA* gene expression confirms its role as a transcriptional activator, as was, in fact, predicted from its homology to the AraC family of regulatory proteins (25). Activation of *fptA* by PchR is, however, dependent upon the presence of pyochelin, the protein repressing *fptA* expression in its absence. This dependence was shown conclusively by the increase in *fptA* expression upon inactivation of *pchR* in a pyochelin-deficient strain and by the observed repression of *fptA-lacZ* expression by the cloned *pchR* gene in *E. coli.*

The ability to function both in repression and activation of gene expression is unusual in bacterial regulatory proteins. A number of examples do, however, exist (44), the best-studied example being AraC, which regulates the expression of genes involved in the metabolism (*araBAD*) and transport (*araFGH*) of arabinose (48). Most studies have focused on the regulation of the *araBAD* operon, whose expression is repressed by AraC in the absence of arabinose and activated when arabinose is present (48). Repression appears to be a function of loop formation of DNA upstream of *araBAD* that is mediated by an AraC dimer binding to two half sites, $araO_2$ and $araI_1$, 210 bp apart (36). Although the exact mechanism by which arabinose causes induction of the system is unclear, it appears that arabinose interacts directly with AraC, causing a conformational change in the protein which ameliorates loop formation and enhances binding to adjacent half sites ($araI_1$ and $araI_2$) whose occupancy facilitates *ara* gene expression (35, 36). Evidence suggests that arabinose (the so-called effector) interacts with the N-terminal portion of the protein, while the C-terminal domain mediates binding to the DNA (37). This property appears to be shared by other members of the AraC family of regulators (45).

It is unclear, however, whether the loop model proposed for AraC applies to PchR-mediated regulation of *fptA-pchR* expression. There is, for example, no data supporting such a model for regulation by other members of the AraC family, although the multiplicity of binding sites for VirF upstream of *yop* gene promoters in *Yersinia* spp. is at least suggestive of such a model (57). Two partially conserved heptameric repeat sequences, CGAGGAA and CGTGGAT, were identified upstream of the putative −35 region of *fptA* and could conceivably represent two half sites for the binding of PchR monomers. The $araI_1$ and $araI_2$ repeats which function in AraC binding are similarly situated near the *araBAD* −35 region (36). Moreover, XylS, an AraC family regulator of TOL plasmid genes involved in the degradation of benzoate and *m*-toluate, also binds to direct repeats occurring immediately upstream of the −35 region of its target gene (31). Still, the absence of available sequence further upstream of *fptA* precludes the identification of additional binding sites which would, of course, be necessary should looping be invoked as a regulatory mechanism. Nonetheless, the fact that the repeat sequences also occur upstream of the putative −35 region of the autoregulated *pchR* gene is highly suggestive that whatever the mechanism of regulation, these sequences function in PchR binding. Repeated attempts at overproducing and purifying PchR to assess this hypothesis have so far proved unsuc-

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                                                    -35
fptA CGAGGAAAGTTCCGCGACGGTCGTGGATCGATAGAGAAAGACCGGCAATCGAAA
                                                -35
pchR CGAGGAAGTCATGCGATCTCCGTGGATGCGGTCGATTGCCA
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FIG. 5. Identification of conserved sequences upstream of *fptA* and *pchR*. Conserved heptameric sequences (underscored boldface) are shown relative to the putative −35 promoter regions of *fptA* and *pchR* (underscored). The sequences were derived from references 5 (*fptA*) and 25 (*pchR*).

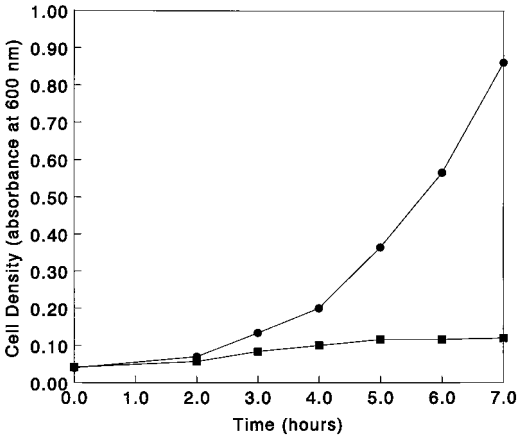

FIG. 6. Growth of *P. aeruginosa* DH54 in iron-deficient minimal medium with (■) or without (●) exogenously added pyochelin (0.5 mM).

cessful, owing to a stubborn instability of the overexpressed protein. Indeed, pulse labelling of the overexpressed protein in preliminary studies yielded a very broad PchR band on autoradiographs (25) indicative of protein degradation during the short (5-min) pulse period. Additional attempts with a variety of expression systems have confirmed this instability (24).

The inducibility of *pchR* expression by pyochelin (pyochelin-producing strains exhibit substantially more *pchR-lacZ* activity than do pyochelin-deficient strains) indicates that *pchR* expression, like *fptA* expression, responds to this siderophore. However, and in contrast to *fptA*, the siderophore does not produce a PchR-dependent activation of *pchR* gene expression. This result can be concluded from the observed increase in *pchR* expression resulting from the mutational loss of *pchR* in a pyochelin-producing or pyochelin-deficient background. Although *pchR* expression is enhanced by pyochelin, the fact that expression increases in both backgrounds upon removal of *pchR* indicates that it functions solely as a repressor with regard to *pchR* regulation.

The negative autoregulation of *pchR* is a property shared not only by AraC (11) and some other AraC family regulators (12) but also by many transcriptional activators (44). Presumably, this property allows the cell to maintain a somewhat constant and low level of activator in order to retain precise control of target gene expression. Unlike AraC, however, which represses *araC* expression in the presence or absence of inducer (arabinose), PchR actually activates *pchR* expression in the presence of inducer (pyochelin), causing repression only when the inducer is absent. In the presence of pyochelin, this serves to increase the levels of PchR and, as a consequence, FptA, which would only be required when pyochelin is present.

The dependence of *fptA* and *pchR* expression on pyochelin could be manifest in a number of ways. One possibility has PchR interacting directly with pyochelin (or perhaps ferric pyochelin), presumably via the nonconserved N terminus, with such interaction either converting the repressor form of the protein to an activator in the case of *fptA* expression or alleviating repression in the case of *pchR*. Whether this interaction involves the antagonization of PchR binding to target DNA or an alteration in binding site occupancy (as is the case in AraC regulation of *ara* gene expression [35, 36]) is unclear. Still, if PchR interacts directly with pyochelin, it probably does so only following the siderophore chelation of iron and transport into the cell. This caveat is important, since pyochelin-dependent upregulation of *fptA* only occurs in the absence of chelators with superior iron-binding abilities (e.g., pyoverdin and enterobactin). Wild-type cells producing both pyoverdin and pyochelin, for example, preferentially synthesize the ferric pyoverdin receptor, FpvA, at the expense of FptA (42), and the addition of enterobactin to pyoverdin-deficient, pyochelin-producing cells activates the expression of the PfeA ferric enterobactin receptor, again at the expense of FptA (43). The implication is that pyochelin must be able to chelate iron in order to stimulate FptA production. One way to assess successful chelation would be the transport of the iron-pyochelin complex into the cell, where it can be detected by appropriate regulatory molecules like PchR.

It seems likely that the well-conserved (in *P. aeruginosa*) phenomenon of siderophore-dependent expression of siderophore receptor synthesis (13, 20, 43) is a response to the problem of choosing which of several available chelators to use in facilitating iron acquisition. In a particular environment, whichever endogenous or heterologous siderophore is ultimately successful in chelating iron (and thus of particular use) would return to the cell via specific transport systems and turn on the expression of transport, including receptor, genes.

Although invoking a direct interaction between pyochelin and PchR would follow the pattern established by several AraC-type regulators which bind effectors directly (45, 48), such an explanation does not address the observed effects of an FptA knockout on *fptA* gene expression. In contrast to the almost total lack of *fptA* expression seen in a pyochelin-deficient strain, an FptA$^-$ strain showed reduced (threefold) but still substantial expression of *fptA*. That such mutants are iron starved in the presence of pyochelin argues against the existence of a second receptor which could be responsible for residual uptake of pyochelin and, thus, activation of *fptA* expression. Given that FptA$^-$ and pyochelin-deficient strains should be uniformly lacking in intracellular pyochelin, it is unlikely that the pyochelin influence on receptor gene expression is manifest by an interaction directly with PchR. Certainly, it is not necessary. The noted pH dependence of virulence gene expression in *Vibrio cholerae*, for example, is mediated by

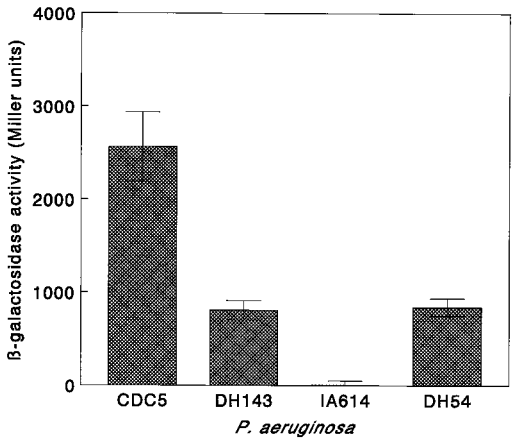

FIG. 7. Involvement of FptA in *fptA* gene expression. *P. aeruginosa* CDC5 (Pch$^+$ FptA$^+$), DH143 (Pch$^+$ FptA$^-$), IA614 (Pch$^-$ FptA$^+$), and DH54 (Pch$^-$ FptA$^-$) harboring pDH9 (*fptA-lacZ*) were grown overnight at 37°C in chloramphenicol-containing BHI (without EDDHA), diluted 100-fold in the same medium, and grown to early-log phase. EDDHA was added to a final concentration of 25 μg/ml, and growth was allowed to continue for 2 h before the cultures were assayed for β-galactosidase activity. The values reported represent the means of three determinations ± the standard deviations (error bars) and have been corrected for background values obtained with *lacZ* fusion vector pMP190 without the insert.

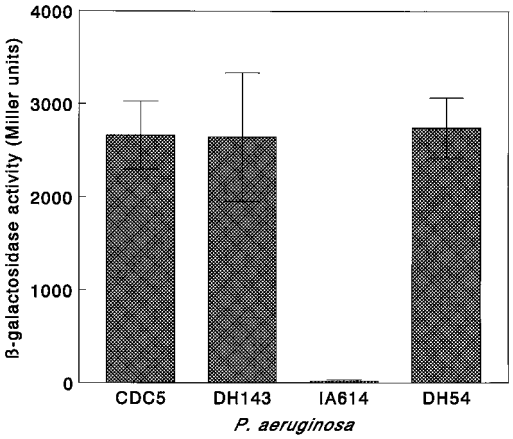

FIG. 8. Influence of FptA on *pchR* gene expression. *P. aeruginosa* CDC5, DH143, IA614, and DH54 harboring pDH10 (*pchR-lacZ*) were cultured and assayed for β-galactosidase activity as described in the legend to Fig. 6. The values reported represent the means of three determinations ± the standard deviations (error bars) and have been corrected for background values obtained with *lacZ* fusion vector pMP190 without the insert.

ToxR, a membrane-associated transcriptional activator which activates expression of *toxT*, whose product is an AraC family activator of the virulence genes themselves (27). Moreover, ToxR does not appear to activate *toxT* directly, as ToxR binding sequences are absent from the *toxT* promoter region (27). In this case, the primary signal eliciting activation is not the final effector interacting with the AraC-type regulator.

Taking into account the observed influence of FptA on *fptA* gene expression, it is possible that an interaction between pyochelin or ferric pyochelin and FptA initiates some sort of signal transduction cascade leading either to release of an effector, which interacts with PchR to effect activation or repression, or perhaps to direct interaction between the terminal element of the cascade and PchR. In this way, transport of the siderophore into the cell is not required for the cell to assess iron chelation by pyochelin and upregulate the *fptA* receptor gene. Pseudobactin-mediated activation of *pupB* receptor gene expression in *P. putida* (33) and citrate-mediated induction of the ferric dicitrate uptake system in *E. coli* (23) both involve an interaction of the iron-siderophore complex with the corresponding cell surface receptor which initiates a signal transduction cascade ending ultimately in the activation of receptor gene expression. In both cases, transport of the iron-siderophore complex is not required for the upregulation. Moreover, preliminary data suggests that the observed pyoverdin-dependent upregulation of FpvA in *P. aeruginosa* (20) requires the receptor itself (42), perhaps involving a similar mechanism.

Activation of *fptA* by PchR requires both FptA and PchR, the absence of either resulting in basal levels of *fptA* expression. Interestingly, the apparent repression of *fptA* (and *pchR*) which is observed in a pyochelin-deficient strain also requires both FptA and PchR, since lack of either restores the same basal level of *fptA* expression. Thus, in the absence of either of these proteins, *fptA* expression no longer responds to the presence or absence of pyochelin. This outcome suggests that FptA likely senses the pyochelin status of the cell culture and either communicates it to PchR directly or influences *pchR* expression accordingly. *fpvA* knockout mutations do not adversely affect *pchR* transcription and, in fact, alleviate the apparent repression of *pchR* seen in pyochelin-deficient strains. FptA may, however, affect *pchR* expression at the level of translation. In this vein, the *pchR* gene appears to possess a long untranslated leader sequence (25) reminiscent, in fact, of the sequences of AraC (56) and other members of the AraC family (10, 29). Although the *pchR* promoter has yet to be unambiguously determined, the recent demonstration that the *P. aeruginosa* Fur protein binds to the previously identified Fur box (54) overlapping a putative −10 region argues that our general placement of the promoter region is accurate and that transcription likely initiates substantially upstream of the *pchR* coding region. The role of this leader sequence in the case of AraC is uncertain, although it has been speculated that it could play a role in the posttranscriptional regulation of *araC* expression. Certainly, posttranscriptional regulation of regulatory gene expression is not uncommon (45). Although a *pchR-lacZ* translational fusion was constructed to assess the influence of an *fptA* mutation on *pchR* translation, the results proved inconclusive. The translational fusion failed to exhibit the normal pyochelin *pchR*-dependent regulation (42), possibly because the N-terminal PchR portion of the fusion protein interfered with the activity of the native PchR protein.

If FptA modulates PchR activity directly in response to the presence and absence of pyochelin, it would have to provide a signal in both instances, since the repressor as well as activator activity of PchR is apparently dependent on FptA. Perhaps FptA is able to bind both pyochelin and ferric pyochelin, providing the appropriate stimulus to PchR. The observation that the cloned *pchR* gene represses *fptA-lacZ* in *E. coli* in the absence of FptA would seem to contradict the FptA dependence of PchR repressor activity. The presence of *pchR* on a multicopy vector in *E. coli* (as opposed to a single copy in the *P. aeruginosa* chromosome) might, however, override the need for FptA for PchR repressor activity.

Finally, the observed growth deficiency of pyochelin-producing strains lacking FptA and the growth inhibitory effect of pyochelin on FptA$^-$ cells suggest that FptA is the sole route by which ferric pyochelin enters the cell and is, thus, the sole receptor for ferric pyochelin. This conclusion brings into question the role of the previously described 14-kDa outer membrane ferric pyochelin-binding protein (51) in ferric pyochelin uptake. The low molecular mass and weak iron regulation of this protein are certainly atypical of siderophore receptors, and it now seems unlikely that this protein plays any direct role in pyochelin-mediated iron transport in *P. aeruginosa.*

ACKNOWLEDGMENTS

This work was supported by an operating grant from the Medical Research Council of Canada to K.P. K.P. is a Natural Sciences and Engineering Research Council University Research Fellow. D.E.H. is the recipient of a Canadian Cystic Fibrosis Foundation Studentship.

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