Adaptations of *Pseudomonas aeruginosa* to the Cystic Fibrosis Lung Environment Can Include Deregulation of *zwf*, Encoding Glucose-6-Phosphate Dehydrogenase

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Cystic fibrosis (CF) patients are highly susceptible to chronic pulmonary disease caused by mucoid *Pseudomonas aeruginosa* strains that overproduce the exopolysaccharide alginate. We showed here that a mutation in *zwf*, encoding glucose-6-phosphate dehydrogenase (G6PDH), leads to a ~90% reduction in alginate production in the mucoid, CF isolate, *P. aeruginosa* FRD1. The main regulator of alginate, sigma-22 encoded by *algT* (*algU*), plays a small but demonstrable role in the induction of *zwf* expression in *P. aeruginosa*. However, G6PDH activity and *zwf* expression were higher in FRD1 strains than in PAO1 strains. In PAO1, *zwf* expression and G6PDH activity are known to be subject to catabolite repression by succinate. In contrast, FRD1 *zwf* expression and G6PDH activity were shown to be refractory to such catabolite repression. This was apparently not due to a defect in the catabolite repression control (Crc) protein. Such relaxed control of *zwf* was found to be common among several examined CF isolates but was not seen in other strains of clinical and environmental origin. Two sets of clonal isolates from individual CF patient indicated that the resident *P. aeruginosa* strain underwent an adaptive change that deregulated *zwf* expression. We hypothesized that high-level, unregulated G6PDH activity provided a survival advantage to *P. aeruginosa* within the lung environment. Interestingly, *zwf* expression in *P. aeruginosa* was shown to be required for its resistance to human sputum. This study illustrates that adaptation to the CF pulmonary environment by *P. aeruginosa* can include altered regulation of basic metabolic activities, including carbon catabolism.

*Pseudomonas aeruginosa* is an important opportunistic and nosocomial bacterial pathogen that contributes to a high rate of fatality among cystic fibrosis (CF) patients. During pathogenesis, the CF lung environment promotes and selects for multiple phenotypic and genotypic alterations in *P. aeruginosa* (29). The most obvious and predominant alteration selected within the CF lung is overproduction of the exopolysaccharide alginate (7). The appearance of these alginate-overproducing strains, also known as mucoid variants, correlates with the establishment of a chronic lung infection and a poor prognosis for the CF patient (8). The roles for alginate in virulence are varied and include neutralization of oxygen radicals (38), inhibition of phagocytosis (30), inhibition of antibiotic penetration (10), and inhibition of complement activation (32). Although a significant number of *P. aeruginosa* isolates from adult CF patients are mucoid (7), presentations of the mucoid phenotype in each isolate differ with respect to amount of alginate produced, stability of the phenotype, and growth conditions that promote alginate production. These variations imply that there are multiple factors that affect alginate production, which may be influenced by the CF lung environment. Given the correlation of alginate overproduction with pulmonary deterioration in CF patients, a better understanding of alginate regulation and production may suggest strategies for down-regulating alginate within the CF lung environment (15).

Production of alginate is an energy-costly process that diverts carbon sources from being utilized for energy and growth towards alginate production. The fact that the majority of *P. aeruginosa* CF isolates produce copious amount of alginate suggests that these isolates require alginate production in vivo. Carbon metabolism and alginate production are intimately related such that defects in carbon catabolism have dramatic effects on alginate production (27). Much of our current knowledge regarding carbon catabolism in *P. aeruginosa* is derived from studies with nonmucoid strain PAO1, a wound isolate, whereas much of our current knowledge of alginate production is derived from CF isolates like FRD1. In PAO1, the genes that encode enzymes for the major carbon catabolic pathway are organized into several operons that comprise the hexose regulon (hex-regulon). The hex-regulon is induced by growth on carbon sources such as glucose, gluconate, and glycerol but not by succinate and other intermediates of the tricarboxylic acid (TCA) cycle (12, 14). To date, only two regulatory proteins that control the hex-regulon in PAO1 have been identified, and both are repressors: Crc (catabolite repression control) and HexR (2, 42). However, a molecular mechanism by which Crc mediates catabolite repression in *P. aeruginosa* has not yet been elucidated, and the physiological role of HexR in carbon catabolism is unknown. Moreover, neither of these regulators, or even carbon metabolism in gen-
eral, have been extensively investigated in CF isolates of *P. aeruginosa*, which are reported to differ extensively from non-CF isolates in a number of characteristics (4, 6, 9, 16, 17, 22, 43, 48). In this study, a key enzyme of carbon catabolism and the Entner-Doudoroff pathway, glucose-6-phosphate dehydrogenase (G6PDH, or Zwf) was examined in a mucoid CF non-CF isolates in a number of characteristics (4, 6, 9, 16, 17, 22, 43, 48). In this study, a key enzyme of carbon catabolism and the Entner-Doudoroff pathway, glucose-6-phosphate dehydrogenase (G6PDH, or Zwf) was examined in a mucoid CF isolate because of its potential role in alginate overproduction.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and media.** Bacterial strains and plasmids used in this study are listed in Table 1. Unless otherwise indicated, bacteria were grown in L broth, L broth without NaCl, or L broth supplemented with appropriate antibiotics at 37°C with aeration. No carbon-E minimal medium (NCE) (5) was supplemented with 0.1% Casamino Acids and with glycerol (40 mM), succinate (40 mM), or glycerol and succinate as the carbon source(s). Basal salts medium was supplemented with succinate (40 mM) and lactamide (20 mM). L agar without NaCl was supplemented with 8% sucrose for use in negative selection of *sacB*. *Pseudomonas* isolation agar supplemented with antibiotics at 37°C with aeration. No carbon-E minimal medium (NCE) (5) was supplemented with appropriate antibiotics was used to select for *P. aeruginosa* transconjugants and to counterselect *Escherichia coli*. The following amounts of antibiotics were used in this study (per milliliter): 100 μg ampicillin for *E. coli*, 125 μg carbenicillin for *P. aeruginosa*, and 20 and 180 μg gentamicin (Gm) for *E. coli* and *P. aeruginosa*, respectively.

**DNA manipulations, transformations, and conjugations.** *E. coli* strain DH10B was routinely used as a host strain for cloning. DNA was introduced into *E. coli* by electroporation and into *P. aeruginosa* by conjugation as previously described (41). Plasmids were purified with Qiaquick spin miniprep columns made by Qiagen (Valencia, CA). DNA fragments were excised from agarose gels and purified using the Qiaex II DNA gel extraction system (Qiagen) according to the manufacturer’s instructions. Restriction enzymes and DNA modification enzymes were purchased from New England Biolabs (Beverly, MA). Either *Pfu* from Stratagene (La Jolla, CA) or *Taq* from New England Biolabs was used for PCR amplification of DNA. Oligonucleotides were purchased from Operon, Inc. (Alameda, CA), or Integrated DNA Technologies, Inc. (Coraville, IA).

**Construction of zwf and crc mutants.** Derivatives of FRD1 and PAO1 with a mutation in *zwf* were constructed as follows: a 1.55-kb fragment containing *zwf* was PCR amplified from FRD1 with *Pfu*, digested with EcoRI and XbaI and then cloned into pBluescript (41) between the EcoRI-XbaI sites. A gentamicin resistance cassette isolated from pUCG1 (35) as a BamHI fragment was then cloned into the internal BamHI site within *zwf* to disrupt the open reading frame (ORF). Next, an origin of transfer (oriT) from pLS217 was added as an EcoRI

Table 1. Bacterial strains and plasmids

<table>
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<th>Strain(s) or plasmid</th>
<th>Genotype, relevant characteristics</th>
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<tr>
<td>PAO1</td>
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<td>PDO300</td>
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</tr>
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Plasmids

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<td>pEX100T</td>
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<td>pLS214</td>
<td>pUC19 with morT at HindIII</td>
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<tr>
<td>pLS217</td>
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<td>pLS594</td>
<td>zwf-lacZ transcriptional fusion in pSS223</td>
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<td>pLS1436</td>
<td>Δsac1::aacCI in pEX100T</td>
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<tr>
<td>pLS1446</td>
<td>crc complementing plasmid</td>
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<td>pLS1051</td>
<td>crc-lacZ translational fusion in pSS361</td>
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<td>pSS23</td>
<td>lacZ operon fusion vector</td>
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<td>pSS1517</td>
<td>zwf complementing plasmid, cis</td>
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<td>ColE1 Amp’ Gm’</td>
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<tr>
<td>pLS1155</td>
<td>Regulatable promoter/repressor system</td>
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<td>pLS1393</td>
<td>Regulatable crc upon plasmid integration</td>
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</table>

Abbreviations used for genetic markers are described by Holloway et al. (11). ApR, ampicillin resistance. Alternate strain names are shown in parentheses.
fragment. The final construct, pLS635, was conjugated into FRD1 and PAO1, and potential zwf mutants were isolated as gentamicin-resistant (Gm') and carbenicillin-sensitive colonies. The presence of the mutant allele and the absence of the wild-type allele were verified by PCR analysis. To complement the zwf mutation in trans, zwf was PCR amplified with Pfu and cloned into the P. aeruginosa E. coli shuttle vector, pUCP19, at a 1,850-bp EcoRI-HindIII fragment. The resulting plasmid, pSS354, was converted into a mobilizable plasmid, pSS366, by the addition of a motT (40) of plasmid RP4. To complement the zwf mutation in cis, zwf was PCR amplified with Pfu and cloned into pBK* as a 2,380-bp EcoRI-Smal fragment. The resulting plasmid, pLS1515, was converted to a mobilizable plasmid, pLS1517, by the addition of a mini-oriT (motT) to the HindIII site. pSS366 and pLS1517 were introduced into P. aeruginosa via triparental mating. To construct a crc mutant in P. aeruginosa, an internal 343-bp fragment was deleted from the crc coding region by using the splicing by overlap extension technique (SOEing) (45). First, an 820-bp fragment, 5' to and containing the first 180 bp of the crc ORF, was PCR amplified from FRD1 with Pfu. Concurrently, a 999-bp fragment, 3' to and containing the last 255 bp of the crc ORF was PCR amplified with Pfu. The two fragments shared approximately 20 bp of overlap so that they could be joined by SOE PCR. In the second round of PCR amplification, the fragments described above served as DNA templates by use of the 5' primer of the first fragment and the 3' primer of the second fragment to generate a 1,819-bp product. This product, with 343 bp deleted from the crc coding sequence, was cloned into pEX100T, which carries a carbenicillin resistance gene and a counterselectable marker, sacB (36), to generate pLS1436. Following the conjugation of pLS1436 into P. aeruginosa, merodiploid colonies with the plasmid integrated into the P. aeruginosa chromosome via homologous recombination were selected for their resistance to carbenicillin. The merodiploids were subsequently resolved by selecting for growth on medium containing sucrose to promote the loss of plasmid DNA sequences carrying sacB. This resulted in allelic exchange between the wild-type crc and Δcrc alleles. The presence of the mutant allele and the loss of plasmid and the wild-type copy of crc in the putative crc mutants were verified by PCR. To complement the crc mutation, the crc coding sequence, along with approximately 800 bp of upstream sequence and 80 bp of downstream sequence, was PCR amplified from FRD1 by using the first fragment and cloned into pUCP19 as a BamHI-EcoRI fragment. A 2,380-bp fragment isolated from pLS214 was then cloned as a HindIII fragment to generate pLS1446 and the plasmid was introduced into P. aeruginosa crc mutants via conjugation. The complemented FRD1crc and PAO1crc mutants were designated FRD1crc+ (LS1447) and PAO1crc+ (LS1448), respectively.

Construction of transcriptional and translational fusions. To construct the zwf::lacZ and crc::lacZ fusions, DNA fragments containing the promoter for the genes and a portion of the 5' coding sequence were PCR amplified from FRD1 genome using Pfu DNA polymerase, digested with the appropriate enzymes and cloned into either pSS223 for a transcriptional fusion (zwf::lacZ) or pSS361 for a translational fusion (crc::lacZ). pSS361 is a mobilizable lacZ translational fusion vector that can replicate in P. aeruginosa to a mobilizable plasmid, pLS1517, by the addition of a mini-oriT (motT) to the HindIII site. pSS366 and pLS1517 were introduced into P. aeruginosa via triparental mating. To construct the zwf::lacZ fusion, dnaJ mutants were chosen on CM agar plates supplemented with 20 mM glycerol and containing 5 μg/ml of chloramphenicol to select for a mutation in an essential gene. The CM-resistant colonies were picked and re-tested to verify the absence of the zwf::lacZ fusion. The presence of the zwf::lacZ fusion was confirmed by PCR and restriction digestion before they were conjugated into P. aeruginosa.

Biochemical assays. Alginate was isolated from P. aeruginosa culture supernatants that were dialyzed against distilled water as previously described (41), and alginate (i.e., uronic acid) level was quantified by the carbazole method (13) using Macrocystis pyrifera alginate (Sigma) as a standard. G6PDH activity was determined as described previously (18), and β-galactosidase activities were performed as described by Miller (25). Pyocyanin was quantified from 20-h cultures and its mucoid derivative, PDO300, were also tested. A mutant in the alginate biosynthetic operon (1), did not significantly affect the activation (i.e., derepression) of sigma-22, which is also known as AlgT or AlgU. This alternative extracytoplasmic-function sigma factor is at the top of a hierarchy of regulators for alginate biosynthesis (49). However, mucoid FRD1 (with a mucA22 allele) contained G6PDH levels that were only about 30% higher than those of the nonmucoid FRD1 mutant when grown in L broth (P < 0.001) (Fig. 1). To test whether disruption of the alginate biosynthesis pathway affected G6PDH activity, an FRD1algD nonmucoid mutant was also tested. However, a mutation in algD, the first gene in the alginate biosynthetic operon (1), did not significantly affect G6PDH activity (P > 0.05) in FRD1. As described above, the FRD1algD mutant contained no G6PDH activity. Thus, there was only a minor correlation between alginate overproduction and G6PDH levels by comparing these FRD1 derivatives. The G6PDH activities of the nonmucoid non-CF isolate, PAO1, and its mucoid mucA22 mutant derivative, PDO300, were also compared. PDO300 displayed a slightly higher level of G6PDH activity than its nonmucoid parent, PAO1 (P < 0.01) (Fig. 1). However, it was striking that the FRD1 derivatives contained about 10-fold more G6PDH activity than the PAO1 derivatives when grown in L broth (Fig. 1). This suggested that FRD1, which had undergone adaptation to the CF pulmonary environment, may have undergone a genetic change that up-regulated G6PDH activity.

Catabolite repression of zwf transcription. To study the transcriptional control of zwf, a zwf::lacZ transcriptional fusion was constructed and designated pLS94. The results showed that L broth-grown P. aeruginosa strains expressed zwf::lacZ in a manner that closely mimicked the G6PDH activity patterns described above. Mucoid FRD1 contained approximately 40%
The presence of a TCA cycle intermediate, such as succinate, re-
clease in the CF strain, FRD1, and its derivatives. This was a
phenotype much like a crc mutant (46).

**Role of Crc in PAO1 and FRD1.** *P. aeruginosa* PAO1 is
known to preferentially metabolize organic acids and TCA
cycle intermediates, such as succinate, before metabolizing
nonorganic acids, such as glycerol, or glucose (2). Further-
more, expression of zwf and other genes involved in catabolism
of hexoses are repressed in the presence of preferred carbon
sources in PAO1 (18). Catabolite repression of zwf in PAO1 is
mediated by the catabolite repression control protein (Crc)
(46) and at the transcriptional level (19), although the mech-
anism has not been deduced.

To address the role of Crc in FRD1, we constructed Δcrc
mutants of FRD1 and PAO1 (see Materials and Methods).
The FRD1Δcrc and PAO1Δcrc mutants overproduced a blue pig-
mament on agar plates and in liquid culture, which has been
observed previously with other crc mutants (31). This blue
coloration appears to be due to the overproduction of pyocy-
amin (data not shown). This phenotype was complemented by
crc in trans on plasmid pLS1446. We compared G6PDH activ-
ities of the wild type and the crc mutant when the bacteria were
grown in the presence of succinate (preferred carbon source),
glycerol (nonpreferred carbon source), or a combination of
succinate and glycerol. In agreement with published results,
the level of G6PDH activity was high in PAO1 with glycerol
and was repressed by succinate plus glycerol (Fig. 3A). As
previously described (46), this catabolite repression of Zwf
required Crc because G6PDH activity was not severely re-
pressed in the PAO1Δcrc mutant by succinate plus glycerol in
PAO1 (Fig. 3A).

In contrast, there was little phenotypic difference between
FRD1 and FRD1Δcrc. G6PDH remained high in the presence of
succinate plus glycerol in both strains, as if parent strain
FRD1 lacked a functional Crc control mechanism (Fig. 3B).
Also, G6PDH remained relatively high in both FRD1 and
FRD1crc when grown with succinate alone, which strongly represses zwf transcription in PAO1. Because Crc-mediated control of G6PDH activity appeared to be defective in FRD1, we tested for catabolite repression control of amidase. Amidase activity is derepressed in PAO1\textsuperscript{crc} compared to PAO1 when grown in the presence of succinate (preferred substrate) and lactamide (inducing substrate) (Fig. 4) (20). Although we observed slightly higher amidase activity in FRD1 compared to PAO1, we also observed that loss of Crc in FRD1 led to increased amidase activity. Therefore, deregulation of G6PDH activity in FRD1 appears to be independent of Crc regulation when FRD1 is grown in L broth. To determine whether Crc may be altered in FRD1, a crc-lac\textsuperscript{Z} fusion was constructed (pLS1051), but little difference in the expression levels was observed in FRD1 and PAO1 in L broth over time (data not shown). We then cloned the FRD crc gene and sequenced it from /H\textsubscript{110}0 to 80 bp downstream of the crc ORF. However, the sequence analysis revealed only two conserved changes compared to crc from PAO1, which did not change any amino acids, and these were located at nucleotides 606 (C to T) and 684 (A to G) in the coding sequence. This suggests that expression of crc in FRD1 is not defective. Studies to identify the molecular mechanism for this apparent deregulation of zwf in FRD1 are in progress.

High-level G6PDH activity correlates with adaptation to the CF lung. We next looked at a collection of CF isolates of \textit{P. aeruginosa} to see if a high level of expression of zwf in the presence of succinate (i.e., zwf deregulation) was a common trait among such strains. We compared G6PDH activity following growth with succinate as a sole carbon source in a variety of \textit{P. aeruginosa} isolates: 10 CF isolates, 5 non-CF clinical isolates, and 6 environmental isolates. Like FRD1, half of the CF isolates exhibited a high level of G6PDH activity (defined as >35 mIU/mg of protein). None of the other 5 clinical or 6 environmental strains exhibited this alteration in zwf control (Fig. 5). In that 8 of the 10 CF isolates tested were mucoid but not all of the mucoid isolates had high G6PDH activity, this small survey suggests that a high level of unregulated G6PDH activity is a trait acquired separately from overproduction of alginate.

We also examined alginate production by mucoid CF strain PA2192, which retained the normal catabolite repression phenotype of zwf when grown in succinate (Fig. 5, lane 6). It accumulated \(
\text{~}835 \pm 125 \mu g/\text{ml}
\) in an L broth culture, which is comparable to FRD1. A PA2192zwf mutant was also constructed, and it accumulated much-reduced levels of alginate (\(\leq 25 \mu g/\text{ml}\)), much like an FRD1zwf mutant. Thus, zwf was required for high-level alginate production in both classes of CF strains. Also, normally regulated G6PDH activity can still support substantial alginate production, at least under laboratory conditions. This led us to consider the possibility that deregulated G6PDH could have other selective advan-
tages (besides alginate production) for P. aeruginosa while growing within the CF lung.

G6PDH activity in sequential CF clonal isolates shows selection in vivo for unregulated zwf. Three sets of sequential P. aeruginosa isolates that had been collected from individual CF patients were obtained (21). RAPD analysis of these isolates indicates that the isolates are clonal from each patient but different between patients (data not shown). We observed that all of the isolates recovered from patient 13 retained low levels of G6PDH activity throughout the infection (Fig. 6). In contrast, the early isolates from patients 12 and 17 displayed low levels of G6PDH activity, while the later isolates displayed high levels of G6PDH activity, suggesting a conversion in vivo to the higher production of G6PDH. From patient 12, we observed a moderate increase in G6PDH activity between the ages of 8 and 9.6 years, while from patient 17, we observed a ~5-fold increase between the ages of 3.2 and 5. In both patient 12 and patient 17, the levels of G6PDH activity remained high in isolates from subsequent years (Fig. 6) following the conversion. Interestingly, in both patients 12 and 17 the conversion to high levels of G6PDH activity may have preceded the conversion to a dominantly mucoid phenotype, suggesting that increased G6PDH confers some selective advantage to these isolates within the CF lung.

A defect in zwf causes sputum sensitivity. Previous studies suggest an association between G6PDH and alginate in protecting P. aeruginosa from oxidative stress (18, 38). However, in this study, FRD1 and FRD1zwf showed equal sensitivity to paraquat and hydrogen peroxide (data not shown). In that the FRD1zwf mutant was partially defective for alginate production, this suggests that neither excess alginate nor G6PDH activity contributed to increased resistance to oxidative stress in FRD1 under these laboratory-tested conditions.

In an attempt to explore the selection for high-level G6PDH activity in P. aeruginosa while in the CF lung environment, we tested the effect of lung sputum on the growth of several P. aeruginosa isolates. We observed that lung sputum, either isolated from CF or non-CF individuals, inhibited the growth of FRD1zwf and PAO1zwf mutants but not the parental strains (Fig. 7). Complementation of zwf in cis restored the ability of PAO1 and FRD1 to grow in the presence of sputum (data not shown). The inhibitory effect appeared to be specifically associated with loss of zwf, in that a variety of other FRD1 mutants were not inhibited by sputum, including algT, algD, and crc mutants (data not shown). In that G6PDH appears to be required for maximum protection from lung sputum, it is plausible that the selection for deregulated high-level G6PDH activity in the CF lung may be associated with the phenomenon. Identification of the specific inhibitor in sputum or whether growth on sputum leads to a build up of a toxic intermediate within the zwf mutants is currently under investigation.

**FIG. 6.** Comparison of sequential isolates for unregulated G6PDH activity. G6PDH activity was measured from cells grown to an OD_{600} of approximately 1.0 in LB. The data represent the averages (±standard deviations) of two independent experiments conducted in duplicate. The approximate ages of the patients from which the isolates were collected are shown beneath the graph. The mucoid phenotypes of the isolates as observed on agar plates are shown.

**FIG. 7.** Effect of sputum on the growth of P. aeruginosa. NCE agar plates were seeded with P. aeruginosa and centrally inoculated with sputum. Shown is growth of the bacteria following an overnight incubation at 32°C.

**DISCUSSION**

The Entner-Doudoroff pathway is an alternative to the Emden-Meyerhof glycolytic pathway in a diverse group of bacteria, including Pseudomonas sp., and it provides both energy and metabolic precursors for many biosynthetic processes. Here, we established that a mutation in zwf (encoding G6PDH, the first enzyme of the Entner-Doudoroff pathway) leads to a ~90% reduction in alginate production in the mucoid, CF isolate of P. aeruginosa, FRD1. This effect was presumably due to a reduction in the pool of fructose-6-phosphate, the primary precursor of alginate. Consistent with this model is the observation that there were no differences between FRD1 and
FRD1 zwf for expression on an algD-lacZ transcriptional fusion over a growth cycle (data not shown). Mucoid strains produce large amounts of alginate, which is expensive in terms of carbon and energy, and so we began to explore the possibility that mucoid strains contain increased levels of G6PDH to supply sufficient amounts of precursor for the alginate pathway. However, an algD mutation in FRD1, which blocked the biosynthetic pathway for alginate, did not significantly affect G6PDH levels, indicating that this drain on the pool of metabolic sugar precursors did not affect G6PDH levels. Also examined was a FRD1 derivative with a mutation in algT, encoding sigma-22, the master regulator of alginate biosynthesis, and this defect did reduce G6PDH by ~30%. Also tested was the effect of a mucA mutation in strain PAO1, which inactivated the anti-sigma factor of sigma-22 to increase the level of active sigma-22 in the cell and results in alginate gene activation. A modest increase in G6PDH activity was observed in mucA PAO1 mucA compared to the wild type. Thus, sigma-22 apparently has a small role in the induction of zwf expression in P. aeruginosa, which is likely to be indirect because no obvious sigma-22 consensus sequence could be identified upstream of the zwf coding region.

The striking observation from the data described above was that the levels of G6PDH activity and zwf expression were both severalfold higher in FRD1 than in PAO1 background strains when grown in L broth. Although caution is always advised when making interstrain comparisons, this led us to examine whether zwf in strain FRD1 was still subject to catabolite repression as it is in PAO1. As expected for PAO1, levels of zwf expression and G6PDH activity were high following growth with glycerol but low with succinate or glycerol plus succinate, as previously described (46). However, in FRD1, levels of zwf expression and G6PDH activity remained high with succinate or glycerol plus succinate, suggesting that zwf expression was possibly deregulated and constitutively expressed. Although a mutation affecting Crc or its expression would produce these phenotypes (46), no evidence could be found here to support a Crc defect in FRD1. However, we cannot rule out the possibility that Crc activity remained high with succinate or glycerol plus succinate, as previously described (46). Furthermore, CF isolates of P. aeruginosa appear to utilize a different set of virulence determinants and pathogenic mechanisms to cause disease than non-CF isolates (37). Presumably, the hostile environment of the CF lung not only induces mutations in P. aeruginosa but also selects those mutants best able to survive and persist. Therefore, it is not unreasonable to expect that basic metabolic activities, such as regulation of carbon catabolism, might also be altered in CF isolates of P. aeruginosa, as demonstrated in this study. However, because basic metabolic processes and not just virulence determinants are altered in CF P. aeruginosa, these isolates may respond differently than non-CF isolates to treatments that were developed for non-CF isolates. Thus, in order to develop more effective treatments for pulmonary infections in CF patients, it is important to further characterize the physiology and metabolism of CF isolates of P. aeruginosa.

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