Promoter Recognition and Activation by the Global Response Regulator CbrB in Pseudomonas aeruginosa

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In Pseudomonas aeruginosa, the CbrA/CbrB two-component system is instrumental in the maintenance of the carbon-nitrogen balance and for growth on carbon sources that are energetically less favorable than the preferred dicarboxylate substrates. The CbrA/CbrB system drives the expression of the small RNA CrcZ, which antagonizes the repressing effects of the catabolite repression control protein Crc, an RNA-binding protein. Dicarboxylates appear to cause carbon catabolite repression by inhibiting the activity of the CbrA/CbrB system, resulting in reduced cbrZ expression. Here we have identified a conserved palindromic nucleotide sequence that is present in upstream activating sequences (UASs) of promoters under positive control by CbrB and σ54 RNA polymerase, especially in the UAS of the cbrZ promoter. Evidence for recognition of this palindromic sequence by CbrB was obtained in vivo from mutational analysis of the cbrZ promoter and in vitro from electrophoretic mobility shift assays using cbrZ promoter fragments and purified CbrB protein truncated at the N terminus. Integration host factor (IHF) was required for cbrZ expression. CbrB also activated the lipA (lipase) promoter, albeit less effectively, apparently by interacting with a similar but less conserved palindromic sequence in the UAS of the lipA promoter. Based on these results and previously published data, a consensus CbrB recognition sequence is proposed. This sequence has similarity to the consensus NtrC recognition sequence, which is relevant for nitrogen control.

Pseudomonas aeruginosa, like other fluorescent pseudomonads, is a metabolically versatile bacterium; it utilizes more than 100 different organic substrates for growth (37). This versatility requires a complex regulatory network that ensures the cellular carbon-nitrogen balance and determines the order in which growth substrates are degraded. In general, substrates that yield high energy and promote fast growth are degraded preferentially. For instance, intermediates of the tricarboxylic acid (TCA) cycle such as succinate prevent glucose degradation in P. aeruginosa (21). As a result, growth on a mixture of succinate and glucose is biphasic (diauxic) because the bacterium utilizes first succinate and then glucose (39). The underlying mechanism of carbon catabolite repression involves the CbrA/CbrB two-component system (16, 30), the small RNA (sRNA) CrcZ (36), and the RNA-binding protein Crc (6, 22, 26, 33) as key regulatory elements. They are conserved in fluorescent pseudomonads (38; Pseudomonas Genome Database).

Mutational inactivation of the CbrA/CbrB two-component system has vast consequences in fluorescent pseudomonads. In P. aeruginosa, Pseudomonas fluorescens, and Pseudomonas putida, cbrA and cbrB mutants no longer grow on a large number of carbon and nitrogen sources, suffer from a carbon-nitrogen imbalance, and are affected in biofilm development and stress tolerance (1, 19, 29, 30, 40, 41). The signals that interact with the membrane-bound CbrA sensor are unknown, but it appears that TCA cycle intermediates have an inhibitory effect on CbrA/CbrB activity (16). The CbrB protein is a transcriptional activator for σ54 RNA polymerase and belongs to the NtrC family of response regulators (30). Genetic evidence indicates that CbrB positively controls the expression of the crcZ sRNA gene in P. aeruginosa (36), the lipA (lipase) gene in Pseudomonas alcaligenes (7, 18), and the hutU (histidine utilization) operon of P. aeruginosa and P. fluorescens (16, 40). The fact that cbrB and crcZ mutants of P. aeruginosa have similar, but not identical, phenotypes suggests that most, but not all, activities of the CbrA/CbrB system are mediated by the sRNA CrcZ (36). CrcZ has high affinity for the Crc protein and, when in excess, prevents Crc from acting as a translational repressor of target mRNAs. Typical Crc targets are mRNAs encoding porins, uptake systems, and enzymes involved in the degradation of less-preferred substrates (20, 27, 28). In P. aeruginosa, CrcZ levels are lower during growth on succinate than during growth on less-preferred substrates such as glucose or mannitol, reflecting the activity of the CbrA/CbrB system as a master regulator of carbon catabolite repression (36). The growth handicap of a crcZ mutant on numerous substrates appears to be a consequence of permanent translational repression of target mRNAs by the Crc protein.

The CbrA/CbrB two-component system has moderate amino acid sequence similarity to the well-characterized NtrB/NtrC regulatory system, which controls nitrogen assimilation in Gram-negative bacteria (14, 30, 32). Importantly, mutations that partially suppress a cbrAB deletion have been mapped to ntrB or ntrC in P. aeruginosa (16, 19), suggesting that both two-component systems may functionally overlap. NtrC is a bacterial enhancer binding protein (bEBP). When activated by phosphorylation, NtrC binds to upstream activating sequences (UASs) in NtrC-
TABLE 1. Bacterial strains and plasmids used in this study

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<th>Strain or plasmid</th>
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<td>This study (Fig. 5)</td>
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<td>pUC28</td>
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* Ap', ampicillin resistance; Gm', gentamicin resistance; Te', tetracycline resistance.

dependent σ54 promoters (2, 32). Mechanistic aspects of this process have been studied mainly in enteric bacteria (10). In P. putida, NtrC binding sites have been found at −170 to −40 relative to the transcription start sites of various genes whose expression is activated by NtrC (13, 14). The interaction between NtrC bound to a UAS and σ54 RNA polymerase is often facilitated by the integration host factor (IHF) protein, which binds the DNA between the UAS and the −24 and −12 promoter sequences recognized by σ54 RNA polymerase (10). In the present study, we have obtained evidence for specific CbrB-DNA interaction, allowing us to localize CbrB recognition sites in the crcZ and lipA promoters of P. aeruginosa. We have also found that activation of the crcZ promoter requires IHF.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The strains used in this study are listed in Table 1. P. aeruginosa was grown in Luria broth (LB) (34) at 37°C or in a basal salts medium (BSM) containing 30.8 mM K2HPO4, 19.3 mM KH2PO4, 15 mM (NH4)2SO4, 1 mM MgCl2, and 2 mM FeSO4 amended with either 40 mM succinate or 40 mM mannitol (36). When required, antibiotics were added to the medium at the following concentrations: ampicillin, 100 μg ml−1; tetracycline, 30 μg ml−1 for Escherichia coli or 100 μg ml−1 for P. aeruginosa. Construction of plasmids. The primers used for plasmid construction are listed in Table S1 in the supplemental material. Plasmids carrying a transcriptional crcZ-lacZ fusion were obtained as follows. Primers V3_pcnBlacZfs and crcZ-lacZ rev were used to amplify a 183-bp PCR product from chromosomal DNA of strain PAO1. The resulting crcZ promoter fragment was digested with EcoRI and PstI and cloned into pME6016 cut with the same enzymes to give
Primer cbrZ_fw, EcoRI and ccrZ-lacZ_rev were used to amplify a 160-bp PCR product from PAO1 chromosomal DNA. Similarly, this PCR product was digested with EcoRI and PstI and inserted into pME6016 to produce pME9842. Primers cbrZ_rev, EcoRI and ccrZ-lacZ_rev were used to amplify a 138-bp PCR product from PAO1 chromosomal DNA. Again, the PCR product was digested with EcoRI and PstI and inserted into pME6016 to produce pME9834.

To construct plasmids carrying mutated motifs in the ccrZ UAS, the 171-bp ccrZ promoter region was subcloned as an EcoRI-PstI fragment into vector pUC28 to give pME9835. Mutations in the upstream motif (Mut1) were introduced into pME9835 using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) with mutagenesis primers Motif_1_fw and Motif_1_rev. The parental DNA template was digested with DpnI, and the mutated plasmid was introduced into E. coli XL1-Blue by transformation, generating pME9838. Mutations in the downstream motif (Mut2) were introduced into pME9835 using mutagenesis primers Motif_2_fw and Motif_2_rev. The mutated plasmid was termed pME9837. Mutations in both motifs (Mut1,2) were introduced into pME9837 using mutagenesis primers Motif_1_fw and Motif_1_rev, generating pME9839. Plasmids pME9838, pME9837, and pME9839 were digested with EcoRI and PstI, and the resulting 171-bp restriction fragments were inserted into pME6016 at the corresponding sites to give pME9845, pME9843, and pME9834. The –24 box mutations in the ccrZ promoter were introduced into pME9835, again by the QuickChange site-directed mutagenesis method, with mutagenesis primers rop-nmut1_dir and rop-nmut1_rev; this generated pME9825, which was digested with EcoRI and PstI. The mutated 171-bp restriction fragment was cloned into pME6016 at the same sites, giving pME9826.

Primers carrying a translational lipA9_EcoRI and lipA1 from PAO1 chromosomal DNA was digested with EcoRI and BamHI and inserted into pME6013 to give pME9831. A 219-bp PCR product obtained with primers crcZ7fw_EcoRI and crcZ-lacZrev were used to amplify a 1,549-bp PCR product with plasmid pSU300 as template. This placed the cloned cbrB promoter behind the lacZ promoter.

For the overexpression of His-tagged, truncated ‘cbrB, primers ArgU-3 and ArgU-4 were used to amplify a PCR product of 1,446 bp. This PCR product was digested with BglII and EcoRI and inserted into pBAD/HisB at the corresponding sites, giving pME9845. The –24 box mutations in the ccrZ promoter were introduced into pME9835, again by the QuickChange site-directed mutagenesis method, with mutagenesis primers rop-nmut1_dir and rop-nmut1_rev; this generated pME9825, which was digested with EcoRI and PstI. The mutated 171-bp restriction fragment was cloned into pME6016 at the same sites, giving pME9826. The resulting plasmid was termed pME9837. Mutations in both motifs (Mut1,2) were introduced into pME9837 using mutagenesis primers Motif_1_fw and Motif_1_rev, generating pME9839. Plasmids pME9838, pME9837, and pME9839 were digested with EcoRI and PstI, and the resulting 171-bp restriction fragments were inserted into pME6016 at the corresponding sites to give pME9845, pME9843, and pME9834. The –24 box mutations in the ccrZ promoter were introduced into pME9835, again by the QuickChange site-directed mutagenesis method, with mutagenesis primers rop-nmut1_dir and rop-nmut1_rev; this generated pME9825, which was digested with EcoRI and PstI. The mutated 171-bp restriction fragment was cloned into pME6016 at the same sites, giving pME9826.

Primers carrying a translational lipA9_EcoRI and lipA1 from PAO1 chromosomal DNA was digested with EcoRI and BamHI and inserted into pME6013 to give pME9831. A 219-bp PCR product obtained with primers crcZ7fw_EcoRI and crcZ-lacZrev were used to amplify a 1,549-bp PCR product with plasmid pSU300 as template. This placed the cloned cbrB promoter behind the lacZ promoter.

**Electrophoretic mobility shift assays.** DNA fragments covering wild-type or mutated promoter regulatory regions were PCR amplified using designed primers (see Table S1 in the supplemental material). The DNA probes were prepared by labeling with [γ-32P]ATP and T4 polynucleotide kinase (34). Radioactively labeled DNA probes were allowed to interact with different concentrations of purified His-CbrB in a 20-μl reaction mixture containing 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, 5% (vol/vol) glycerol, 150 μg/ml acetylated bovine serum albumin, and 40 ng unlabeled pUC18 vector as nonspecific DNA.

**RESULTS**

**Mutational analysis of the crcZ promoter region.** The crcZ promoter of *P. aeruginosa* contains conserved -24/−12 boxes (TG GCCA CGN CTGCT) that are typical of σ54 promoters (consensus, TGGCCAGN T TGCAG, where the most highly conserved nucleotides are in boldface and N indicates any nucleotide [3]) (Fig. 1A). A transcriptional lacZ fusion to the predicted +1 transcription start site of the crcZ gene (on pME9805) was highly expressed in wild-type PAO1 (Fig. 1B), whereas the expression of the same construct was <1% in both an ropN and a cbrB mutant (Fig. 1C). The lacZ construct used contained 161 bp of the crcZ promoter region. When the crcZ promoter region was extended to 1,561 bp, which includes the upstream cbrB gene except for the first 12 codons (on pME9806; Fig. 1A), lacZ expression was unchanged in the wild type (data not shown), indicating that the crcZ promoter activity depends essentially on the 161-bp segment. Mutation of the -24 box in the crcZ-lacZ fusion construct (pME9826; Fig. 1A) resulted in <1% expression (Fig. 1C), confirming that the crcZ promoter is recognized by σ54 RNA polymerase.

A deletion removing the region from -161 to -116 (on pME9834) abolished crcZ-lacZ expression almost entirely (Fig. 1B). This region contains a sequence of dyad symmetry (Fig. 1A). Base change mutations in both parts of this palindrome (Mut1,2; on pME9845) also had a drastic negative effect on crcZ-lacZ expression (Fig. 1B). Deletion of the region extending from -161 to -138 (pME9842) or mutations in the upstream element of the palindrome (Mut1; on pME9845) reduced crcZ-lacZ expression by about 70%, compared with the wild-type value. Mutations in the downstream element (Mut2; on pME9843) resulted in the loss of about half of the lacZ activity (Fig. 1B). These data qualify the palindromic sequence as a UAS and suggest that it may be recognized by the CbrB response regulator.

**Construction and activity of His-tagged CbrB.** An amino acid sequence alignment of CbrB with related bEBPs reveals an N-terminal regulatory domain with a conserved aspartate residue that is predicted to accept phosphate from CbrA, a central domain, and a C-terminal helix-turn-helix domain for DNA binding (Fig. 2A). The central domain contains all of the motifs that are typical of bEBPs interacting with σ54 RNA polymerase: Walker A and Walker B motifs, which are required for ATP binding and hydrolysis, respectively; a GAFTGA element, which defines a sequence interacting with σ54, and a switch Asn and an R finger motif, which are involved in oligomerization and nucleotide binding, respectively (10). The former three subdomains are identical in CbrB and NtrC of *P. aeruginosa*, whereas the latter two subdomains are similar (Fig. 2A). Overall, CbrB and NtrC have 43% amino acid se-
sequence identity in a window of 387 residues. In some members of the NtrC family, the regulatory domain inhibits the activity of the bEBP and truncation of the N terminus can enhance this activity (11, 31). We therefore constructed a full-length, as well as a truncated, version of His-tagged CbrB. The truncated CbrB protein lacks the 13 N-terminal amino acid residues of CbrB (Fig. 2A), and its construction is described in Materials and Methods. Whereas the full-length construct in the expression vector pME6032 was active in vivo, we were unable to demonstrate specific DNA binding in vitro (data not shown). In contrast, the truncated His<sub>6</sub>-CbrB construct, when expressed from the induced tac promoter in pME6032, had partial activity as an activator of crcZ expression in vivo (Fig. 2B) and allowed detection of CbrB binding to the UAS (see below).

CbrB protein binds to a palindromic UAS located at −151 to −125 from the crcZ transcription start site. A wild-type crcZ promoter fragment of 183 bp interacted with the purified truncated His-CbrB protein in an electrophoretic mobility shift

FIG. 1. Sequence of the crcZ promoter region and recognition sites for CbrB. (A) In the nucleotide sequence of the crcZ promoter region, the 45-bp UAS region is highlighted in gray. The cbrB stop codon, the mutations in motifs M1 and M2 of the UAS, and the −24 and −12 elements of the σ<sup>54</sup> promoter are all shown in boxes. Inverted arrows show a palindromic sequence in the UAS region; arrows with solid lines below boldface nucleotides indicate the extent of the CbrB consensus binding site as defined in Fig. 6. Dashed inverted arrows show a putative ρ-independent terminator of the cbrB transcript. A conserved IHF binding site (nucleotides in boldface and italics) is underlined. The arrow at +1 shows the start of crcZ transcription. In the schematic representation of the crcZ-lacZ transcriptional fusions carried by different plasmids, Mut1, Mut2, and Mut1,2 designate mutations in motifs M1 and/or M2, respectively. σ<sup>54</sup>mut indicates that the −24 promoter element GG was mutated into TT. (B) β-Galactosidase activities were determined in wild-type strain PAO1 carrying crcZ-lacZ transcriptional fusions on pME9805 (filled circles), pME9834 (filled squares), pME9842 (filled triangles), pME9843 (open squares), pME9845 (open circles), pME9843 (open squares), or pME9846 (open triangles). (C) β-Galactosidase activities of crcZ-lacZ carried by pME9805 were determined in the cbrB deletion mutant PAO6711 (closed circles) and in the rpoN deletion mutant PAO6358 (open circles). β-Galactosidase activities of crcZ-lacZ carried by pME9826 (containing a mutated −24 promoter element) were measured in wild-type (wt) PAO1 (closed circles connected with dotted lines).
assay, and the formation of protein-DNA complexes was seen (Fig. 3). Deletion of both elements of the palindromic UAS (H9004 M1,2) abolished CbrB binding, whereas either deletion of the upstream element (H9004 M1) or mutation of the downstream element (Mut2) resulted in diminished CbrB binding (Fig. 1A and 3). These data confirm the results obtained with the crcZ transcriptional fusions and indicate that a single element in the UAS is sufficient for CbrB binding. However, both elements are needed for strong binding and full activation of the crcZ promoter.

IHF is required for crcZ expression. Contact between bEBPs and RNA polymerase is often facilitated by IHF, a DNA-bending protein that is structurally and functionally conserved between enteric bacteria and P. aeruginosa (8). A sequence resembling the IHF consensus binding site, AATCAAN_4TTG (23), occurs in the crcZ promoter region at −60 to −48 (Fig. 1A). We therefore tested the crcZ-lacZ fusion (on pME9805) in the IHF-negative mutant PAO6766. At the end of exponential growth in LB, the expression was very low (100 ± 15 Miller units), compared to that in the wild type (35,600 ± 2,600 Miller units), indicating that IHF is required for the activation of the crcZ promoter. It is likely that this activation is due to direct binding of IHF to the proposed IHF binding site, but indirect effects of IHF on crcZ expression also remain possible.

Autoregulation of cbrB expression. It has been noted previously that cbrB expression is not constitutive but varies depending on the growth medium, with succinate exerting catabolite repression (30). We found that a cbrB-lacZ fusion
carried by pME9814 in wild-type PAO1 was expressed more strongly at the end of growth than during the exponential phase in rich medium (Fig. 4). In the cbrB mutant PAO6711, the same fusion was expressed at elevated levels during exponential growth (Fig. 4), suggesting negative autoregulation of cbrB. The cbrB promoter region contains a potential CbrB binding site, which is located between –21 and +7 (relative to the +1 transcription start site; reference 30) and which is weakly conserved (ATGGAAGN$_4$GATAACCA [conserved nucleotides are in boldface] in comparison with the CbrB binding site found in the crcZ upstream region (CTGGTAC N$_4$GTAACCA). However, we did not investigate the role of the putative autoregulatory site further and autoregulation might involve additional regulatory elements.

**Positive CbrB control of the lipA promoter.** In *P. alcaligenes*, the expression of the lipA gene coding for secreted lipase is positively controlled by RpoN and by the LipQ/LipR two-component system. LipQ and LipR are homologues of *P. aeruginosa* CbrA and CbrB, respectively (9, 18). At –130 to –112 of the lipA promoter, a UAS has been proposed as a LipR binding site (7). In *P. aeruginosa*, lipA expression also requires RpoN and LipR (= CbrB) (17), but molecular details of the lipA promoter and its putative UAS have not been studied. We constructed several translational lipA-lacZ fusions, with and without the UAS (Fig. 5A). Two constructs containing the UAS (pME7260 and pME9831) showed 7- to 10-fold higher expression than did a construct lacking the UAS (pME9844) in wild-type PAO1 (Fig. 5B). In the cbrB mutant PAO6711, lacZ expression directed by pME7260 was low, confirming the requirement for CbrB (Fig. 5B). The requirement for RpoN was corroborated both by mutation of the host rpoN gene and by mutations in the –24 box of the lipA promoter. Both types of mutation strongly diminished lipA expression to <1% of the wild-type level (Fig. 5B).

We then asked whether lipA expression was under catabolite repression control, as would be predicted from the involvement of CbrB as an activator of the lipA promoter. When wild-type cells harboring a lipA-lacZ fusion with an intact UAS (on pME9831) were grown in succinate minimal medium, expression was about 10-fold lower than that in mannitol-grown cells (Fig. 5C). We verified that this differential expression was not due to translational regulation via Cre (data not shown). Importantly, a lipA-lacZ construct lacking the UAS (on pME9844) did not give rise to derepressed β-galactosidase levels in mannitol-grown cells and was expressed at low levels throughout growth (Fig. 5C). We conclude that CbrB mediates catabolite repression of lipA gene expression, probably by interacting directly with the UAS, although the possibility of an indirect activation mechanism is not entirely excluded.

In an electrophoretic mobility shift assay, the purified His-CbrB protein interacted with the wild-type lipA promoter region (corresponding to pME9831) at the highest protein concentration used but caused no band shift of a promoter fragment lacking the UAS (corresponding to pME9844) (see Fig. S2 in the supplemental material). We suspect that the relatively low affinity of His-CbrB for the UAS in the lipA promoter reflects a suboptimal recognition sequence in comparison with the UAS in the crcZ promoter (Fig. 6).

**DISCUSSION**

The CbrA/CbrB two-component system was discovered as a master regulator that is necessary for growth of *P. aeruginosa* on a large range of carbon and nitrogen sources such as histidine, proline, arginine, alanine, polyamines, benzoate, or mannitol (30). In independent work, the homologous LipQ/LipR two-component system was described as an activator of lipase expression in an industrial strain of *P. alcaligenes* (9), which is typically grown in soybean oil medium for lipase production.
All of the substrates mentioned sustain the growth of pseudomonads to high cell population densities but are energetically less favorable than C4 dicarboxylates, glutamate, and aspartate, which are preferred substrates of pseudomonads (30, 33). An initial simple model pictured CbrB as a general transcriptional activator of genes that encode catabolic enzymes for the mineralization of less-preferred substrates (16). As it appears now, this regulatory model still applies to the lipAH operons of P. alcaligenes and P. aeruginosa and to the hutUH1TH2IG operon of P. aeruginosa. Previous genetic studies have indicated that CbrB recognizes a UAS and activates the expression of the lipAH operon of P. alcaligenes and of the hut operon of P. aeruginosa (16, 18). However, it has recently become clear that a majority of catabolic pathways are not under direct transcriptional control by CbrB but are regulated indirectly via the sRNA CrcZ and the RNA-binding protein Crc in P. aeruginosa (36). CbrB strongly activates the promoter of the crcZ gene, and the resulting elevated levels of CrcZ account for sequestration of the Crc protein, which then no longer translationally represses mRNAs coding for catabolic enzymes (36).

The powerful activation of the crcZ promoter by CbrB and the strict dyad symmetry in the UAS have provided us with a lead to define the CbrB recognition sequence in P. aeruginosa. By introducing point mutations and deletions into the UAS of crcZ, we have demonstrated that a palindromic sequence with a 12-bp spacer binds His-CbrB both in vivo and in vitro (Fig. 1 and 3). In the lipA promoter of P. aeruginosa, a similar palindrome occurs but with a 3-bp spacer (Fig. 5 and 6). Our deletion analysis confirms that this sequence is indeed required...
cTGTTACC  N_{12} cGTACAg  crcZ (P. aeruginosa)

gcGTTTCg  N_{3} cGTACAAa  lipA (P. aeruginosa)

tGTTTCCc  N_{3} gGTACAc  lipA (P. alcaligenes)

gTGTTAcC  N_{3} gGCCCAa  hutU (P. aeruginosa)

cTGTTAcC  N_{3/12} cGTACAg  CbrB consensus

TG CACC  N_{5} gGT GCA  NtrC consensus (E. coli)

CG CACC  N_{5} gGT GCA  NtrC consensus (P. putida)

FIG. 6. The proposed consensus CbrB recognition site is similar to the consensus NtrC recognition site. The CbrB binding sites in the crcZ and lipA promoters of P. aeruginosa were determined in this work (Fig. 1, 3, and 5; see Fig. S2 in the supplemental material). Mutational analysis of the lipA promoter of P. alcaligenes (7) and preliminary data on the hutU promoter of P. aeruginosa (16) reveal similar conserved palindromic sequences. Alignment of their sequences leads us to propose a consensus CbrB binding site, in which the spacer consists of 3 or 12 variable nucleotides (designated N3 and N12, respectively). The consensus CbrB binding site has similarity to the consensus NtrC binding sites of enteric bacteria (2) and P. putida (13, 14).

for CbrB-dependent lipA promoter activation (Fig. 5). The lipA promoter of P. alcaligenes also contains a similar palindrome with a 3-bp spacer, and mutational analysis has previously suggested its function as a UAS (7). In the case of the P. aeruginosa hutU operon, a CbrB binding site has been tentatively localized to the region extending from −240 to −160 (16). In this UAS, only the upstream element is fully conserved and the downstream element, which is separated by a 3-bp spacer, has three mismatches (Fig. 6). Nevertheless, this UAS may be expected to bind CbrB, as it appears that a single upstream or downstream element is sufficient for some CbrB binding in the crcZ promoter (Fig. 1 and 3). The combined analysis of these promoters allows us to propose a consensus CbrB recognition sequence in which the spacer may consist of either 12 bp or 3 bp (Fig. 6). The consensus NtrC binding site, as defined in enteric bacteria and in P. putida (2, 14), is similar to the CbrB recognition site: four nucleotides in each half-site are identical. However, the spacer for NtrC (N5) differs from the spacer for CbrB (N3 or N12) (Fig. 6). The overall similarity of CbrB and NtrC recognition sites may explain why a mutant form of ntrC has been found that can suppress a cbrB deletion for growth of P. aeruginosa on histidine (16).

In some members of the NtrC family, such as DctD and XylR, the N-terminal signal input domain inhibits the function of the N-terminal regulatory domain and its interaction with the CbrA sensor kinase.

By showing that CbrB activates the lipA promoter of P. aeruginosa and in this way controls succinate-dependent catabolite repression of lipase expression (Fig. 5; see Fig. S2 in the supplemental material), we have obtained further support for the model of Itoh et al. (16), which stipulates that CbrB regulates carbon sources and other preferred carbon sources inhibit the activity of the CbrA/CbrB two-component system, whereas growth media containing energetically suboptimal carbon sources are conducive to CbrA/CbrB activity. In turn, high CbrA/CbrB activity favors the expression of catabolic pathways for less-preferred substrates (including lipase-dependent utilization of lipids), whereas low CbrA/CbrB activity results in global catabolite repression of these pathways. As a corollary, the model proposes that the CbrA sensor must be able to integrate a multitude of nutritional signals. The molecular mechanisms by which CbrA achieves this function are not clear yet. Conceivably, the CbrA/CbrB system might receive input from accessory sensors and engage in cross talk with other two-component systems, especially NtrB/NtrC, in order to optimize carbon source utilization and to maintain the carbon-nitrogen ratio.

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