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Transcriptional Regulation of the *cydDC* Operon, Encoding a Heterodimeric ABC Transporter Required for Assembly of Cytochromes *c* and *bd* in *Escherichia coli* K-12: Regulation by Oxygen and Alternative Electron Acceptors

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The expression of the *cydDC* operon was investigated by using a chromosomal Φ(*cydD-lacZ*) transcriptional fusion and primer extension analysis. A single transcriptional start site was found for *cydD* located 68 bp upstream of the translational start site, and Northern blot analysis confirmed that *cydDC* is transcribed as a polycistronic message independently of the upstream gene *trxB*. *cydDC* was highly expressed under aerobic growth conditions and during anaerobic growth with alternative electron acceptors. Aerobic expression was independent of ArcA and Fnr, but induction of *cydDC* by nitrate and nitrite was dependent on NarL and Fnr.

Like almost all bacteria, the enteric bacterium *Escherichia coli* has to cope with constantly changing conditions in the environment to optimize its growth rate and yield. *E. coli* adapts to fluctuations in oxygen supply by using two well-characterized membrane-bound terminal oxidases for aerobic respiration, cytochromes *bo′* and *bd* (15). One of these oxidases, cytochrome *bd*, is expressed maximally at low aeration (7% air saturation) as determined by *cydA-lacZ* activity, but at 0% air saturation of the growth medium, the level of this oxidase is decreased to 60% of its maximum expression (44). ArcA was shown to be a positive regulator of *cydAB* expression under these conditions, but Fnr functioned as a repressor (44). It has also been shown that cytochrome *bd* is induced when *E. coli* is grown under alkaline or other unfavorable growth conditions (3, 6, 9).

In addition to the structural genes *cydA* and *cydB*, which encode the two subunits of the oxidase, two further genes, *cydD* and *cydC*, encoding a heterodimeric ATP-binding cassette (ABC-type) transporter, are required for a functional cytochrome *bd* in *E. coli* (16, 30–32). Mutants defective in *cydD* or *cydC* are sensitive to hydrogen peroxide and are unable to exit stationary phase and resume aerobic growth at high temperatures (11, 37). However, recent studies (19, 36) have suggested that it is the lack of a functional cytochrome *bd* caused by mutations in *cydD* or *cydC* that leads to the observed phenotypes of stationary-phase mortality and sensitivity to oxidative stress.

Mutations in *cydDC* do not influence the transcription of *cydAB* or the assembly of CydA or CydB polypeptides into the membrane (4). The oxidase polypeptides are assembled but lack the distinctive chlorin heme *d* and presumably hemes b_{595} and b_{558} (30). Periplasmic cytochromes (i.e., cytochrome *c* and cytochrome b_{562}) are also significantly reduced in *cydDC* mutants (18, 30). At present, neither the function of nor the substrate for the CydDC ABC transporter is known. The goal of the present work was to understand how expression of the *cydDC* operon is regulated and to define a role for this transporter in the growth physiology of *E. coli*. We report here the use of a chromosomal operon fusion to study *cydDC* expression under various conditions of oxygen supply and in the presence of alternative electron acceptors.

Identification of the transcriptional start site for *cydD*. *E. coli* K-12 strains, plasmids, and λ specialized transducing bacteriophage used in this study are described in Table 1. Two oligonucleotide primers complementary to regions within *cydD* were used to map the *E. coli* K-12 *cydD* transcriptional start site. Total RNA was prepared by hot phenol extraction of cells grown in Luria-Bertani (LB) medium of initial pH 7.0 (27) and harvested during exponential or stationary phase. Ten nanograms of 5′-end labelled (35) oligonucleotides GC1 (5′-CTTT TTGACGAGATTTATTCATTG-3′) and GC2 (5′-GCCCAG CAGACGACAAATATT-3′) was mixed with 10 μg of RNA in 10 μl of primer extension buffer (50 mM Tris-HCl [pH 8.3], 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM each deoxynucleoside triphosphate, 0.5 mM spermidine), heated at 75°C for 3 min, and cooled to room temperature for 10 min. Ten microliters of a prewarmed reverse transcriptase extension mix (1 U of avian myeloblastosis virus reverse transcriptase [Promega] in primer extension buffer containing 5.6 mM sodium pyrophosphate) was added to the annealed primer-RNA and incubated at 42°C for 30 min. Nucleic acids were ethanol precipitated and resuspended in formamide loading dye and separated on a 6% denaturing polyacrylamide gel. The size of the primer-extended product was calculated by running a known sequence ladder (M13mp18 DNA sequenced with primer −40 [United States Biochemicals]). Northern blot analysis was performed as described previously (35).

Figure 1a shows a major extension product with primer GC1, indicating that the 5′ end of the *cydD* mRNA lies 68 bp upstream of the *cydD* translational start site and is initiated at a guanidine nucleotide (Fig. 1b). Upstream of the transcriptional start point, there is a potential promoter sequence, GA CTATG (positions −11 to −5), resembling the consensus Pribnow box, TATAATG (8). A putative −35 region was found, the sequence 17 to 18 bp upstream of the presumptive −10 box being AAAGTA (positions −35 to −30). A higher

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TABLE 1. Strains, plasmids, and phage used in this study

Strain, plasmid, or phage	Relevant genotype	Reference or source
E. coli strains		
CG05	Φ(*cydC-lacZ*)Kan	16
ECL585	*arcA1 zjj*::Tn*10*	23
GS015	*rpoS*::Tn*10*	2
JD215	*htrD*::mini-Tn*10*	11
MK1010	IN(*rrnD-rrnE*)*1 cya*::Kan	24
QC1732	F^- Δ(*argF-lac*)*U169* Δ*fur*::Kan	43
RK4353	*araD139* Δ(*argF-lac*)*U169 gyrA219 non-9 rpsL150*	38
RK5278	Same as RK4353 but *narL215*::Tn*10*	V. J. Stewart
RKP3546	Same as VJS676 but Φ(*cydD-lacZ*)	This study
RKP3575	Same as RKP3546 but *fnr-271*::Tn*10*	This study
RKP3576	Same as RKP3546 but *arcA1*	This study
RKP3579	Same as RKP3546 but *rpoS*::Tn*10*	This study
RKP3580	Same as RKP3546 but *narL215*::Tn*10*	This study
RKP3582	Same as RKP3546 but *cya*	This study
RKP3584	Same as RKP3546 but *cydC*	This study
RKP3609	Same as RKP3546 but *cydAB*::Kan	This study
RKP3614	Same as RKP3546 but Δ*fur*::Kan	This study
RKP3636	Same as RKP3546 but *surA*	This study
RKP3655	Same as RKP3546 but *narP253*::Tn*10*d(Cm)	This study
RKP3658	Same as RKP3546 but *narL215*::Tn*10 narP253*::Tn*10*d(Cm)	This study
RKP3659	Same as RKP3546 but *cydD*	This study
RKP3695	Same as RKP3546 but pRP33 (*cydDC*$^+$)	This study
UNF3502	Δ*cydAB*::Kan	12
VJS1741	*fnr-271*::Tn*10*	V. J. Stewart
VJS4322	*narP253*::Tn*10*d(Cm)	V. J. Stewart
VJS676	Δ(*argF-lacZ*)*U169*	V. J. Stewart
ZK126	Δ*surA3*::Kan	25
Plasmids		
pRP33	*cydC*$^+$ *cydD*$^+$ in 5.3-kb *Cla*I fragment in pBR328, Ap^r	31
pRS528	Ap^r *lacZ*$^+$ *lacY*$^+$ *lacA*$^+$	38
pGC01	0.52-kb PCR product containing the *cydD* promoter region in pRS528	This study
Phage		
λGMC1	Φ(*cydD-lacZ*) (gene fusion)	This study
λRS45	*bla′-lacZ*$_{SC}$ *att*$^+$ *int*$^+$ *imm*^{21}	38

level of transcription was observed in cells grown under aerobic conditions than in cells grown anaerobically with glucose (compare lanes 1 and 2 in Fig. 1a). However, high levels of *cydD* transcription were observed in anaerobically grown cells if glycerol and nitrate were provided (data not shown). Because of the poor matches of the −35 and −10 sites to consensus sequences, we proceeded to investigate the possibility that *trxB* and the *cydDC* genes might form an operon. Northern blot analysis confirmed that *cydDC* was being transcribed as a polycistronic message under aerobic growth conditions and that mutations in *cydD* were polar on the expression of *cydC* (data not shown). Mutations in the upstream gene *trxB* had no effect on the expression of *cydDC* (data not shown). Delaney et al. (11) also demonstrated that *trxB* was transcribed as a monocistronic message, and a plasmid containing a *trxB* minimal complementing clone confirmed that *trxB* can be expressed in the absence of *cydDC* (34). Furthermore, between the *trxB* and *cydD* open reading frames is a rho-independent terminator sequence, suggesting that *trxB* transcription terminates at this site (Fig. 1b). No inverted repeat pattern was identified following the *cydD* open reading frame, and the stop codon of *cydD* (TAA) is arranged in frame and next to the start codon of *cydC* (ATG). Based on these observations, *cydDC* is transcribed as polycistronic message and this expression is not dependent on the upstream gene *trxB*.

Expression of Φ(*cydD-lacZ*) is maximal in exponentially growing cells under aerobic conditions. Since the discovery of *cydD* (*htrD*) (11, 32) and *cydC* (*surB*) (16, 37), no studies regarding the regulation and expression of these genes have been made. Delaney et al. (11) reported limited studies of the expression of a Φ(*htrD-lacZ*) operon fusion, but their interpretation was problematic since the operon organization of *cydD* and *cydC* had not been established and the gene product was unidentified. In the present study, an operon fusion of *cydD* to *lacZ* was constructed on a plasmid and then transferred to λ phage by recombination in vivo, by the method of Simons et al. (38). Standard methods were used for restriction endonuclease digestion, ligation of DNA, and DNA purification (26, 27). Transformation of bacteria with plasmid DNA was done by the single-step method of Chung et al. (7). A 522-bp PCR fragment containing 360 bp upstream of the translational start site of *cydD* was generated with primers GC3 (5′-AAAAGGATCCATCGGTCACAGCCCGAATACTGCGATTTTC-3′) and GC4 (5′-GTTGCAGAATTCGCGCCATGAACCAGGCCTGGGCAATGA-3′). Restriction sites are underlined. The amplified fragment was then cut with *Bam*HI and *Eco*RI, isolated from a low-melting-point agarose gel (1%, wt/vol) with the Bio-Rad (Hercules, Calif.) Prep-a-Gene kit, and ligated into vector pRS528, previously digested with *Eco*RI and *Bam*HI. The resulting plasmid containing the *cydD* promoter was se-

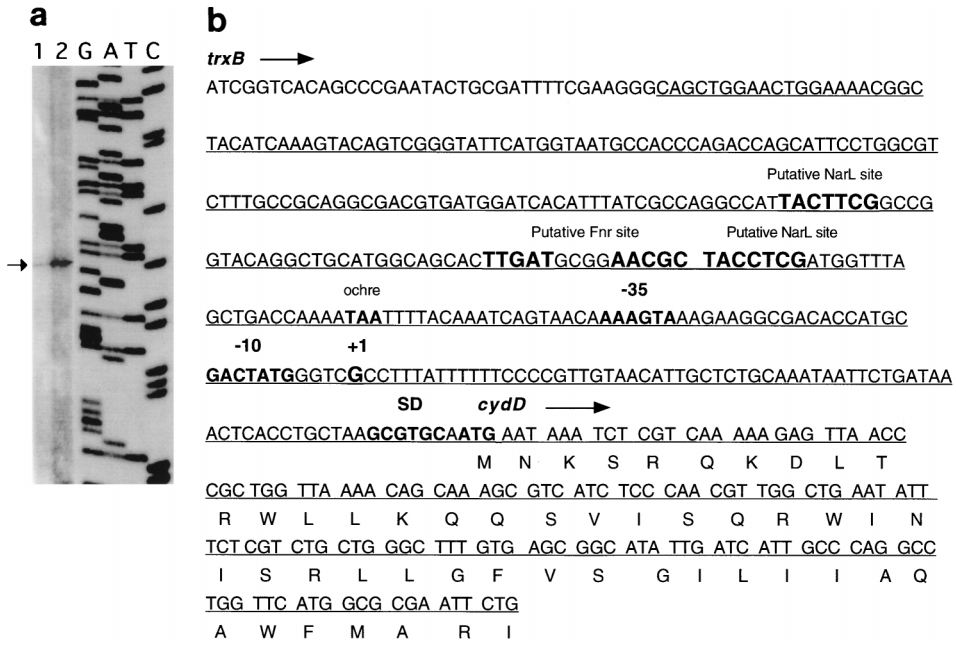

FIG. 1. (a) Primer extension analysis of the *cydD* transcript. A known nucleotide sequence ladder was used to estimate the size of the extended product, which is indicated by an arrow. Total RNA (10 μg) from mid-logarithmic-phase cells grown anaerobically (lane 1) and aerobically (lane 2) are shown. (b) Nucleotide sequence of the 522-bp *Bam*HI-*Eco*RI fragment (underlined) containing the putative *cydD* promoter. The translational start site is indicated as +1, near the putative ribosome binding site (SD). The putative −35 (AAAGTA) and −10 (GACTATG) sequences and putative Fnr and NarL binding sites are shown in boldface type. The amino acid translation product of *cydD* appears below the DNA sequence. The 3′ end of the *trxB* gene and the stop codon (ochre) are indicated.

quenced to confirm that the sequence of the insert in the amplified fragment was identical to that of the *cydD* promoter. The required recombinant plasmid (pGC01) was isolated by transformation of strain RK4353 (Δ*lac*). The fusion was recombined onto λRS45 to make λGMC1. Several single-copy fusions to the chromosome of VJS676 (Δ*lac*) were isolated and verified with β-galactosidase assays and Ter tests as described before (42). One such fusion strain (RKP3546) was used for the experiments described in this paper, but other fusions gave similar results. Mutant alleles introduced into strain RKP3546 by bacteriophage P1-mediated transduction (26) (Table 1) were *arcA* (ECL585), *cya* (MK1010), *cydAB* (UNF3502), *cydC* (CG05), *cydD* (JD215), *fnr* (VJS1741), *fur* (QC1732), *narL* (RK5278), *narP* (VJS4322), *rpoS* (GS015), and *surA* (ZK126).

Cells were grown in LB broth, initial pH 7.0, or in 3-(*N*-morpholino)propanesulfonic acid (MOPS)-buffered minimal medium with 40 mM glucose as the sole carbon source (40). Other carbon sources were added to 40 mM. The initial pH of MOPS-buffered medium was 7.4. Tetracycline, chloramphenicol, kanamycin, and ampicillin were used at final concentrations of 20, 25, 75, and 150 μg ml^{-1}, respectively. Nitric oxide was prepared and used as described previously (29). Culture optical density at 600 nm (OD_{600}) was measured with a Pye-Unicam SP6-550 spectrophotometer using culture samples diluted with medium to bring the OD_{600} to below 0.7 in cells with a 1-cm path length. All cultures were grown at 37°C. Aerated cultures were grown with shaking (200 rpm) in 250-ml conical flasks containing one-fifth of their own volume of medium. Anaerobic cultures for β-galactosidase assays were grown in screw-capped glass tubes (total volume, 15 ml) (41), filled to the brim and containing two glass beads (approximately 1 mm in diameter) to aid OD measurements by resuspension of cells that had sedimented during static culture. After growth to mid-exponential phase ($OD_{600} < 0.5$), chloramphenicol (100 μg/ml) or spectinomycin (for chloramphenicol-resistant strains [300 μg/ml]) was added and incubation continued for 5 min before harvest, to prevent adaptation to anaerobiosis or other change. β-Galactosidase assays were carried out at room temperature, around 21°C, as previously described (29). All experiments were carried out a minimum of three times; for each, three independent assays were done and the mean values for a typical experiment are shown in the results. The values obtained in independent experiments did not differ by more than 15%.

When strain RKP3546 [Φ(*cydD-lacZ*)] was grown in LB medium or minimal medium containing glucose under aerobic growth conditions, the level of Φ(*cydD-lacZ*) expression (Miller units; i.e., specific activity) was approximately 500 in exponentially growing cells (Table 2). When Φ(*cydD-lacZ*) expression was monitored throughout the growth cycle it was found that the level of Φ(*cydD-lacZ*) expression declined to around 200 Miller units as cell growth slowed and cells entered stationary phase (Table 2). Cells that were monitored well into stationary phase (12 h) exhibited no increase in the level of Φ(*cydD-lacZ*) expression (data not shown). Stationary-phase cells (12 h) reinoculated again into fresh medium showed an identical pattern of Φ(*cydD-lacZ*) expression. Because the level of Φ(*cydD-lacZ*) expression varied throughout the growth cycle, the level of Φ(*cydD-lacZ*) expression was compared in cultures of identical optical density to make quantitative comparisons. Delaney et al. (11) also reported that the level of *cydD* expression was low at high cell densities. Based on these results it appears as though *cydD* expression is not coordinately regulated with *cydAB* expression, which is maximal at high cell densities (13).

When cells were grown under anaerobic conditions in minimal medium, the level of Φ(*cydD-lacZ*) expression was fivefold lower in exponential phase than in aerobically grown cultures (Table 2). This basal level did not change throughout the growth cycle, and no stationary-phase-dependent induction was noted. A null mutation in *rpoS*::Tn*10* (strain RKP3579)

TABLE 2. Effects of alternative electron acceptors on the expression of Φ(*cydD-lacZ*) during growth of strain RKP3546 in minimal medium

Electron acceptor[a]	β-Galactosidase activity (Miller units) in medium with[b]:		
	Glucose (40 mM)	Glycerol (40 mM)	Sorbitol (40 mM)
Aerobic cultures			
Oxygen, exponential	530	600	590
Oxygen, stationary	230	250	240
Anaerobic cultures[c]			
None	110	NG	140
Nitrate	160	370	330
Nitrite	150	200	380
Fumarate	160	230	270
TMAO	130	140	170
Nitric oxide	120	130	140

[a] All alternative electron acceptors were added at 25 mM with the exception of nitrite and nitric oxide, which were tested at final concentrations of 5 mM and 50 μM, respectively.

[b] Cells were grown in minimal medium, and samples were collected during exponential growth ($OD_{600} < 0.5$) and at stationary phase ($OD_{600} > 2.0$) under the growth conditions tested. All values are means of three independent determinations. NG, no growth.

[c] All anaerobic cultures were grown to exponential phase.

had no effect on the expression of *cydD* under aerobic or anaerobic conditions (data not shown). Elevated *cydD* expression under aerobic growth conditions was also observed when primer extension analysis of the *cydD* transcript was performed (Fig. 1a). Neither an *arcA1* (strain RKP3576) nor an *fnr*::Tn*10* (strain RKP3575) mutation had any significant effect on the expression of Φ(*cydD-lacZ*) under aerobic or anaerobic conditions (data not shown). During aerobic growth in minimal medium, the level of Φ(*cydD-lacZ*) expression was not significantly affected by the nature of the carbon source supplied (Table 2) and the decreased expression of Φ(*cydD-lacZ*) in stationary phase was evident in all cases. Furthermore, an insertional mutation of *cya* (encoding adenyl cyclase) (strain RKP3582) had no significant effect on Φ(*cydD-lacZ*) expression during growth in minimal medium (data not shown).

Previous studies have shown that the level of cytochrome *bd* is expressed maximally under microaerobic growth conditions (13, 44). Because the level of Φ(*cydD-lacZ*) was low under anaerobic conditions, this result suggested that the transcription of *cydD* is also regulated by oxygen tension. To investigate this possibility further, the level of Φ(*cydD-lacZ*) was determined under various oxygen transfer rates used for cell growth. The oxygen transfer coefficient, K_La, was determined for shake flasks containing different volumes of solution by the sodium sulfite method (17). In a typical experiment, the volume of culture medium (LB) was varied from 5 to 50 ml in 250-ml conical flasks to alter K_La, a measure of the efficiency of oxygen absorption. Samples from cultures having different K_La values were taken during the logarithmic phase of growth. The level of Φ(*cydD-lacZ*) activity increased as the oxygen transfer rate increased in exponentially growing cultures (Fig. 2), indicating that oxygen tension was regulating Φ(*cydD-lacZ*) expression. Delaney et al. (11) reported that a transcriptional Φ(*htrD-lacZ*) (*cydD*) fusion showed maximal expression in aerobically growing cultures and that this expression decreased when cultures were transferred to anaerobic conditions. Those authors also demonstrated that this regulation was not *arcA*- or *fnr*-dependent under the growth conditions they used.

These results suggest that the transcription of *cydD* is regulated by oxygen or redox state. To clearly differentiate the effect of oxygen per se from that of a positive redox potential, the effect of hexacyanoferrate(III) and hexacyanoferrate(II) on the expression of *cydD* was studied (45). Experiments with hexacyanoferrate(III) [E'_0 of hexacyanoferrate(II/III), +360 mV] were performed as described previously (45). In the presence of 60 mM hexacyanoferrate(III) (redox potential, +500 mV) under anaerobic conditions, no effect on the expression of *cydD* was noted compared with controls in which no hexacyanoferrate was added (data not shown). Furthermore, addition of hexacyanoferrate(II) of the same concentration (redox potential, +180 mV) did not alter *cydD* expression compared with controls with no addition (data not shown). These results indicate that positive redox potential is not sufficient to increase *cydD* expression under anaerobic growth conditions.

Φ(*cydD-lacZ*) expression is increased under anaerobic growth conditions in the presence of alternative electron acceptors. When the electron acceptors nitrate, nitrite, or fumarate were provided during anaerobic growth with glucose as the sole carbon source, there was a 1.5-fold increase in the level of Φ(*cydD-lacZ*) expression above that where no electron acceptor had been added (Table 2). However, when cells were supplied with glycerol or sorbitol as the sole carbon source and an alternative electron acceptor, there was a significantly higher increase (two- to threefold) in the level of Φ(*cydD-lacZ*) expression. Nitrate and nitrite were the most effective inducers of Φ(*cydD-lacZ*) expression when sorbitol was supplied as the sole carbon source (Table 2). It may be significant that mutants defective in *cydD* fail to synthesize periplasmic *c*-type cytochromes (18, 30), which are expressed in *E. coli* only under anaerobic conditions with electron acceptors such as nitrite. Thus, *cydD* may play an important role during anaerobic growth on nitrate and nitrite. Nitric oxide and trimethylamine-*N*-oxide (TMAO) (Table 2) were ineffective at causing a significant increase in the expression of Φ(*cydD-lacZ*) under these conditions. The lack of induction by TMAO is surprising given the fact that other electron acceptors were effective at inducing Φ(*cydD-lacZ*). The TMAO reductase of *E. coli* has a *c*-type cytochrome but, unlike other *c*-type cytochrome-containing enzymes, is not induced by nitrate or nitrite and does not require a functional Fnr protein (15). The effect of *cydD* mutations on the synthesis of the TMAO reductase is not known.

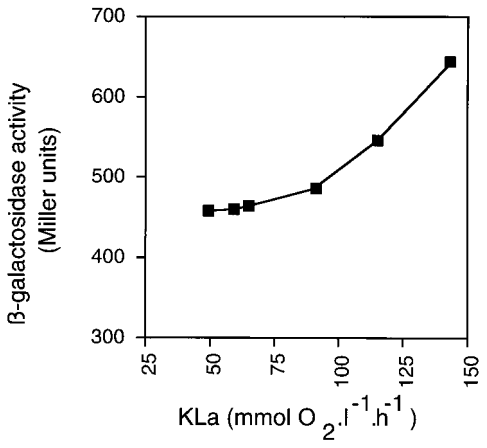

FIG. 2. Effect of oxygen supply rate (K_La) on the expression of Φ(*cydD-lacZ*) in strain RKP3546. Samples were taken throughout the exponential phase of growth ($OD_{600} < 0.5$).

TABLE 3. Effects of nitrate and nitrite and of mutant *nar* and *fnr* alleles on anaerobic Φ(*cydD-lacZ*) expression

Strain and genotype	β-Galactosidase activity (Miller units) with[a]:		
	No acceptor	Nitrate	Nitrite
RKP3546 (wild-type)	120	340	360
RKP3580 *narL*	110	120	140
RKP3655 *narP*	120	270	260
RKP3658 *narL narP*	100	120	130
RKP3575 *fnr*	110	130	110

[a] Samples were taken during exponential growth ($OD_{600} < 0.5$) under the growth conditions tested. All cells were grown in minimal medium supplemented with sorbitol and 0.1% Casamino Acids and the indicated additions. Nitrate and nitrite were added at final concentrations of 25 and 5 mM, respectively. All results are expressed as means of three independent determinations.

Nitrate and nitrite induction of *cydD* is dependent on NarL and Fnr. Nitrate and nitrite gene expression in *E. coli* is mediated by a two-component regulatory system composed of homologous membrane-bound sensor proteins (NarX and NarQ) and homologous DNA-binding response regulators (NarL and NarP) (33, 41). We therefore tested whether mutations in *narL* or *narP* had any effect on nitrate- or nitrite-induced Φ(*cydD-lacZ*) expression during anaerobic growth (Table 3). For these experiments, Casamino Acids (0.1%) were added to the growth medium to allow increased cell growth. The *narL* allele, whether introduced alone (strain RKP3580) or with the *narP* allele (strain RKP3658), reduced Φ(*cydD-lacZ*) expression in cells grown with nitrate or nitrite two- to threefold (Table 3). A mutation in *narP* (strain RKP3655) decreased the expression of *cydD* by only about 20%. When the level of Φ(*cydD-lacZ*) was studied in an *fnr* mutant (strain RKP3575) grown anaerobically in the presence of nitrate or nitrite, the level of Φ(*cydD-lacZ*) expression was again significantly reduced. It should be noted that the effects of these mutations are far less than for most Fnr- and NarL-regulated genes (33, 39, 40, 41), and therefore the effects on Φ(*cydD-lacZ*) expression may be indirect.

Various target operons regulated by nitrate appear to have different specificities for NarL and NarP. For example, the nitrate reductase genes *narGHJI* are regulated exclusively by NarL and are not responsive to NarP as observed here for *cydD*, while nitrate induction of the *fdnGHI* operon is regulated predominantly by NarL but is also responsive in part to NarP (33, 39). Examination of the promoter sequence of *cydD* revealed putative binding sites for NarL that closely resembled the consensus sequence, 5′-TACYNMT-3′ (where Y is C or T and M is A or C) (10). One site, 5′-TACTTCGG-3′, is centered 195 bp upstream of the *cydD* translation start codon. A second potential NarL site, 5′-TACCTCG-3′, is centered 148 bp upstream of the *cydD* translation start codon. All known NarL- and NarP-regulated target operons are also controlled by the Fnr protein in response to anaerobiosis (15) as reported here for *cydD*. Inspection of the DNA upstream of *cydD* reveals one potential Fnr-binding site (5′-TTGAT-N_4-AACGC-3′) centered 161 bp upstream of the *cydD* translation start codon which closely resembles the consensus, 5′-TTGAT-N_4-ATCAA-3′ (21). Further work is needed to confirm the identity of these putative binding sites for NarL and Fnr.

Effects of *surA* and *cyd* mutations on aerobic Φ(*cydD-lacZ*) expression. Delaney et al. (11) reported that mutations in *cydD* reduced the expression of *htrD*, but it was not then recognized that these genes are synonymous. Those authors hypothesized that the gene product of *cydD* was necessary for "*htrD*" expression. We confirmed that a mutation in *cydD* (strain RKP3659) caused a decrease in Φ(*cydD-lacZ*) expression (Table 4). Furthermore, a mutation in *cydC* (strain RKP3584) also caused a decrease in the levels of Φ(*cydD-lacZ*) expression. This suggests that the entire *cydDC* operon is required for its own expression. Overexpression of the CydDC transporter on a multicopy plasmid (pRP33) did not affect Φ(*cydD-lacZ*) expression. Mutations in *cydAB* (strain RKP3609) did not affect the expression of Φ(*cydD-lacZ*), suggesting that these operons are not coordinately regulated and that the structural polypeptides of the oxidase are not required for Φ(*cydD-lacZ*) expression. Conversely, the expression of Φ(*cydA-lacZ*) is unaffected by mutations in *cydD* or *cydC* (data not shown), in agreement with the results of Bebbington and Williams (4). Lazar and Kolter (25) recently reported that a periplasmic protein, SurA, is required for the folding of some outer membrane proteins (OmpA, OmpF, and LamB). When the expression of Φ(*cydD-lacZ*) was monitored in a *surA* background (strain RKP3636), the level of Φ(*cydD-lacZ*) expression was reduced significantly. Because the product of *cydDC* regulated the expression of *cydDC*, this may indicate that whatever is being exported by the CydDC transporter may require correct folding by SurA before it is active. Hence the inactive protein or molecule may block further export.

Gaballa et al. (14) have recently described an operon, *cytABC*, in *Pseudomonas fluorescens* that is required for cytochrome *c* biogenesis and export of pyoverdine, a siderophore that mediates high-affinity iron uptake in fluorescent pseudomonads. This operon has a high degree of similarity to previously described bacterial cytochrome *c* biogenesis genes (1, 5, 20, 28). One of these gene products, CytA, an inner membrane protein of the ABC transporter superfamily (14, 22), has been proposed to transport both heme and pyoverdine from the cytoplasm to the periplasm (14). We cannot eliminate a role for CydDC in iron transport (uptake), but the following results suggest that this is an unlikely role for this transporter. Increasing concentrations of 2,2′-dipyridyl (0.05 to 0.50 mM) did not cause an increase in the expression of Φ(*cydD-lacZ*) either aerobically or anaerobically (data not shown), and *cydDC* expression was not regulated by the repressor protein Fur (data not shown).

Conclusions. The CydD-CydC proteins are members of the ABC (ATP-binding cassette) superfamily of membrane transporters (22, 31) and most closely resemble those ABC transporters involved in export (e.g., the HlyB hemolysin exporter protein and the major histocompatibility complex peptide transporter). It is therefore tempting to speculate that CydD-CydC is involved in some export function. Consistent with this hypothesis are the observations that *cydD* mutants are unable to synthesize periplasmic *c*-type cytochromes (18, 30), cyto-

TABLE 4. Effects of *surA* and *cyd* mutations on aerobic Φ(*cydD-lacZ*) expression

Strain	Genotype	β-Galactosidase activity (Miller units)[a]	
		With O_2	Without O_2
RKP3546	Wild type	502	100
RKP3609	*cydAB*	643	124
RKP3659	*cydD*	77	100
RKP3584	*cydC*	64	111
RKP3636	*surA*	59	96
RKP3695	pRP33 (*cydDC*$^+$)	570	120

[a] Strains were cultivated in LB medium containing glucose. Cells were assayed during the exponential phase of growth ($OD_{600} < 0.5$).

chrome b_{562}, and cytochrome b_5 (18). The expression of the *cydDC* operon increases when a functional respiratory chain is needed for growth, i.e., when cells are grown aerobically or under anaerobic growth conditions with an alternative electron acceptor such as nitrate or nitrite. Because *cydDC* mutants lack both cytochrome *bd* and periplasmic cytochrome *c* for anaerobic growth on nitrate and nitrite, this operon plays an important role in the facultative growth of *E. coli.*

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