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Regulation of Expression of the Cytochrome *d* Terminal Oxidase in *Escherichia coli* Is Transcriptional

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The cytochrome *d* complex is one of the two terminal oxidases in the aerobic respiratory system of *Escherichia coli*. This enzyme is not present in cells grown with high levels of dissolved oxygen in the culture medium but accumulates after mid-exponential growth, reaching high levels in stationary-phase cells. In this study, the transcriptional activity of the *cyd* operon, encoding the two subunits of the enzyme, was examined under a variety of growth conditions. This was accomplished by the use of a chromosomal operon fusion, *cyd-lacZ*, generated in vivo by a λ p*lac*-Mu hopper bacteriophage and also by the use of a *cyd-lacZ* protein fusion created in vitro on a plasmid, transferred onto a lambda transducing phage, and examined as a single-copy lysogen. Transcription of the gene fusions was monitored by determination of β-galactosidase activity. The data clearly show that *cyd* is transcriptionally regulated and that induction is observed when the culture reaches a sufficient cell density so as to substantially reduce the steady-state levels of dissolved oxygen. The transcriptional activity is also regulated by other growth conditions, including the carbon source. The turn-on of *cyd* under semianaerobic conditions does not require the *fnr* gene product, cyclic AMP, or the cyclic AMP-binding protein.

The aerobic respiratory chain of *Escherichia coli* contains two terminal oxidases, the cytrochrome *o* complex and the cytochrome *d* complex (1, 12). These membrane-bound enzymes each catalyze the oxidation of ubiquinol-8 and the reduction of oxygen to water. Under laboratory growth conditions, these enzymes are redundant. Mutants lacking either enzyme grow normally (2, 8). The two oxidases are encoded by the *cyo* and *cyd* operons, which have been mapped to min 10.2 and 16.5, respectively, on the genetic linkage map of *E. coli* (2, 8). Simultaneous mutations in both *cyo* and *cyd* result in the inability of the cells to grow aerobically on nonfermentable substrates such as DL-lactate or succinate (2).

Although the two enzymes appear redundant under the conditions examined to date, their genetic regulation is quite different. The cytochrome *o* complex is predominant when the cells are grown with high oxygen levels (23). At low oxygen tension, the cytochrome *d* complex accumulates (15, 23). These results have been observed in batch cultures (15) and under continuous culture conditions in a chemostat (23). Possibly, the higher affinity of the cytochrome *d* complex for oxygen (K_m = 0.024 μM) compared with that of the cytochrome *o* complex (K_m = 0.2 μM) explains why this enzyme is favored under semianaerobic conditions (23). It has also been observed that cytochrome *d* is induced when cells are sulfate limited (22) or are grown with sublethal concentrations of cyanide (1a) so as to reduce the rate of electron transport. The amount of cytochrome *d* in the membrane of *E. coli* is also dependent on the carbon source, being maximal when cells are grown with DL-lactate.

The purpose of this study was to qualitatively demonstrate that the amount of cytochrome *d* present under various growth conditions is largely, if not entirely, due to transcriptional regulation of *cyd*. This operon was transcriptionally inactive at high oxygen levels, became maximally active after mid-exponential growth, and remained active, even under anaerobic growth conditions when there was no obvious need for the enzyme, except perhaps as an oxygen scavenger. The regulation of gene expression by oxygen is being investigated in a number of laboratories, both in procaryotes (e.g., see references 13, 20, and 26) and in yeast cells (17, 27). Several, although not all, of the genes encoding electron transport enzymes which are required for anaerobic respiration are positively regulated by the *fnr* gene product in *E. coli* (21) (*oxrA* in *Salmonella typhimurium* [13]). In the present report, it is shown that the *fnr* gene product is not required for the transcriptional activation of *cyd*.

MATERIALS AND METHODS

Bacteria, bacteriophages, and genetic procedures. The sources and properties of the various bacterial strains and phages used in this work are listed in Table 1. Transduction with phages P1 *cml* or P1 *kc* were performed as described by Miller (19). Aerobic growth complementation of *cyo* and *cyd* strains by λRG148 and pNG2, respectively, has been described previously (7).

Media and growth conditions. The rich aerobic lactate-succinate and anaerobic glycerol-nitrate growth media used throughout this work have been described previously (7). The aerobic DL-lactate, succinate, and glucose media, used for measuring the cytochrome *d* concentration in membrane preparations and the β-galactosidase activity of the operon and protein fusions, consisted of the minimal A medium of Miller (19) plus 1 μg of vitamin B_1 per ml, 0.5 mg of ferrous sulfate per liter, and 0.15% (wt/vol) Casamino Acids (Difco Laboratories), with the indicated carbon sources (final concentration, 0.3%) substituted for sodium citrate.

For β-galactosidase activity measurements, cells were grown in the indicated media at 37°C. Samples of an overnight culture were diluted 100-fold into 50 ml of growth medium in 250-ml sidearm conical flasks. Cultures were vigorously aerated by shaking at 350 rpm. Antibiotics were included when indicated: 50 μg of kanamycin per ml and 20 μg of tetracycline per ml. Ampicillin was added to a final concentration of 100 μg/ml for plasmid-carrying strains or 40 μg/ml for lambda lysogens.

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TABLE 1. Bacterial strains, phages, and plasmid used in this study

Strain, phage, or plasmid	Description	Reference or origin
Strains[a]		
CC118	*araD139* Δ(*ara leu*)*7697*, Δ*lacX74* Δ*phoA20 galE galK thi rpsE rpoB argE*(Am) *recA1*	18
CG11	RG139 Φ(*cyd-lacZ*$^+$)	This study
CG40	TP2006 Φ(*cyd-lacZ*$^+$)	P1(CG11) × TP2006; this study
CG42	TP2100 Φ(*cyd-lacZ*$^+$)	P1(CG11) × TP2100; this study
CG44	TP2139 Φ(*cyd-lacZ*$^+$)	P1(CG11) × TP2139; this study
JRG861a	*fnr-1 trpA9761 gal-25 strA195* λ$^-$	CGSC 5519[b]
RG84N	*nadA thi* Str^r *cydA2 recA*	This laboratory
RG139	*cyo* Δ(*argF-lac*)*U169 zba*::Tn*10 thi*	7
TP2139	Δ*crp* Δ*lacX74 xyl ilvA argH1*	24
TP2006	Δ*cya* Δ*lacX74 glp*	24
TP2100	Δ*lacX74 xyl ilvA argH1*	24
Phages		
λ p*lac*-Mu53	*imm*λ *'trp' lacZ*$^+$ *lacY*$^+$ *lacA' 'uvrD' Xho*::*kan* Mu (*c*I ts62 *ner*$^+$ *A*$^+$ *S*$^+$)	G. M. Weinstock[c]
λ pMu-507	*c*I857 *S*am7 Mu(*c*I ts62 *ner*$^+$ *A*$^+$ *B*$^+$)	G. M. Weinstock[c]
λRG148	λSE6 (7) carrying the *cyo* gene, *c*I857	This laboratory
Plasmid		
pNG2	pBR322 Δ(*Eco*RI-*Pvu*I) carrying *cyd*	9

[a] The sex of all strains was F$^-$.
[b] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[c] Hopper kit provided by G. M. Weinstock, Laboratory of Genetics and Recombinant DNA, NCI-Frederick Cancer Research Facility, Frederick, Md.

The Lac$^+$ phenotype was scored by using either lactose-MacConkey indicator plates or plates which contained 40 μg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) per ml (19).

Isolating *lacZ* operon fusions in the *cyd* locus. The in vivo creation of random *lacZ* operon fusions in the chromosome of *E. coli* with λ p*lac*-Mu53 and the isolation of colonies that grow on rich anaerobic but not on rich aerobic plates have been described previously (7). The method is based on the work of Bremer et al. (3). The *E. coli* strain which was used, RG139, lacks cytochrome *o*, so that inserts into *cyd* would be expected to prevent aerobic growth on nonfermentable substrates. From the colonies with an "aerobic-minus" phenotype, colonies were selected whose ability for aerobic growth was restored by complementation with the plasmid pNG2, which carries *cyd*, or with λRG148, which carries *cyo*. Membranes prepared from these strains did not contain cytochrome *d*, as judged by spectroscopic analysis (8). This phenotype is expected for insertions in the chromosomal *cyd* locus. Among strains carrying the presumed *cyd-lacZ* fusions was one strain which was Lac$^+$ on lactose-MacConkey plates. This strain, CG11, was used for the approximate mapping of the *lacZ* fusion within the *cyd* locus and to examine the transcriptional regulation of *cyd*.

Mapping the *cyd-lacZ* operon fusion. Plasmids containing overlapping fragments of the *cyd* gene were constructed by ligating restriction fragments from plasmid pNG2 (9), containing *cyd*, into the polylinker of a smaller plasmid, pZF19U (D. A. Mead and B. Kemper, unpublished data) (Fig. 1). These were used to transform strain CG11 by a high-efficiency method (10), using ampicillin resistance (Ap^r) for plasmid selection, with all growth accomplished anaerobically in an Oxoid anaerobic jar that generates an atmosphere of hydrogen gas. Stable transformants were grown anaerobically in 1 to 2 ml of rich glycerol-nitrate medium for 12 to 16 h, and samples were plated on rich lactate-succinate agar plates and incubated aerobically at 37°C for 1 to 2 days to check for aerobic transformants. To provide a measure of the recombination frequency, viable cell counts were determined by plating appropriate dilutions and growing them anaerobically. Transformation of the fusion strain with the plasmid vector lacking *cyd* sequences served as a control to check the reversion frequency. Recombinants which restored the ability of strain CG11 to grow aerobically were checked for loss of the λ p*lac*-Mu prophage by demonstration of renewed kanamycin sensitivity. In addition, spectra of membrane preparations were obtained to confirm restoration of cytochrome *d*.

Construction of pGC101. The 1.19-kilobase (kb) *Nru*I-*Nco*I Klenow blunt-end DNA fragment from pNG2 (9), containing the putative promoter of the *cyd* operon and the first 74 codons of the gene encoding subunit I, and the 6.25-kb *Sma*I-*Bam*HI Klenow blunt-end fragment from pMLB1034 (25) were ligated at the *Sma*I-*Nru*I and *Nco*I-*Bam*HI junctions to form plasmid pGC101 carrying the in-frame protein fusion *cyd-lacZ* (Fig. 2). Plasmid pGC101 was isolated from blue colonies on Luria-Bertani (LB) plates containing X-Gal plus ampicillin (150 μg/ml) after transform-

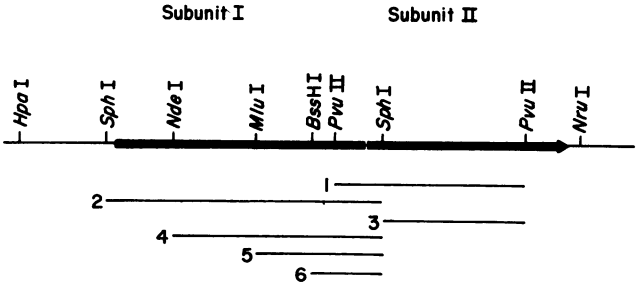

FIG. 1. Approximate mapping of the *cyd-lacZ* operon fusion of strain CG11. Marker rescue experiments were performed with plasmids containing the fragments of the *cyd* gene indicated by numbered lines. The fusion is located within the DNA region defined by *Nde*I-*Mlu*I.

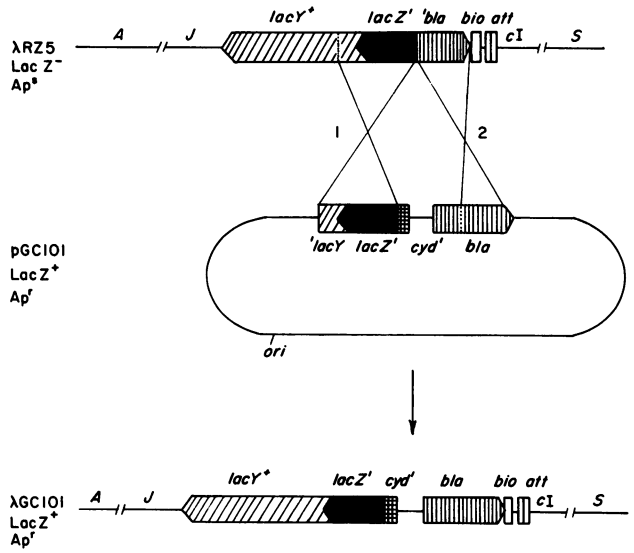

FIG. 2. Transfer of the *cyd-lacZ* protein fusion from pGC101 to the chromosome through λRZ5. The fusion was crossed into the lambda transducing phage by a double recombination event. Recombination 1 occurred between the homologous regions of either *lacY* or *lacZ*, and recombination 2 occurred within the region of *bla* homology. The resulting recombinant phage, λGC101, was used for the formation of lysogens. The phenotypes exhibited by strains carrying the particular phage or plasmid are shown on the left.

ing strain CC118 with the ligation mixture. The sequence of the fusion junction between codon 74 of subunit I of *cyd* (left, underlined) and codon 9 of *lacZ* (right, underlined), as well as the two codons resulting from the polylinker of pMLB1034, is shown below. This was determined by DNA sequencing (11).

5'....GGT.CTG.ACC.ATG.GAT.CCC.GTC.GTT.TTA.CAA.....3'

Creation of single-copy lambda lysogens of the *cyd-lacZ* protein fusion. To avoid problems associated with multicopy plasmids, a single copy of the *cyd-lacZ* protein fusion in pGC101 was transferred in vivo to the λRZ5 transducing phage (20) by double recombination between the *bla* and *lacZ* homologous regions of λRZ5 and pGC101 (Fig. 2). Phage λRZ5 was used to infect strain TP2100 carrying pGC101, and the resulting lysate was used to transduce TP2100 (*cya*$^+$ *crp*$^+$) and TP2006 (*cya crp*$^+$) to ampicillin resistance (Ap^r) and Lac$^+$ on LB plates containing X-Gal (40 μg/ml) and ampicillin (40 μg/ml). Strain TP2006 was grown on LB medium plus 5 mM cyclic AMP (cAMP) before it was infected with the lysate since the *lamB* gene product of the maltose regulon that is required for λ attachment is derepressed by cAMP (25). For each strain construction approximately 10 independent lysogens were grown and assayed for β-galactosidase expression to screen single from multiple lambda lysogens. Lysogens containing a single copy of λGC101 were used for *cyd-lacZ* expression analysis.

Spectroscopic and other methods. Membrane preparations and biochemical and spectroscopic procedures used in this work have been described previously (9, 16). The spectroscopic method used for the determination of the cytochrome *d* terminal oxidase concentration in the membrane fraction of the various strains used in this work has been described elsewhere (8).

Enzyme assays. β-Galactosidase assays were performed in duplicate as described by Miller (19), with the following modifications. Small samples of cells (1 to 2 ml) were drawn every 2 h during growth and after centrifugation at 10,000 × *g* for 5 min were suspended in 0.1 ml of ice-cold Z buffer (0.1 M $NaPO_4$, 1 mM $MgSO_4$, 10 mM KCl, 50 mM β-mercaptoethanol [pH 7.0]). Samples of these cells were then diluted in Z buffer to the appropriate density for enzyme assay in a final volume of 1 ml. The cells were made permeable by the addition of 2 drops of chloroform and 1 drop of 0.1% (wt/vol) sodium dodecyl sulfate and mixed by vortexing for 10 s. The samples were brought to 28°C by placing the tubes in a circulating water bath for 5 min, and the reaction was initiated by the addition of 0.2 ml of *o*-nitrophenyl-β-D-galactopyranoside (4 mg/ml in 0.1 M $NaPO_4$ [pH 7.0]). The reaction was stopped by the addition of 0.5 ml of 1 M Na_2CO_3. Whole-cell protein concentration was determined by using a correspondence of 0.25 mg/U of optical density at 600 nm for whole-cell suspensions. This was confirmed with cells harvested at the early exponential, mid-exponential, and late stationary phases of growth by a modification of the method of Lowry (14). All growth curve β-galactosidase activity data for each fusion strain represent averages of three independent experiments. Maximum levels of the β-galactosidase activity of the *cyd-lacZ* fusions were determined as averages of the values of five independent experiments with strains grown until the early stationary phase (optical density at 600 nm of 1.1 to 1.2). Cell density measurements were made with a Cary 219 spectrophotometer (Varian).

RESULTS

Mapping the *cyd-lacZ* operon fusion. Insertion of prophage λ *plac*-Mu53 into strain RG139 (*cyo*) yielded a strain, CG11, which is unable to grow aerobically, presumably due to interruption of the *cyd* gene. Transduction by phage P1 of the prophage into strain TP2100 resulted in a strain, CG42, that lacks cytochrome *d* by spectroscopic criteria but which can grow aerobically due to the presence of cytochrome *o* (*cyo*$^+$).

Marker rescue experiments with plasmids that carry various restriction fragments of *cyd* confirmed that the prophage is inserted in *cyd*. Plasmids 2 (restriction fragment *Sph*I to *Sph*I) and 4 (restriction fragment *Nde*I to *Sph*I) were able to restore the wild-type function of the insertionally mutated *cyd* gene in CG11 (Fig. 1). The frequency of recovery of aerobic growth per transformant was 3.7×10^{-7} for fragment 2 and 1.4×10^{-7} for fragment 4. The reported frequencies are averages from six independent experiments. These low frequencies may have been due to (i) the relatively small size of restriction fragments 2 and 4 (1.7 and 1.2 kb, respectively) in relation to the size of the prophage insertion (~50 kb) or (ii) the position of the prophage insertion in relation to the ends of the fragments. These experiments demonstrated that the fusion junction is located within the restriction fragment *Nde*I-*Mlu*I of the gene encoding subunit I (Fig. 1).

Factors affecting expression of *cyd*. The *cyd-lacZ* operon and protein fusions were used to monitor transcriptional activity of the *cyd* promoter under various conditions. The data in Fig. 3A demonstrate that the amount of cytochrome *d* in the membrane is dependent on the growth medium and conditions. This has previously been well documented (12), including the fact that cytochrome *d* is induced in cells grown aerobically only at low oxygen tension, e.g., when the cell density is high (12, 15, 23).

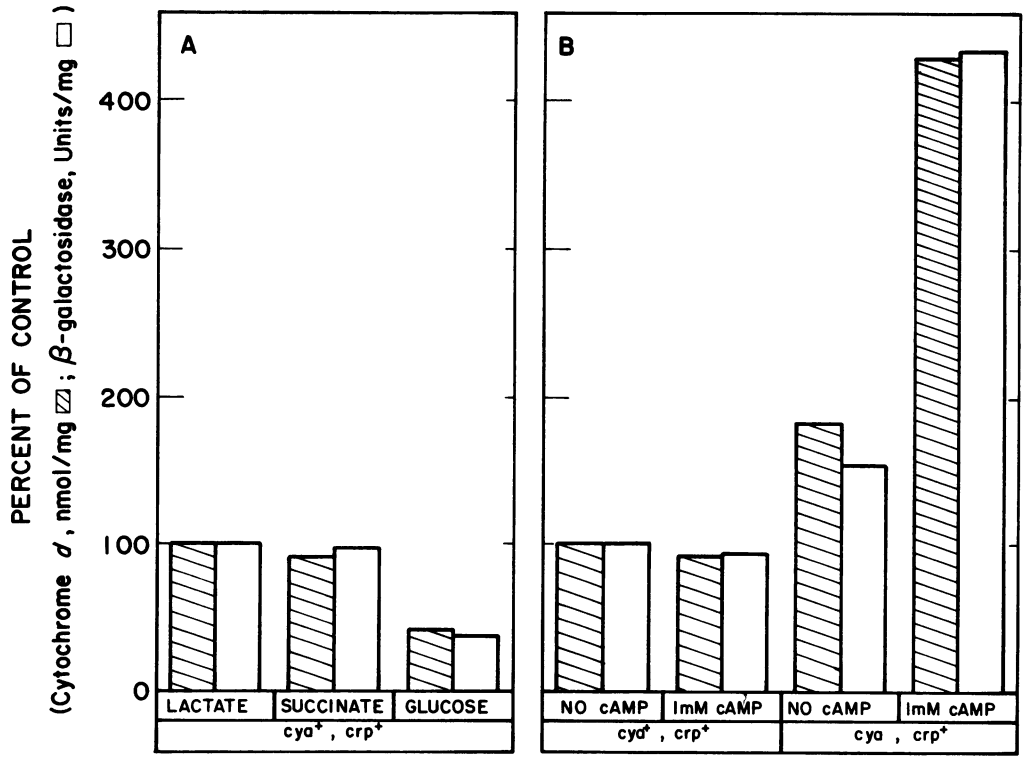

FIG. 3. Percent expression of protein fusion *cyd-lacZ* and production of cytochrome *d* as a function of carbon source (A) and cAMP (B). (A) Percent cytochrome *d* concentration in strain TP2100 (*cya*$^+$ *crp*$^+$) and β-galactosidase activity in the lambda lysogen strain TP2100(λGC101) measured in aerobic lactate, succinate, and glucose basal growth media. (B) Percent cytochrome *d* concentration in strains TP2100 (*cya*$^+$ *crp*$^+$) and TP2006 (*cya crp*$^+$) and β-galactosidase activity in lambda lysogen strains TP2100(λGC101) and TP2006(λGC101) measured in lactate basal growth medium with or without 1 mM cAMP. The cytochrome *d* levels and the β-galactosidase activity were measured at the late exponential to early stationary growth phase (cell density, $A_{600} \sim 1.1$ to 1.2). A 100% cytochrome *d* concentration in lactate basal medium corresponds to 0.28 nmol of heme *d* per mg of membrane protein; 100% β-galactosidase activity in lactate basal medium corresponds to 1,255 U/mg of whole-cell protein.

Most of the studies used TP2100(λGC101), which carries *cyd*$^+$ and also contains a prophage which has a single copy of a gene encoding a *cyd-lacZ* protein fusion. The fusion junction is located near the amino-terminal end of the proximal subunit in the *cyd* operon, and the fusion product is a soluble, cytoplasmic protein not bound to the membrane (data not shown). Overnight growths of this strain were prepared by using DL-lactate, succinate, and glucose as carbon sources. The data in Fig. 3A show high levels of cytochrome *d* when the cells were grown with the nonfermentable substrates and about half of this amount when the cells were grown with glucose. The β-galactosidase activity from the *cyd-lacZ* fusion product showed the same pattern (Fig. 3A). The specific activity of β-galactosidase in the cells was measured as a function of growth time (Fig. 4B). The results show a dramatic increase in transcription of the *cyd-lacZ* gene fusion during the mid-exponential growth phase. After the cell density reached about 1 absorbance unit at 600 nm, the transcriptional activity reached its maximal level, which was then maintained.

Similar results were obtained both with the chromosomal operon fusion (Fig. 4A) and with a multicopy plasmid encoding the protein fusion (Fig. 4C). The results obtained with a *lac*$^+$ strain, RG84N, in which the *lac* operon was induced by the inclusion of IPTG (isopropyl-β-D-thiogalactopyranoside) in the growth medium are shown in Fig. 4D. In this instance, the β-galactosidase levels were highest during the early exponential phase of growth, in contrast to the data obtained when expression was regulated by the *cyd* promotor. Several genes have been implicated in regulating the expression of genes which are turned on under anaerobic conditions, most notably *fnr* (21). Strain JRG861a, which carries *fnr*, was examined and clearly was able to synthesize cytochrome *d*, as determined by spectroscopic criteria (data not shown). Hence, *fnr* is not absolutely required for *cyd* expression. The possibility that *fnr* might influence the rate of transcription of *cyd* was not investigated.

The fact that the operon fusion and the lysogen-borne protein fusion (single copy) were induced in a similar fashion suggests that the presence of the wild-type *cyd* allele has no influence on the expression of the gene fusions. This was confirmed by examining the expression of the gene fusions in strains harboring a multicopy plasmid (pNG2) carrying the *cyd* operon. The presence of the plasmid had no effect on the expression of the gene fusions. This suggests that the *cyd* gene is not regulated by its own translation products.

The data in Fig. 3B confirm previous puzzling observations (4–6) concerning the effects of cAMP on the expression of cytochrome *d*. Strains unable to make cAMP (*cya*) or which lack the cAMP-binding protein (*crp*) produced cytochrome *d* in overnight growths at levels 1.5- to 2-fold higher than did isogenic strains (data not shown). The same effect was seen in the expression of the *cyd-lacZ* protein fusion in *cya*$^+$ and *cya* mutant strains. This suggests a possible role of cAMP as a repressor of the *cyd* operon, as implied previously (6). However, the addition of exogenous cAMP to

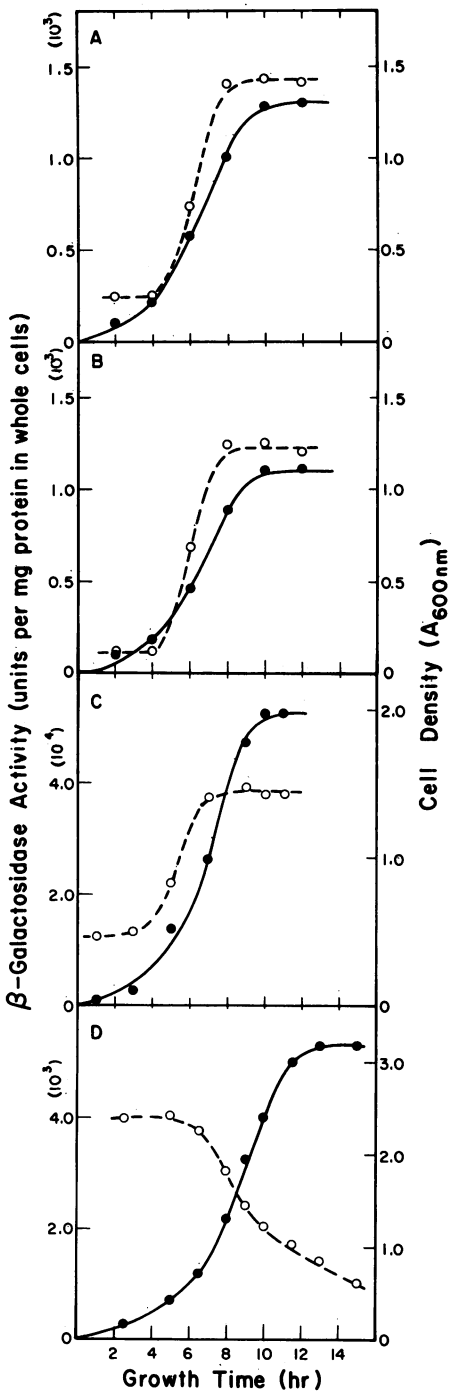

FIG. 4. Expression of *cyc-lacZ* operon and protein fusions as a function of growth time in lactate basal medium. (A) Strain CG42 carrying a chromosomal operon fusion; (B) strain TP2100 carrying a protein fusion in a λGC101 lysogen; (C) strain TP2100 transformed with pGC101 carrying a protein fusion; (D) control strain RG84N grown in glucose basal medium supplemented with 1 mM IPTG. - - - -, β-Galactosidase activity; ——, cell density.

strain TP2006 (*cya*) did not reduce the levels of either cytochrome *d* or the protein fusion but instead resulted in a further increase in both by 2.5- to 3-fold (Fig. 3B). Similar results have been reported previously (4). In contrast, exogenous cAMP had no effect on the expression of *cyd* in strain TP2100 (*cya*$^+$ *crp*$^+$) or TP2139 (*cya*$^+$ *crp*). No attempt will be made to explain these data, but they indicate an effect, either direct or indirect, on *cyd* transcription which is mediated by cAMP.

One artifact was encountered in these experiments relating to the chromosomal operon fusion. It was P1 transduced into strains TP2006 (*cya*) and TP2139 (*crp*), and the cAMP dependence of expression was examined. Expression of the operon fusion in the *cya* genetic background was absolutely dependent on the addition of exogenous cAMP. This is in contrast to results obtained with the protein fusion (single or multiple copy) or by directly measuring cytochrome *d*, which show that expression was doubled in the absence of cAMP. As expected, the addition of cAMP to the growth medium restored the expression of the *cyd-lacZ* operon fusion in strain CG40 (*cya*), which was induced at the mid-exponential growth phase. In a consistent manner, the *cyd-lacZ* operon fusion was not expressed in CG44 (*crp*), and exogenous cAMP had no effect. It can only be concluded that the operon fusion created or unmasked sites which made expression absolutely dependent on cAMP and its binding protein in a manner not observed with either *cyd* or the independently generated protein fusion.

DISCUSSION

The major conclusion of this work is that the regulation of the amount of cytochrome *d* in *E. coli* under various growth conditions is primarily at the level of transcription of the *cyd* operon. The most interesting phenomenon was the relatively sudden turn-on of transcription during aerobic growth once the cell density reached a sufficient level ($A_{600} \simeq 0.5$). Cytochrome *d* expression reaches its maximum during the mid-exponential growth phase, and the enzyme continues to accumulate into the stationary phase. Previous work using continuous cultures has shown that this effect is due to a decrease in oxygen tension in the culture medium because of the increase in cell density (23). This induction under quasi-anaerobic conditions was not influenced by the *fnr* gene, known to be required for expression of electron transfer systems utilized under anaerobic growth conditions (21).

Relatively subtle but real effects were observed due to differences in the growth medium, with aerobic growth in DL-lactate and succinate resulting in maximum expression of *cyd*. It is not yet clear whether this modulation was due to a direct or indirect effect on *cyd*. The data confirm early reports on the effects of cAMP (4–6), although these effects are complex and have not been demonstrated to be physiologically relevant. One interesting, but puzzling, artifact was the false indication obtained with the operon fusion that *cyd* transcription is dependent on cAMP and the *crp* gene product. This was not observed when the amount of cytochrome *d* present in membranes from *cya* or *crp* strains was monitored, and the cAMP dependence was not observed with the *cyd-lacZ* protein fusion on a multicopy plasmid or lambda lysogen. It is not known whether this artifact was due to the specific operon fusion which was examined or whether it is a more general phenomenon of *cyd-lacZ* fusions generated in vivo by this method.

The gene fusions described in this report will be particularly useful in extending the study of the transcriptional regulation of *cyd*. In particular, these fusions will be used to genetically define other genes required for the expression of *cyd* under conditions of low aeration and to define the target DNA sequences in the 5′-flanking region upstream of the *cyd* operon which are responsible for these effects.

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LITERATURE CITED

1. **Anraku, Y., and R. B. Gennis.** 1987. The aerobic respiratory chain of *Escherichia coli*. Trends Biochem. Sci. **12**:262–266.
1a. **Ashcroft, J. R., and B. A. Haddock.** 1975. Synthesis of alternative membrane-bound redox carriers during aerobic growth of *Escherichia coli* in the presence of potassium cyanide. Biochem. J. **148**:349–352.
2. **Au, D. C.-T., R. M. Lorence, and R. B. Gennis.** 1985. Isolation and characterization of an *Escherichia coli* mutant lacking the cytochrome *o* terminal oxidase. J. Bacteriol. **161**:123–127.
3. **Bremer, E., T. J. Silhavy, and G. M. Weinstock.** 1985. Transposable λ *plac*Mu bacteriophages for creating *lacZ* operon fusions and kanamycin resistance insertions in *Escherichia coli*. J. Bacteriol. **162**:1092–1099.
4. **Broman, R. L., W. J. Dobrogosz, and D. C. White.** 1974. Stimulation of cytochrome synthesis in *Escherichia coli* by cyclic AMP. Arch. Biochem. Biophys. **162**:595–601.
5. **Dallas, W. S., Y. Tseng, and W. J. Dobrogosz.** 1976. Regulation of membrane functions and fatty acid composition in *Escherichia coli* by cyclic AMP receptor protein. Arch. Biochem. Biophys. **175**:295–302.
6. **Daoud, M. S., and B. A. Haddock.** 1976. Electron transport in mutants of *Escherichia coli* deficient in their ability to synthesize adenosine 3′:5′-cyclic monophosphate and the catabolite-gene activator protein. Biochem. Soc. Trans. **4**:711.
7. **Georgiou, D. C., H. Fang, and R. B. Gennis.** 1987. Identification of the *cydC* locus required for expression of the functional form of the cytochrome *d* terminal oxidase complex in *Escherichia coli*. J. Bacteriol. **169**:2107–2112.
8. **Green, G. N., and R. B. Gennis.** 1983. Isolation and characterization of an *Escherichia coli* mutant lacking cytochrome *d* terminal oxidase. J. Bacteriol. **154**:1269–1275.
9. **Green. G. N., J. E. Kranz, and R. B. Gennis.** 1984. Cloning the *cyd* gene locus coding for the cytrochrome *d* complex of *Escherichia coli*. Gene **32**:99–106.
10. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
11. **Hattori, M., and Y. Sakaki.** 1986. Dideoxy sequencing method using denatured plasmid templates. Anal. Biochem. **152**:232–238.
12. **Ingledew, W. J., and R. K. Poole.** 1984. The respiratory chains of *Escherichia coli*. Microbiol. Rev. **48**:222–271.
13. **Jamieson, D. J., and C. F. Higgins.** 1986. Two genetically distinct pathways for transcriptional regulation of anaerobic gene expression in *Salmonella typhimurium*. J. Bacteriol. **168**: 389–397.
14. **Koland, J. G., J. Miller, and R. B. Gennis.** 1984. Reconstitution of the membrane-bound, ubiquinone-dependent pyruvate oxidase respiratory chain of *Escherichia coli* with the cytochrome *d* terminal oxidase. Biochemistry **23**:445–453.
15. **Kranz, R. G., C. A. Barassi, and R. B. Gennis.** 1984. Immunological analysis of the heme proteins present in aerobically grown *Escherichia coli*. J. Bacteriol. **158**:1191–1194.
16. **Kranz, R. G., and R. B. Gennis.** 1982. Isoelectric focusing and crossed immunoelectrophoresis of heme proteins in the *Escherichia coli* cytoplasmic membrane. J. Bacteriol. **150**:36–45.
17. **Lowry, C. V., and R. H. Lieber.** 1986. Negative regulation of the *Saccharomyces cerevisiae ANB1* gene by heme, as mediated by the *ROX1* gene product. Mol. Cell. Biol. **6**:4145–4148.
18. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export signals. Proc. Natl. Acad. Sci. USA **82**: 8129–8133.
19. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Ostrow, K. S., T. J. Silhavy, and S. Garrett.** 1986. *cis*-acting sites required for osmoregulation of *ompF* expression in *Escherichia coli* K-12. J. Bacteriol. **168**:1165–1171.
21. **Pascal, M.-C., V. Bonnefoy, M. Fons, and M. Chippaux.** 1986. Use of gene fusions to study the expression of *fnr*, the regulatory gene of anaerobic electron transfer in *Escherichia coli*. FEMS Microbiol. Lett. **36**:35–39.
22. **Poole, R. K., and B. A. Haddock.** 1975. Effects of sulphate-limited growth in continuous culture on the electron-transport chain and energy conservation in *Escherichia coli* K12. Biochem. J. **152**:537–546.
23. **Rice, C. W., and W. P. Hempfling.** 1978. Oxygen-limited continuous culture and respiratory energy conservation in *Escherichia coli*. J. Bacteriol. **134**:115–124.
24. **Roy, A., C. Haziza, and A. Danchin.** 1983. Regulation of adenylate cyclase synthesis in *Escherichia coli*: nucleotide sequence of the control region. EMBO J. **2**:791–797.
25. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Winkelman, J. W., and D. P. Clark.** 1986. Anaerobically induced genes of *Escherichia coli*. J. Bacteriol. **167**:362–367.
27. **Zagorec, M., and R. Labbe-Bois.** 1986. Negative control of yeast coproporphyrinogen oxidase synthesis by heme and oxygen. J. Biol. Chem. **261**:2506–2509.