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Effect of Microaerophilic Cell Growth Conditions on Expression of the Aerobic (*cyoABCDE* and *cydAB*) and Anaerobic (*narGHJI*, *frdABCD*, and *dmsABC*) Respiratory Pathway Genes in *Escherichia coli*

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Escherichia coli **varies the synthesis of many of its respiratory enzymes in response to oxygen availability. These enzymes include cytochrome *o* oxidase (*cyoABCDE*) and cytochrome *d* oxidase (*cydAB*), used during aerobic cell growth, and a fumarate reductase (*frdABCD*), dimethyl sulfoxide/trimethylamine oxide reductase (*dmsABC*), and nitrate reductase (*narGHJI*), used during anaerobic respiratory conditions. To determine how different levels of oxygen affect the expression of each operon, strains containing *cyo-lacZ*, *cyd-lacZ*, *frdA-lacZ*, *dmsA-lacZ*, and *narG-lacZ* fusions were grown in continuous culture at various degrees of air saturation. The basal-level expression of the anaerobic respiratory genes, *frdABCD*, *dmsABC*, and *narGHJI*, occurred when the air saturation of the medium was above 20%; as the saturation was reduced to below 10% (ca. 2% oxygen), the expression rapidly increased and reached a maximal level at 0% air. In contrast, *cyoABCDE* gene expression was lowest under anaerobic conditions while *cyd-lacZ* expression was about 40% of its maximum level. When the oxygen level was raised into the microaerophilic range (ca. 7% air saturation) *cyd-lacZ* expression was maximal while *cyo-lacZ* expression was elevated by about fivefold. As the air level was raised to above 20% saturation, *cyd-lacZ* expression fell to a basal level while *cyo-lacZ* expression was increased to its maximum level. The role of the Fnr and ArcA regulatory proteins in this microaerophilic control of respiratory gene expression was documented: whereas Fnr function as an aerobic/anaerobic switch in the range of 0 to 10% air saturation, ArcA exerted its control in the 10 to 20% range. These two transcriptional regulators coordinate the hierarchial control of respiratory pathway gene expression in *E. coli* to ensure the optimal use of oxygen in the cell environment.**

The bacterium *Escherichia coli* can respire either aerobically or anaerobically by using a variety of terminal electron acceptors for electron transport-linked phosphorylation reactions (5–7). In the presence of oxygen, the preferred electron acceptor, *E. coli* can respire by using either of two distinct cytochrome oxidases, cytochrome *o* oxidase and cytochrome *d* oxidase. They are encoded by the *cyoABCDE* and *cydAB* operons, respectively. *E. coli* can also synthesize a number of anaerobic respiratory enzymes that include a nitrate-regulated nitrate reductase (*narGHJI*), a fumarate reductase (*frdABCD*), and a broad-substrate-specificity dimethyl sulfoxide/trimethylamine oxide (DMSO/TMSO) reductase (*dmsABC*), among others. These enzymes are produced in significant amounts only when oxygen becomes limiting in the environment of the cells. The presence of one or more of the anaerobic terminal electron acceptors, primarily nitrate, can further modulate gene expression (5). These electron transport-linked phosphorylation reactions are energetically more favorable to the cell than are the fermentative reactions, in which ATP must be obtained by substrate-level phosphorylation.

The regulation of the aerobic and anaerobic respiratory pathway genes by oxygen is mediated by two distinct regulatory systems composed of the *fnr* and the *arcA-arcB* gene products (5, 6, 10, 11, 20). Fnr, a transcriptional regulator of each of the respiratory pathway genes, becomes activated when cells are shifted from aerobic to anaerobic growth conditions. Under anaerobic conditions, Fnr is a transcriptional repressor of the aerobic respiratory genes, *cyoABCDE* and *cydAB*, and a transcriptional activator of the anaerobic respiratory pathway genes, *frdABCD*, *dmsABC*, and *narGHJI* (1, 2, 12, 17). The mechanism by which Fnr senses the anaerobic state is still unclear, although its cysteine-rich N-terminal domain is essential for this process (13, 14, 18). The protein contains several iron atoms that may participate in a redox-sensing function to interconvert Fnr from an inactive DNA-binding conformation present under aerobic cell growth conditions to the active form under anaerobic conditions (14, 18, 20). Whereas prior studies have thoroughly documented the roles of Fnr in aerobic-anaerobic gene regulation under air-saturating versus fully anaerobic conditions (6, 18), little is known concerning how Fnr functions under conditions of oxygen limitation.

The ArcA and ArcB proteins constitute a second regulatory element for controlling aerobic-anaerobic gene expression in *E. coli*. They form a two-component regulatory system in which ArcB is an environmental sensor of the cell growth conditions. ArcB functions to activate ArcA during anaerobic conditions via protein phosphorylation reactions (7, 11). ArcA, when activated, can then bind to regulatory DNA sites to mediate both positive and negative control of gene expression (8, 9): *cyoABCDE* expression is repressed under anaerobic conditions, while *cydAB* expression is activated (3, 4, 8). The anaerobic respiratory pathway genes are unaffected by the ArcA ArcB regulatory system (3).

In previous studies by Rice and Hempfling, the level of

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cytochrome *o* oxidase was shown to be highest in the cell under oxygen-saturating conditions while the cytochrome *d* oxidase level was low (16). Because oxygen was limited, the amount of cytochrome *d* oxidase in the cell increased whereas the level of cytochrome *o* oxidase was lowered. These observations correlate well with the biochemical properties of the two oxidases, since cytochrome *o* oxidase has a weaker affinity for oxygen and cytochrome *d* oxidase has a stronger affinity (16). *E. coli* apparently synthesizes cytochrome *d* oxidase to scavenge oxygen during oxygen-limiting conditions.

In this study, we have used continuous-culture techniques to examine the expression of the aerobic and anaerobic respiratory pathway genes in *E. coli* during cell growth at different oxygen levels. We also examined the roles of the *fnr* and the *arcA* gene products in this process. The results of these studies demonstrate that expression of the aerobic and anaerobic respiratory pathway genes is coordinately controlled under microaerophilic conditions to ensure a hierarchial pattern of gene expression in response to oxygen availability.

MATERIALS AND METHODS

Bacteria, plasmids, and phages. All experiments were performed with *E. coli* MC4100 [$F^-\Delta$ (*argF'-'lac*)*U169 araD139* Δ*lacU169 rpsL150 deoC1 relA1 flbB5301 rbsR ptsF25*] or the Δ*fnr* PC2 (3) or Δ*arcA* PC35 (3) strains derived from it. The *lacZ* reporter fusions used to monitor expression of the aerobic- and anaerobic-pathway genes were λJ100 [Φ(*frdA-lacZ*) *lacY*$^+$ *lacA*$^+$] (12), λPC25 [Φ(*dmsA-lacZ*) *lacY*$^+$ *lacA*$^+$] (2), λPC50 [Φ(*narG-lacZ*) *lacY*$^+$ *lacA*$^+$] (17), λGC101 [Φ(*cydA-lacZ*) *lacY*$^+$ *lacA*$^+$] (1), and λVLH114 [Φ(*cyoA-lacZ*) *lacY*$^+$ *lacA*$^+$] (1). Each fusion was contained in the chromosome at λatt and thus was wild type for each electron transport pathway.

Cell growth. For continuous-culture experiments, a Mouse bioreactor (Queue Corp., Parkersburg, W.V.) was fitted with a 2-liter vessel and operated at a 1-liter liquid working volume as previously described (19). A modified Vogel-Bonner medium (pH 6.5) supplemented with Casamino Acids (0.25 mg/liter) and glucose (2.25 mM) was used to limit cell growth (i.e., carbon-limited medium). Aerobic continuous culture conditions were maintained by saturating the culture medium with sterile air at a flow rate of 200 ml/min, and anaerobic conditions were maintained by continuously sparging the vessel with oxygen-free nitrogen at a flow rate of 200 ml/min (19). To vary the degree of air saturation of the medium, the vessel was sparged with a stream of premixed gas in which the proportion of compressed air (21% O_2) and compressed nitrogen (99.98%) was controlled by using a manifold with precalibrated flow meters for each gas. The percent air saturation of the medium was monitored by an Ingold oxygen probe (model 1046), which was calibrated with 100% air and 99.98% N_2 (0% air) prior to inoculation of the vessel in each experiment. When the chemostat was shifted to a new aeration level, steady state was generally achieved within five reactor residence times. This was confirmed by assaying the β-galactosidase activity of harvested cells as an indicator that gene expression had reached equilibrium. The chemostat was maintained under the same conditions until the β-galactosidase values varied no more than 5%. The β-galactosidase value obtained for each continuous-culture condition was independently determined at least twice, and there was less than 10% variation in β-galactosidase activity. In control experiments performed at 5% air saturation, the flow rate was increased from 200 to 300 ml/min or decreased to 100 ml/min to determine if the gas flow rate affected β-galactosidase levels. Gene expression was unaffected. Thus, the rate of oxygen uptake by the cells was not limiting under these conditions. Another control experiment was performed by increasing the vessel stirring rate to 600 rpm, and gene expression was unaffected.

During the experiments, the chemostat was maintained at a medium flow rate (F) of 10 ml/min (cell growth rate, k = 0.6/h). This corresponded to a cell doubling time (g) of 70 min. The number of cell doublings per hour (μ) is equal to 1/g. The cell generation time (g) is equal to (1n 2)/k (15). Between experiments, the chemostat was maintained at a flow rate of 2 ml/min (doubling time, of 5.8 h).

To monitor strain stability and purity, chemostat samples were collected daily and spread on 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) indicator plates to check for loss of the *lacZ* fusion or for strain contamination. To ensure that no deleterious mutations had occurred in the *lacZ* indicator strain, cell samples were taken from the chemostat periodically and grown in batch culture for β-galactosidase assays to confirm that the expression of each *lacZ* fusion was wild type. Minimal glucose–X-Gal plates (19) for strain testing were prepared with Difco Bacto Agar (15 g/liter) and X-Gal (40 μg/ml).

β-Galactosidase assays. β-galactosidase assays were performed as previously described (1). For cell sampling, 10 ml of culture was collected and placed on ice. The cells were then harvested by centrifugation, and β-galactosidase levels were determined. One unit of β-galactosidase is defined as the hydrolysis of 1 nmol of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) per min per mg of protein.

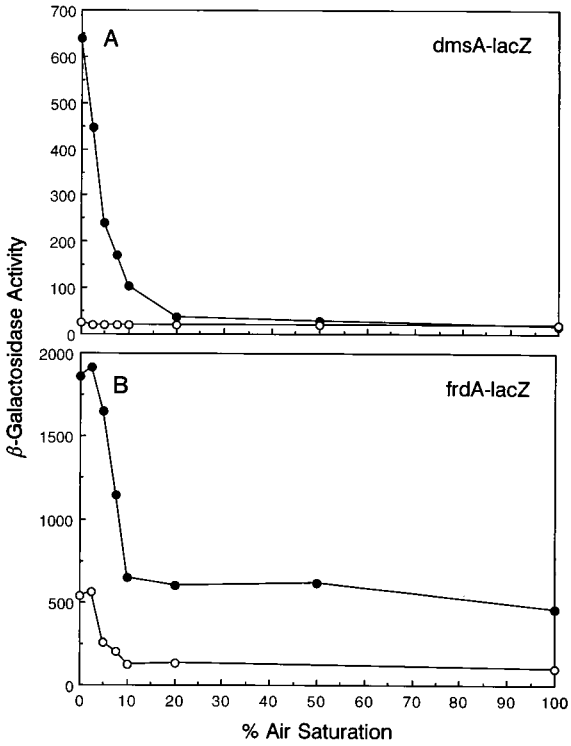

FIG. 1. Effect of oxygen on *dmsA-lacZ* (A) and *frdA-lacZ* (B) expression in a wild-type and an *fnr* strain. The amount of air present in the gas stream used for cell growth is expressed as percent air saturation, where the oxygen content is 0.21 of the percent air saturation. (A) *dmsA-lacZ* expression in wild-type (●) and *fnr* (○) strains. (B) *frdA-lacZ* expression in wild-type (●) and *fnr* (○) strains.

Materials. ONPG was purchased from Sigma Chemical Co., St. Louis, Mo. X-Gal was obtained from International Biotechnologies, Inc., New Haven, Conn. Nitrogen gas (99.98% purity) and compressed air (medical grade) were purchased from AIRCO Inc. All other chemicals used were of reagent grade.

RESULTS AND DISCUSSION

Effect of oxygen levels on *dmsA-lacZ* expression. To determine how *dmsABC* operon expression varies in the cell during growth at different levels of oxygen, a *dmsA-lacZ* reporter strain was grown in continuous culture at degrees of air saturation from 0 to 100% (Fig. 1A). The percent air saturation of the medium was controlled by sparging the vessel with a stream of premixed air and nitrogen. A dissolved-oxygen electrode was also used to directly monitor the percent air saturation of the medium. This allowed us to confirm when the vessel had reached equilibrium. β-Galactosidase assays were performed at intervals to determine when a steady-state level of gene expression had been achieved.

The maximum level of *dmsA-lacZ* expression was seen only under completely anaerobic conditions (i.e., 100% nitrogen gas). When air was introduced into the gas stream in trace amounts (i.e., 4%, vol/vol), *dmsA-lacZ* expression was reduced by half. As the level of air in the premixed gas stream was elevated to above 10%, *dmsA-lacZ* expression was further reduced to the basal level seen at high oxygen levels. The basal level was about 3% of the maximum level of *dmsA-lacZ* expression seen under completely anaerobic conditions. In control experiments, the fermentor stirring rate and the vessel gassing rate were independently varied to confirm that the level of oxygen in the medium was in equilibrium (see Materials and Methods).

We also examined the role of the *fnr* gene product in regu-

lating *dmsA-lacZ* expression in response to oxygen (Fig. 1A). In a Δ*fnr* strain, the activation of *dmsA-lacZ* expression was completely abolished: expression remained at the basal aerobic level under all conditions tested. These results are consistent with results of previous batch culture experiments in which anaerobic *dmsA-lacZ* expression was impaired in a *fnr* mutant (2). From these chemostat experiments, it appears that the cell is detecting oxygen in the microaerophilic range to control *dmsABC* gene expression. At above 10% air saturation, Fnr protein apparently exists in an inactive form inside the cell, and as the level of air saturation is reduced to 5%, Fnr is converted to a partially activated form that acts to induce *dmsABC* gene expression to half the maximal level. As the oxygen level is reduced further, the proportion of Fnr in the active form increases as monitored by Fnr-dependent *dmsA-lacZ* expression. In these experiments, the midpoint for transition of Fnr from an inactive to active form occurs when cells are grown at an air saturation level of 4 to 5%; this corresponds to an oxygen level in the cell environment of 0.8 to 1% (ca. 10 μM) as determined with a dissolved-oxygen probe.

Effect of varying the oxygen levels on *frdA-lacZ* expression. By using the above continuous-culture techniques, the pattern of anaerobic induction of *frdABCD* operon expression was also examined: *frdA-lacZ* expression was lowest during oxygen-rich cell growth conditions (Fig. 1B). Half-maximal gene induction occurred at about 7% air saturation (i.e., 1.5% dissolved oxygen), while *frdA-lacZ* expression was highest when cells were grown at an air saturation level of 2.5% or below (0.5 to 0% oxygen). The range of *frdA-lacZ* expression was 4-fold in these chemostat experiments, whereas it was 12-fold in batch culture (12). This difference is due in part to growth rate control of *frdABCD* gene expression (19).

To evaluate the contribution of the *fnr* gene product on the induction of *frdA-lacZ* expression, a Δ*fnr* strain was grown under the same conditions described above for the *fnr*$^+$ strain studies (Fig. 1B). The level of *frdA-lacZ* expression was about fivefold lower in the *fnr* strain than in the wild-type strain for each level of aeration examined. This pattern was in contrast to the induction pattern seen for *dmsA-lacZ* expression, for which Fnr-dependent control occurred only under oxygen-limiting conditions. These results suggest that some protein(s) in addition to Fnr is needed to regulate *frdABCD* gene expression in response to anaerobiosis. Noteworthy in these studies, the induction of both anaerobic gene fusions occurred in the same microaerophilic range of oxygen (i.e., air saturation of 0 to 10% [Fig. 1A]). Since fumarate and TMAO have little influence on *frdA-lacZ* and *dmsA-lacZ* expression (2, 12), respectively, we did not further examine the effect of these respiratory substrates on gene expression in continuous culture.

Effect of oxygen on *narG-lacZ* expression. The *narGHJI* operon encodes an oxygen- and nitrate-regulated nitrate reductase complex whose role is to catalyze anaerobic respiration with nitrate as a terminal electron acceptor. When *narG-lacZ* expression was examined at the different air saturation levels used in the *dmsA-lacZ* and *frdA-lacZ* studies, the pattern was nearly identical to that seen for the *dmsABC* operon (Fig. 2A and 1A). As anticipated, gene expression was lowest at high aeration: when the percent air saturation was reduced from 10 to 0%, expression was increased by sevenfold (Fig. 2A). Half-maximal expression occurred at 5% air saturation (ca. 1% oxygen) when nitrate was absent, but when nitrate was present it occurred at 2.5% (ca. 0.5% oxygen [Fig. 2B insert]). The presence of nitrate also caused a dramatic induction of *narG-lacZ* expression: when nitrate was added at 2.25 mM in the medium feed, *narG-lacZ* expression was elevated by 90-fold (Fig. 2B). This induction process was also Fnr dependent (reference 17 and data not shown). Finally, when nitrate was present under oxygen-rich conditions, the level of *narG-lacZ* expression was about twofold above that seen when no nitrate was present: thus, nitrate control of *narGHJI* expression is operative under both aerobic and anaerobic cell growth conditions.

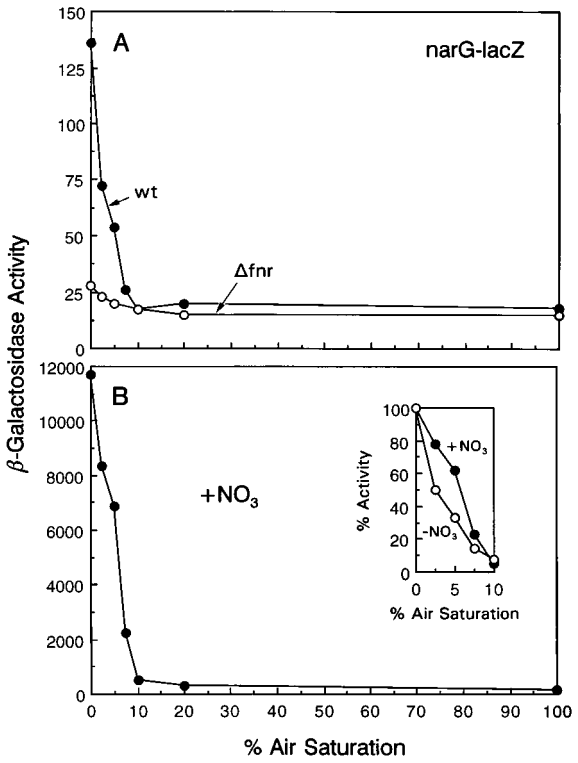

FIG. 2. Effect of oxygen on *narG-lacZ* expression in the absence (A) and presence (B) of added nitrate. Air saturation is expressed as percent air in the gas stream used for cell growth. The O_2 content of 100% air-saturated medium is equal to 21% or approximately 230 μM. The wild-type (wt) and *fnr* (ΔFnr) strains are indicated by the solid and open symbols, respectively. The insert in panel B shows the difference in *narG-lacZ* expression in cells grown with (●) and without (○) nitrate additions. Data are from the activity levels shown in panels A and B.

Effect of oxygen on *cyd-lacZ* expression. It was previously demonstrated that the level of cytochrome *d* oxidase in the cell is highest under microaerophilic growth conditions (16). We wished to (i) determine if this process is controlled in part by transcription of the *cydAB* genes and (ii) identify the roles of the *fnr* and *arcA* gene products in regulating *cydAB* gene expression in response to oxygen availability. When a *cyd-lacZ* reporter fusion was tested over the range of 100 to 0% air saturation (Fig. 3), expression was at a low basal level from 100 to 20% saturation. As the percent air saturation was reduced further, *cyd-lacZ* expression increased rapidly, until it achieved a maximum at 7% air saturation (ca. eightfold induction over aerobic levels). When the air was decreased to 0% saturation, *cyd-lacZ* expression was lowered to 60% of the maximum expression. These findings demonstrate that a major control of cytochrome *d* oxidase synthesis in response to microaerophilic growth occurs at the level of *cydAB* transcription.

On the basis of batch culture *cyd-lacZ* gene expression studies, it has been proposed that *cyd-lacZ* expression is activated by the *arcA* gene product (3, 4, 8) and repressed by the *fnr* gene product (1, 3). We examined the roles of these two regulatory proteins on *cydAB* gene expression at different oxygen levels in

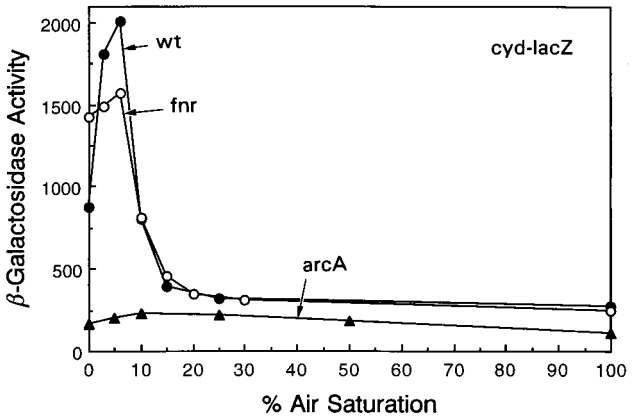

FIG. 3. Effect of oxygen on *cyd-lacZ* expression in a wild-type (wt) strain, an Δ*arcA* strain, and a *fnr* strain. The level of oxygen used for cell growth is expressed as the percent air saturation of the medium.

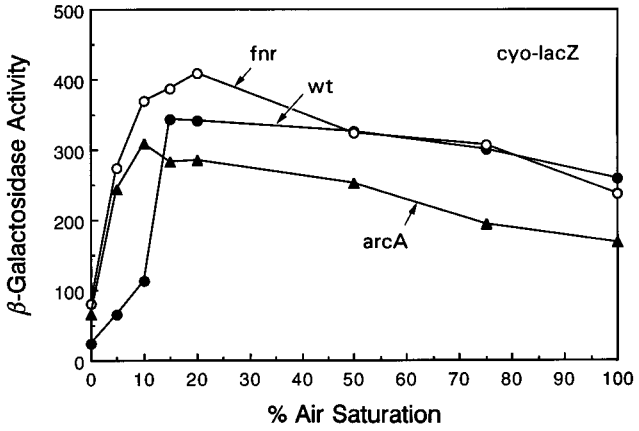

FIG. 4. Effect of oxygen on *cyo-lacZ* expression in a wild-type (wt) strain, an Δ*arcA* strain, and a *fnr* strain. The level of oxygen used for cell growth is expressed as the percent air saturation of the medium.

continuous culture (Fig. 3). The activation of *cyd-lacZ* expression was still seen in a Δ*fnr* strain, but it was abolished in an Δ*arcA* strain. These findings support the proposed role of ArcA as a positive regulator in *cydAB* expression. Second, as the level of *cyd-lacZ* gene expression in the *fnr* strain grown at 0% air saturation was higher than in the wild-type strain, it is consistent with Fnr functioning as a repressor of *cyd-lacZ* expression under anaerobic conditions (1). These results are in contrast to those of Fu et al., who proposed that Fnr is an activator of *cyd* gene expression (4). Interestingly, as the peak level of gene expression in the wild-type strain grown at 7% air saturation was slightly reduced in the Δ*fnr* strain, Fnr may also contribute slightly to peak *cydAB* expression. The microaerophilic induction of *cyd-lacZ* expression occurred in the air saturation range between 7 and 15%. This range was higher than the range for Fnr-dependent activation of *dmsA-lacZ*, *frdA-lacZ*, and *narG-lacZ* gene expression (ca. 5% air saturation [Fig. 1 and 2]).

Effect of oxygen on *cyo-lacZ* expression. The pattern of *cyo-lacZ* expression at different oxygen levels differed significantly from that seen for *cyd-lacZ* expression (Fig. 4). Over the range from 100 to 15% air saturation, *cyo-lacZ* expression was optimal. However, when the air saturation level was reduced to 0%, *cyo-lacZ* expression dropped by 10-fold. These findings indicate (i) that the control of cytochrome *o* oxidase synthesis occurs in part at the level of *cyoABCDE* operon transcription and (ii) that *cyoABCDE* and *cydAB* operon expression is coordinately controlled in a reciprocal fashion under microaerophilic conditions.

The ArcA and Fnr proteins are negative regulators of *cyoABCDE* transcription (1, 3, 4, 8). Their contribution to *cyo-lacZ* expression was also tested in continuous culture (Fig. 4). As the percent air saturation was reduced from 15 to 0% in the Δ*arcA* and Δ*fnr* strains, *cyo-lacZ* expression was higher than in the wild-type strain at each corresponding oxygen concentration. These results are in support of the roles for ArcA and Fnr as repressors of *cyo-lacZ* expression. Additionally, these data indicate that Fnr and ArcA can act independently of each other to control *cyo-lacZ* expression, in contrast to the requirement of ArcA for Fnr control of *cyd-lacZ* expression (Fig. 3). Finally, as *cyo-lacZ* expression was lower in the *arcA* strain than in the wild-type strain at each level of oxygen examined from 10 to 100% air saturation, it suggests that ArcA can also act to stimulate *cyo-lacZ* expression. Whether this effect is direct or indirect is unknown. In batch culture, *cyo-lacZ* expression is further derepressed in a *fnr arcA* double mutant compared with the *arcA* or *fnr* mutant (3).

The respiratory pathway genes are coordinately regulated in response to microaerophilic cell growth conditions. In this study, we used continuous-culture techniques to examine the effect of oxygen on anaerobic and aerobic respiratory pathway gene expression in *E. coli*. Transcription of each of the anaerobic respiratory operons, *frdABCD*, *dmsABC*, and *narGHJI*, was increased as the percent air saturation used for cell growth was lowered below 10%. This corresponds to a dissolved-oxygen level of 2%, or about 20 μM O_2, because air-saturated medium contains 21% oxygen (ca. 220 μM dissolved oxygen). The expression of the *dmsABC* and *narGHJI* operons appears to be slightly more sensitive to oxygen than does the expression of the *frdABCD* operon. Maximum *frdA-lacZ* expression was achieved at 2.5% air saturation (0.5% oxygen), while *dmsA-lacZ* and *narG-lacZ* expression occurred maximally only when oxygen was fully depleted. The fact that anaerobic induction of the *frdABCD* genes is also partially Fnr independent (Fig. 1B) compared with *dmsABC* and *narGHJI* gene expression (Fig. 1A and 2A) suggests that fumarate reductase may be utilized for cell metabolism under slightly different conditions from those when the nitrate and the DMSO reductases are produced. These last two respiratory enzymes are used solely for cell respiration, whereas fumarate reductase is also involved in the branched tricarboxylic acid cycle (i.e., noncyclic) reactions (6). The Fnr-independent effect on *frdABCD* expression has been documented previously (12). Lastly, from batch culture experiments, both *frdABCD* and *dmsABC* gene expression appears to be independent of *arcA* (3).

The ArcA and Fnr regulatory proteins appear to operate at different oxygen levels. Why does the cell possess two aerobic/anaerobic regulators of gene expression? From the results of the present chemostat studies, it appears that Fnr and ArcA operate in vivo to detect somewhat different degrees of air saturation. Fnr activates *dmsABC*, *narGHJI*, and *frdABCD* gene expression and represses *cydAB* and *cyoABCDE* gene expression when the level of air saturation is in the range of 0 to 10% saturation. In contrast, ArcA operates as a repressor of *cyoABCDE* expression and as an activator of *cydAB* expression when the air saturation is in the range of 10 to 20%. This dual control of the *cydAB* and *cyoABCDE* genes by the ArcA and Fnr regulatory proteins provides an effective way to microaerophilically control the synthesis of the two alternative aerobic

respiratory enzymes. The synthesis of the cytochrome *o* oxidase is switched off as the oxygen in the cell environment is limited (in the range of ca. 20 to 5% air saturation) (Fig. 4). Simultaneously, cytochrome *d* oxidase synthesis is switched on. The level of *cydAB* expression reaches a maximum at an air saturation level of about 7% (Fig. 4). As the air saturation is further reduced (i.e., below 5%), *cydAB* expression is lowered. However, the cell continues to synthesize significant amounts of cytochrome *d* oxidase under anaerobic conditions (ca. 60% of its maximum level). This apparently allows the cell to remain poised to respire with O_2 should it be encountered.

When the levels of cytochrome *d* and *o* oxidase enzymes each drop when oxygen becomes limiting, the synthesis of the anaerobic respiratory enzymes (e.g., nitrate reductase, fumarate reductase, and DMSO reductase) is switched on in a highly coordinated fashion (Fig. 1 to 3). The Fnr regulatory protein coordinates this major switch between aerobic and anaerobic modes of cell respiration. It will be of interest to determine if other Fnr-controlled genes in *E. coli* including *sdhCDAB*, *fumC*, *ndh*, *sodA*, and *pfl* genes respond to the same oxygen range as shown in this study (6).

Although the present study did not address which environmental signal(s) are detected by the ArcA/ArcB and Fnr regulatory proteins, it appears that the ArcA/ArcB and Fnr regulatory proteins operationally detect different thresholds of oxygen in the culture medium. ArcA is less sensitive, while Fnr is more sensitive. Regardless of which signal is detected by ArcA and Fnr, the mechanism of signal detection must differ for each protein, given that the proteins do not contain similar or conserved protein domains (6). Many other bacterial species have Fnr-like and ArcA-like regulatory proteins for controlling a variety of metabolic functions ranging from respiration to nitrogen fixation and bioluminescence. Whether these analogous proteins respond within similar ranges of oxygen will be of interest to determine: the ability to detect the microaerophilic growth state is critical for survival and adaptation of many microorganisms.

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