

MINIREVIEW

Control of Electron Flow in *Escherichia coli*: Coordinated Transcription of Respiratory Pathway Genes

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INTRODUCTION

Escherichia coli has proven to be a productive system to examine basic regulatory processes at the genetic and molecular levels and, by inference, to understand related processes in other bacteria and in higher organisms. Until recently, our knowledge of the control of electron transport pathways was quite limited, in contrast to our relatively detailed knowledge of the mechanisms involved in control of carbon flow through catabolic and anabolic pathways. The purpose of this minireview is to summarize recent developments in the area of electron transport pathway gene control in *E. coli*. The emerging picture reveals the existence of a highly organized network of overlapping regulatory elements, which in large measure account for the seemingly complex regulation involved in control of aerobic and anaerobic cell metabolism.

RESPIRATORY PATHWAYS IN *E. COLI*

E. coli exhibits diverse respiratory abilities. It synthesizes at least two distinct cytochrome oxidases (cytochrome *o* oxidase and cytochrome *d* oxidase) during aerobic growth and can produce at least an additional five terminal oxidoreductases for anaerobic respiration with the alternative electron acceptors, nitrate, dimethyl sulfoxide (DMSO), trimethylamine-*N*-oxide (TMAO), and fumarate (Table 1). Each enzyme is able to couple oxidation of NADH to a variety of other electron donors via the cellular quinone pool (i.e., ubiquinone or menaquinone). The two enzyme complexes for oxygen reduction, encoded by the *cyoABCDE* and *cydAB* operons, exhibit quite different substrate affinities and reaction rates. The affinity for oxygen of cytochrome *o* oxidase ($K_m = 1.4$ to $2.9 \mu\text{M}$) is lower than that of cytochrome *d* oxidase ($K_m = 0.23$ to $0.38 \mu\text{M}$), whereas cytochrome *d* oxidase exhibits a low velocity and cytochrome *o* oxidase exhibits a high velocity (35).

Anaerobically, the cell synthesizes two distinct dissimilatory nitrate reductases for conversion of nitrate to nitrite. These nitrate reductases include the major respiratory enzyme encoded by the *narGHJI* operon and the enzyme that has been described as a cryptic nitrate reductase (encoded by *narZYWV*) which is present at relatively low levels in the cell (3, 46). For anaerobic respiration to either DMSO or TMAO, *E. coli* synthesizes an enzyme with a broad substrate specificity, called DMSO/TMAO reductase encoded by the *dmsABC* operon (2, 55). TMAO is commonly found in a variety of marine fishes that utilize it as an osmoregulatory agent. DMSO, an oxidation product of methylated sulfides in marine and freshwater environments, is probably encountered along with TMAO by *E. coli* cells as a result of human

dietary habits. The *E. coli* TMAO/DMSO reductase can also utilize a variety of other sulfoxides and amine-*N*-oxides besides TMAO and DMSO (55). An additional enzyme with specificity for TMAO, encoded by *torA*, has been described (34, 41) but has not been studied well at either the biochemical or genetic level. *E. coli* also possesses a fumarate reductase encoded by the *frdABCD* operon that catalyzes the reduction of fumarate to succinate (1). It is biochemically similar to but genetically distinct from succinate dehydrogenase. This second enzyme is used during aerobic cell metabolism and performs the physiological role of catalyzing the reverse reaction to fumarate reductase (1).

It has been known for a number of years that *E. coli* controls the synthesis of its respiratory pathway enzymes in response to aerobic and anaerobic cell growth conditions and that this control depends on whether the alternative anaerobic respiratory substrates are present. During aerobic culture, the cytochrome *o* oxidase and cytochrome *d* oxidase enzymes are present at relatively high levels in the cell, while the anaerobic respiratory enzymes are produced only sparingly (35, 46). Cytochrome *o* oxidase is the predominant enzyme under oxygen-rich conditions and it exhibits decreased abundance as cytochrome *d* oxidase levels rise when oxygen becomes limiting (37). Upon oxygen depletion, synthesis of the anaerobic functioning enzymes allows the energetically less favorable electron acceptors to be used for respiratory metabolism in lieu of fermentation. Synthesis of the anaerobic oxidoreductase enzymes is nitrate dependent; nitrate reductase levels are elevated in the presence of this preferred electron acceptor, while levels of the DMSO/TMAO reductase and fumarate reductase remain low. Only when nitrate is depleted from the medium or is absent, do the remaining anaerobic respiratory enzymes accumulate to optimal levels. In the absence of any of these alternative anaerobic electron acceptor substrates, the cell resorts to a mixed-acid fermentation. This strategy for respiratory protein synthesis is apparently maximized for cell growth and efficiently allocates use of the limited space available in the cytoplasmic membrane.

The potential free energy available from the reduction of the alternative respiratory substrates can be correlated with the synthesis of the alternative respiratory enzymes in the cell (Table 1). Thus, a hierarchy for substrate use is ordered as follows: oxygen > nitrate > DMSO > TMAO > fumarate. When several electron acceptors are present, the energetically preferred acceptor would be expected to be used before the second, and so forth. Unfortunately, a rigorous test of this scheme, namely, a quantitative evaluation of the amount of each terminal oxidoreductase in the cell under different conditions, is difficult because of the

TABLE 1. Characteristics of electron transport systems in *E. coli*

Electron acceptor	$\delta G^{\circ a}$ (kJ/mol)	Terminal respiratory enzyme	Operon	Chromosomal location (min)
O ₂	-233	Cytochrome <i>o</i> oxidase	<i>cyoABCDE</i>	10
O ₂	-233	Cytochrome <i>d</i> oxidase	<i>cydAB</i>	17
NO ₃ ⁻	-144	Nitrate reductase	<i>narGHJI</i>	27
NO ₃ ⁻	-144	Nitrate reductase	<i>narZYWV</i>	33
DMSO	-92	DMSO/TMAO reductase	<i>dmsABC</i>	20
TMAO	-87	TMAO reductase	<i>torA</i>	28
Fumarate	-67	Fumarate reductase	<i>frdABCD</i>	94

^a Free energy calculated by using NADH as an electron donor to the indicated electron acceptor.

presence of multiple respiratory enzymes in the cell that use the same alternate electron acceptor.

TRANSCRIPTIONAL CONTROL OF RESPIRATORY GENE EXPRESSION

With the knowledge that respiratory enzyme levels vary, depending on the mode of cell growth, recent interest has focused on examining the basis of this regulatory control using molecular and genetic approaches.

Regulation in response to anaerobiosis. A general response to a switch between aerobic and anaerobic growth conditions has been established for five of the seven terminal oxidoreductase operons in *E. coli* (6, 8, 15, 22, 44, 45). Typical findings determined by using *lacZ* gene fusion methods (Table 2) reveal that of these five operons, only the cytochrome *o* oxidase operon is expressed optimally during oxygen-rich growth conditions. Under anaerobic conditions, *cyo-lacZ* transcription is reduced by over 150-fold, while the cytochrome *d* oxidase (*cydAB*) genes are expressed about threefold higher than under aerobic conditions (6, 9, 15). Fu et al. (12) recently demonstrated that a *cyd-lacZ* reporter fusion is optimally expressed under microaerophilic growth conditions. This finding is consistent with the high oxygen affinity of the cytochrome *d* enzyme. The threefold elevation in the level of *cydAB* expression observed when oxygen is absent (versus when oxygen is present) suggests that the cell remains poised and ready to scavenge oxygen as it may be encountered in the environment.

The levels of expression of the anaerobic respiratory pathway genes, *narGHJI*, *dmsABC*, and *frdABCD*, are elevated by 25-, 65-, and 10-fold, respectively, upon shift to oxygen-free conditions, as revealed by *lacZ* reporter fusions (Table 2) (8, 22, 23, 44, 45). Thus, in the absence of any of the substrates for anaerobic electron transport, the cell appears well poised to respire in the likelihood that any one of the electron acceptor is encountered. This cellular strat-

TABLE 2. Aerobic versus anaerobic expression of the terminal respiratory pathway genes of *E. coli*

Fusion	Activity ^a (fold)	
	O ₂ present	O ₂ absent
<i>cyoA-lacZ</i>	156	1
<i>cydA-lacZ</i>	1	3
<i>narG-lacZ</i>	1	25
<i>dmsA-lacZ</i>	1	65
<i>frdA-lacZ</i>	1	10

^a Cells were grown in minimal medium containing glucose.

TABLE 3. Effect of respiratory substrates on anaerobic gene expression^a

Electron acceptor	Change in gene expression		
	<i>frdA-lacZ</i>	<i>dmsA-lacZ</i>	<i>narG-lacZ</i>
Nitrate	25-fold decrease	12-fold decrease	10-fold increase
TMAO	NC ^b	NC	NC
Fumarate	1.5-fold increase	NC	NC

^a Cells were grown anaerobically and harvested in mid-logarithmic growth phase. The indicated electron acceptor was added to minimal medium containing glucose at an initial concentration of 40 mM.

^b NC, no change in expression.

egy reflects a genetic design to carry out anaerobic respiratory metabolism, rather than fermentation.

Regulation in response to nitrate availability. Nitrate availability has been known for a number of years to stimulate nitrate reductase synthesis and to suppress fumarate reductase and TMAO reductase synthesis. Different investigators using different types of *lacZ* reporter fusions have demonstrated that this nitrate control is manifested at the level of gene transcription (5, 8, 22, 23, 29, 45). Typical expression data, shown in Table 3, demonstrate that the cellular response to availability of nitrate is to further elevate the potential for nitrate respiration by elevating *narGHJI* transcription 10-fold. Simultaneously, the cell reduces its ability to reduce TMAO and fumarate by 12- and 25-fold, respectively; these levels are still above the activities seen under aerobic growth conditions. The presence of fumarate, DMSO, and TMAO do not affect the expression of the *narGHJI*, *dmsABC*, and *frdABCD* operons to a significant degree, with the exception of a 1.5-fold stimulation of *frdABCD* expression by fumarate (23). TMAO is known to induce synthesis of a *dmsABC*-independent TMAO reductase activity (41, 53), which is possibly encoded by *torA*.

TRANSCRIPTIONAL REGULATORS

The NarX, NarL, and NarQ regulatory system. The products of at least three genes, *narX*, *narL*, and *narQ*, are required to regulate respiratory gene expression in response to nitrate availability (Fig. 1; Table 4). The *narL* gene was originally identified by its requirement for synthesis of nitrate reductase (45), and additional roles were later demonstrated for repression of fumarate reductase and TMAO reductase synthesis (8, 17, 24, 25). By using a *frdA-lacZ* genetic screening method, an independent *narL* allele originally called *frdR* (24) and a second nitrate regulator gene, *narX* (*narR* [25]), located near *narL*, were identified on the basis of the loss of nitrate-dependent repression of this fusion. The *narX* mutations are pleiotropic; nitrate repression of TMAO reductase and alcohol dehydrogenase synthesis and nitrate induction of nitrate reductase synthesis are severely altered (25, 26). However, on the basis of *narX* deletion studies, Egan and Stewart (10) concluded that the *narX* gene product was nonessential for nitrate control. As a result, the existence of another regulator gene was proposed and was named *narQ*. *narQ* mutants that are defective in nitrate repression of *frdA-lacZ* expression were subsequently isolated (4). Analysis of these mutants reveals that NarQ and NarX can independently sense and respond to nitrate availability, although each requires NarL to effect nitrate-dependent transcription (4). *narQ* mutants with similar properties have been recently reported by Rabin and Stewart (36).

TABLE 4. Regulatory genes for electron transport pathway control in *E. coli*

Regulatory gene [alternate designation(s)]	Location (min)	Protein	Regulatory roles
<i>fnr</i> (<i>nirA</i> , <i>nirR</i>)	30	Fnr	Anaerobic activator, repressor
<i>narL</i> (<i>frdR</i>)	27	NarL	Receiver-regulator from NarX and NarQ
<i>narX</i> (<i>narR</i>)	27	NarX	Sensor-transmitter for NO ₃ ⁻ and MoO ₂ ⁻
<i>narQ</i>	53	NarQ	Sensor-transmitter for NO ₃ ⁻
<i>arcB</i>	69	ArcB	Anaerobic sensor-transmitter for ArcA
<i>arcA</i> (<i>dye</i> , <i>sfrA</i> , <i>fexA</i> , <i>cpxC</i> , <i>seg</i> , <i>msp</i>)	100	ArcA	Receiver-regulator from ArcB

The DNA sequence analysis of *narL* and *narX* genes (13, 31, 47) indicates that they make up a two-component regulatory system similar to many other bacterial regulator genes like *ntrB/ntrC* and *envZ/ompR* (38, 48). By analogy to these regulators, NarL functions as a response regulator that binds DNA, while NarX is a membrane-bound sensor-transmitter for detecting nitrate availability. The DNA sequence analysis of *narQ* reveals high (58%) amino acid similarity to NarX (4) and identifies it as a second membrane-bound sensor-transmitter of nitrate, consistent with the genetic findings (4). Strains defective in either *narQ* or *narX* alone exhibit no apparent regulatory defects, whereas mutants defective in both *narQ* and *narX* are fully inactive for nitrate-dependent repression or activation. Interestingly, analysis of the original *narX* mutants (25) reveal they are dominant negative to *narQ*⁺ (4). For all conditions tested, this regulation requires a functional *narL* gene product (4, 10, 11, 26, 36). These findings suggest that the *narX* and *narQ* products have complementary sensor-transmitter functions for nitrate detection and can work independently to activate NarL, thus eliciting nitrate-dependent transcription of anaerobic electron transport and certain fermentation functions. By analogy to other bacterial two-component regulatory systems, NarX and NarQ are proposed to have kinase activity and phosphorylate NarL in response to the environmental signal, nitrate. The phosphorylated NarL is thought to be the transcriptionally active form for eliciting both repression and activation of gene expression (4, 11, 25, 26).

The nitrate regulatory network (Fig. 1) controls expression of the anaerobic oxidoreductase genes during both anaerobic and aerobic conditions. Under aerobic conditions, the *frdABCD*, *dmsABC*, and *narGHJI* operons are regulated by two- to threefold by nitrate availability (4, 8, 23, 26). Under anaerobic conditions, this regulation occurs over a much wider range (Table 3) and in part reflects the role of Fnr as an anaerobic activator of these genes. Note that *narL* and *narX* mutations have no direct effect on expression of either of the two aerobic function respiratory operons, *cyoABCDE* or *cydAB* (9).

Anaerobic regulation by Fnr. The participation of the *fnr* gene product in respiratory gene expression is shown in Fig. 1 and extends to each terminal oxidoreductase operon. Fnr functions as a transcriptional activator of *frdABCD* (23, 28), *dmsABC* (8), and *narGHJI* (5, 45) expression under anaerobic conditions. It also represses *cyoABCDE* and *cydAB* transcription under anaerobic conditions (6, 9, 12). The magnitude of positive and negative control by Fnr varies considerably; it ranges from a 65-fold activation of *dmsABC* expression to 4- to 5-fold activation for *frdABCD* expression. Fnr represses *cyoABCDE* operon expression by 35-fold and *cydAB* expression by 12-fold (6).

The location of Fnr-binding sites within the regulatory elements for several operons have been identified (29, 43, 51, 54), while the locations of other sites have been proposed on

the basis of the presence of a Fnr consensus binding site immediately 5' of the -35 site for RNA polymerase recognition at the promoters (43, 51). Sites for Fnr interaction at the *cydAB* and *cyoABCDE* promoters, however, are less evident. The proposed mechanism of transcription activation by Fnr is based in large part on its similarity to catabolite gene activator protein of *E. coli* (40, 43, 51). The two proteins are similar in predicted protein structure and in DNA recognition determinants. Two excellent recent review articles thoroughly discuss the experimental evidence in support of this model and the reader is directed to them for further details (43, 51).

The means by which Fnr senses the anaerobic state is not known, although its N-terminal domain has been implicated in this process (27, 30, 39, 44, 49). The protein contains four essential cysteine residues that are thought to aid in binding iron to form a redox or oxygen-sensitive center. Upon a shift from aerobic to anaerobic conditions, a conformational change presumably occurs in the Fnr protein to permit it to recognize and bind DNA, thus activating or repressing transcription. Whether the protein responds to changes in the redox state of the cell, as has been proposed (52), or interacts directly with oxygen is unclear. An oxidation-reduction sensing model has strong support based on in vivo gene expression experiments using redox agents to alter the cell's environment (52). The lack of a suitable biological assay to purify Fnr in an active state and to study its biochemical properties in vitro has proved frustrating.

The ArcA-ArcB regulon. A second level of aerobic-anaerobic control exists for regulating two of the terminal respiratory operons, *cyoABCDE* and *cydAB*, and is mediated by *arcA* and *arcB* gene products (Fig. 1). The ArcA and ArcB proteins make up a two-component regulatory system (14, 18, 21), distinct from the NarX, NarQ, and NarL system that responds to anaerobiosis. On the basis of its amino acid sequence, ArcB functions as a sensor-transmitter and communicates with ArcA, the receiver-regulator, to mediate transcriptional control of *cyoABCDE* and *cydAB* (14, 18, 19, 21). ArcA and ArcB also coordinate synthesis of tricarboxylic acid cycle enzymes (14, 18). In vitro studies demonstrate that ArcB is autophosphorylated and can subsequently transfer phosphate via its kinase activity to ArcA (20); the phosphorylated form of ArcA is proposed to bind DNA and mediate transcription. The nature of the signal, the means of signal detection, and how the signal is transduced to activate ArcB kinase activity are unclear. The presence of two trans-membrane-spanning regions near the N terminus of ArcB suggests that the signal is either membrane generated or associated. It is also not known which DNA site(s) is recognized by ArcA to effect transcriptional regulation in response to anaerobiosis.

The *cydAB* and *cyoABCDE* operons are responsive to ArcA control in quite different ways (Fig. 1). Whereas ArcA can repress *cyoABCDE* expression by 70-fold during anaer-

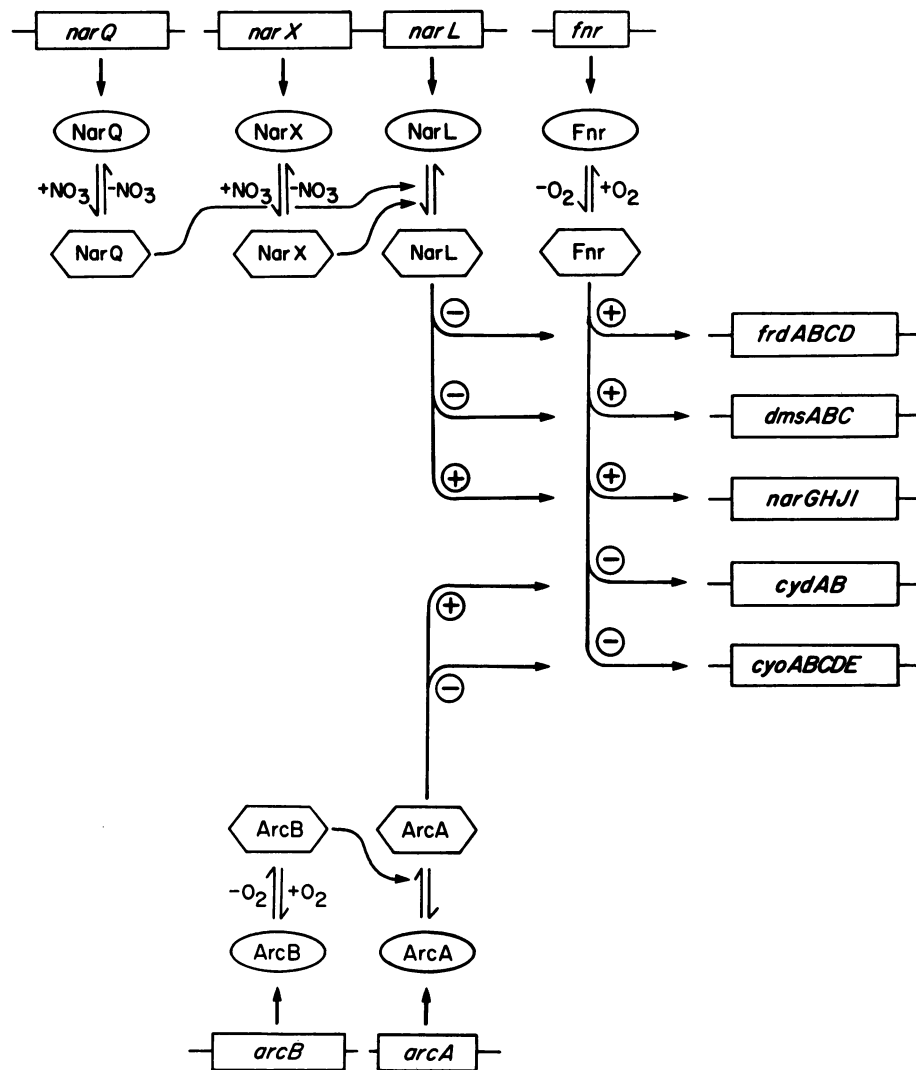


FIG. 1. Regulatory scheme for control of the aerobic and anaerobic respiratory pathway operons in *E. coli*. In response to availability of alternative electron acceptors, the three global regulators provide the indicated negative and positive transcriptional control to coordinate enzyme synthesis. The three regulatory networks are as follows: (i) Fnr-dependent control in response to anaerobiosis, (ii) ArcA-ArcB-dependent control for aerobic-anaerobic response, and (iii) NarL-, NarQ-, and NarX-dependent control for response to nitrate and molybdate availability. The following genes encode the subunits for the indicated enzymes: *frdABCD*, fumarate reductase; *narGHJI*, nitrate reductase; *dmsABC*, DMSO/TMAO reductase; *cydAB*, cytochrome *d* oxidase; and *cyoABCDE*, cytochrome *o* oxidase. Positive control (transcription activation) \oplus and negative control (transcriptional repression) \ominus of the genes are indicated. Control by additional proposed regulators is not shown in this scheme.

obic growth (9, 12, 15), it activates *cydAB* expression by 3-fold aerobically and 12-fold anaerobically (9, 15). Together, Fnr and ArcA repress *cyoABCDE* expression by 90-fold, depending on the cell culture conditions, and these two proteins appear to mediate this control independently of one another (9). In contrast, Fnr control of *cydAB* expression is dependent on active ArcA protein (9). For this high-affinity cytochrome oxidase, the 12-fold Fnr-dependent repression of *cydAB* expression seen under anaerobic conditions is abolished in a strain with *arcA* deleted (9, 15). It is interesting to speculate that the enhanced expression of *cydA-lacZ* under microaerophilic conditions reported by Fu et al. (12) can be explained by the activation of *cydAB* expression by ArcA as the cells encounter microaerophilic conditions, which is then repressed fourfold by Fnr as the

cells become further limited for oxygen (6). This model predicts different thresholds for sensing of a common signal by Fnr and ArcA-ArcB, or alternatively, the detection of two different signals (one by Fnr and another by ArcA and ArcB) that report somewhat different microaerophilic or anaerobic conditions within the cell. This explanation would rationalize the apparent redundancy need for two anaerobic sensors-regulators in *E. coli*. Note that an additional level of aerobic and anaerobic control of *cydAB* and *cyoABCDE* expression remains unexplained, as evidenced by the observed residual expression seen in strains with both *fnr* and *arcA* deleted (9). This and other observations regarding *frdABCD* expression (23) suggest the presence of a third means to regulate gene expression in *E. coli* in response to anaerobiosis (9).

Regulation of the regulators. For control of *fnr* expression, Fnr mildly autoregulates its synthesis at the level of transcription; lowered *fnr* expression occurs under anaerobic conditions (23, 33, 42). An estimate of the level of Fnr protein in the cell, based on protein determinations, gives a value of 2,400 molecules per cell, assuming a monomeric form for Fnr (50). On the basis of the expression of an in-frame *fnr-lacZ* gene fusion, it is estimated that Fnr varies from 2,500 monomers per cell during aerobic growth to 1,600 monomers per cell under anaerobic conditions (7).

The *arcA* gene is also mildly autoregulated in response to anaerobic growth (32). From gene fusion studies, ArcA appears to be a relatively abundant protein in the cell (ca. 6,600 to 8,800 monomers per cell during anaerobic and aerobic conditions). As yet, little is known about regulation of *arcB* expression. For control of the *narXL* genes which are cotranscribed in a complex operon (10), the presence of nitrate appears to elevate *narX* and *narL* expression by two- to threefold although the concentration of either protein in the cell is unknown. Thus, as each regulatory protein appears to be present in relatively constant amounts under all growth conditions, it is the presence of the cellular signal each regulatory protein detects which triggers the formation of the functionally active state to control respiratory gene expression. The cell appears poised to respond quickly to changes in the environmental stimuli.

Control of electron transport pathways by metals and cofactors. Iron and, in several cases, molybdate ions are required for synthesis of each terminal oxidoreductase. Cytochrome *o* oxidase, cytochrome *d* oxidase, and nitrate reductase contain two, three, and four heme molecules per complex, respectively. The nitrate reductase, DMSO: TMAO reductase, and fumarate reductase complexes contain 16, 12, and 9 non-heme irons, while the nitrate and DMSO/TMAO reductases also contain one molybdopterin cofactor apiece. Whereas a role for iron has been shown in transcriptional control of *frdABCD* (7, 44, 49), *narGHJI* (7), and *dmsABC* (7) expression, a molecular basis for this regulation has yet to be elucidated. Likewise, molybdate limitation results in abnormal control of the anaerobic respiratory genes (8, 16, 26). This molybdate control appears to be mediated, in part, through the NarX, NarL, and NarQ regulatory system (16, 26) in which NarX may directly participate in molybdate sensing in addition to its role in nitrate sensing (26).

Conclusions. The pathways for electron transport and energy generation in *E. coli* are highly regulated at the level of transcription. Each operon responds to the availability of electron acceptors in a manner that can be rationalized only partly by the principle of optimizing potential energy production. Oxygen and nitrate are the cell's preferred substrates for detection and consumption, respectively; in their absence, the cell induces synthesis of the DMSO-, TMAO-, and fumarate-utilizing oxidoreductases. Each of the respiratory operons also appears to be regulated by essential metals needed for synthesis of cofactors found in the respiratory enzymes they encode. Together, these studies demonstrate a highly organized network of overlapping regulatory elements for controlling electron flow in *E. coli*. These studies may also provide insight regarding control of other aerobically and anaerobically expressed genes in *E. coli* which include uptake of respiratory substrates, control of mixed-acid fermentation pathways, and control of cofactor biosynthesis including heme and molybdopterin cofactors.

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