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Effects of Nitrate Respiration on Expression of the Arc-Controlled Operons Encoding Succinate Dehydrogenase and Flavin-Linked L-Lactate Dehydrogenase

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Expression of *sdhCDAB* (encoding succinate dehydrogenase) and *lctD* (encoding the flavin-linked L-lactate dehydrogenase) is elevated aerobically and repressed anaerobically in *Escherichia coli*. The repression is initiated by autophosphorylation of the sensor protein ArcB, followed by phosphoryl group transfer to the regulator ArcA. ArcA-P, a global transcriptional regulator, then prevents *sdh* and *lct* expression. The stimulus for ArcB is not O_2 deficiency per se. In vitro experiments showed that ArcB phosphorylation is enhanced by pyruvate, D-lactate, acetate, and NADH, the concentrations of which are likely to increase with the lack of an effective exogenous electron sink. In addition to their aerobic function, the two primary dehydrogenases also have roles in anaerobic nitrate respiration. Results presented here indicate that the increase of *sdh* and *lct* expression by nitrate depended on its chemical reduction, which in turn diminished the ArcA-P pool. Unexpectedly, a mutation in the *fnr* gene (encoding a global regulator involved in anaerobic metabolism) also alleviated the anaerobic repressions. Mutations in *arcB* or *arcA* were epistatic over that of *fnr*. Moreover, since this relief was counteracted by pyruvate in the growth medium, Fnr appears to affect formation of stimuli for ArcB. It is possible that Fnr also indirectly affects some of the other members of the *arcA* modulon, e.g., *cyoABCDE* (encoding the cytochrome *o* complex), *cydAB* (encoding the cytochrome *d* complex), and *sodA* (encoding the manganese-dependent superoxide dismutase).

Three global regulatory systems, Fnr, NarX/NarL, and ArcB/ArcA, control expression of operons encoding enzymes involved in respiration and fermentation in *Escherichia coli* (10, 25, 28, 36, 38). In general, Fnr anaerobically activates expression of numerous operons involved in fermentation and anaerobic respiration, such as those that encode nitrate, fumarate, and TMAO (trimethylamine *N*-oxide) reductases. An N-terminal cysteine cluster is known to be the key sensing element of Fnr, although the identity of the signal(s) remains to be established (9, 37, 43).

NarX/NarL belongs to a family of two-component regulatory systems (11, 30, 41). Upon stimulation by nitrate, the sensor undergoes autophosphorylation and becomes a protein kinase for its cognate regulator, NarL (10, 34, 38, 46). Phosphorylated NarL (NarL-P) is thought to activate critical operons for nitrate respiration, *narGHJI* (encoding the major nitrate reductase) and *fdhGHI* (encoding formate dehydrogenase N) (1). In contrast, NarL-P is thought to repress other operons for anaerobic respirations involving electron acceptors with standard oxidation-reduction potentials lower than that of nitrate, i.e., those that are less effective in metabolic energy generation. These operons include *frdABCD*, encoding fumarate reductase, and *dmsABC*, encoding dimethyl sulfoxide reductase (10, 22, 38). The differential behavior of the NarX/NarL system toward its targets thus allows the cell to carry out the most profitable mode of anaerobic respiration.

ArcB/ArcA is another two-component regulatory system. ArcB is the membrane sensor comprising both a transmitter domain and a receiver domain, and ArcA is the cognate regulator. Some metabolites (notably pyruvate, D-lactate, acetate, and NADH), accumulating during O_2 deficiency, enhance the phosphorylation of both the transmitter and receiver domains of ArcB. The doubly phosphorylated ArcB is a highly effective kinase of ArcA (13). ArcA-P appears to be a negative transcriptional regulator of the majority of target operons encoding enzymes involved in aerobic respiratory pathways, like *sdhCDAB* (encoding the succinate dehydrogenase complex) and *lctD* (encoding the flavoprotein complex L-lactate dehydrogenase). In a few cases, however, ArcA-P appears to serve as a positive regulator. Example target operons are those that encode enzymes in microaerobic or fermentative metabolism, such as cytochrome *d* and pyruvate formate lyase. ArcA also happens to be required for expression of *traY* of the F plasmid (23–25). It is worth mentioning that even in vigorously aerated cells, there is still a significant basal level of ArcA-P. This conclusion was reached by comparing the extent of aerobic expression of *sdh* in wild-type cells with that of an *arcA2* null mutant (24).

Although Fnr, NarX/NarL, and ArcB/ArcA each have their own unique set of target operons, the three systems exert their influence in a physiologically integrative manner. Part of this cooperation is achieved through overlapping controls of certain target promoters. For instance, Fnr and NarL-P jointly activate the promoter of *narGHJI* (encoding the major nitrate reductase) at sites located, respectively, about 41 and 200 bp upstream of the transcription start site (7, 27). In contrast, Fnr activates the promoters of *frd*, *nir*, and *dms*, which are repressed by NarL-P. In each case, the expected Fnr and NarL control boxes were found (31, 44). Other examples are the joint Fnr/ArcA regulation of the promoters of *pfl* (encoding pyruvate formate lyase), *cydAB* (encoding the cytochrome *d* complex), and *cyoABCDE* (encoding the cytochrome *o* complex) (5, 8, 33), although the mechanisms of Fnr action might not all be direct. An extreme example of multiple control is provided by the *sodA* operon (encoding the Mn-dependent

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TABLE 1. *E. coli* K-12 strains

Strain	Genotype or phenotype	Source, reference, or construction
MC4100	*araD139* Δ(*argF-lac*) *U169 rpsL150 relA1 deoC1 flb-5301 ptsF15*	M. J. Casadaban
RK5278	*narL215*::Tn*10*	40
LCB898	*pfl-1*	B. Bachmann; 45
CAG18478	*zbi-1230*::Tn*10*	C. A. Gross
ECL512	*fnr-1 zci*::Tn*10*	18
ECL525	*araD139* Δ(*argF-lac*) *U169 rpsL150 relA1 deoC1 flb-5301 ptsF15 frd-101*	21
ECL547	*sdh*$^+$ φ(*sdh-lac*) (otherwise like ECL525)	21
BW6164	*thr-43*::Tn*10*	B. L. Wanner
ECL552	*narL215*::Tn*10*	20, 40
ECL562	*moe-103 zbi-624*::Tn*10*	19
ECL566	*moe-103 zbi-624*::Tn*10*	16
ECL589	*sdh*$^+$ φ(*sdh-lac*) *arcA2* (spontaneous mutant from ECL547)	17
ECL590	*sdh*$^+$ φ(*sdh-lac*) *arcB1* (spontaneous mutant from ECL547)	14
ECL618	*arcA2 zjj*::Tn*10*	17
ECL901	φ(*lctD-lac*) (otherwise like ECL525)	6
ECL902	φ(*lctD-lac*) *fnr-1 zci*::Tn*10*	P1 (ECL512) × ECL901
ECL904	φ(*lctD-lac*) *moe-103 zbi-624*::Tn*10*	P1 (ECL566) × ECL901
ECL905	φ(*lctD-lac*) *narL215*::Tn*10*	P1 (ECL552) × ECL901
ECL906	φ(*lctD-lac*) *arcA2 zjj*::Tn*10*	P1 (ECL618) × ECL901
ECL907	φ(*lctD-lac*) *arcA2 fnr-1 zci*::Tn*10*	P1 (ECL512) × P1 (ECL618) × P1 (BW6164) × ECL901
ECL908	φ(*lct-lac*) *arcA2 moe-103 zbi-624*::Tn*10*	P1 (ECL566) × P1 (ECL618) × P1 (BW6164) × ECL901
ECL910	φ(*lctD-lac*) *arcB1*	P1 (ECL901) × ECL972
ECL911	φ(*lctD-lac*) *arcB1 moe-103 zbi-624*::Tn*10*	P1 (ECL562) × ECL910
ECL912	φ(*lctD-lac*) *arcB1 fnr-1 zci*::Tn*10*	P1 (ECL512) × ECL910
ECL913	*sdh*$^+$ φ(*sdh-lac*) *narL215*::Tn*10*	P1 (RK5278) × ECL547
ECL914	*sdh*$^+$ φ(*sdh-lac*) *moe-103 zbi-624*::Tn*10*	P1 (ECL562) × ECL547
ECL915	*sdh*$^+$ φ(*sdh-lac*) *fnr-1 zci*::Tn*10*	P1 (ECL512) × ECL547
ECL916	*sdh*$^+$ φ(*sdh-lac*) *arcA2 moe-103 zbi-624*::Tn*10*	P1 (ECL562) × ECL589
ECL917	*sdh*$^+$ φ(*sdh-lac*) *arcA2 fnr-1 zci*::Tn*10*	P1 (ECL512) × ECL589
ECL918	*sdh*$^+$ φ(*sdh-lac*) *arcB1 moe-103 zbi-624*::Tn*10*	P1 (ECL562) × ECL590
ECL919	*sdh*$^+$ φ(*sdh-lac*) *arcB1 fnr-1 zci*::Tn*10*	P1 (ECL512) × ECL590
ECL920	φ(*lctD-lac*) *pfl-1 zbi-1230*::Tn*10*	P1 transduction to ECL901
ECL921	*sdh*$^+$ φ(*sdh-lac*) *pfl-1 zbi-1230*::Tn*10*	P1 transduction to ECL547
ECL972	*arcB1* (otherwise like ECL525)	24

superoxide dismutase), in which at least five *trans*-acting elements, Fnr, ArcA, SoxS, SoxQ, and Fur (3, 12), are implicated.

In this study, we analyzed how expressions of the *sdh* and *lct* operons, typically regarded to be involved in aerobic metabolism, can also be recruited for anaerobic nitrate respiration.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study were *E. coli* K-12 derivatives, the genotypes and sources of which are given in Table 1. Unless otherwise specified, mutant alleles were introduced by P1 transduction, exploiting a closely linked Tn*10* marker. Inheritance of the desired mutations was confirmed by the phenotypes as follows: *arcA* or *arcB* by sensitivity to toluidine blue in the agar (26), *moe* or *fnr* by anaerobic growth failure on glycerol-nitrate, *narL* by retarded anaerobic growth on glycerol-nitrate, and *pfl* by failure to produce gas anaerobically on glucose-LB medium and failure to grow anaerobically on glucose mineral medium.

Growth of cells. Overnight cultures in LB medium were used to inoculate defined mineral media (20). When used, the following concentrations of supplements were added: glucose, 10 mM; acetate, 5 mM; L-lactate, 20 mM; pyruvate, 20 mM; succinate, 20 mM; D-xylose, 20 mM; nitrate, 20 mM; fumarate, 20 mM; TMAO, 20 mM; casein acid hydrolysate (CAA), 0.5%; kanamycin, 40 μg/ml; and ampicillin, 100 μg/ml.

For anaerobic incubation of cultures on agar, the GasPak Anaerobic system (BBL) was used. For monitoring anaerobic growth rates in liquid media, the seeding culture was spun down and resuspended in 0.1 M MOPS [3-(*N*-morpholino)propanesulfonate] buffer (pH 7.6). An appropriate volume of the suspension was diluted with mineral medium to give a cell density of about 10 Klett units (no. 45 filter) in screw-cap test tubes (13 by 100 mm, 9 ml) filled to capacity. To prime growth on the principal carbon and energy source, 0.01% CAA was included (20).

For determining β-galactosidase specific activity in anaerobically grown cells, the culture tubes were left undisturbed for 16 h at 37°C, and cell growth was monitored with a Klett Summerson colorimeter. For cultivation of the *pfl* mutant, about 3 days of incubation was allowed because of the impaired growth rate.

To determine the specific enzyme activity in aerobically grown cells, the inoculum was added to 55-ml test tubes containing 2 ml of the same defined medium used for anaerobic cultivation, giving an initial density of 10 Klett units. The tubes were placed in a rack rotating at 330 rpm until the culture reached mid-exponential phase.

Harvested anaerobic and aerobic cultures were chilled on ice before centrifugation, and the cell pellet was resuspended in 2 ml of 0.9% NaCl. The preparations were kept in a cold room and assayed for enzyme activity within a few hours.

β-Galactosidase activity assay. Enzyme activity was assayed by measuring the rate of *o*-nitrophenyl-β-D-galactopyranoside hydrolysis (29) in cells rendered permeable by the addition of 2 drops of chloroform and 1 drop of 0.1% sodium dodecyl

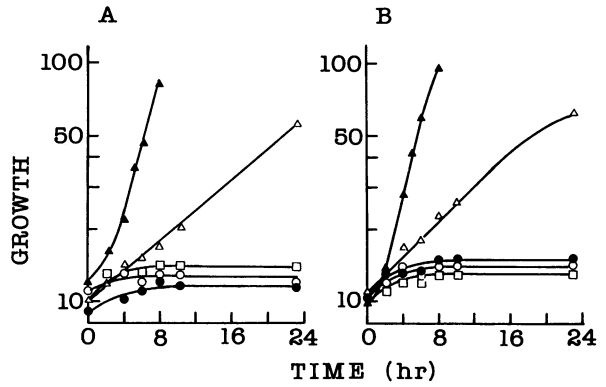

FIG. 1. Strain MC4100 was grown in succinate mineral medium (A) and L-lactate mineral medium (B) without (○) or with an electron acceptor (fumarate [●], TMAO [□], nitrate [△], or O_2 [▲]). Cell growth is expressed as Klett units in a logarithmic scale.

TABLE 2. Effects of O_2, nitrate, and various mutations on ϕ(*sdh-lac*) expression[a]

Strain	Relevant genotype	β-Galactosidase activity (U)			
		$-O_2$		$+O_2$	
		$-NO_3$	$+NO_3$	$-NO_3$	$+NO_3$
ECL547	Parent	15	260	690	730
ECL913	*narL*	33	210	680	720
ECL914	*moe*	38	28	740	780
ECL915	*fnr*	220	200	720	760
ECL589	*arcA2*	1,900	1,300	1,500	1,500
ECL916	*arcA2 moe*	2,300	2,000	1,500	1,400
ECL917	*arcA2 fnr*	2,000	2,200	1,400	1,500
ECL590	*arcB1*	1,000	670	940	1,010
ECL918	*arcB1 moe*	1,300	1,300	900	1,000
ECL919	*arcB1 fnr*	1,200	1,700	810	950

[a] Cells were grown aerobically or anaerobically in CAA-xylose medium in the presence or absence of nitrate.

sulfate. Specific activity of β-galactosidase was expressed in Miller units at room temperature.

DNA sequencing of the *arcA2* allele. The mutant allele (17) in the mutant chromosome was amplified by PCR (24). The DNA fragment was then cloned into pBluescript for nucleotide sequencing. The double-stranded plasmid was denatured for 5 min at 85°C in 0.2 N NaOH–4 mM EDTA (42). Nucleotide sequences of the plasmid were determined by the dideoxynucleotide method with a modified T7 DNA polymerase system (Sequenase; United States Biochemical), using [^{35}S]ATP.

RESULTS

Effect of anaerobic electron acceptors on growth rates with succinate or L-lactate as the carbon and energy source. Wild-type strain MC4100 was first tested for growth in a succinate mineral medium supplemented with 0.01% CAA but without an added electron acceptor. The strain grew slowly during the first 8 h and then stopped (Fig. 1). This limited growth appears to be supported by the O_2 ($E^{0\prime}$ = +0.82 V) initially dissolved in the medium. No stimulation of cell growth occurred when either fumarate ($E^{0\prime}$ = +0.03 V) or TMAO ($E^{0\prime}$ = +0.13 V) was present as an electron acceptor. However, nitrate ($E^{0\prime}$ = +0.43 V) permitted growth with a doubling time of about 9.5 h, which was about four times longer than that observed in the aerated culture. A similar growth pattern was observed with L-lactate. It thus appears that nitrate has a redox potential high enough to partially relieve the anaerobic repression of the *sdh* and *lct* operons.

Expressions of ϕ(*sdh-lac*) and ϕ(*lct-lac*) in various mutant strains in the presence and absence of nitrate. To analyze the nitrate effect on *sdh* expression, we first compared the β-galactosidase activities of ϕ(*sdh-lac*) in strains with different genetic backgrounds grown on xylose-CAA medium under anaerobic versus aerobic conditions (Table 2). Nitrate exerted no effect on aerobic expression irrespective of the genetic background. Anaerobically, nitrate raised the activity level 17-fold (from 15 to 260 U) in wild-type cells.

When an isogenic strain (ECL913) lacking NarL was examined, the nitrate effect was diminished but not abolished. Since NarL-P is required for transcriptional activation of *narGHJI*, encoding the major nitrate reductase, but not of *narZYWV*, encoding the minor nitrate reductase (2, 38), the persistence of this effect suggested that it was caused by the chemical reduction process. This kind of effect was suggested in several previous studies in other contexts (16, 39). To test the hypothesis, we used a mutant *moe-103* strain (ECL914) blocked in the synthesis of the molybdenum cofactor required for catalysis by both the major and the minor nitrate reductases. The expression of *sdh* was no longer affected by nitrate in this mutant.

If anaerobic derepression of ϕ(*sdh-lac*) by nitrate was dependent on its rate of reduction, then a null *fnr* mutation preventing *narGHJI* but not *narZYWV* expression (2) should mimic the phenotype of the *narL* mutation. When strain ECL915 (*fnr-1*) was tested, anaerobic repression of ϕ(*sdh-lac*) was also partially lifted. Surprisingly, this lifting of repression occurred even in the absence of nitrate (see section below on Fnr action).

In strain ECL589 bearing the *arcA2* null allele (insertion of an IS*10* element in the codon 170), the anaerobic β-galactosidase activity level was greatly elevated (about 100 times higher than that of the isogenic parent strain, as expected from previous studies) in the presence or absence of nitrate. Similar results were obtained with the null mutation *arcB1* (with an IS*10* insertion in the codon upstream of the conserved His-292 residue (24). It is important to note that both of the *arc* mutations were epistatic to the *moe* and *fnr* mutations.

To test the behavior of another target operon of ArcA, expression of ϕ(*lct-lac*) was examined in a manner parallel to that described for ϕ(*sdh-lac*). Anaerobically, the wild-type strain ECL901 showed a repressed β-galactosidase activity level of 6 U (Table 3). This activity level was increased about 14-fold by nitrate. Effects of *narL*, *moe*, *fnr*, *arcA2*, and *arcB1* mutations on ϕ(*lct-lac*) expression resembled those on ϕ(*sdh-lac*).

Further studies of Fnr action on ϕ(*sdh-lac*) and ϕ(*lct-lac*). For exploring whether the Fnr effect already described was direct or indirect, the growth medium was tested with pyruvate, known to stimulate the ArcB signal transduction process in vitro (13). Although expressions of ϕ(*sdh-lac*) and ϕ(*lct-lac*) were only slightly (but consistently) lowered by the metabolite in the wild-type background, strong repressions occurred in the presence of the *fnr-1* mutation (Tables 4 and 5). Such repressions, however, were not observed in the *arcB1*, *arcB1 fnr-1*, *arcA2*, or *arcA2 fnr-1* background. These results could be most simply explained if the lack of Fnr contracted the pool of metabolites enhancing ArcA-P formation. Other variations in expression of the two operons presented in the tables are not readily explicable.

TABLE 3. Effects of O_2, nitrate, and various mutations on φ(*lct-lac*) expression[a]

Strain	Relevant genotype	β-Galactosidase activity (U)			
		−O_2		+O_2	
		−NO_3	+NO_3	−NO_3	+NO_3
ECL901	Parent	6	82	820	950
ECL905	*narL*	7	39	820	890
ECL904	*moe*	13	17	820	970
ECL902	*fnr*	72	67	800	940
ECL906	*arcA2*	1,300	1,400	1,400	1,500
ECL908	*arcA2 moe*	1,600	1,600	1,400	1,700
ECL907	*arcA2 fnr*	1,500	1,700	1,400	1,600
ECL910	*arcB1*	1,200	1,200	1,500	1,700
ECL911	*arcB1 moe*	1,700	1,800	1,500	1,700
ECL912	*arcB1 fnr*	1,800	1,400	1,500	1,700

[a] Cells were grown aerobically or anaerobically in CAA–xylose–L-lactate medium in the presence or absence of nitrate.

Effects of *pfl* mutation and metabolites on expression of φ(*sdh-lac*) and φ(*lct-lac*). Since pyruvate-formate lyase (encoded by *pfl*) is required for the production of acetate from pyruvate, and expression of the gene depends strongly on activation by Fnr (32), we also examined the effect of a *pfl* mutation on φ(*sdh-lac*) or φ(*lct-lac*) expression (Table 6). Negligible effects were observed. Also, exogenous pyruvate exercised little influence in *pfl* mutants, and there seemed to be no significant influence of exogenous D-lactate or acetate in the wild-type and *fnr* mutants studied.

DISCUSSION

Nitrate respiration and level of ArcA-P. The strongest hint that chemical reduction of nitrate permitted significant anaerobic expression of *sdh* and *lct* was provided by the indispensability of the molybdenum cofactor but not of NarL. The epistatic effect of *arc* mutations (especially striking with the *arcA2* null allele) on the other mutations studied suggests that the level of ArcA-P ultimately determines the degree of expression of the two operons. The repressive influence of pyruvate (a demonstrated in vitro stimulator of ArcB phosphorylation) under anaerobic growth conditions supported this view. More definitive evidence for the proposed model, however, awaits chemical determination of the intracellular concentration of pyruvate, as well as other possible effectors, such as D-lactate, acetate, and NADH, under appropriate conditions.

Indirect role of Fnr in expression of φ(*sdh-lac*) and φ(*lct-lac*). Permissive anaerobic expression of the two operons by the *fnr-1* mutation and the antagonism of pyruvate (Tables 4 to 6) suggest that Fnr, like NarL, exerts its effect by changing metabolism rather than by acting directly on the promoters of the two operons. Once more, relative rates of substrate uptake and their internal concentrations must be determined before the different in vivo patterns of pyruvate, L-lactate, and acetate effects can be interpreted. It is also possible that reactivities of the metabolites with ArcB are not the same in vitro and in vivo. Other considerations to be entertained are the importance of the NADH or NAD concentration and the existence of additional control elements (13). If there is indeed a direct action of Fnr on the two operons, available data would indicate that the magnitude is small.

Possible roles of Fnr in expression of φ(*cyo-lac*), φ(*cyd-lac*), and φ(*sodA-lac*). Anaerobic expression of φ(*cyo-lac*) is increased by either an *arcA2* or an *fnr* mutation (4, 15). In one study, the effect of Δ*arcA* was reported to be stronger than that of Δ*fnr*, and the strongest effect was observed with the double deletion. When multiple copies of *fnr*$^+$, however, were introduced into the Δ*arcA* host, the fusion expression was not strongly decreased (5). This model, invoking Fnr and ArcA as independent repressors, deserves to be reviewed in the light of the data collected from our studies of *sdh* and *lct*.

The φ(*cyd-lac*) expression is maximal under microaerobic conditions. On the basis of genetic analysis, we previously proposed that both ArcA and Fnr are activators of the operon. According to the model, as O_2 tension diminishes, the concentration of ArcA-P starts to increase before that of functional Fnr. Thus, competition for the *cyd* promoter by ArcA-P and Fnr as activators would become more severe as anoxia becomes stricter. Assuming that Fnr is less effective than ArcA-P

TABLE 4. Effects of pyruvate on φ(*sdh-lac*) expression[a]

Strain	Relevant genotype	β-Galactosidase activity (U)			
		−O_2		+O_2	
		−Pyr	+Pyr	−Pyr	+Pyr
ECL547	Parent	15	10	680	680
ECL915	*fnr*	220	49	560	510
ECL589	*arcA2*	1,600	1,600	1,200	1,200
ECL917	*arcA2 fnr*	1,800	1,700	ND[b]	ND
ECL590	*arcB1*	840	440	ND	ND
ECL919	*arcB1 fnr*	1,000	770	ND	ND

[a] Cells were grown aerobically or anaerobically in CAA-xylose medium in the presence or absence of pyruvate.
[b] ND, not determined.

TABLE 5. Effects of pyruvate on φ(*lct-lac*) expression[a]

Strain	Relevant genotype	β-Galactosidase activity (U)			
		−O_2		+O_2	
		−Pyr	+Pyr	−Pyr	+Pyr
ECL901	Parent	6	4	820	750
ECL902	*fnr*	110	13	850	790
ECL906	*arcA2*	1,400	1,400	1,400	1,300
ECL907	*arcA2 fnr*	1,600	1,500	ND[b]	ND
ECL910	*arcB1*	940	980	ND	ND
ECL912	*arcB1 fnr*	1,600	1,400	ND	ND

[a] Cells were grown aerobically or anaerobically in CAA–xylose–D-lactate in the presence or absence of pyruvate.
[b] ND, not determined.

TABLE 6. Effect of metabolites and *pfl-1* mutation on anaerobic expression of φ(*sdh-lac*) and φ(*lct-lac*)[a]

Strain	Relevant genotype	β-Galactosidase activity (U)			
		None	Pyruvate	D-Lactate	Acetate
ECL547	φ(*sdh-lac*)	18	9	15	13
ECL915	φ(*sdh-lac*) *fnr*	210	49	160	210
ECL921	φ(*sdh-lac*) *pfl*	22	16	ND[b]	ND
ECL901	φ(*lct-lac*)	8	8	9	6
ECL902	φ(*lct-lac*) *fnr*	121	21	37	44
ECL920	φ(*lct-lac*) *pfl*	11	17	ND	ND

[a] Cells were grown anaerobically in MOPS-CAA-xylose medium for φ(*sdh-lac*) expression and in CAA–xylose–D-lactate medium for φ(*lct-lac*) expression with a suitable metabolite (pyruvate, D-lactate, or acetate).
[b] ND, not determined.

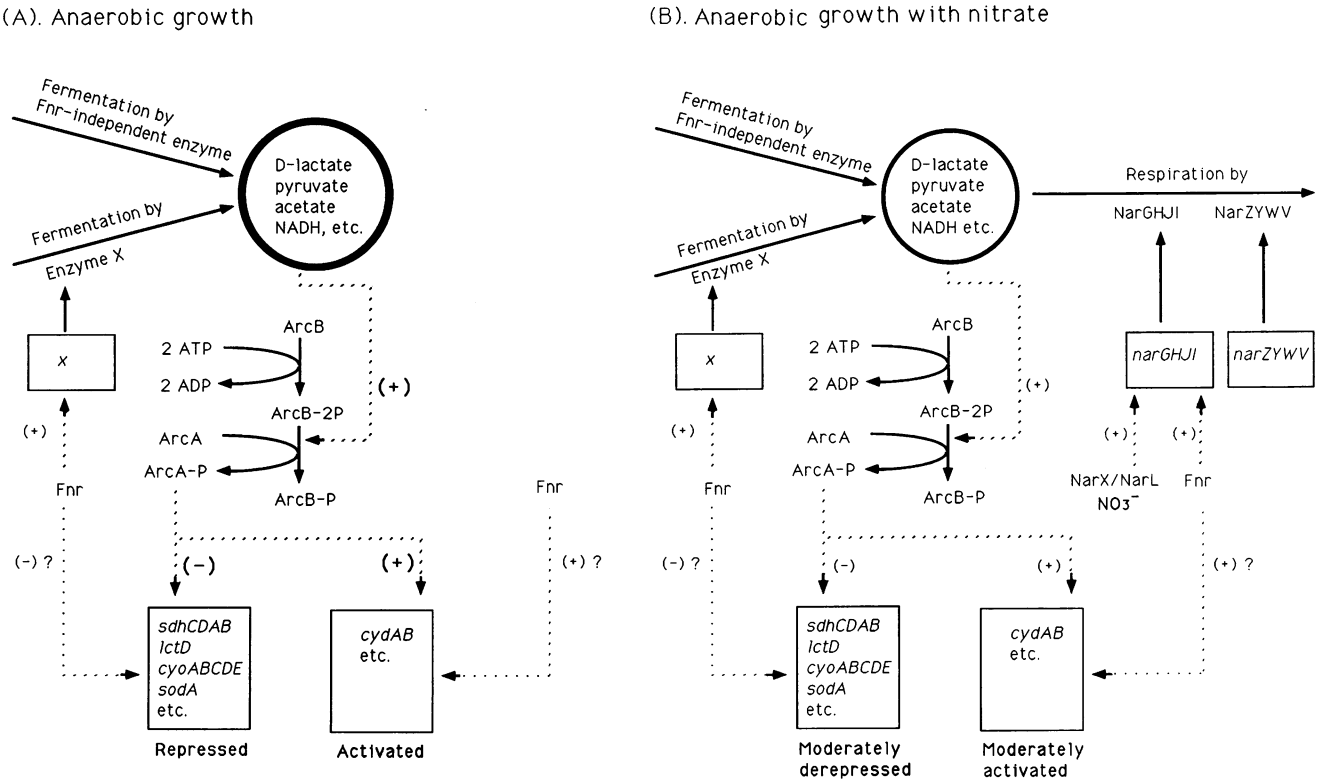

FIG. 2. Schematic illustration of regulatory networks during anaerobic growth (fermentation) (A), anaerobic nitrate respiration (B), and aerobic respiration (C). Metabolites in circles are putative effectors of the Arc system, and the width of each circle indicates the relative abundance of these metabolites. Boxes enclose genetic symbols. Symbols: ····, regulatory circuits: (+), activation; (−), repression; (+)? and (−)?, possible indirect control.

in activating transcription, the point of peak target expression would represent a shift of dominance by the two control elements (8). An analogous model was postulated in another study, but in that case Fnr was assumed to be the repressor (5).

From what has been found in this study and from evidence that the regulatory function of Fnr in *cyd* expression required ArcA (5), it now seems more probable that the role of Fnr in *cyd* regulation is also indirect. Thus, it becomes moot to ask where real Fnr boxes can be identified in the promoters of *sdh*, *lct*, and *cyd*. It should be mentioned that a convincing model invoking a single regulator to explain optimal expression of *ompF* has been proposed. This model is based on finding multiple recognition sites in *ompF* with different affinities for OmpR-P, interacting through the looping DNA (35).

There are two conflicting reports on the activating effects of Fnr and ArcA-P on ϕ(*sodA-lac*) expression. In one study, an *fnr* mutation exerted an effect stronger than that of the *arcA1* mutation, and the effects of the two mutations were additive (12). In the other study, an *arcA* mutation was stronger than an *fnr* mutation, and the effects were not additive. Furthermore, after the putative Fnr binding site was removed, the *fnr* mutation remained effective (3). Results from the second report on *sodA* control seem analogous to those of the present study; i.e., the Fnr effect is indirect.

Critical redox potential on expression of operons encoding enzymes involved in aerobic and anaerobic respirations. There are two differences between ArcB/ArcA and Fnr meriting close attention. Although the ArcB/ArcA and Fnr systems both come to play anaerobically, Fnr may sense the redox potential, whereas ArcB probably senses cellular concentrations of metabolites. More critical is the difference in sensitivity of the two

systems to the redox parameter. The cellular redox conditions should correspond to the property of the exogenous electron acceptor: fumarate ($E^{0\prime}$ = +0.03 V), TMAO ($E^{0\prime}$ = +0.13 V), or nitrate ($E^{0\prime}$ = +0.42 V). Above +0.51 V, Fnr almost completely shifts from the functional to the nonfunctional form. Such a condition pertains when there is adequate O_2 ($E^{0\prime}$ = +0.82) (43). Since Fnr plays a role only under anaerobic respiratory or fermentative conditions, the midpoint potential of +0.5 V is well poised. In contrast, the lack of a sharp borderline for Arc control seems also adaptively fitting. It makes possible the functioning of *sdh* and *lct* both aerobically with O_2 and anaerobically with nitrate. A schematic presentation is given to illustrate how the three global controls—Arc, Fnr, and Nar—operate in an integrative manner during aerobic respiration, anaerobic respiration, and fermentation (Fig. 2).

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