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Genetics

arcA (*dye*), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways

(global control/anaerobic repression/modulon)

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ABSTRACT **In *Escherichia coli* the levels of numerous enzymes associated with aerobic metabolism are decreased during anaerobic growth. In an *arcA* mutant the anaerobic levels of these enzymes are increased. The enzymes, which are encoded by different regulons, include members that belong to the tricarboxylic acid cycle, the glyoxylate shunt, the pathway for fatty acid degradation, several dehydrogenases of the flavoprotein class, and the cytochrome *o* oxidase complex. Transductional crosses placed the *arcA* gene near min 0 on the chromosomal map. Complementation tests showed that the *arcA* gene corresponded to the *dye* gene, which is also known as *fexA*, *msp*, *seg*, or *sfrA* because of various phenotypic properties [Bachmann, B. (1983) *Microbiol. Rev.* 47, 180–230]. A *dye*-deletion mutant was derepressed in the aerobic enzyme system. The term modulon is proposed to describe a set of regulons that are subject to a common transcriptional control.**

In facultative anaerobes such as *Escherichia coli*, the network for electron transport is organized in such a way that the energetically most favorable pathway is used. On the basis of the regulatory patterns of gene expression, these networks appear to be three main hierarchical systems. The aerobic respiratory pathways conduct the flow of electrons (or reducing equivalents) to O_2 ($E^{\circ\prime}$ = +0.82 V). In the absence of O_2, nitrate ($E^{\circ\prime}$ = +0.42 V) can be used. In this first system ubiquinone ($E^{\circ\prime}$ = +0.1 V) acts as an adapter and passes the electrons to the O_2 or nitrate acceptor chain. The next preferred system conducts electrons to terminal acceptors such as trimethylamine *N*-oxide ($E^{\circ\prime}$ = +0.13 V) or fumarate ($E^{\circ\prime}$ = +0.03 V), with menaquinone ($E^{\circ\prime}$ = −0.074 V) as the adapter. Both respiratory pathways supply the cell with metabolic energy by generating proton-motive force across the cytoplasmic membrane. As the operation of these pathways is curtailed by the shortage of exogenous electron acceptors, the cell relies increasingly on the energetically least rewarding system, in which redox reactions are balanced by fermentative dismutation of carbon and energy sources. The pyridine nucleotides serve as the adapter for the transfer of electrons, and substrate-level phosphorylation becomes the principal means of energy generation. The choice of the redox pathways is biased in favor of those that provide the greatest difference in midpoint potential between the initial electron donor and the terminal acceptor, for this difference limits the amount of energy that can be harnessed (for reviews see refs. 1 and 2).

The flow of electrons through the three main redox systems and the channeling within each is guided by a complex set of controls that include the regulation of gene expression. Thus, O_2 respiration interferes with induction of the anaerobic respiratory system. The mechanism of this control is beginning to be understood. Expression of several genes, including those encoding nitrate, trimethylamine *N*-oxide, and fumarate reductases, depends on the pleiotropic transcriptional activator encoded by the *fnr* gene. Aerobic repression of this system appears to result from a deficiency of functional Fnr protein (ref. 3; for a review, see ref. 2). In the absence of O_2, nitrate becomes the dominant electron acceptor by inhibiting the induction of fumarate and trimethylamine *N*-oxide reductases. In this control, nitrate and a molybdenum compound act as corepressors (4). The apo-repressor may be encoded by the *narL* gene (5), which also acts as an activator for the synthesis of nitrate reductase (6). The mechanisms by which enzymes that produce fermentation end products are controlled by O_2 and nitrate remain obscure.

In this study, we explored the overall regulation of a number of enzymes of aerobic metabolism that are lower when there is a lack of O_2 (for examples, see refs. 7–11). Assuming that these might all be subject to a transregulator, we approached the problem by searching for mutations that simultaneously increase the anaerobic expression of the *sdh* operon encoding succinate dehydrogenase and a tandem hybrid operon ϕ(*sdh-lac*) formed by protein fusion of *lacZ* to an *sdh* gene.

MATERIALS AND METHODS

Bacterial and Phage Strains. All strains are *E. coli* K-12 derivatives. Their sources and genotypes are given in Table 1, except for the F′ strain 71-18 used for testing sensitivity to M13 (16) and those of the Hfr kit used for mapping (12).

Basic genetic manipulations were carried out by standard procedures (60). For the isolation of mutants with a ϕ(*sdh-lac*) fusion, cells of strain ECL525 with an *frd* deletion (to eliminate the slight succinate dehydrogenase activity contributed by fumarate reductase) in addition to the *lac* deletion were infected with λ*plac*Mu9(*kan*) and the helper λMu507 (17) and spread on succinate/5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal)/kanamycin agar supplemented with 0.25% tryptone and 0.1% yeast extract. The colonies with putative ϕ(*sdh-lac*) were tentatively identified by their small size (possibly because of failure to use succinate) and blue color (hydrolysis of X-Gal, indicative of chromosomal integration of *lacZ*). Candidate clones were screened for loss of ability to grow on succinate but not on glucose. To purify the presumptive ϕ(*sdh-lac*) genetically, it was transduced into strain ECL525 with phage P1 by selecting for kanamycin resistance. The fusion in the transductant ECL546 was verified by locating the fusion in the *sdh* region and by showing that the cell extract contained no succinate dehydrogenase activity. To construct a *sdh*$^+$ and ϕ(*sdh-lac*) merodiploid, a λ lysate from strain ECL546 was used to infect strain ECL525. The lysogens were purified on succinate/2,3,5-triphenyltetrazolium chloride agar (18) sup-

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Abbreviation: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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

Strain	Relevant genotype	Derivation	Source or ref.
BW6144	*thr-43*::Tn*10*		B. Bachmann, ref. 12
RK4923	*zaa-625*::Tn*10*		Ref. 13
SP314	Δ*(deoD-dye)253*		B. Bachmann, ref. 14
MC4100	*araD139* Δ*(argF-lac)U169 rpsL150 relA1 deoC1 flb-5301 ptsF25*		Laboratory collection
ECL388	*ϕ(frd-lac)*		Ref. 3
ECL510	Δ*frd-101 zjd*::Tn*10*		Ref. 15
ECL525	Δ*frd-101*	P1(ECL510) × MC4100 and deletion of Tn*10*	This work
ECL546	*ϕ(sdh-lac)*	Infection of ECL525 by λ p*lac*Mu9 and transduction of fusion to ECL525	This work
ECL547	*sdh*$^+$ *ϕ(sdh-lac)*	Infection of ECL525 with λ lysate of strain ECL546	This work
ECL549	*sdh*$^+$ *ϕ(sdh-lac) arcA1*	Spontaneous mutant of ECL547	This work
ECL550	*chlG101*		Ref. 4
ECL584	*sdh*$^+$ *ϕ(sdh-lac) zjj*::Tn*10*	P1(Tn*10* pool of MC4100) × ECL549	This work
ECL585	*sdh*$^+$ *ϕ(sdh-lac) arcA1 zjj*::Tn*10*	Transduction of *arcA1* to ECL547	This work
ECL586	*ϕ(frd-lac) arcA1 zjj*::Tn*10*	Transduction of *arcA1* to ECL388	This work
ECL587	*sdh*$^+$ *ϕ(sdh-lac)* Δ*(deoD-dye)253 zjj*::Tn*10*	Transduction of Δ*(deoD-dye)253* to ECL547	This work
ECL588	*chlG101 thr-43*::Tn*10*	P1(BW6144) × ECL550	This work

The *ϕ(sdh-lac)* listed throughout refers to the fusion *sdh-1*::λp*lac*Mu9, and the *ϕ(frd-lac)* refers to *frd-101*::λp1(209).

plemented with X-Gal and seeded with λ*cI*h80. On this agar, colonies that are Sdh$^+$ and Lac$^-$ appear red, and colonies that are Sdh$^-$ and Lac$^+$ appear blue. A purple lysogen, ECL547, appeared. Its genotype, expected to be *sdh*$^+$, *ϕ(sdh-lac)*, was confirmed by the enzymic regulatory patterns. Transductional analysis showed that the Sdh$^+$ and Lac$^+$ phenotypes were 85% linked.

Resistance to phage M13 was tested with the indicator F′ strain 71-18 that received the regulatory mutation by transduction. For testing complementation of the *arcA* mutation, the following two pBR322 plasmids were used: pMW2 with a 6.2-kilobase chromosomal fragment of the *phoM-dye-thr* region and pMW2-488 shown by endonuclease restriction mapping to have a *dye*::Tn*5* insertion (M. R. Wilmes and B. L. Wanner, personal communication).

Culture Medium. For enzyme assays the cells were grown aerobically or anaerobically on xylose mineral medium (5). When indicated, the following supplements were added: L-alanine at 100 mM; glycerol at 20 mM, pyruvate at 20 mM, succinate at 20 mM, fumarate at 20 mM, glucose at 10 mM, xylose at 10 mM, nitrate at 10 mM, trimethylamine *N*-oxide at 10 mM, oleate at 2 mM, L-lactate at 2 mM, ampicillin at 50 μg/ml, kanamycin at 25 μg/ml, sodium molybdate at 1 μM, sodium selenite at 1 μM, tetracycline at 10 μg/ml, toluidine blue at 0.2 mg/ml, 2,3,5-triphenyltetrazolium chloride at 25 μg/ml, and X-Gal at 40 μg/ml.

Enzyme Assays. Cells washed with 10 mM potassium phosphate (pH 7.0) were sonically disrupted. For the assay of formate dehydrogenase, 1 mM DL-dithiothreitol was added. Unbroken cells were removed by centrifugation at 10,000 × *g* for 30 min. The crude sonic extract was used to determine the activities of aconitase (19), pyruvate dehydrogenase (20), fumarase (21), succinate dehydrogenase (22), L-lactate dehydrogenase with 10 mM substrate (23), D-lactate dehydrogenase with 10 mM substrate (23), citrate synthase (24), isocitrate lyase (25), D-amino acid dehydrogenase with Q_o as coenzyme (26), nitrate reductase (5), trimethylamine *N*-oxide reductase (5), acyl-CoA dehydrogenase with 0.5 mM myristoyl-CoA as substrate (23), 2-oxoglutarate dehydrogenase (27), and formate dehydrogenase (28). To measure the activities of malate dehydrogenase (29), isocitrate dehydrogenase (30), 3-hydroxyacyl-CoA dehydrogenase (31), D-lactate:NAD$^+$ oxidoreductase (32), and ethanol:NAD$^+$ oxidoreductase (33) the extract was further clarified by ultracentrifugation at 100,000 × *g* for 60 min. The particulate fraction was resuspended by blending in 10 mM potassium phosphate buffer containing 1% Triton X-100 and used for the assay of ubiquinol oxidase activity (34). All enzyme activities were determined at 30°C except that of ubiquinol oxidase, which was assayed at room temperature. Protein concentrations were estimated with bovine serum albumin as standard. Specific activities are expressed as nmol per min per mg of protein; that of β-galactosidase activity was assayed and expressed in units according to Miller (35).

RESULTS

Parallel Expression of *sdh*$^+$ and *ϕ(sdh-lac)* in the Merodiploid Strain ECL547. The presence in the same cell of the wild-type *sdh* operon and the hybrid operon in which the promoter region of the *sdh* operon controls the *lac* structural genes provided an opportunity to test the step at which respiratory control is exerted on succinate dehydrogenase. The β-galactosidase activity in strain ECL547 was 70 units when growth was anaerobic and 700 units when growth was aerobic. The aerobic activity of succinate dehydrogenase was also higher than the anaerobic activity by a factor of 10. Thus, the change in succinate dehydrogenase activity is entirely attributable to transcriptional regulation. In addition to respiratory control, succinate dehydrogenase is also subject to catabolite repression (7, 8). When cells of strain ECL547 were grown in the standard xylose medium, the aerobic level of β-galactosidase was reduced by a factor of 3 by added glucose. In the presence of 5 mM cAMP, glucose reduced the level only by a factor of 1.3. Thus, the glucose effect is also due to transcriptional control. The addition of succinate to the xylose medium did not change succinate dehydrogenase or β-galactosidase levels, regardless of the respiratory condition of growth.

Selection of Mutants with Increased Anaerobic Expression of *ϕ(sdh-lac)*. Cells of strain ECL547 were spread on MacConkey/1% lactose agar plates and incubated anaerobically. After 5 days, 20% of the colonies showed papillae. One papilla was picked from each colony, spread on the same type of medium, and incubated anaerobically for a day. Cells from the papillae gave round red colonies of increased size, in contrast to the flat, pale, and smaller colonies of parental cells. The papillae-derived clones were grown anaerobically and screened for succinate dehydrogenase activity. Two mutants that synthesized both β-galactosidase and

succinate dehydrogenase were kept. One of these mutants, strain ECL549, was used for this study.

Pleiotropic Effects of the Regulatory Mutation. To minimize the risk that the complex phenotype had resulted from multiple genetic changes, we transferred the mutation in strain ECL549 responsible for the altered expression of *sdh* into a strain with a fresh genetic background. This was accomplished by cotransduction of the mutation with a closely linked Tn*10*. To obtain a cell line containing this Tn*10*, we transduced mutant ECL549 with a P1 lysate of a random Tn*10* pool of strain MC4100 and isolated a tetracycline-resistant transductant (ECL584) with a wild-type phenotype of ϕ(sdh-lac). The Tn*10* of ECL584 (*zjj*::Tn*10*) was transduced back into mutant strain ECL549. The mutation in strain ECL549 was then transduced together with the *zjj*::Tn*10* into the parent strain ECL547. Thus, strain ECL585 was obtained. This mutant strain expressed its ϕ(*sdh-lac*) at an anaerobic level 20 times higher than the parental level. A parallel change occurred in the level of succinate dehydrogenase. Since succinate dehydrogenase is a part of the tricarboxylic acid cycle and a part of an electron transport chain, we compared the wild-type and mutant levels of several other enzymes having functions related to the cycle or to electron transport (Fig. 1 and Table 2).

The anaerobic levels of citrate synthase, aconitase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase, and malate dehydrogenase were also strikingly increased by the mutation. The level of pyruvate dehydrogenase was reported to be slightly decreased in wild-type cells by anaerobic growth (38, 39). In the mutant the enzyme level was not diminished by anaerobiosis. The glyoxylate shunt is mediated by isocitrate lyase and malate synthase encoded by the *ace* operon. We found that the activity of isocitrate lyase was lowered by anaerobic growth in the wild-type strain but not in the mutant.

The pathway for degradation of long-chain fatty acids was also examined by measuring two of the enzymes. The anaerobic level of acyl-CoA dehydrogenase (product of *fadE* at min 5) was insensitive to the respiratory growth conditions in both wild-type and mutant strains. In contrast, the anaerobic level of 3-hydroxyacyl-CoA dehydrogenase (product of *fadB* at min 86) was 7 times lower than the aerobic level in wild-type cells, but this control was relaxed in the mutant.

Anaerobic growth decreased the levels of L-lactate dehydrogenase, the formate dehydrogenase known as FDH_N (*N* indicates that it also has a role in nitrate respiration), and D-amino acid dehydrogenase in the wild-type, but not in the mutant, strain. All the aerobic enzymes tested are parts of pathways that use ubiquinone as an intermediate acceptor. The activity of ubiquinol oxidase was severely decreased by anaerobic growth in wild-type cells but not in the mutant.

Ubiquinol can be oxidized by the cytochrome *o* complex with a low affinity for O_2 or by the cytochrome *d* complex with a high affinity for O_2 (reviewed in ref. 1). To assess the relative contributions of the two terminal cytochromes to the oxidase activity, we took advantage of the fact that cytochrome *o* is ≈200 times more sensitive to KCN inhibition than cytochrome *d* (40). On the basis of inhibition during a 2-min preincubation with 0.08 mM KCN, it appeared that cytochrome *o* contributed ≈80% of the total oxidase activity from aerobically grown wild-type cells or from mutant cells grown either aerobically or anaerobically.

In contrast to the regulatory patterns of enzymes associated with aerobic respiration, the enzymes involved in anaerobic respiration—as represented by the reductases of nitrate, fumarate, and trimethylamine *N*-oxide—were unaffected by the mutation. The levels of the two NAD-linked fermentation enzymes studied were also unaltered.

Among the 13 enzymes whose anaerobic levels were found to be raised by the mutation, in several cases (notably

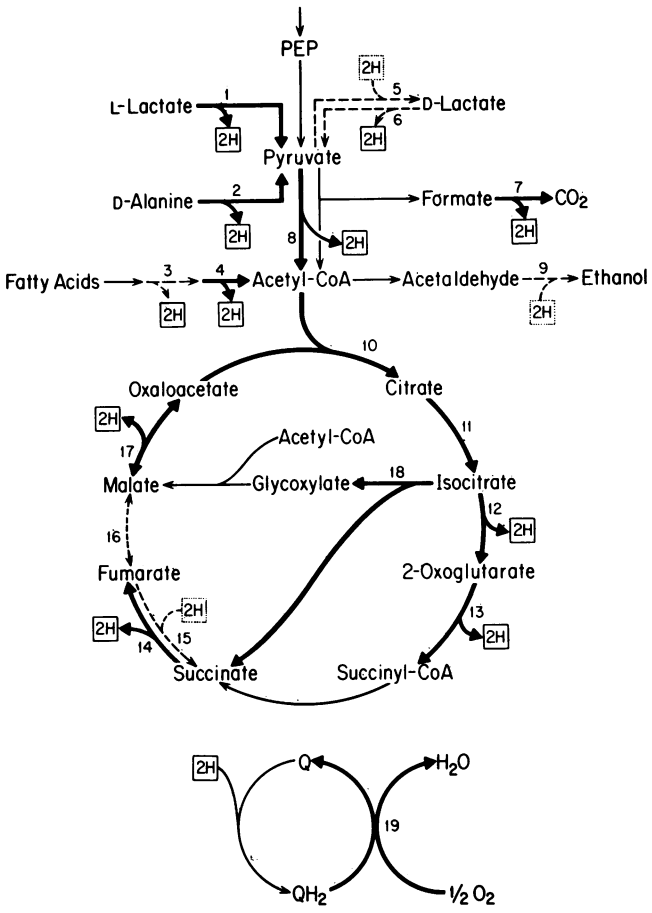

FIG. 1. Metabolic network studied. The numbers show the reactions of the enzymes assayed as follows: 1, L-lactate dehydrogenase (flavoprotein); 2, D-amino acid dehydrogenase (flavoprotein); 3, acyl-CoA dehydrogenase (flavoprotein); 4, 3-hydroxyacetyl-CoA dehydrogenase (NAD^+-linked); 5, D-lactate:NAD^+ oxidoreductase; 6, D-lactate dehydrogenase (flavoprotein); 7, formate dehydrogenase (the FDH_N enzyme); 8, pyruvate dehydrogenase; 9, ethanol:NAD^+ oxidoreductase; 10, citrate synthase; 11, aconitase; 12, isocitrate dehydrogenase; 13, 2-oxoglutarate dehydrogenase; 14, succinate dehydrogenase; 15, fumarate reductase; 16, fumarase; 17, malate dehydrogenase; 18, isocitrate lyase; and 19, ubiquinone-1 oxidase. Thick arrows indicate reactions catalyzed by enzymes with levels significantly reduced by anaerobic growth. Dashed lines and arrows indicate reactions catalyzed by enzymes with levels not significantly affected by the respiratory state of growth. "2H" boxed by solid lines represents reducing equivalents yielded by the reaction, and 2H boxed by dotted lines represents reducing equivalents consumed by the reaction.

aconitase, isocitrate lyase, and L-lactate dehydrogenase) the anaerobic level surprisingly exceeded the aerobic level. The extensive effect of the regulatory mutation on enzymes of aerobic metabolism led us to adopt *arc* (acronym for aerobic respiration control) as the gene symbol. The gene examined in this study will be referred to as *arcA*, since we have discovered an unlinked *arcB* gene mutation that gave a similar phenotype (unpublished results).

Mapping of the *arcA* gene. Transduction experiments showed that the mutation was not linked to *sdh*, and mating experiments with several different Hfr strains (12) showed that it mapped in the 90- to 5-min region. P1 crosses with strain RK4923 (zaa-625::Tn*10*) as donor showed that the mutation was 75% (73/97) linked to the tetracycline resistance marker. This placed the mutation within the 99- to 1-min region. In a three-factor cross with strain ECL588 (*chlG101 thr-43*::Tn*10*) as donor, 41% (128/313) were chlo-

Table 2. Enzyme activities of parent and mutant strains grown anaerobically or aerobically

Enzyme	Reaction shown in Fig. 1, no.	% specific activity of aerobically grown parental cells: Parent (ECL547) − O_2	Mutant (ECL585) − O_2	Mutant (ECL585) + O_2
β-Galactosidase [φ(*sdh-lac*)]	—	10	190	170
Succinate dehydrogenase	14	10	170	160
Citrate synthase	10	23	240	180
Aconitase	11	23	230	110
Isocitrate dehydrogenase	12	16	86	170
2-Oxoglutarate dehydrogenase	13	12	170	120
Malate dehydrogenase	17	30	150	170
Fumarase	16	140	210	220
Pyruvate dehydrogenase	8	57	97	86
Isocitrate lyase	18	43	190	100
Acyl-CoA dehydrogenase	3	72	130	110
3-Hydroxyacyl-CoA dehydrogenase	4	14	380	710
L-Lactate dehydrogenase	1	4	350	110
Formate dehydrogenase	7	23	92	130
D-Amino acid dehydrogenase	2	10	75	130
D-Lactate dehydrogenase	6	60	77	110
Ubiquinol oxidase	19	5	130	85
Nitrate reductase	—	6700	6700	33
Trimethylamine *N*-oxide reductase	—	1500	1200	160
β-Galactosidase [φ(*frd-lac*)]	15	1500	1100	110
D-Lactate:NAD^+ oxidoreductase	5	79	88	120
Ethanol:NAD^+ oxidoreductase	9	180	170	110

Absolute specific activity units in aerobically grown wild-type cells were as follows: β-galactosidase [φ(*sdh-lac*)], 720; succinate dehydrogenase, 52; citrate synthase, 110; aconitase, 31; isocitrate dehydrogenase, 370; 2-oxoglutarate dehydrogenase, 41; malate dehydrogenase, 11,000; fumarase, 250 (induced with fumarate); pyruvate dehydrogenase (induced with pyruvate), 35; isocitrate lyase [induced with oleate dissolved in the detergent polyoxyethylene 20 cetyl ether (36)], 7; acyl-CoA dehydrogenase [induced with oleate dissolved in the detergent polyoxyethylene 20 cetyl ether (36)], 11; 3-hydroxyacyl-CoA dehydrogenase [induced with oleate dissolved in the detergent polyoxyethylene 20 cetyl ether (36)], 65; L-lactate dehydrogenase (induced with L-lactate), 27; formate dehydrogenase (supplemented with selenite and molybdate), 83; D-amino acid dehydrogenase [induced with L-alanine (37)], 20; D-lactate dehydrogenase, 100; ubiquinol oxidase, 47,000; nitrate reductase (induced with nitrate), 21; trimethylamine *N*-oxide reductase (induced with trimethylamine *N*-oxide), 120; β-galactosidase [φ(*frd-lac*)] (induced with fumarate; in this experiment strain ECL388 served as the parent and strain ECL586 served as the mutant), 88; D-lactate:NAD^+ oxidoreductase, 420; ethanol:NAD^+ oxidoreductase, 28.

rate resistant (*chlG*) (41) and 94% (294/313) showed the wild-type φ(*sdh-lac*) control. Among the tetracycline-resistant and chlorate-resistant transductants, 5% (6/128) inherited the mutant regulatory gene. Thus, the mutation should be ≈0.04 min counterclockwise to *thr* (42).

Identity of the *arcA* Gene as the *dye* Gene. The *dye* gene, whose loss leads to sensitivity to dyes such as toluidine blue, maps near the *thr* operon (41). We found that the strain ECL547 (*arcA*$^+$) is dye-resistant, and the mutant ECL549 (*arcA1*) is dye-sensitive on tryptone/toluidine-blue agar (14). Mutations in the *dye* gene also confer resistance to male-specific phages (see *Discussion*). The *arcA* mutant acquired resistance to the male-specific phage M13. Finally, complementation tests of strain ECL585 (*arcA1*) with the plasmids pMW2 (*dye*$^+$) and pMW2-488 (*dye*::Tn*5*) were done. Five ampicillin-resistant transformants of each plasmid were tested for dye sensitivity by counting colony-forming units on toluidine agar and comparing them to those on LB (Luria–Bertani) agar. For pMW2-transformed cells, all were dye-resistant, whereas for pMW2-488-transformed cells, all were dye-sensitive. Scoring for anaerobic expression of φ(*sdh-lac*) also showed complementation of *arcA1* by pMW2 but not by pMW2-488.

The arcA Product Mediates Anaerobic Repression. To see whether the *arcA* gene product mediates positive or negative control of the aerobic enzymes, a deletion extending from *dye* through *deoD* (14) was transduced from strain SP314 to strain ECL547 [*sdh*$^+$ φ(*sdh-lac*)] with the *zjj*:Tn*10* as a linked marker. When a transductant, ECL587, was grown anaerobically, elevated activities of β-galactosidase (1600 units), 2-oxoglutarate dehydrogenase (56 units), and L-lactate dehydrogenase (80 units) were found. Hence, in wild-type cells the lower level of aerobic enzymes in anaerobically grown cells was the result of repression exerted either directly or indirectly by the *arcA* product.

DISCUSSION

The *dye* gene was so named because deletions in it caused sensitivity to certain dyes (14). On the basis of altered protein profiles of inner and outer membranes in such mutants, the gene is thought to affect the synthesis of envelope proteins (43, 44). The *dye* product was identified as a polypeptide of M_r 29,000 in a soluble fraction (45), and the DNA sequence indicates a product of 238 amino acid residues (44). The same gene was first called *seg* for segregation, because mutations in it affected the replication or segregation of F plasmids (46, 47). It is also known as *fexA* for F expression (48), and the lack of F pilus would account for the resistance to male-specific phages noted in a study referring to the gene as *msp* (49). Other designations were *sfrA* for sex-factor regulation (50–52) and *cpxC* for conjugative plasmid expression (53). Studies on deletions of *dye*, however, distinguished it from the nearby *cet* (for colicin E-2) in which mutations conferred tolerance to ColE2 (49).

We prefer *arcA* as the symbol for this pleiotropic regulatory gene, because its central role now appears to be in the regulation of the aerobic respiration system that embraces many target genes. The actual list of genes belonging to the *arc* system will probably be extended considerably by future studies. Obvious candidates are genes that specify certain components in aerotaxis, permeases for substrates of aerobic pathways, and the biosynthetic pathway for ubiquinone, whose level is greatly lowered by anaerobiosis (reviewed in ref. 1). A rewarding way to search further might be to use as a guide the biochemical activities of mitochondria (widely considered as descendants of aerobic bacterial endosymbionts) that also occur in *E. coli*.

A clue to the mechanism of *arcA* regulation may be that its DNA sequence (44) is 40% similar to *ompR* (54). The latter is involved in regulating the expression of *ompF* and *ompC*, which specify outer membrane porins (54–56). In this regulatory process the *ompR* product functions in conjunction with the *envZ* product. Both genes belong to a single operon, with *ompR* encoding a cytoplasmic protein and *envZ* encoding an envelope protein (54, 56). The *envZ* product possibly serves as the sensor and transmits the stimulus to the *ompR* product (56). Like the *ompR* product, the *arcA* (*dye*) gene product is a soluble protein (44). By analogy, the product of this gene may also act in conjunction with a membrane protein (see for example the regulation of proline dehydrogenase) (57). Such a protein would have to be encoded by a separately expressed gene such as *arcB*, since *arcA* is the sole member of its operon (44).

A preliminary search for the signal for the *arc* system excluded the direct action of O_2. In an φ(*sdh-lac*) strain, to

which the *frd* operon (encoding fumarate reductase) was restored to allow anaerobic growth with fumarate as the exogenous electron acceptor, it was found that the level of β-galactosidase varied under various growth conditions according to the midpotential of the terminal electron acceptor available: 720 units with O_2, 190 units with nitrate, 110 units with fumarate, and 76 units with no exogenous acceptor (fermentation of xylose). These results, however, can be explained by two other kinds of signals.

As the operation of the redox system shifts from aerobic to anaerobic respiration and from anaerobic respiration to fermentation there may be a graded decrease in the difference of electrical potential across the cytoplasmic membrane (less negative inside) or in the pH gradient across that membrane (less alkaline inside). A protein in the plasma membrane, such as an element of the electron transport chain that can exist in an oxidized or a reduced form (e.g., a cytochrome) may be able to sense one of these changes. An alternative kind of signal would invoke a more classical mechanism. The cellular redox state is likely to influence the ratio of the oxidized to reduced forms of a small molecule in the aerobic electron transport chain, such as ubiquinone. One of them might serve as the effector.

The discovery of the *arc* system adds another example of global control. In studying hierarchical control of gene expression, the term regulon was originally used to designate a set of operons under the control of a common effector and regulatory protein (58). Since the *arc* system includes several enzymes that are induced by different effectors—e.g., 3-hydroxyacyl-CoA dehydrogenase inducible by oleate (31), L-lactate dehydrogenase inducible by its specific substrate (59), and D-amino acid dehydrogenase inducible by L-alanine (37)—it is not a regulon. We propose the term modulon to describe a group of operons and/or regulons that are under the modulation of a common pleiotropic regulatory protein (possibly even a specialized σ protein). Under this definition the classical system of catabolite repression would be the *crp* modulon, and the group of operons involved in anaerobic respiration would be the *fnr* modulon. Many operons could have overlapping controls as members in different modulons. The *sdh* operon is an example. Such a system of nomenclature has the advantage of revealing both the organizational level of transcriptional control and the critical regulatory gene.

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