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Aerobic Regulation of the *sucABCD* Genes of *Escherichia coli*, Which Encode α-Ketoglutarate Dehydrogenase and Succinyl Coenzyme A Synthetase: Roles of ArcA, Fnr, and the Upstream *sdhCDAB* Promoter

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The *sucABCD* genes of *Escherichia coli* encode subunits for two enzymes of the tricarboxylic acid (TCA) cycle, α-ketoglutarate dehydrogenase (*sucAB*) and succinyl coenzyme A synthetase (*sucCD*). To examine how these genes are expressed in response to changes in oxygen and carbon availability, a set of *sucA-lacZ*, *sucC-lacZ*, *sdhCDAB-sucA-lacZ*, and *sdhC-lacZ* fusions were constructed and analyzed in vivo. While the expression of a *sucA-lacZ* fusion was low under all cell growth conditions tested, the expression of the *sucA* gene from the upstream *sdhC* promoter was considerably higher and varied by up to 14-fold depending on the carbon substrate used. Expression of the *sdhCDAB-sucA-lacZ* fusion varied by fourfold in response to oxygen. In contrast, no expression was seen from a *sucC-lacZ* reporter fusion, indicating that no promoter immediately precedes the *sucCD* genes. Taken together, these findings demonstrate that the oxygen and carbon control of *sucABCD* gene expression occurs by transcriptional regulation of the upstream *sdhC* promoter. The weaker *sucA* promoter provides an additional low constitutive level of *sucABCD* gene expression to supplement transcription from the *sdhC* promoter. The negative control of *sucABCD* gene expression seen under anaerobic conditions, like that for the *sdhCDAB* genes, is provided by the *arcA* and *fnr* gene products. These findings establish that the differential expression of eight genes for three of the TCA cycle enzymes in *E. coli* is controlled from one regulatory element.

The two tricarboxylic acid (TCA) cycle enzymes in *Escherichia coli*, α-ketoglutarate dehydrogenase and succinyl coenzyme A synthetase (succinyl-CoA synthetase), are encoded by the*sucAB* and *sucCD* genes, respectively (4). α-Ketoglutarate dehydrogenase catalyzes the oxidative decarboxylation of α-ketoglutarate to generate succinyl-CoA and carbon dioxide, along with the production of NADH plus H^+ (20). Succinyl-CoA synthetase catalyzes the interconversion of succinyl-CoA and succinate, and this interconversion is accompanied by the production or hydrolysis of GTP (10, 15). Both TCA cycle enzymes participate in the cyclic flow of carbon from acetyl-CoA to carbon dioxide during aerobic cell growth conditions (4). This process provides reducing equivalents in the form of NADH for subsequent use in electron transport-linked phosphorylation reactions. Succinyl-CoA synthetase also participates in the noncyclic or branched pathway operative during anaerobic cell growth conditions to provide carbon intermediates for cell biosynthetic reactions (10, 15). The results of enzyme assays and two-dimensional protein gel electrophoresis studies indicate that the cellular synthesis of α-ketoglutarate dehydrogenase is suppressed by anaerobiosis and glucose and is induced by acetate or other oxidized carbon sources (1, 5, 24).

The *sucABCD* genes are located at 16.7 min in the *E. coli* chromosome near the genes for two other TCA cycle enzymes, succinate dehydrogenase (*sdhCDAB*) and citrate synthase (*gltA*) (Fig. 1) (25). Little information is available concerning the regulation of the *sucABCD* genes under differing cell growth conditions. S1 nuclease mapping studies identified a large *sucABCD* gene transcript as well as a smaller *sucAB* transcript (26). Because no *sucCD*-specific transcript was observed, it was concluded that the *sucCD* genes are transcribed from a putative *sucA* promoter. Additionally, no evidence for the transcription of the *sucABCD* genes from the upstream *sdhCDAB* operon was observed. In more recent studies, *arcA* strains were shown to have elevated levels of α-ketoglutarate dehydrogenase activity and other TCA cycle enzymes during anaerobic cell growth conditions (7, 9, 12). ArcA protein in combination with the ArcB protein forms a two-component regulatory system that also modulates the aerobic-anaerobic expression of other genes that encode cytochrome-*d* oxidase, cytochrome-*o* oxidase, pyruvate formate lyase, and superoxide dismutase (7, 12).

To investigate how the *sucABCD* genes of *E. coli* are expressed in response to changes in oxygen and carbon availability, several *sucA-lacZ*, *sucC-lacZ*, *sdhCDAB-sucA-lacZ*, and *sdhC-lacZ* fusions were constructed and analyzed in vivo. The *sucA* gene was found to be expressed from a low-level constitutive *sucA* promoter as well as from the stronger upstream *sdhC* promoter of the succinate dehydrogenase (*sdhCDAB*) operon. The aerobic-anaerobic control of the *sucABCD* operon is provided by the *arcA* and *fnr* gene products, which act at the upstream *sdhC* promoter (8, 19). Thus, the synthesis of three TCA cycle enzymes is coordinated by the transcription of the *sdhCDAB*, *sucAB*, and *sucCD* genes from the oxygen- and carbon-controlled *sdhC* promoter.

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TABLE 1. Strains, plasmids, and phages

Strain, phage, or plasmid	Derived from	Genotype or phenotype	Source or reference
Strains			
MC4100		F⁻ *araD139* Δ(*argF-lac*)*U169* *rpsL150 relA1 flb-5301* *deoC1 ptsF25 rbsR*	22
PC2	MC4100	Δ*fnr*	3
PC35	MC4100	Δ*arcA*, Kan^r	3
SJP2	MC4100	*fur*	19
SJP3	MC4100	*himA*	19
SJP4	MC4100	*fis*	19
Phages			
λRZ5		*lacZ*	23
λSJP33	λRZ5	Φ(*sdhC-lacZ*) *lacY*$^+$ *lacA*$^+$ (Hyb) (gene fusion)	19
λSJP42	λRZ5	Φ(*sucA-lacZ*) *lacY*$^+$ *lacA*$^+$ (Hyb) (gene fusion)	This study
λSJP43	λRZ5	Φ(*sucA-lacZ*$^+$) *lacY*$^+$ *lacA*$^+$ (operon fusion)	This study
λSJP61	λRZ5	Φ(*sdhCDAB-sucA-lacZ*$^+$) *lacY*$^+$ *lacA*$^+$ (operon fusion)	This study
Plasmids			
pJTSD1	pTZ19	*sdh*::Tn*10*	18
pSDH	pBR322	*sdhC*$^+$*D*$^+$*A*$^+$*B*$^+$	19
pGEM11f(+)			Promega
pRS414		*lacZ lacY*$^+$ *lacA*$^+$	23
pRS415		*lacZ*$^+$ *lacY*$^+$ *lacA*$^+$	23
pSJP39	pGEM11f(+)	*sucA*	This study
pSJP42	pRS414	Φ(*sucA-lacZ*) *lacY*$^+$ *lacA*$^+$ (Hyb)	This study
pSJP43	pRS415	Φ(*sucA-lacZ*$^+$) *lacY*$^+$ *lacA*$^+$	This study
pSJP61	pRS415	Φ(*sdhCDAB-sucA-lacZ*$^+$) *lacY*$^+$ *lacA*$^+$	This study
pGC60	pUC18	Φ(*sucB-sucC*)	This study
pGC61	pRS414	Φ(*sucC-lacZ*) *lacY*$^+$ *lacA*$^+$ (Hyb)	This study
pGC62	pRS415	Φ(*sucC-lacZ*$^+$) *lacY*$^+$ *lacA*$^+$	This study

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The genotypes of the *E. coli* K-12 strains, plasmids, and bacteriophages used in this study are listed in Table 1. The *arcA*, *himA*, and *fis* strains were constructed by introducing the indicated mutations into strain MC4100 bacteriophage λSJP43 (*sucA-lacZ*) or MC4100 bacteriophage λSJP61 (*sdhCDAB-sucA-lacZ*) by P1 transduction followed by selection for the appropriate drug resistance (13). The PC2 (*fnr*) λSJP43 and the PC2 (*fnr*) λSJP61 lysogens were constructed by infecting strain PC2 with a high-titer lysate of λSJP43 or λSJP61 as previously described (23).

Construction of *sucA-lacZ*, *sucC-lacZ*, and *sdhCDAB-sucA-lacZ* fusions. A 1.375-kb *Xho*I-*Bam*HI DNA fragment (Fig. 1) containing the sequence encoding the 235-amino-acid C-terminal region of SdhB, the 300-bp *sdhB-sucA* intergenic region, and the sequence encoding the 123-amino-acid N-terminal region of SucA was isolated from plasmid pJTSD1 (18) and cloned into pGEM11f(+) (Promega Inc.) to give pSJP39. The *Eco*RI-*Bam*HI fragment of pSJP39 containing the above DNA region was then transferred into pRS414 and pRS415 to generate the *sucA-lacZ* protein (pSJP42) and the *sucA-lacZ* operon (pSJP43) plasmids, respectively. An *sdhCDAB-sucA-lacZ* fusion was also constructed to test if the upstream *sdhC* regulatory region affects *sucABCD* gene expression. This plasmid, designated pSJP61, contains the *sdhCDAB* operon and the associated 555-bp upstream regulatory region, in addition to the same region of *sdhC-sucA* present on plasmid pSJP42. It was made by inserting the 4.5-kb *Bam*HI fragment from pSDH (19) into the *Bam*HI site of pRS415 (Fig. 1). The intended junctions of the above *lacZ* fusions were confirmed by double-stranded DNA sequence analysis (21). The *sucA-lacZ*, *sucA-lacZ*$^+$, and *sdhCDAB-sucA-lacZ*$^+$ fusions were then transferred onto λRZ5 (23) to give λSJP42, λSJP43, and λSJP61, respectively. Single lysogens containing each fusion were made as previously described (23).

A *sucC-lacZ* fusion was also constructed to test if the *sucAB-sucCD* intergenic region contained a promoter for expression of the downstream *sucCD* genes. A 418-bp *Eco*RI-*Bam*HI fragment was inserted into the gene and operon fusion vectors pRS414 and pRS415 to give the *sucC-lacZ* plasmids, pGC61 and pGC62, respectively. The *Eco*RI and *Bam*HI sites were introduced at position 59 prior to the end of *sucB* and at 75 bp into *sucC* by standard PCR methods, and the resulting DNA fragments were cloned into pUC18. The DNA was sequenced to confirm the intended wild-type sequence.

Cell growth. For strain manipulations and maintenance, cells were grown in Luria broth (LB) or on solid media. When required, ampicillin was added to the medium at a concentration of 100 mg/liter. For β-galactosidase assays, cells were grown in glucose (40 mM) minimal medium (pH 7.0) (2), unless otherwise indicated. For assay of cells grown on other carbon sources, each compound was added at 40 mM. Buffered LB (50 mM KPO_4, pH 7.0) was made with glucose (40 mM) or pyruvate (30 mM) supplements as indicated in Table 3 (3).

Aerobic and anaerobic cell growth was performed as previously described (2). High-level aeration of cultures during aerobic growth was accomplished by shaking 10-ml culture volumes in 150-ml flasks. Flasks or tubes containing the indicated medium were inoculated from the overnight cultures grown under the same conditions, and the cells were allowed to double four or five times in the exponential phase prior to harvesting for analysis (optical density at 600 nm (OD_{600}) of 0.4-0.5; Kontron Uvikon 810 spectrophotometer). Anaerobic cultures were harvested at an OD_{600} of 0.25. Trimethylamine-*N*-oxide (TMAO), sodium nitrate, and fumarate were added at a final concentration of 40 mM (2). For iron limitation studies, 2,2′-dipyridyl and/or ferrous sulfate was added at a final concentration of 150 μM or 80 μM, respectively (19).

β-Galactosidase assay. β-Galactosidase levels were determined by hydrolysis of *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) as previously described (2). Protein concentrations were estimated by assuming that a culture OD_{600} of 1.4 corresponds to 150 μg of protein per ml as previously described (13). β-Galactosidase levels are expressed as nanomoles of ONPG hydrolyzed per minute per milligram of protein (13). β-Galactosidase values represent the averages of at least four experiments with a variation of no more than ±5% from the mean.

Materials. ONPG and ampicillin were purchased from Sigma Chemical Co., St. Louis, Mo. All other chemicals used were of reagent grade.

RESULTS

Effects of oxygen and other electron acceptors on *sucA-lacZ* expression. To determine how oxygen availability affects*sucABCD* gene expression, a *sucA-lacZ* operon fusion (λSJP43) that contained a 1.37-kb DNA fragment spanning the intergenic region between *sdhCDAB* and *sucABCD* was constructed (Fig. 1) and analyzed in vivo in single copy. During aerobic growth, *sucA-lacZ* expression was about one-half that seen during anaerobic cell growth in a glucose minimal medium (Table 2). This was unanticipated since the *sucABCD* gene products, α-ketoglutarate dehydrogenase and succinyl-CoA synthetase, are more abundant during aerobic cell growth conditions than during anaerobic conditions (1, 5, 9). The addition of any of the anaerobic electron acceptors, nitrate, TMAO, or fumarate, did not further elevate *sucA-lacZ* expression (Table 2). When glycerol was substituted for glucose as the carbon source, *sucA-lacZ* expression was elevated by twofold for each condition tested. An identical pattern of gene expression was observed for a *sucA-lacZ* gene fusion (i.e., λSJP42) except that the level of β-galactosidase was always about 10-fold lower for each cell growth condition tested (data

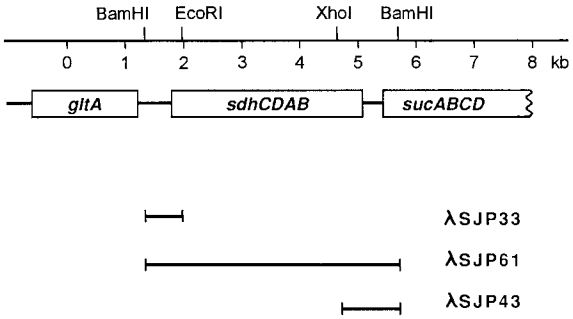

FIG. 1. Physical map of the *gltA-sdhCDAB-sucABCD* gene region. The DNA fragments used to construct the *sdhC-lacZ* (λSJP33), *sucA-lacZ* (λSJP43), and *sdhCDAB-sucA-lacZ* (λSJP61) fusions are indicated below the map. Additional *lacZ* fusions are described in Materials and Methods and Table 1.

TABLE 2. Effects of respiratory substrates on *sucA-lacZ* gene expression

Addition[a]	β-Galactosidase activity[b] in medium containing:	
	Glucose	Glycerol
None	650	NG[c]
Oxygen	380	570
Nitrate	610	1,020
TMAO	570	1,380
Fumarate	630	1,290

[a] Cells containing λSJP43 were grown in a minimal glucose- or minimal glycerol-containing medium either aerobically or anaerobically as described in the text. Sodium nitrate, TMAO, or fumarate was added at an initial concentration of 40 mM.

[b] Units are nanomoles of ONPG hydrolyzed per minute per milligram of protein.

[c] NG, no growth.

not shown). This suggests that the translation of the *sucA* gene is considerably less efficient than is the translation of the *lacZ*$^+$ gene.

The *sucABCD* genes are also cotranscribed from the *sdhC* promoter. The fact that the level of α-ketoglutarate dehydrogenase activity was inversely related to *sucA* gene expression during aerobic versus anaerobic conditions (1) (Table 2) suggested that *sucA* gene expression must occur from an additional upstream promoter(s). To test this, a second fusion was constructed that contained the *sdhCDAB* operon and its associated regulatory region in addition to the *sucA* gene region present in the *sucA-lacZ* fusion (Fig. 1; λSJP61). During aerobic cell growth, expression of the *sdhCDAB-sucA-lacZ* reporter fusion was eightfold higher than that of the *sucA-lacZ* fusion and was twofold higher during anaerobic conditions (Table 3). Thus, the *sdhC* promoter makes a major contribution to the aerobic expression of the *sucAB* genes, while it also supplements the anaerobic expression. A third *lacZ* reporter fusion that contained only the *sdhC* promoter region (*sdhC-lacZ*) was nearly equal in its expression aerobically (3,600 nmol of ONPG hydrolyzed/min/mg of protein). Interestingly, under anaerobic conditions the combined level of *sucA* gene expression was roughly additive to that provided by the *sdhC* and *sucA* promoters, although alternative interpretations can also be made.

To determine if the *sucCD* genes located downstream of *sucAB* are expressed from their own promoter, a 418-bp DNA fragment containing the 3′ end of *sucB* and the 5′ end of *sucC* was used to construct the *sucC-lacZ* gene and operon fusions, designated pGC60 and pGC61, respectively (see Materials and Methods). These plasmids were introduced into a *pcnB* strain to lower the plasmid copy number to 1 or 2 (11). When cells containing the operon fusion were grown aerobically or anaerobically in buffered LB, *sucC-lacZ* expression was barely detectable (ca. 18 to 45 nmol of ONPG hydrolyzed/min/mg of protein). Thus, the intergenic *sucB-sucC* region does not appear to contain a promoter of significant strength; rather, these data suggest that the *sucCD* genes are expressed from the upstream *sucA* and *sdhC* promoters, as was previously proposed from mRNA studies (26). However, this study did not directly test if other promoters exist further upstream within *sucAB*.

Effects of carbon substrates on *sucA-lacZ* and *sdhCDAB-sucA-lacZ* expression. Because the level of α-ketoglutarate dehydrogenase activity in *E. coli* was also shown to vary depending on the type of carbon compound used for cell growth (1, 5, 24), we asked whether this control is provided by either the *sdhC* or the *sucA* promoter. Cells containing the *sucA-lacZ* (λSJP43) or the *sdhCDAB-sucA-lacZ* fusion (λSJP 61) were grown in a minimal medium with different carbon compounds added or in a rich medium (LB medium). Aerobic expression of the *sucA-lacZ* fusion did not vary by more than about 2.5-fold in response to carbon type (Table 3). In contrast, *sdhCDAB-sucA-lacZ* expression varied over a 14-fold range during aerobic conditions and over a 5-fold range during anaerobic conditions. The highest level of gene expression occurred in a minimal acetate medium. Notably, under these conditions *sucA* expression as revealed by the *sdhCDAB-sucA-lacZ* fusion was 35-fold higher than that from the *sucA-lacZ* fusion (Table 3). The lowest level of *sucA* expression was seen in cells grown in a glucose minimal medium or in a buffered LB medium with glucose added. Thus, catabolite control of *sucABCD* gene expression is provided by the *sdhC* promoter located 3.7 kb upstream.

Effects of the *arcA*, *fnr*, *himA*, and *fis* gene products on *sucA-lacZ* expression. Since the ArcA and/or the Fnr proteins are known to regulate several TCA cycle genes, including the *sdhCDAB*, *mdh*, *gltA*, *acnA*, and *fumAC* genes, in *E. coli* in response to anaerobiosis (6, 8, 16–19, 27), we examined the effects of *arcA* and *fnr* gene deletions on *sucA-lacZ* and *sdhCDAB-sucA-lacZ* expression (Table 4). In neither the *arcA* strain nor the *fnr* strain did *sucA-lacZ* expression vary significantly compared to that of the wild-type strain grown under aerobic or anaerobic conditions. In contrast, *sdhCDAB-sucA-lacZ* expression was derepressed in an *arcA* mutant by about 10-fold under anaerobic conditions and by 2.5-fold during aerobic conditions. In the Δ*fnr* strain, *sdhCDAB-sucA-lacZ* expression was derepressed by about twofold under anaerobic conditions only. Thus, the *sucABCD* genes are negatively regulated by both the ArcA and Fnr proteins. This pattern is similar to that observed for a *sdhC-lacZ* reporter fusion (Table 4).

The IHF protein is also involved in regulating *sucABCD* gene expression (Table 4). In a *himA* mutant with a defective α-subunit of IHF, β-galactosidase activity was elevated by three- to fourfold when either the *sucA-lacZ* or the *sdhCDAB-*

TABLE 3. Effects of carbon substrates on *sucA-lacZ* and *sdhCDAB-sucA-lacZ* expression

Medium[a]	β-Galactosidase activity[b] for:			
	sucA-lacZ		*sdhCDAB-sucA-lacZ*	
	$+O_2$	$-O_2$	$+O_2$	$-O_2$
Minimal glucose	380	650	2,970	1,340
Minimal galactose	390	870	5,390	1,970
Minimal xylose	440	890	7,090	2,450
Minimal glycerol	570	NG[c]	6,290	NG
Minimal acetate	660	NG	23,000	NG
Minimal succinate	620	NG	11,100	NG
Minimal fumarate	600	NG	11,400	NG
Buffered LB	420	630	3,890	1,680
Buffered LB + glucose	250	450	1,670	480
Buffered LB + pyruvate	320	320	ND[d]	ND

[a] Cells containing λSJP43 or λSJP61 were grown in a minimal medium (pH 7.0) with the indicated additions except when buffered LB was used. Aerobic and anaerobic cultures were grown as described in the text.

[b] Units are nanomoles of ONPG hydrolyzed per minute per milligram of protein.

[c] NG, no growth.

[d] ND, not determined.

TABLE 4. Effects of *arcA*, *fnr*, *himA*, and *fis* mutations on *sucA-lacZ* and *sdhCDAB-sucA-lacZ* expression

Genotype[a]	β-Galactosidase activity[b] for:					
	sucA-lacZ		*sdhCDAB sucA-lacZ*		*sdhC-lacZ*[c]	
	$+O_2$	$-O_2$	$+O_2$	$-O_2$	$+O_2$	$-O_2$
Wild type	380	650	2,970	1,340	3,530	320
arcA	340	530	7,610	12,600	9,050	22,500
fnr	330	640	2,510	2,270	3,420	1,490
himA	1,550	2,600	3,380	3,720	2,340	410
fis	310	580	ND[d]	ND	2,030	430

[a] Cells containing λSJP43 or λSJP61 were grown in a minimal glucose medium under aerobic or anaerobic conditions as described in the text.
[b] Units are nanomoles of ONPG hydrolyzed per minute per milligram of protein.
[c] Data are as reported in reference 19.
[d] ND, not determined.

sucA-lacZ fusion was present, as compared to the β-galactosidase activity of wild-type strains when grown under anaerobic conditions. Since *sdhC-lacZ* expression was not significantly altered in a *himA* mutant (19), this suggests that the IHF control is exerted primarily from the *sucA* promoter. A *fis* mutant in which another general DNA-binding protein, Fis, was defective was not significantly altered in gene expression under the conditions tested.

Effect of iron availability on *sucA-lacZ* expression. Cellular iron levels have been shown to affect the expression of several TCA cycle genes including *acnA* and *fumC* (17, 27). To determine if the iron limitation or iron excess condition affects *sucABCD* expression, cells containing the *sucA-lacZ* fusion were grown in minimal glucose medium under conditions previously used for the *fumC* studies (17). When the iron chelator 2,2′ dipyridyl was present during aerobic or anaerobic cell growth, *sucA-lacZ* expression was not significantly altered (data not shown). The addition of Fe^{2+} iron also did not alter *sucA-lacZ* expression. Previous studies have established that the *sdhC* promoter is only modestly controlled by iron limitation (19).

DISCUSSION

The transcription of the *sucABCD* genes that encode subunits of α-ketoglutarate dehydrogenase (*sucAB*) and succinyl-CoA synthetase (*sucCD*) of *E. coli* occurs from two distinct promoter elements (Fig. 2). One is located near the start of the *sucA* gene and provides a relatively low constitutive level of *sucABCD* gene expression. The second promoter is located 3.7 kb upstream and regulates both the *sdhCDAB* and the *sucABCD* operons in response to oxygen and carbon availability (this study). The two global regulatory proteins, Fnr and ArcA, modulate this aerobic-anaerobic control of *sucABCD* gene expression in a pattern similar to that seen for the *sdhCDAB* genes (8, 19). The level of *sucA* transcription occurring by read-through from the *sdhCDAB* operon appears to be high as revealed by the β-galactosidase activities in the *sdhCDAB-sucA-lacZ* and *sdhC-lacZ* reporter strains (Table 4). Whereas the physiological role of the *sdhC* promoter is to provide for the oxygen and carbon control of *sucABCD* gene expression, the primary role of the weaker *sucA* promoter is to provide for a level of *sucABCD* gene expression during anaerobic conditions that is higher than that provided by the *sdhC* promoter (Table 4). Over the range of conditions tested, *sucA* gene expression varied by 48-fold (Table 3).

Prior S1 nuclease mapping studies of the *sucABCD* gene region indicated two distinct mRNA transcripts (26). The smaller 4.4-kb mRNA species corresponds to the *sucAB* gene region, while an mRNA species greater than 4.4 kb apparently corresponds to the entire *sucABCD* gene region. On the basis of these findings, the *sucABCD* genes were proposed to be expressed from a putative *sucA* promoter since no larger transcript that extended from the *sdhCDAB* gene region through the *sucABCD* region was observed (26). A putative 5′ end of the *sucA* mRNA is proposed at 199 bp prior to the translational start site of the gene. The fact that the S1 nuclease studies did not reveal a large *sdhABCD-sucABCD* transcript originating from the upstream *sdhC* promoter suggests that this mRNA was either specifically processed by an endonuclease or that, alternatively, it was rapidly degraded during the isolation of cellular mRNA prior to the S1 nuclease studies. The presence of several potential mRNA secondary structures in this region was noted (26). Expression from the *sucA* promoter was relatively constant regardless of the type of medium used for cell growth (Table 3). The fact that the control of this promoter was independent of ArcA and Fnr (Table 4) suggests that the prior observation by Iuchi et al. (9) that an *arcA* mutant was elevated for α-ketoglutarate dehydrogenase activity when grown under anaerobic conditions is accounted for by the regulated expression of the *sucAB* genes from the upstream *sdhC* promoter. By implication, the *sucCD* genes are similarly controlled.

It is evident from the present studies that transcription originating from the *sucA* promoter alone cannot account for the variation in α-ketoglutarate dehydrogenase levels seen in cells grown aerobically versus those grown anaerobically (1, 5). However, when the contribution of the upstream *sdhC* promoter is taken into consideration, the pattern of *sucA* gene expression, as measured with an *sdhCDAB-sucA-lacZ* fusion, is similar but not identical to that previously reported for *sdhC-lacZ* fusion λSJP33 (Table 4) (19). Expression of the *sdhCDAB* operon varied over a 10-fold range depending on oxygen availability and over a 14-fold range depending on the type of carbon used for cell growth. The ArcA and Fnr proteins provide anaerobic control of *sucA* expression at a distance as revealed by analysis of the larger *sdhCDAB-sucA-lacZ* fusion (Fig. 2, Table 4). Since expression of the *sucA* promoter is not affected in an *arcA* mutant, transcription read-through from the upstream *sdhC* promoter into *sucAB* accounts for the elevated level of

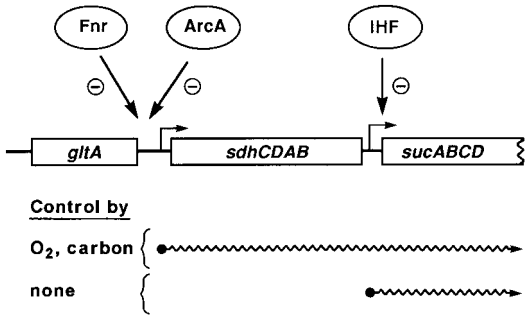

FIG. 2. Proposed model for regulation of the *sdhCDAB* and *sucABCD* genes by the ArcA, Fnr, and IHF proteins. The *sucABCD* genes are transcribed from both the weaker *sucA* promoter and from the stronger oxygen- and carbon-regulated upstream *sdhC* promoter. The mRNA transcript originating from each operon is indicated as a wavy line. Control of the *sucABCD* genes by Fnr and ArcA is from the *sdhC* promoter, while IHF control is primarily from the more weakly expressed *sucA* promoter. Negative control of gene expression is indicated by an encircled minus sign. It is not evident if Fnr control is direct or indirect.

α-ketoglutarate dehydrogenase activity previously reported for *arcA* strains (9).

Strikingly, the levels of the two α-ketoglutarate dehydrogenase subunits, E1 and E2, as revealed by two-dimensional gel protein studies (24) correlate remarkably well to the level of *sucAB* transcription reported for the *sdhCDAB-sucA-lacZ* fusion (Tables 2 and 3). These two independent studies employed slightly different cell growth conditions but gave the same relative level of aerobic-anaerobic changes in gene expression and in the amounts of the two protein subunits. A similar pattern was also seen when cells were grown aerobically in media with either glucose or acetate as the carbon source (ca. 9- to 10-fold). Thus, the regulated transcription of *sucAB* from the *sdhC* and *sucA* promoters appears to account for the observed variation in α-ketoglutarate dehydrogenase subunit synthesis (24). The cell has employed a regulatory strategy involving two promoters of differing strengths and responses as a means to coordinate the synthesis of three of the TCA cycle enzymes as cell growth conditions change. A related two-promoter strategy is used for the expression of the *fumA* and *fumC* genes of *E. coli* (17), but in this case the downstream promoter also responds to oxygen superoxide radical, and iron availability.

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