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Aerobic Regulation of Isocitrate Dehydrogenase Gene (*icd*) Expression in *Escherichia coli* by the *arcA* and *fnr* Gene Products

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Isocitrate dehydrogenase, the *icd* gene product, has been studied extensively regarding the regulation of enzymatic activity and its relationship to the metabolic flux between the tricarboxylic acid cycle and the glyoxylate bypass. In this study, the transcriptional regulation of *icd* gene expression was monitored by using an *icd-lacZ* gene fusion and shown to vary over a 15-fold range in response to changes in oxygen and carbon availability. Anaerobic cell growth resulted in fivefold-lower *icd-lacZ* expression than during aerobic growth. This negative control is mediated by the *arcA* and *fnr* gene products. When different carbon compounds were used for cell growth, *icd-lacZ* expression varied threefold. The results of continuous cell culture studies indicated that this control may be due to variations in cell growth rate rather than to catabolite repression. DNase I footprinting at the *icd* promoter revealed a 42-bp ArcA-phosphate-protected region that overlaps the start site of *icd* transcription. Phosphorylation of ArcA considerably enhanced its binding to DNA, while ArcA-phosphate exhibited an apparent dissociation value of approximately 0.1 μM. Based on these studies, ArcA appears to function as a classical repressor of transcription by binding at a site overlapping the *icd* promoter during anaerobic cell growth conditions.

The tricarboxylic acid (TCA) cycle enzyme isocitrate dehydrogenase (ICDH; EC 1.1.1.42) catalyzes the conversion of isocitrate to α-ketoglutarate, with concomitant production of NADH and carbon dioxide (3, 15). In *Escherichia coli*, ICDH activity is regulated by posttranslational modification involving a phosphorylation of serine 113 within the homodimeric protein (16, 17, 34, 36). This reaction is catalyzed by the AceK protein, isocitrate dehydrogenase kinase/phosphatase, to inactivate ICDH under conditions when the cell is grown on acetate or fatty acids. AceK also serves as a protein phosphatase that restores ICDH activity under alternative cell growth conditions when glucose or its saccharide precursors are present. The modulation of ICDH enzyme activity in the cell aids in maintaining the optimal amounts of TCA cycle intermediates, since this enzyme is at the branch point for carbon flow to the glyoxylate bypass pathway (3, 14). In the competing reactions, isocitrate lyase converts part of the isocitrate pool to glyoxylate and succinate, while malate synthase then combines glyoxylate with acetyl coenzyme A to form malate. Thus, when *E. coli* is grown on acetate or its direct precursors, the glyoxylate bypass reactions makes it possible for the cell to generate four-carbon compounds needed for biosynthetic reactions while also balancing its needs for energy via TCA cycle-derived NADH and FADH.

Little is known about the control of isocitrate dehydrogenase (*icd*) gene expression in *E. coli* under different cell growth conditions. Early studies by Gray et al. demonstrated that ICDH enzyme activity varied over a 10-fold range depending on the availability of oxygen and the composition of the cell growth medium (8). Subsequent work by Iuchi and Lin demonstrated that *arcA* mutants exhibit elevated ICDH enzyme levels under anaerobic conditions (12). ArcA in combination with the ArcB protein has been shown to be global regulator of other TCA cycle genes, including *gltA*, *sdhCABA*, *mdh*, *fumA*, *fumC*, and *acnA*, during anaerobic cell growth conditions (9, 11, 22, 23, 24, 25, 37).

To examine how the *E. coli icd* gene is regulated, an *icd-lacZ* reporter fusion was constructed and analyzed in vivo under a variety of cell growth conditions. A fivefold aerobic anaerobic response that was provided by the *arcA* and *fnr* gene products was seen. The location of an ArcA binding site that overlaps the start site of *icd* transcription was identified by DNase footprinting experiments. A regulatory mechanism whereby ArcA-phosphate binds to the *icd* promoter element to prevent transcription by RNA polymerase during anaerobic cell growth conditions is proposed.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The genotypes of the *E. coli* K-12 strains, plasmids, and bacteriophages used are listed in Table 1. The *arcA* and *himA* strains were constructed by introducing the indicated mutation into strain MC4100 λSJP69 (*icd-lacZ*) by P1 transduction followed by selection for the appropriate drug resistance (20, 30). The PC2 (*fnr*) λSJP69 lysogen and GLC03 (*arcA fnr*) λSJP69 lysogens were constructed by infecting PC2 and GLC03 with a high-titer λSJP69 lysate as previously described (31). To construct an *icd-lacZ* fusion, a 300-bp fragment containing the 5′ end of *icd* gene (123 bp) and the upstream 342-bp regulatory region was generated by PCR using chromosomal DNA of strain MC4100 as a template. The fragment was cloned into the *Sma*I site of plasmid pRS415 (31) to give the *icd-lacZ* operon fusion designated pSJP69, and the DNA sequence was confirmed. The fusion was transferred to λRS45 to generate λSJP69, which was then introduced into MC4100 as previously described (31).

Cell growth. For strain manipulations and cell maintenance, cells were grown in Luria broth (LB) or on solid LB medium (20). When required, ampicillin and

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TABLE 1. Bacterial strains, phages and plasmids

Strain, phage, or plasmid	Derived from:	Genotype or phenotype	Source
Bacterial strains			
MC4100		F$^-$ *araD139* Δ(*argF-lac*)*U169* *rpsl 150 relA1 flb-5301 deoC1 ptsF25 rbsR*	30
PC2	MC4100	Δ*fnr-2*	2
PC35	MC4100	Δ*arcA35* Kan^r	24
GLCO3	PC2	Δ*fnr-2* Δ*arc35* Kan^r	This study
SJP3	MC4100	*himA*Δ*82*	24
IS1	MC4100	Δ(*narXL*)	29
Phages			
λSJP69	λRS45	Φ(*icd-lacZ*)	This study
λRS45		*lacZ*	31
Plasmids			
pGLC63	pUC18	*icd* promoter fragment	This study
pSJP69	pRS415	Φ(*icd-lacZ*$^+$) *lacY*$^+$ *lacA*$^+$ (operon fusion)	This study
pRS415		*lacZ*$^+$ *lacY*$^+$ *lacA*$^+$	31

kanamycin were added to the medium at concentrations of 100 and 50 mg/liter, respectively. For β-galactosidase assay, cells were grown in a phosphate-buffered minimal medium (pH 7.0) with glucose (40 mM) unless otherwise indicated (2). 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 as indicated elsewhere (23).

Aerobic and anaerobic growth was carried out as previously described (2). 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 under mid-log exponential phase prior to harvesting for analysis (optical density at 600 nm [OD_{600}] of 0.4 to 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.

β-Galactosidase assay. β-Galactosidase levels were determined by hydrolysis of *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) as previously described (2). Protein concentration was estimated by assuming that a culture absorbance of 1.4 at an OD_{600} corresponds to 150 mg of protein per ml as previously described (20). Units of β-galactosidase are expressed as nanomoles of ONPG hydrolyzed per minute per milligram of protein (2). β-Galactosidase values represent averages of at least five independent determinations that varied by less than 10%.

Primer extension analysis. Total cellular mRNA was isolated from MC4100 grown aerobically in LB medium with a Qiagen RNeasy Total RNA kit. Primer extension reactions were performed with a synthetic oligonucleotide complementary to positions +13 to −9 of the *icd* gene relative to the start of translation (13). The primers were radiolabeled with [γ-^{32}P]ATP (Andotek Life Sciences Company, Irvine, Calif.). DNA sequencing reactions were performed with a high-copy-number plasmid containing *icd* promoter sequences (pGLC63) as the template to provide a nucleotide sequence ladder for comparison.

DNase I footprinting. DNase I footprinting to locate the ArcA site at the *icd* promoter was performed as described previously (28, 29). For these experiments, a 300-bp *Eco*RI-*Bam*HI fragment was used: it was isolated from pSJP69 that contained the *icd* promoter region extending from nucleotides −164 bp to +137 bp relative to the transcription start site (Fig. 1). The labeled fragment (2 nM) was preincubated with increasing amounts of purified ArcA or ArcA-phosphate (0.06 to 0.62 μM) for 10 min at 22°C. The reaction mixture (30 μl) contained 50 mM morpholinepropanesulfonic acid chloride (pH 7.0), 200 mM potassium chloride, 7 mM magnesium chloride, 5% glycerol, and 40 μg of bovine serum albumin per ml. The reaction was allowed to proceed for 2 min at 22 C. Xylene cyanol (0.25%)–bromphenol blue (0.25%) in 50% glycerol (3 μl) was then added to the reaction mixture to stop the digestion. Samples were run on an 8% polyacrylamide gel containing 7 M urea. The G sequencing reaction was performed by a modification of the Maxam-Gilbert sequencing reaction (26). The ArcA protein was purified from *E. coli* JM101 that contained plasmid pSJP13 with the *arcA* gene under control of the IPTG-inducible promoter on pQE32 (Qiagen). Six histidine codons were included at the N terminus so the resulting protein could be purified by affinity chromatography. Induced cells (3 g [wet weight]) were resuspended in 12 ml of 50 mM Tris-HCl (pH 7.5)–300 mM potassium chloride–1 mM dithiothreitol–1 mM magnesium chloride–50 μg of phenylmethylsulfonyl fluoride per ml–10 μg of RNase A per ml and passed through a French pressure cell twice at 540 lb/in^2. DNA was sheared by 15 s of sonication on ice, and the extract was centrifuged at 8,000 rpm for 10 min to remove cell debris. A 50% ammonium sulfate pellet was prepared as follows. The protein was resuspended in 6.0 ml of Tris-KCl buffer (50 mM Tris-HCl [pH 7.5], 200 mM KCl) and loaded onto a Qiagen Ni-nitrilotriacetic acid resin (1 ml) equilibrated in Tris-KCl buffer. The column was washed with 10 ml of Tris-KCl buffer, and the ArcA protein was eluted with 2 ml of Tris-KCl buffer containing 0.5 M imidazole. Following dialysis to remove the imidazole, the protein was stored in 50 mM Tris-HCl (pH 7.5)–200 mM KCl–1 mM $MgCl_2$–10% glycerol at −70°C until used. Protein concentrations were determined by the Coomassie brilliant blue G-250 assay kit (Bio-Rad), with bovine serum albumin as the standard.

Continuous culture cell growth. A Queue Mouse bioreactor (Queue Corporation, Parkersburg, W.Va.) fitted with a 2-liter vessel and operated at a 1-liter liquid working volume was used as previously described (35). The medium was a modified Vogel-Bonner medium (pH 6.5) supplemented with Casamino Acids (0.25 mg/liter; Difco, Inc.), and glucose (2.25 mM) was used to limit cell growth (i.e., carbon-limited medium [35]). Aerobic and anaerobic culture conditions were maintained by controlling the composition of the sterile gas used for sparging the vessel (35). When the chemostat was shifted to a new growth condition, steady state was generally achieved within five reactor residence times. The chemostat was maintained at the same condition until the β-galactosidase values varied no more than 5%. During the experiments, the chemostat was maintained at a medium flow rate of 10 ml/min (cell growth rate [k] = 0.6 per h).

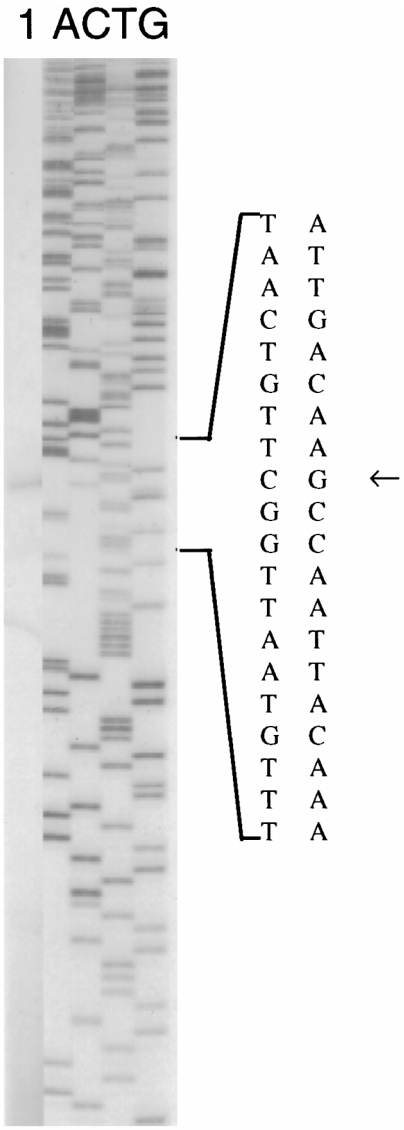

FIG. 1. Location of the in vivo mRNA 5′ terminus of the *icd* transcript. Land 1 represents the primer extension reaction using mRNA prepared from cells grown aerobically. Lanes A, C, T, and G show the DNA sequencing reaction products from the corresponding region within the *icd* regulatory region. The arrow indicates the position corresponding to the observed 5′ end of the *icd* mRNA. The primer corresponded to nucleotide positions +13 to −9 relative to the start of *icd* translation.

TABLE 2. Effects of alternative electron acceptors on *icd-lacZ* gene expression

Electron acceptor added[a]	β-Galactosidase activity (nmol of ONPG hydrolyzed/min/mg of protein)	
	Glucose	Glycerol
None	1,200	NG[b]
Oxygen	6,400	7,100
Nitrate	2,700	4,000
TMAO	1,500	5,500
Fumarate	1,500	4,000

[a] Cells were grown in a glucose minimal or glycerol minimal medium either aerobically or anaerobically as described in the text. Sodium nitrate, TMAO, or sodium fumarate was added at an initial concentration of 40 mM.
[b] NG, no growth.

This corresponded to a cell doubling time of 69 min. The cell generation time is equal to ln2/*k* (21).

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

RESULTS AND DISCUSSION

Effects of oxygen and other electron acceptors on *icd-lacZ* expression. To determine how *icd* gene expression varies in response to oxygen availability, an *icd-lacZ* fusion was constructed and inserted in the chromosome in single copy, and the resulting lysogen was grown in a glucose minimal medium under aerobic and anaerobic conditions. Expression of the *icd-lacZ* fusion was fivefold higher during aerobic cell growth conditions than during anaerobic conditions (Table 2). To evaluate if any of the anaerobic electron acceptors, nitrate, TMAO, and fumarate, further affect anaerobic *icd-lacZ* expression, each compound was added to a glucose minimal medium at a final concentration of 40 mM, and cells were grown as described above. When nitrate was present, *icd-lacZ* expression was about twofold higher than in glucose medium alone (i.e., fermentation conditions). Neither TMAO nor fumarate significantly affected *icd-lacZ* expression (Table 2). When glycerol was used in place of glucose, *icd-lacZ* expression was elevated by 1.5- to 3-fold, depending on which anaerobic electron acceptor was present. The presence of oxygen increased gene expression less than twofold in this medium relative to a nitrate glycerol medium.

Effects of carbon substrates used for cell growth on *icd-lacZ* expression. Since *icd-lacZ* gene expression varied depending on whether glucose or glycerol was used for cell growth, we examined how other carbon compounds affect *icd* gene expression (Table 3). During aerobic conditions, *icd-lacZ* expression varied over a threefold range: expression was lowest when a buffered LB medium or a minimal glucose medium was used and highest when acetate was used as the carbon substrate. During anaerobic conditions, *icd-lacZ* expression also varied 3-fold in response to carbon type: gene expression was always 1.5- to 6-fold higher during aerobic conditions in the corresponding medium. Over the range of conditions tested, *icd-lacZ* expression varied 15-fold.

Effects of *arcA*, *fnr*, and *himA* gene products on *icd-lacZ* expression. The two global regulatory proteins ArcA and Fnr negatively regulate many of the TCA cycle genes in response to anaerobiosis (10, 18). To document the role of each regulator in *icd* gene expression, isogenic strains that contained a deletion in either the *arcA* gene or the *fnr* gene or in both genes were constructed (Table 4). During anaerobic conditions, *icd-lacZ* expression was elevated 2.5-fold in the *fnr* strain and 10-fold in the *arcA* strain compared to the wild-type parent. Thus, ArcA and Fnr function as negative regulators of *icd* gene expression. Expression in the *arcA* mutant was also elevated about twofold during aerobic growth. In the *arcA fnr* double-mutant strain, expression was also 2-fold higher under aerobic conditions and 12-fold higher under anaerobic conditions. The anaerobic control of *icd* expression by ArcA and that by Fnr appear to occur relatively independently of each other. A similar pattern of ArcA- and Fnr-dependent gene expression was previously reported for several other TCA cycle genes, including *sdhCDAB* and *fumA* (23, 25), although it is not clear if this control occur directly or indirectly.

TABLE 3. Effects of different carbon compounds and medium richness on *icd-lacZ* expression

Medium[a]	β-Galactosidase activity (nmol of ONPG hydrolyzed/min/mg of protein)	
	$+O_2$	$-O_2$
Glucose	6,400	1,000
Galactose	7,000	2,100
Xylose	8,200	2,000
Fumarate	12,000	NG[b]
Glycerol	8,200	NG
Succinate	14,000	NG
Acetate	15,000	NG
Buffered LB	4,500	3,360

[a] Cells were grown in a minimal medium with indicated carbon additions or in buffered LB either aerobically or anaerobically as described in the text.
[b] NG, no growth.

Integration host factor protein (IHF) acts to control the expression of many genes in *E. coli* (7). A defect in the *himA* gene that encodes one of the subunits of IHF resulted in a threefold elevation of *icd-lacZ* expression during anaerobic cell growth (Table 4). During aerobic cell growth, this effect was less pronounced. IHF appears to aid in modulating ICDH synthesis by altering the level of *icd* transcription.

To test whether the nitrate two-component regulatory system affects *icd* expression, a *narXL* deletion strain (IS1) containing the *icd-lacZ* fusion was grown anaerobically with or without nitrate present. The β-galactosidase levels were not significantly different in the mutant compared to the MC4100 parental strain for any condition tested (data not shown).

Location of the *icd* mRNA 5′ end. Primer extension reactions were performed to locate the start site for *icd* transcription. A single 5′ mRNA terminus corresponding to a position 115 nucleotides upstream of *icd* translation start was identified (Fig. 1).

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

Strain[a] (genotype)	β-Galactosidase activity (nmol of ONPG hydrolyzed/min/mg of protein)	
	$+O_2$	$+O_2$
MC4100 (wildtype)	6,400	1,000
PC35 (*arcA*)	11,000	12,000
PC2 (*fnr*)	6,000	2,500
GLC03 (*arcA fnr*)	11,200	15,000
SJP3 (*himA*)	8,000	3,000

[a] Cells were grown in glucose minimal medium.

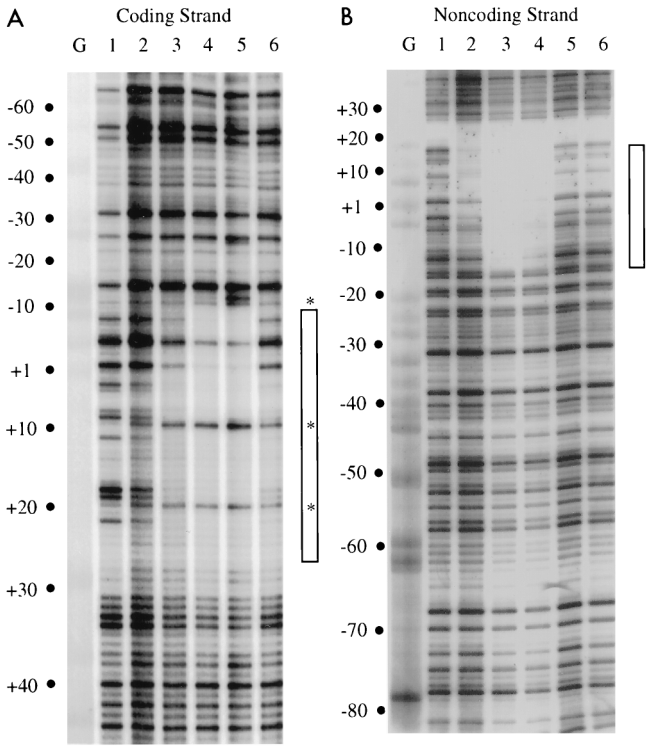

FIG. 2. DNASe I footprint of ArcA protein binding at the *icd* promoter. (A) Protections with a 300-bp coding strand containing the *icd* promoter fragment; (B) protections with a 300-bp noncoding strand in the *icd* promoter region. Assay conditions are described in Materials and Methods; the number above each lane indicates the relative amount of ArcA protein used in each reaction. The rectangle represents the ArcA-protected region, and asterisks indicate bases showing increased sensitivity to DNase I cleavage upon binding of ArcA-phosphate to the DNA. Numbering of the DNA is relative to the start of *icd* transcription. Lanes: 1, no ArcA protein present; 2, 0.062 μM phosphorylated ArcA; 3, 0.123 μM phosphorylated ArcA; 4, 0.248 μM phosphorylated ArcA; 5, 0.62 μM phosphorylated ArcA. Lane 6 of the coding strand contained 0.62 μM unphosphorylated ArcA; lane 5 of the noncoding strand contained 0.248 μM unphosphorylated ArcA, and lane 6 of the noncoding strand contained 0.62 μM unphosphorylated ArcA.

Location of the ArcA binding site at the *icd* promoter. DNase I footprinting methods were used to locate the ArcA binding site(s) within the *icd* promoter region (Fig. 2). ArcA protein, when prephosphorylated with acetyl phosphate (Materials and Methods), protected a 42-bp region of DNA from positions −15 to +27 bp relative to the start site of *icd* transcription (Fig. 2). The protected region was considerably larger (ca. four turns of B-DNA) than would be expected from binding of a 28-kDa ArcA monomeric protein as purified for use in these experiments, which suggests that multiple ArcA molecules are bound at the *icd* regulatory site. Enhanced sensitivity to DNase I cutting was observed at several positions located either 5′ of or within the ArcA-phosphate protected region (i.e., at positions −11, +9, and +20), which suggests that ArcA-phosphate may alter the DNA topology by bending it. A 42-bp DNase I-protected region was also observed at the other strand at the *icd* promoter (Fig. 2). ArcA protections extended from about positions −15 to +27 relative to the start site of *icd* transcription. No enhanced DNase I cutting was seen on this strand.

ArcA binding at the *icd* promoter was also phosphorylation dependent (Fig. 2). From the DNase I results, an apparent dissociation value of 0.1 μM was estimated for ArcA-phosphate binding a the *icd* ArcA site. No ArcA protected regions of intermediate size were observed when lower levels of protein were used (Fig. 2 and data not shown).

Effect of cell growth rate on *icd* gene expression. Since the type of carbon compound used for cell growth in batch culture caused a threefold variation of *icd-lacZ* gene expression (Table 2), we tested whether varying the cell growth rate had a similar effect. Using continuous cell culture methods where glucose was used to limit cell growth, *icd-lacZ* gene expression was shown to vary threefold when the cell doubling time was changed from 58 min ($k = 0.96$) to 350 min ($k = 0.12$) (Fig. 3). These findings suggest that cell growth rate rather than a mechanism involving catabolite repression acts to control *icd* gene expression.

Regulatory implications. The control of *icd* gene expression is of particular interest because the gene product, ICDH, functions at the branch point between the TCA cycle and the glyoxylate bypass reactions. A balance must be reached between utilization of isocitrate by the glyoxylate bypass when cells are grown on acetate or other low-molecular-weight carbon compounds that lead to acetyl coenzyme A formation and use of isocitrate in the TCA cycle to supply energy and additional carbon intermediates for cell biosynthesis (3, 5, 14). The modulation of ICDH activity occurs by the phosphorylation-dephosphorylation of the protein by AceK (3). In this study, we demonstrate that the potential for metabolic flow through this TCA cycle enzyme is also controlled at the transcriptional level: expression of a *icd-lacZ* fusion varied as much as 15-fold between aerobic and anaerobic cell growth in the different types of culture media tested (Table 3).

The aerobic anaerobic control of *icd* gene transcription is accomplished through the ArcAB and Fnr global regulatory circuits (Table 4). ArcA was shown to bind directly within the *icd* regulatory region, where DNase I footprinting studies revealed a 42-bp ArcA-phosphate-protected region overlapping the start site of *icd* transcription (Fig. 4). A mechanism for repression of *icd* expression is proposed from the DNase I experiments whereby RNA polymerase is unable to either bind or productively transcribe the *icd* gene when ArcA-phosphate is bound at its site overlapping the *icd* promoter. During anaerobic cell growth conditions, this repression is optimal: under some conditions of aerobic cell growth, ArcA appears to still partially repress gene expression (ca. twofold in glucose grown cells [Table 4]). Apparently a portion of the ArcA protein can exist in a phosphorylated state during aerobic growth conditions such that *icd-lacZ* expression remains partly repressed except when cells are grown on substrates such as acetate or succinate (Table 3).

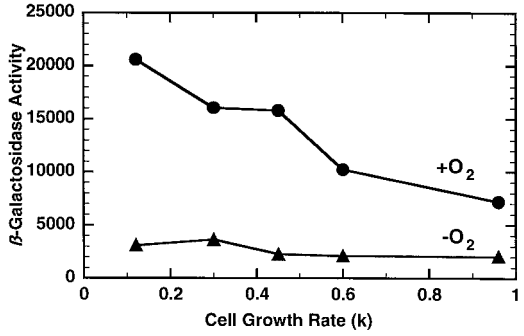

FIG. 3. Effect of cell growth rate on *icd-lacZ* gene expression. Cells were grown at the indicated growth rates under aerobic (●) or anaerobic (▲) conditions as described previously (25). β-Galactosidase activity is expressed as nanomoles of ONPG hydrolyzed per minute per milligram of protein.

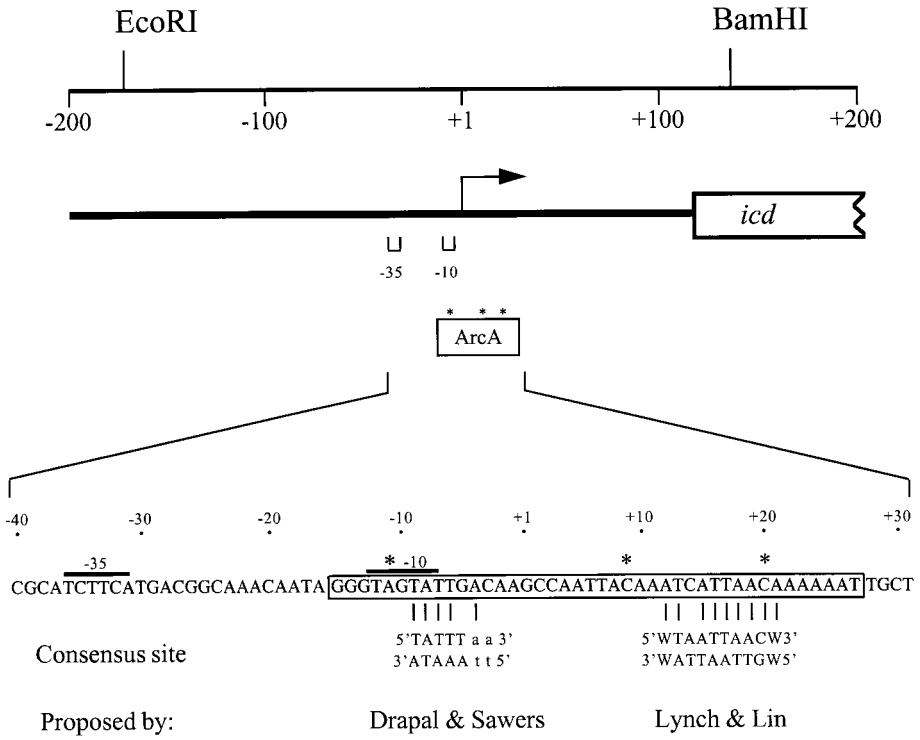

FIG. 4. Location of the ArcA-protected region at the *icd* promoter. The closed box represents the 43-bp ArcA-phosphate-protected region that overlaps the start site (+1) of *icd* transcription; solid bars represent the -35 and -10 regions for RNA-polymerase binding; asterisks mark sites hypersensitive to cutting by DNase I. Numbering of the DNA is relative to the transcription start site (Fig. 1). The *Bam*HI and *Eco*RI sites were introduced during the cloning procedures (Materials and Methods). The proposed ArcA consensus recognition sequences according to Drapel and Sawers (4) and Lynch and Lin (19) are shown at the bottom in alignment to related *icd* sequences.

ArcA consensus binding sites have been proposed from footprinting studies with several ArcA-controlled promoters, including the *pfl* promoter (e.g., TATTTaa, where a represents a poorly conserved A residue [4]) and the *sdh* and *cyd* promoters (e.g., WATTAATTGW, where W equals A or T [19]). Although no exact sequence match to the Drapal and Sawers consensus site is seen for the *icd* promoter, a related sequence (TATTgac) overlaps the −10 region relative to the start site for *icd* transcription (Fig. 4). A sequence proposed by Lynch and Lin (19) is also seen in the *icd* regulatory region from positions +10 to +20 relative to the transcription start (i.e., ATcATTAACA [Fig. 4]). For either ArcA consensus sequence, additional regions of ArcA-phosphate-protected DNA which extend by 2.5 to 3 additional turns of B-DNA are seen 5′ and 3′ of these sites. This result suggests that the ArcA protein-DNA interactions are more complex than can be accounted for by binding of an ArcA monomer at either consensus site. Additional DNA determinants must exist for ArcA recognition and binding to the *icd* DNA site of 42 bp. Interestingly, ArcA protein-DNA protections at other regulatory elements are either similar in size to the *icd* protections (ca. 31 to 90+ bp for *sdh* and 55 bp for *lct*) or larger (ca. 65 bp for *sodA* and up to 94 bp for *pfl* [4, 19, 33]). These different footprints are much larger than those observed for other two-component system response regulators such as OmpR (27). Studies are in progress to explore the molecular basis for these differences.

A mechanism for Fnr control of *icd* gene expression is less clear than for the ArcA protein. Inspection of the DNA sequences near the start site for *icd* mRNA synthesis did not reveal the presence of an Fnr consensus sequence, TTGATnnnnATCAA (32). However, from the present study, it is apparent that Fnr and ArcA can act relatively independently of one another to negatively control *icd* expression (Table 4). Interestingly, while several TCA cycle genes are under dual control by Fnr and ArcA (i.e., *sdhCDAB* and *fumA* [11, 23, 25]), other genes appear to be controlled only by ArcA (*mdh*, *fumC*, and *gltA* [22–24, 37]). Together, these findings do not support the suggestion that Fnr acts indirectly by controlling *arcA* gene expression (1, 6), since this would predict full derepression of *icd*, *mdh*, *fumC*, and *gltA* gene expression in an *arcA* strain.

The change in *icd* gene expression seen when cells are grown on alternative carbon sources is relatively modest (ca. threefold [Table 3]). Since *icd* gene expression was also shown to vary about threefold at different cell growth rates (Fig. 3), it is not evident that the *icd* gene is catabolite controlled as has been suggested (3). A similar effect of cell growth rate was seen for the *gltA*, *mdh*, and *sdhCDAB* genes (22, 24, 25). The TCA cycle genes were shown to vary over a much greater range in response to carbon (*sdhCDAB*, 15-fold; *fumA*, 20-fold; and *gltA*, 14-fold [22–25]) than for *icd* (this study). Additional studies are needed to more fully evaluate the contributions of cell growth rate versus cyclic AMP-dependent catabolite repression of TCA cycle gene expression.

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