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Regulation of the Citrate Synthase (*gltA*) Gene of *Escherichia coli* in Response to Anaerobiosis and Carbon Supply: Role of the *arcA* Gene Product

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As an enzyme of the tricarboxylic acid cycle pathway, citrate synthase participates in the generation of a variety of cellular biosynthetic intermediates and in that of reduced purine nucleotides that are used in energy generation via electron transport-linked phosphorylation reactions. It catalyzes the condensation of oxaloacetate and acetyl coenzyme A to produce citrate plus coenzyme A. In *Escherichia coli* this enzyme is encoded by the *gltA* gene. To investigate how *gltA* expression is regulated, a *gltA-lacZ* operon fusion was constructed and analyzed following aerobic and anaerobic cell growth on various types of culture media. Under aerobic culture conditions, expression was elevated to a level twofold higher than that reached under anaerobic culture conditions. ArcA functions as a repressor of *gltA* expression under each set of conditions: in a Δ*arcA* strain, *gltA-lacZ* expression was elevated to levels two- and eightfold higher than those seen in a wild-type strain under aerobic and anaerobic conditions, respectively. This control is independent of the *fnr* gene product, an alternative anaerobic gene regulator in *E. coli*. When the richness or type of carbon compound used for cell growth was varied, *gltA-lacZ* expression varied by 10- to 14-fold during aerobic and anaerobic growth. This regulation was independent of both the *crp* and *fruR* gene products, suggesting that another regulatory element in *E. coli* is responsible for the observed control. Finally, *gltA-lacZ* expression was shown to be inversely proportional to the cell growth rate. These findings indicate that the regulation of *gltA* gene expression is complex in meeting the differential needs of the cell for biosynthesis and energy generation under various cell culture conditions.

The *Escherichia coli gltA* gene product, citrate synthase (EC 4.1.3.7), catalyzes the condensation of acetyl coenzyme A with oxaloacetate to form citrate. Because of its key position as the first enzyme of the tricarboxylic acid (TCA) cycle, citrate synthase had been assumed to be an important control point for determining the metabolic rate of the cell. On the basis of enzyme activities in wild-type and TCA cycle mutants, a branched pathway was proposed to operate under anaerobic conditions. In the absence of oxygen certain TCA cycle enzymes contribute significantly to cellular biosynthesis rather than to energy generation (18). Citrate synthase must function under both aerobic (TCA cycle) and anaerobic (branched pathway) conditions, as the cell does not possess an alternative enzyme at this step, in contrast to other steps of the cycle (e.g., fumarate reductase and succinate dehydrogenase, and the fumarases). Measurements of citrate synthase levels show that citrate synthase synthesis is suppressed by anaerobiosis and glucose and elevated by the presence of oxygen and acetate (10, 30). Enzyme levels are inversely related to the growth rate (10, 30).

The *E. coli* enzyme is typical of citrate synthases of gram-negative microorganisms in that it is a large enzyme composed of six identical subunits with M_rs of 48,000 (31, 36). Its activity is inhibited by NADH (35), ATP (16), and α-ketoglutarate (38). The gene for the *E. coli* citrate synthase, *gltA*, is located at 16.2 min on the chromosome linkage map (1) and has been cloned and sequenced (11); it encodes a polypeptide of 427 amino acids in size (2, 9, 22). The *gltA* gene is located in a gene cluster encoding three other citric acid cycle enzymes, succinate dehydrogenase (*sdhCDAB*), α-ketoglutarate dehydrogenase (*sucAB*), and succinyl-coenzyme A synthetase (*sucCD*), in which *gltA* is transcribed divergently from the latter three sets of genes (37). Little is known about the mechanisms for control of *gltA* transcription in *E. coli*. Citrate synthase activity is elevated in an *arcA* strain, suggesting that ArcA is a transcriptional regulator of citrate synthase production (14). Transcript studies using S1 nuclease mapping (37) indicate that the *gltA* gene is transcribed from two promoters with mRNA initiation sites located 196 bp (major) and 299 bp (minor) upstream of the *gltA* coding region. A prediction that both of the transcripts are subject to catabolite repression via the CRP protein was made on the basis of the presence of four putative CRP binding sites within the *gltA* promoter region (37).

To examine how the *gltA* gene is controlled in response to anaerobiosis, different carbon substrates, and variations in medium richness, a *gltA-lacZ* fusion was constructed and its expression was examined in vivo. Expression varied by 2-fold depending on oxygen availability and by 10- to 14-fold in response to changes in the richness of the medium and in the type of carbon compound used for cell growth.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The genotypes of the *E. coli* K-12 strains and plasmids and of the bacteriophages used in this study are listed in Table 1. The *arcA*, *himA*, *fis*, *fur*, *fruR*, and *crp* strains were constructed by

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TABLE 1. Bacterial strains, phages, and plasmids

Strain, phage, or plasmid	Strain, phage, or plasmid from which derived	Genotype and/or phenotype	Source or reference
Bacteria			
MC4100		F^- *araD139* Δ(*argF-lac*) U169 *rpsL150 relA1 flb5301 deoC1 ptsF25 rbsR*	28
PC2	MC4100	Δ*fnr*	7
PC35	MC4100	Δ*arcA* Kan^r	24
W3110*fur*::Tn5		*fur*::Tn5	8
SA2777		Δ*crp* Chl^r	Susan Garges
SJP2	MC4100	*fur*::Tn5	This study
SJP3	MC4100	*himA*Δ*82*	This study
SJP4	MC4100	*fis767*	This study
SJP5	MC4100	Δ*crp* Chl^r	This study
SJP7		*fruR*	S.-J. Park
Phages			
M13mp19			20
M13mp1911	M13mp19	1.2-kb *Bam*HI-*Hin*dIII fragment	This study
M13mp1912	M13mp19	Additional *Eco*RI site in M13mp1911	This study
λRZ5			29
λSJP30	λRZ5	Φ(*gltA-lacZ*) $lacY^+$ $lacA^+$	This study
Plasmids			
pJTSD1	pTZ19	*sdh*::Tn*10*	J. Turna
pRS415		$lacZ^+$ $lacY^+$ $lacA^+$	29
pSJP30	pRS415	Φ(*gltA-lacZ*) $lacY^+$ $lacA^+$	This study

introducing various mutations into strain MC4100 λSJP30 (*gltA-lacZ*) by P1 transduction and then selecting for the appropriate drug resistance (21). The PC2 (*fnr*) λSJP30 lysogen was constructed by infecting PC2 with a high-titer λSJP30 lysate as previously described (29).

Construction of a *gltA-lacZ* operon fusion. A 1.2-kb *Bam*HI-*Hin*dIII fragment containing the *gltA* promoter and upstream region was isolated from plasmid pJTSD1 and inserted into M13mp19 to produce M13SJP1911. By oligonucleotide-directed mutagenesis (19), an *Eco*RI site was introduced into the *sdhC* gene at position +93 relative to the translational initiation site (+1) of *sdhC* (Fig. 1); the resulting phage was designated M13SJP1912. The 648-bp *Eco*RI-*Bam*HI fragment of M13SJP1912 was cloned into plasmid pRS415, a promoterless *lacZ* operon fusion vector (29), to produce the *gltA-lacZ* operon fusion plasmid pSJP30. The junction between *gltA* and *lacZ* was confirmed by double-stranded DNA sequence analysis (26). This *gltA-lacZ* fusion was then transferred to λRZ5 (29) to produce λSJP30. The fusion was then inserted in a single copy into the chromosome of MC4100 as previously described (25).

Cell growth. For strain manipulations and maintenance, cells were grown in Luria broth or on solid media. When required, ampicillin and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were added to the medium at concentrations of 100 and 40 mg/liter, respectively. For β-galactosidase assays, cells were grown in glucose (40 mM) minimal medium (pH 7.0) (6), unless otherwise indicated. For assays of cells grown on other carbon sources, each compound was added at 40 mM. Buffered L broth (50 mM KPO_4, pH 7.0) was made with glucose (40 mM) or pyruvate (30 mM) supplements as indicated (7).

Aerobic and anaerobic growth was as previously described (6). High-level aeration of cultures during aerobic growth was accomplished by shaking 10-ml culture volumes in 150-ml flasks. The fact that oxygen saturation had been achieved was verified by control experiments in which larger flasks were used

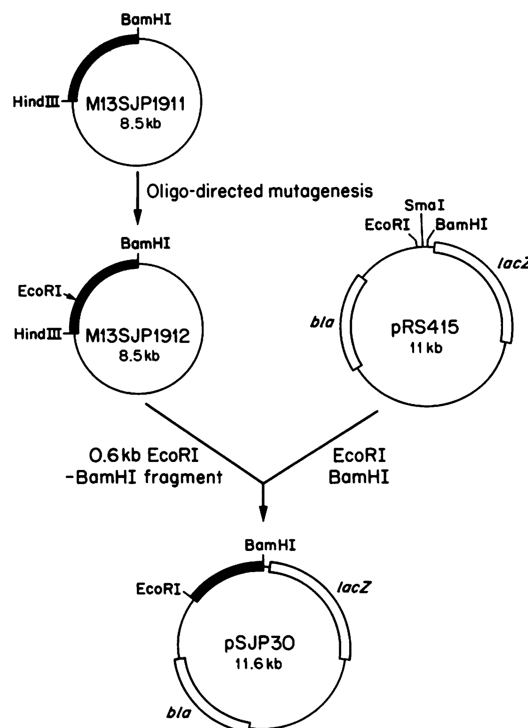

FIG. 1. Construction of the *gltA-lacZ* operon fusion plasmid pSJP30. The 648-bp *Bam*HI-*Eco*RI fragment was isolated from phage M13SJP1912 and inserted into pRS415 to produce the 11.6-kb *gltA-lacZ* plasmid designated pSJP30. The filled-in boxed regions indicate the chromosomal fragment containing the *gltA* promoter region. The open boxed regions represent the locations of the *lacZ* and *bla* genes. Oligo, oligonucleotide.

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

Electron acceptor added[a]	β-Galactosidase activity[b] in minimal medium containing:	
	Glucose	Glycerol
None	2,030	NG[c]
Oxygen	4,680	7,080
Nitrate	2,400	4,710
TMAO	2,690	4,560
Fumarate	2,080	3,590

[a] Cells (MC4100λSJP30) were grown in a minimal medium containing glucose or glycerol either aerobically or anaerobically as described in the text. Sodium nitrate, TMAO, or fumarate was added at an initial concentration of 40 mM.
[b] Nanomoles of ONPG hydrolyzed per min per mg of protein.
[c] NG, no growth.

and the rate of shaking was higher. Flasks or tubes containing various media were inoculated from cultures grown overnight under the same conditions, and the cells were allowed to double four or five times in exponential phase prior to being harvested for analysis (optical density at 600 nm of 0.4 to 0.5; Kontron Uvikon 810 Spectrophotometer). Anaerobic cultures were harvested at an optical density at 600 nm of 0.25. Trimethylamine-*N*-oxide (TMAO), sodium nitrate, and fumarate were added at a final concentration of 40 mM (6). For iron limitation studies, 2,2′-dipyridyl, ferrous sulfate, or both were added at final concentrations of 150 and 80 μM, respectively.

β-Galactosidase assay. β-Galactosidase levels were determined by hydrolysis of *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG) as previously described (6). The protein concentration was estimated by assuming that a culture absorbance of 1.4 at an optical density of 600 nm corresponds to 150 μg of protein per ml as previously described (21). Units of β-galactosidase are expressed as nanomoles of ONPG hydrolyzed per min per mg of protein (21). β-Galactosidase values represent the averages of at least four experiments with a variation of no more than 10% 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 *gltA-lacZ* expression. To determine how oxygen availability affects *gltA-lacZ* expression, cells were grown in a minimal glucose medium under aerobic and anaerobic conditions (Table 2). During aerobic growth, the level of *gltA-lacZ* expression was about twofold higher than that obtained during anaerobic growth; anaerobiosis is clearly not a major effector of *gltA* expression. Additionally, little effect on *gltA-lacZ* expression was observed when the anaerobic electron acceptors, nitrate, TMAO, and fumarate, were added to the glucose-containing medium. However, when glycerol was substituted for glucose as the carbon source, *gltA-lacZ* expression was elevated by about 50 to 70% under anaerobic conditions compared with the level obtained under aerobic conditions; the presence of nitrate or TMAO resulted in levels of β-galactosidase slightly higher than those produced when fumarate was used. Interestingly, the level of *gltA-lacZ* expression was as high during anaerobic growth with glycerol as it was during aerobic growth on a glucose-containing medium. As expected, no cell growth occurred anaerobically when respiratory substrates were omitted from the glycerol-containing culture medium.

TABLE 3. Effect of carbon type and medium richness on *gltA-lacZ* expression

Medium and/or addition[a]	β-Galactosidase activity[b]	
	With O_2	Without O_2
Glucose	4,680	2,030
Galactose	6,070	3,850
Xylose	7,140	3,600
Succinate	8,800	NG[c]
Fumarate	9,250	
Glycerol	7,080	
Acetate	14,800	
Buffered L broth	2,950	1,500
Buffered L broth + glucose	1,060	670
Buffered L broth + pyruvate	2,550	350

[a] Cells (MC4100λSJP30) were grown in a minimal medium (pH 7.0) or in buffered L broth with the indicated additions. Aerobic and anaerobic cultures were grown as described in the text.
[b] Nanomoles of ONPG hydrolyzed per min per mg of protein.
[c] NG, no growth.

Effects of carbon substrates on *gltA-lacZ* expression. Aerobic expression of *gltA-lacZ* varied 14-fold depending on the type of carbon substrate used for cell growth (Table 3); progressively higher levels were produced with each of the following substrates: glucose, galactose, xylose, glycerol, and the TCA intermediates succinate and fumarate. In minimal acetate medium, the level of *gltA-lacZ* expression was the highest (ca. threefold higher than that in a glucose medium). When a buffered L-broth medium was used in place of the glucose-supplemented minimal medium, the level of *gltA-lacZ* expression was lowered by about 40% (Table 3). Addition of glucose to the L-broth medium resulted in even lower levels of *gltA-lacZ* expression, whereas pyruvate addition had little effect during aerobic growth.

During anaerobic cell culture on different types of media *gltA-lacZ* expression varied by 10-fold (Table 3). For each medium tested, the level of *gltA-lacZ* expression was higher during aerobic growth than it was during anaerobic growth.

However, under anaerobic conditions, the presence of pyruvate in the L-broth medium resulted in the lowest level of *gltA-lacZ* expression observed (ca. 5-fold lower than that in L-broth medium alone and 11-fold lower than that in galactose-containing minimal medium). Over the range of aerobic and anaerobic cell growth conditions tested in these studies, *gltA-lacZ* expression varied by about 40-fold.

Effects of the *arcA*, *fnr*, *himA*, and *fis* gene products on *gltA-lacZ* expression. Since the ArcA and Fnr proteins are known to regulate a number of *E. coli* genes in response to anaerobiosis, we examined the effect of deleting each regulatory gene on *gltA-lacZ* expression (Table 4). In the Δ*arcA* strain, the level of *gltA-lacZ* expression was elevated 2.5-fold compared with that in the wild-type strain during aerobic cell growth, and it was elevated 8-fold under conditions of anaerobic growth. ArcA thus appears to function as a repressor of *gltA* expression during both aerobic and anaerobic growth. In contrast, a Δ*fnr* strain did not show any significant change in *gltA-lacZ* expression under anaerobic conditions compared with the wild-type parent. Under oxygen-rich-growth conditions, expression was reduced by 40%. Mutations in either the *himA* or *fis* gene, both of which encode general cellular DNA binding proteins, had either a modest stimulatory effect or a modest inhibitory effect (ca. 20 to 40%) on *gltA* expression depending on the conditions examined (Table 4).

Effects of the *crp* and *fruR* mutations on *gltA-lacZ* expres-

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

Strain (genotype or description)[a]	β-Galactosidase activity[b]	
	With O_2	Without O_2
MC4100 (wt)[c]	4,680	2,030
PC35 (*arcA*)	11,700	16,100
PC2 (*fnr*)	2,630	1,970
SJP3 (*himA*)	4,810	3,500
SJP4 (*fis*)	3,190	2,140

[a] Cells were grown in a glucose minimal medium under aerobic or anaerobic conditions as described in the text.
[b] Nanomoles of ONPG hydrolyzed per min per mg of protein.
[c] wt, wild type.

sion. Because *gltA-lacZ* expression was observed to vary by as much as 14-fold depending on the richness of the cell culture medium during aerobic growth (Table 3), we tested whether the *crp* gene product, Crp, provided this control. A *crp* deletion was introduced into the *gltA-lacZ* lysogen, and the resulting strain was grown both aerobically and anaerobically in different media (Fig. 2A and B). Compared with that in the wild-type parent strain, *gltA-lacZ* expression did not vary by more than 20 to 40% in the Δ*crp* strain for nearly every condition tested; the sole exception occurred when cells were grown anaerobically with galactose as the substrate (Fig. 2B). To determine whether the effects of carbon type and medium richness on *gltA-lacZ* expression are due to the *fruR* gene product, which participates in the control of glucogenesis reactions, a *fruR* gene deletion strain was also tested as described above for the *crp* deletion. The *fruR* gene disruption did not significantly affect *gltA-lacZ* expression (Fig. 2). *E. coli* possesses some other means to regulate *gltA-lacZ* expression in response to

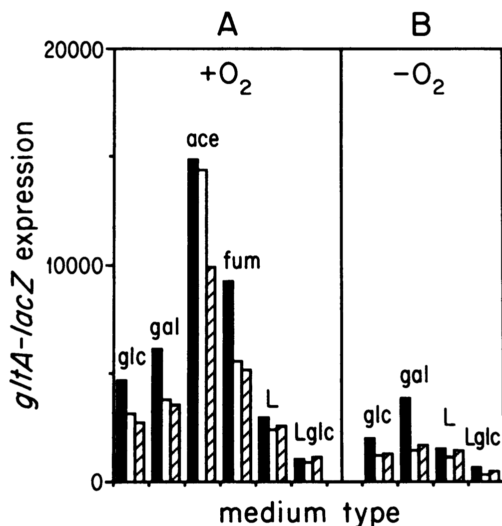

FIG. 2. Effects of Δ*crp* and Δ*fruR* mutations on *gltA-lacZ* expression. (A) Aerobic cell growth. (B) Anaerobic cell growth. The solid bars represent values for the wild-type strain, while the open bars and the hatched bars represent values for the Δ*crp* strain (SJP5) and the Δ*fruR* strain (SJP7), respectively. Cells were grown in a minimal medium supplemented with the indicated carbon compounds (40 mM) or in buffered L-broth medium. glc, glucose; gal, galactose; ace, acetate; fum, fumarate; L, L broth. Data are in nanomoles of ONPG hydrolyzed per min per mg of protein.

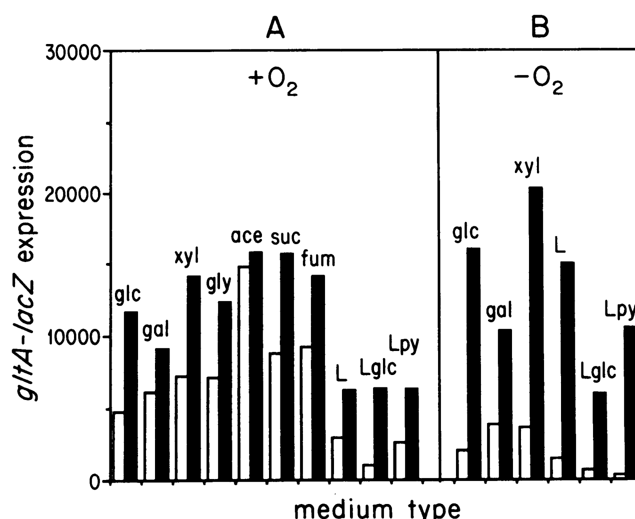

FIG. 3. Effect of a Δ*arcA* mutation on *gltA-lacZ* expression in cells grown on various types of carbon and medium richness. (A) Aerobic cell growth. (B) Anaerobic cell growth. The open bars represent values for the wild-type strain, while the solid bars represent values for the Δ*arcA* strain. Cells were grown in a minimal medium supplemented with the indicated carbon compounds (40 mM) or in buffered L broth. Data are in nanomoles of ONPG hydrolyzed per min per mg of protein. glc, glucose; gal, galactose; xyl, xylose; gly, glycerol; ace, acetate; suc, succinate; fum, fumarate; L, L broth; py, pyruvate.

changes in the carbon type and/or the richness of the medium (e.g., Crp- and FruR-independent catabolite repression).

Effect of *arcA* on *gltA-lacZ* expression in cells grown on alternative substrates. Since the 14-fold range in *gltA-lacZ* expression seen when cells were grown on different carbon compounds was independent of *crp* and *fruR* (Fig. 2), we asked whether the *arcA* gene product may contribute to this control. An *arcA* deletion strain was grown aerobically and anaerobically on each substrate, and β-galactosidase activities were determined (Fig. 3). During aerobic cell culture in most types of media, *gltA-lacZ* expression was increased by about 2-fold in the *arcA* mutant relative to that in the wild-type parent strain (Fig. 3A). The sole exceptions occurred when buffered L-broth glucose medium was used (a sixfold increase in *gltA-lacZ* expression was produced) and when an acetate minimal medium was used (less than 10% difference was observed).

During anaerobic cell culture, the *arcA*-dependent control of *gltA-lacZ* expression was more pronounced than that observed under aerobic conditions, although the derepressed levels were similar (Fig. 3B). The magnitude of the ArcA-dependent repression ranged from 3-fold when galactose minimal medium was used to 30-fold when a buffered L-broth pyruvate medium was used. The highest level of gene expression observed was that produced when the *arcA* strain was grown in a minimal xylose medium (ca. 20,000 U, which is about 60-fold above the level seen for the wild-type strain on buffered L-broth pyruvate medium). It appears that ArcA may contribute to the control observed in response to variations in medium composition (i.e., richness or carbon type). It is not evident whether this effect is direct or indirect.

Effect of iron availability on *gltA-lacZ* expression. To determine if iron-limiting or iron excess growth conditions affect *gltA-lacZ* expression, a wild-type strain and a *fur* mutant defective for repression of iron uptake systems (7) were grown in the presence of the iron chelator 2,2-dipyridyl (Table 5).

TABLE 5. Effect of iron availability on *gltA-lacZ* expression

Addition[a]			β-Galactosidase activity[b] strain:	
O_2	Dipyridyl	Fe^{2+}	MC4100 (wt)	SJP2 (*fur*)
+	−	−	4,680	4,450
+	+	−	4,440	4,660
+	+	+	3,130	4,550
−	−	−	2,030	2,380
−	+	−	3,780	2,760
−	+	+	1,360	1,910

[a] Cells containing λSJP30 were grown in a minimal glucose medium aerobically or anaerobically as described in the text. Dipyridyl and ferrous sulfate were added at initial concentrations of 150 and 80 μM, respectively, as indicated. +, added to medium; −, not added.

[b] Nanomoles of ONPG hydrolyzed per min per mg of protein.

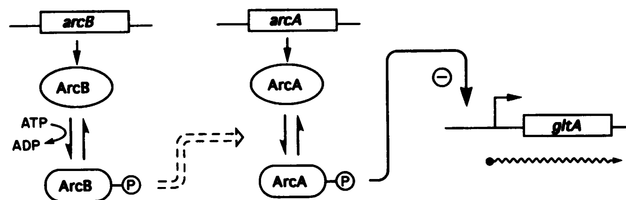

FIG. 5. Scheme for ArcA control of *gltA* gene expression in response to anaerobic cell growth. The activation of ArcA by ArcB is shown by the dotted arrow. A circled P represents covalently modified ArcA or ArcB protein (15). The wavy line represents *gltA* mRNA, while the circled minus symbols indicate negative control of *gltA* gene expression.

Expression of *gltA-lacZ* was not greatly influenced (ca. 1.5-fold) by the iron chelator under either aerobic or anaerobic conditions in either strain. Addition of excess iron resulted in a modest (ca. 30%) reduction in β-galactosidase activity in the wild-type strain following either aerobic or anaerobic growth.

Effect of growth rate on *gltA-lacZ* expression. When cells were grown in different types of media, we noted that the levels of *gltA-lacZ* expression were higher in carbon-poor media than in rich media (Table 2). Cell growth rates on each type of medium were determined, and when the generation times were graphed versus the level of *gltA-lacZ* expression, an inversely proportional relationship was seen (Fig. 4). Thus, the earlier observation that citrate synthase enzyme levels vary inversely with the cell growth rate (10) can be accounted for, in part, by the transcriptional control of *gltA* expression. Whether ArcA participates in this process directly or indirectly is not yet known.

DISCUSSION

In this study we document the effects of different cell growth conditions on transcription of the *gltA* gene that encodes citrate synthase, the first enzyme in the TCA cycle. Expression of the *gltA* gene is complex: one major part of the control of citrate synthase synthesis is shown to occur at the level of gene expression in response to oxygen, and another is shown to

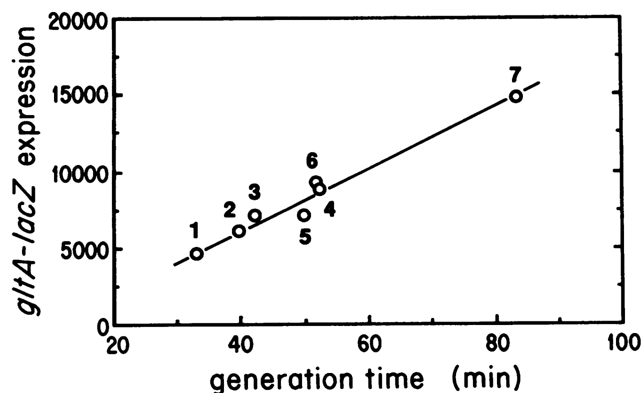

FIG. 4. Effect of cell growth rate on *gltA-lacZ* expression. Cells were grown in various media, and the cell generation time was recorded. The carbon compound used for cell growth was glucose (1), galactose (2), xylose (3), succinate (4), glycerol (5), fumarate (6), or acetate (7).

occur in response to the type of carbon and/or the degree of medium richness used during cell culture.

Aerobic and anaerobic control. The 2-fold aerobic and anaerobic control observed for *gltA* expression under conditions of steady-state cell growth on glucose is considerably less than the 10-fold difference seen for succinate dehydrogenase operon (*sdhCDAB*) expression under identical conditions (25). This pattern is consistent with the notion that the *gltA* gene product must function during both aerobic and anaerobic cell growth, whereas succinate dehydrogenase participates primarily during aerobic cell growth. The fumarate reductase encoded by the *frdABCD* genes catalyzes the reverse reaction for succinate generation and is the predominant enzyme during anaerobic growth (12). The aerobic and anaerobic control of *gltA-lacZ* expression is due primarily to the *arcA* gene product (Table 4; Fig. 5), whereas *sdhCDAB* gene expression is controlled by both ArcA and Fnr (25). The two- to sixfold derepression of *gltA-lacZ* expression seen in the *arcA* strain during aerobic cell growth in various media suggests that ArcA must exist in a partially active state under these conditions. Whereas the magnitude of the ArcA-dependent repression is greatest when a rich medium or a glucose medium is used, the control is reduced when other carbon compounds are used as the substrate. Almost no repression of *gltA* expression is seen when acetate is used for cell growth. Thus, except in the case of growth on acetate, either ArcA must exist in a partly activated state (e.g., a phosphorylated form) which can bind to the DNA, or, alternatively, the nonphosphorylated form of ArcA must be able to effectively bind DNA and regulate gene expression. The latter possibility is less likely, as one would expect to see aerobic repression of *gltA-lacZ* expression regardless of the type of culture medium used. ArcA repression under anaerobic conditions is always greater than that under aerobic conditions. On the basis of the in vitro studies of Iuchi, it is interesting to speculate that ArcA responds to the intracellular accumulation of reduced compounds formed during cell metabolism or of fermentation end products via the ArcB protein (13).

The extent of ArcA control of *gltA* and *sdhCDAB* gene expression under aerobic conditions is in marked contrast to the lack of Fnr control under the same conditions. This alternative anaerobic regulator protein exhibits its control only under conditions of anaerobic cell growth (5, 12, 27). Together, these observations suggest that the cell signal(s) detected by Fnr and the signal(s) detected by ArcA and ArcB must differ or that they act in very different ways. Whereas the oxidation-reduction state of the cell has been proposed to be the signal detected by Fnr on the basis of the studies by Unden and coworkers (32), ArcA appears instead to respond to the

medium richness and type of carbon compound used for cell growth (this study). It is interesting to speculate that this dual control by Fnr and ArcA aids in the coordination of carbon flow with energy generation via the aerobic and anaerobic respiratory pathways in the cell (12).

Control by carbon source. The composition of the culture medium with respect to carbon type and medium richness was shown to be a major variable in the control of *gltA* gene expression (Table 3). This carbon effect is dramatic: a 14-fold range in *gltA-lacZ* expression under aerobic conditions was seen, while a 10-fold effect under anaerobic conditions was seen. For all conditions tested, a 40-fold variation in *gltA-lacZ* expression was seen. *E. coli* is thus able to dynamically regulate the level of *gltA* transcription to vary the production of citrate synthase in the cell for generating biosynthetic intermediates and energy via the TCA cycle and the noncyclic or branched pathway. Since we observed a decrease in the level of *gltA-lacZ* expression during cell growth on glucose compared with levels produced during growth on other six-carbon compounds as well as on five-, four-, three-, and two-carbon compounds (a process commonly referred to as catabolite repression), we evaluated whether the Crp protein participates in this control. The introduction of a *crp* gene deletion into the λSJP30 lysogen did not significantly alter *gltA-lacZ* expression (Fig. 2). In contrast, when the same *crp* deletion was introduced into a $lacZ^+$ strain, it resulted in a 100-fold decrease in $lacZ^+$ gene expression from the *lac* promoter (data not shown). Crp clearly does not effect the 14-fold difference in aerobic *gltA-lacZ* expression due to variations in the medium composition. The previous notion that *gltA* expression is regulated by Crp, based on the presence of putative Crp binding sites in the *gltA* promoter region, must now be revised (37). Expression of *gltA-lacZ* is also independent of the *E. coli fruR* gene product. In the case of *Salmonella typhimurium*, FruR has been shown to be involved in glucose control of the fructose utilization system, which is Crp independent (4, 33). There must therefore be some other means to regulate citrate synthase gene expression in response to medium richness and/or carbon type. It is noteworthy that Crp- and cAMP-independent catabolite repression of gene expression has been demonstrated to occur in other microorganisms, including *Bacillus subtilis*, *Streptomycetes* spp., and *Staphylococcus aureus* (3, 34). These organisms employ a negative regulatory control element to provide catabolite repression, in contrast to the positive regulatory mechanism employed by Crp, which mediates the expression of many sugar utilization operons in *E. coli*. It is interesting to speculate that ArcA may be somehow involved in this carbon control, as an *arcA* mutant exhibits considerably elevated levels of *gltA* expression in most types of media except for those supplemented with L broth (Fig. 3).

Growth rate control. When *gltA-lacZ* expression in *E. coli* cells grown at slow cell doubling times was examined and compared with expression in cells grown at more rapid doubling times, an inverse relationship was observed (Fig. 4). This pattern is opposite that observed for ribosomal protein synthesis (17). Because the *gltA-lacZ* gene expression studies employed a $lacZ^+$ operon fusion, the control of citrate synthase levels in the cell in response to change in the cell growth rate must occur in large part at the level of transcription. It will be of interest to determine how altering the cell growth rate by limiting the carbon supply under continuous cell culture conditions affects *gltA* expression and to contrast this effect with that produced by varying the type of culture medium used for batch culture (this study) (Fig. 4). As little is known about the mechanism responsible for growth rate control, the study of the *gltA* gene may provide a useful model to better understand this phenomenon at the molecular level.

Effects of anaerobic electron acceptors. The presence of another electron acceptor besides oxygen (i.e., nitrate, TMAO, or fumarate) does not elevate *gltA* expression under anaerobic cell culture conditions to a degree different from that seen when such acceptors are absent (e.g., under glucose fermentation conditions; Table 2). However, when cells are grown on glycerol minimal medium under anaerobic respiratory conditions with nitrate, TMAO, or fumarate present, glycerol must be metabolized by respiration as no phosphorylation occurs at the substrate level; *gltA* expression is increased by about twofold and approaches the level observed during aerobic cell growth with glucose (Table 2). These observations support the proposal that the elevated biosynthetic needs of the cell under respiratory conditions lead to an increased level of *gltA-lacZ* expression regardless of the electron acceptor present.

Aerobic and anaerobic functioning of citrate synthase. Citrate synthase participates in both the cyclic (TCA) and noncyclic (branched) pathways for biosynthesis of cellular intermediates and energy generation (e.g., generation of reduced purine nucleotides). It is difficult to separate the complementary roles of the enzyme in each pathway. The branched or noncyclic pathway has been described as an anaerobic pathway for anaplerotic reactions, whereas the TCA cycle is considered to be an aerobic pathway in *E. coli* (23). We can attempt to assess the roles of citrate synthase in these alternative pathways by comparing levels of *gltA-lacZ* expression in cells grown in media of various compositions. During anaerobic growth on a rich L-broth glucose medium, i.e., under conditions of minimal biosynthesis of cellular intermediates, gene expression was about one-third of that which occurred when only glucose was available (i.e., under conditions of increased biosynthesis but limited energy generation; Table 3). The increased level of *gltA* gene expression is thus attributed primarily to cell biosynthetic needs. Similarly, if oxygen is provided during growth on glucose, which produces conditions for elevated biosynthesis and high-level energy generation due the presence of an active aerobic respiratory pathway, *gltA-lacZ* expression is elevated by fourfold compared with the level produced when the medium also contains L-broth constituents, i.e., under conditions in which synthesis of biosynthetic intermediates is presumably reduced. Again, this difference in gene expression appears to be attributable to the demand due to cell biosynthetic needs.

One can easily distinguish between the need for citrate synthase under anaerobic respiration conditions and the need for this enzyme under fermentation conditions (ca. twofold) on the basis of the *gltA-lacZ* expression studies reported here. It will be of considerable interest to determine how the other enzymes of the TCA and noncyclic pathways are controlled under these same conditions.

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