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Involvement of Fnr and ArcA in Anaerobic Expression of the *tdc* Operon of *Escherichia coli*

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Anaerobic expression of the *tdcABC* operon in *Escherichia coli*, as measured by LacZ activity from single-copy *tdc-lacZ* transcriptional and translational fusions, is greatly reduced in strains lacking two global transcriptional regulators, Fnr and ArcA. The nucleotide sequence of the *tdc* promoter around −145 shows significant similarity with the consensus Fnr-binding site; however, extensive base substitutions within this region had no effect on Fnr regulation of the *tdc* genes. A genetic analysis revealed that the effect of Fnr on *tdc* is not mediated via ArcA. Furthermore, addition of cyclic AMP to the anaerobic incubation medium completely restored *tdc* expression in *fnr* and *arcA* mutants as well as in strains harboring mutations in the Fnr- and ArcA-dependent *pfl* gene and the Fnr-regulated *glpA* and *frd* genes. These results, taken together with the earlier finding that *tdc* expression is subject to catabolite repression by intermediary metabolites, strongly suggest that the negative regulatory effects of mutations in the *fnr* and *arcA* genes are mediated physiologically due to accumulation of a metabolite(s) which prevents *tdc* transcription in vivo.

When incubated anaerobically, *Escherichia coli*, a typical facultative anaerobe, represses a wide array of aerobic genes and concomitantly expresses certain others which encode key enzymes and proteins needed for anaerobic metabolism. Two global transcriptional regulators, Fnr (fumarate and nitrate reductase activator) and ArcA (a member of the two-component ArcBA sensor-regulator system), have generally been implicated in activating a large number of anaerobic genes and repressing many aerobic genes in oxygen-limiting growth conditions, respectively (reviewed in references 9, 15, 36, and 40). These regulatory proteins appear to have their individual specificities in regulating groups of genes and/or regulons. However, several operons in *E. coli* are under complex dual control of Fnr and ArcA. Both proteins serve as transcriptional activators of the *pfl* operon (30), whereas Fnr represses the *cydAB* operon while ArcA activates its transcription (9). A recent study on the expression of several aerobic and anaerobic respiratory pathway genes as a function of oxygen concentration revealed that Fnr functions optimally at low oxygen saturation, whereas ArcA controls gene expression at higher levels of oxygen (39). It is interesting that the anaerobic activation of *arcA* transcription is increased three- to fourfold in the presence of Fnr (2). These data clearly suggest that these two regulatory proteins are involved in overall coordination of expression of various anaerobic and aerobic genes in response to the varying oxygen status of the cell.

In molecular terms, Fnr, a sequence-specific DNA binding protein, recognizes a consensus palindromic nucleotide sequence, TTGATNNANATCAA (where N is any base), in the target promoters to activate transcription (36). The active form of Fnr is believed to be a homodimer with an iron-sulfur center which is redox sensitive (8, 16, 19, 27), although a recent model (24) proposed that Fnr exists as monomers, and two monomers occupy the two half-sites of the Fnr recognition sequence in DNA.

Regulation of gene expression by the ArcBA system involves a transphosphorylation event (12, 14). ArcB, the sensor component, undergoes autophosphorylation in response to some metabolic signal and then transfers the phosphate moiety to ArcA. The active phosphorylated form of ArcA (ArcA-P) serves as a regulator to control transcription. Recently, it has been reported that ArcA-P binds specifically to the target promoters of several oxygen-regulated genes, *pfl*, *sodA*, *gltA*, and *lctPRD*, in vitro (4, 22, 38). Although a primary nucleotide sequence, (A/T)GTTAATTA(A/T), required for ArcA-P binding has been derived from computer alignment of various promoters regulated by ArcA, further studies are needed to establish the consensus ArcA-P box (22). How the ArcBA system senses the availability of oxygen is not yet fully understood.

The anaerobically induced *tdc* operon of *E. coli*, which consists of three genes encoding a transcriptional regulator (TdcA), the biodegradative threonine dehydratase (TdcB), and a membrane-associated L-threonine-L-serine permease (TdcC) (Fig. 1A), is implicated in transport and metabolism of the amino acids during anaerobic growth to provide a source of metabolic energy (3, 6, 7, 37). Upstream of *tdcABC* and transcribed in opposite orientation is *tdcR*, which specifies a small protein that is essential for *tdc* transcription (10, 33). In addition to an anaerobic (microaerobic) environment and the two operon-specific transcription factors TdcA and TdcR, the efficient expression of the operon requires two global regulatory proteins, integration host factor (IHF) and the cyclic AMP (cAMP)-catabolite gene activator protein (CAP) complex (42, 44). Genetic and biochemical experiments revealed that TdcA, IHF, and CAP occupy their unique binding sites on *tdcP* at −175, −104, and −41, respectively, with respect to the *tdcP* transcription start site at +1 (Fig. 1B), and act in concert most likely by bending and looping the promoter DNA to form an active transcription complex (10, 42). We continue to examine the *tdc* operon as a well-defined model system to investigate the basic mechanisms underlying anaerobic gene expression. In this report, we present evidence that transcription of the *tdc* operon in vivo is greatly reduced in strains lacking the *fnr* and

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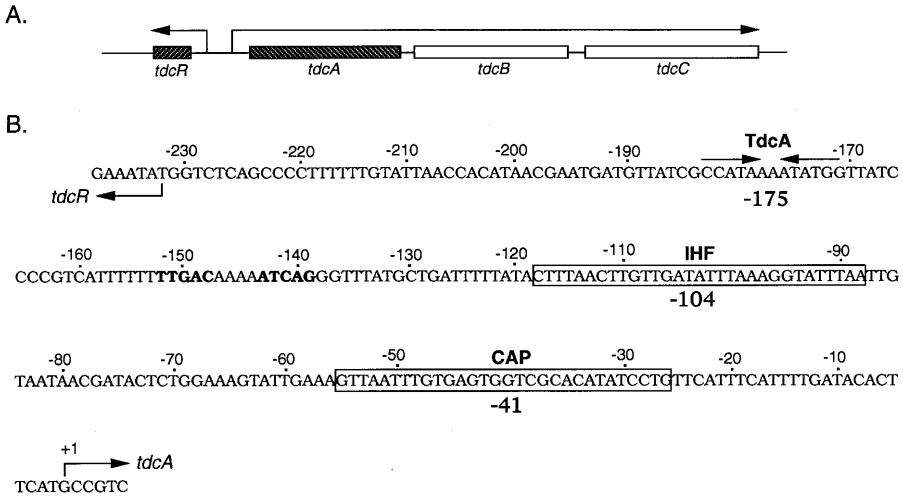

FIG. 1. Organization of the *tdc* operon (A) and nucleotide sequence of the region between *tdcR* and *tdcA* (B). The numbers with minus signs above the sequence represent upstream distance in nucleotides from the transcription start site of *tdcA*, designated +1. The boxed regions designated IHF (−104) and CAP (−41) represent sequences protected by IHF and CAP, respectively, during footprinting. The region TdcA (−175) with inverted repeat signifies the TdcA-binding site. The boldface letters between −152 and −139 in panel B show sequence similarity with the consensus Fnr-binding site.

arcA gene products. Furthermore, both Fnr and ArcA most likely affect *tdc* expression via indirect means by influencing carbon metabolism during anaerobic growth.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The bacterial strains and plasmids used in this study are listed in Table 1. Recombinant *tdc-lacZ* transcriptional and translational fusions of λ phages were constructed by recombining λRZ5 with the corresponding plasmids, and single-copy lysogens in appropriate hosts were obtained as described previously (31). Generalized transduction, using phage P1, was performed by the method of Miller (25). Strains were routinely grown in Luria-Bertani (LB) medium (25). TYE medium used for enzyme induction contained 2% tryptone and 1% yeast extract supplemented with salts and pyridoxine hydrochloride (11). When appropriate, antibiotics were added at the following concentrations: ampicillin, 100 μg ml^{-1}; kanamycin, 50 μg ml^{-1}; and chloramphenicol, 30 μg ml^{-1}.

Plasmid constructions. To isolate promoter mutations containing various base substitutions between −156 and −138, an 1,180-bp *Eco*RI-*Hin*dIII fragment (nucleotides 1197 to 2377) (32) from plasmid pSH241 was subcloned into M13 mp18 and M13 mp19. For specific mutations, synthetic oligodeoxynucleotide primers with appropriate base substitutions (Fig. 2) were used according to the procedure of Kunkel et al. (18) as described elsewhere (42). Putative mutations were screened for the presence of new restriction sites introduced by the mutagenic primers, and their DNA sequences were verified by dideoxy sequence analysis using a Sequenase kit (United States Biochemical). The mutated *Eco*RI-*Hin*dIII fragments were then reintroduced into pSH241.

Plasmid pSC1925 contains the promoterless coding region of $arcA^+$ cloned into an expression vector, pCL1921 (20). Two primers, 5′ AGGTAGCAACCA TGGAGACCCCGC 3′ (forward) and 5′ AAGGATCCACAACGGACGATG

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Genotype or derivation	Reference or source
Strains		
RK4353	*araD139* Δ(*argF-lac*)*U169 rpsL150 deoC1 relA1 ptsF25 flhD5301 gyrA219 non-9*	21
VJS1741	RK4353 *fnr-271*::Tn*10*	21
CAG18552	MG1655 *zej-3144*::Tn*10 kan*	35
QC2003	F^- Δ*lacU169 rpsL* (*sdh-lac*) Δ*fnr zdc-235*::Tn*9*	2
QC2085	F^- Δ*lacU169 rps* Δ*arc*::Tn*10*	2
TL45	MC4100 *glpR2* Δ(*glpT-glpA*)*593 gyrA*	5
MC4100	F^- *araD139* Δ(*argF-lac*)*U169 rpsL150 deoC1 relA1 rbsR ptsF25 flb5301*	34
RM201	MC4100 Δ*pfl-25* Ω (*pfl*::*cat* pACYC184)	29
DW12	MC4100 *zjd*::Tn*10* Δ(*frdABCD*)*102*	41
SCM4105	MC4100 Δ *fnr zdc-235*::Tn*9* [P1 (QC2003) × MC4100]	This study
SCM4200	MC4100 Δ*arc*::Tn*10* [P1 (QC2085) × MC4100]	This study
SCM4220	MC4100 Δ*fnr zdc-235*::Tn*9* Δ*arc*::Tn*10* [P1 (QC2003) × SCM4200]	This study
SCM4250	MC4100 (Δ*glpA*::Ω Kan) [P1(CAG18552) × TL45; P1 (TL45Kan) × MC4100]	This study
Plasmids		
pSH241	$tdcR^+$ $tdcP_{wt}$ $tdcA^+$ *B-lacZ*	33
pYW245	$tdcR^+$ $tdcP_{wt}$-*lacZ*	43
pYW77	Base substitutions in *tdcP* (Fig. 2); otherwise identical to pSH241	This study
pYW83	Base substitutions in *tdcP* (Fig. 2); otherwise identical to pSH241	This study
pYW84	Base substitutions in *tdcP* (Fig. 2); otherwise identical to pSH241	This study
pYW85	Base substitutions in *tdcP* (Fig. 2); otherwise identical to pSH241	This study
pYW86	Base substitutions in *tdcP* (Fig. 2); otherwise identical to pSH241	This study
pCL1921	Spc^r Str^r *lacPO-lacZ*α	20
pSC1925	Promoterless $arcA^+$ cloned into the *Sma*I site of pCL1921	This study

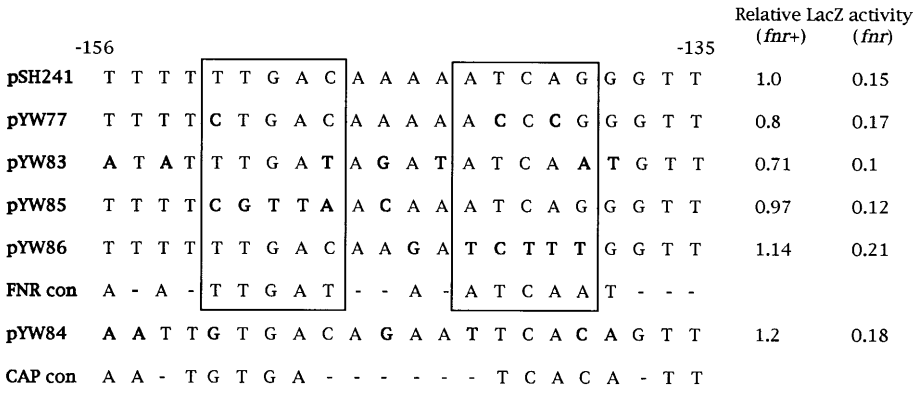

FIG. 2. Nucleotide substitutions between −156 and −135 encompassing the putative Fnr-like sequence in *tdcP* and relative *lacZ* expression (with respect to the wild type) from λ*tdcAB-lacZ* fusions harboring the promoter mutations in *fnr*$^+$ and *fnr* strains. Boldface letters show base substitutions made in the wild-type *tdcP* in pSH241. The boxed regions represent Fnr left half-site and Fnr right half-site. Also shown are the consensus Fnr and CAP recognition sequences.

AGT 3′ (reverse), were used to amplify the *arcA*$^+$ coding region by PCR, using the protocol supplied by Perkin-Elmer Cetus. The PCR product was purified by agarose gel electrophoresis, cut with *Sma*I, and cloned into *Sma*I-cleaved pCL1921. The plasmid was sequenced to verify the construct.

Enzyme induction and assay. Cells were grown aerobically at 37°C for approximately 16 h in LB medium, washed, and resuspended in fresh TYE. Small aliquots of cells were incubated anaerobically at 32° in TYE without shaking for 6 to 8 h. The threonine dehydratase activity of toluene-treated cells was determined as described previously (11). Specific activity was expressed as nanomoles of α-ketobutyrate formed per minute per milligram of protein. Malate dehydrogenase activity in cell extracts was measured spectrophotometrically as described by Kitto (17), and specific activity was expressed as nanomoles of malate oxidized per minute per milligram of protein. β-Galactosidase activity in sodium dodecyl sulfate-chloroform-permeabilized cells was measured as described by Miller, and specific activity was expressed in Miller units (25).

Other methods. All DNA manipulations were carried out as suggested by Sambrook et al. (28). Restriction enzyme digestions of DNA were performed according to the manufacturers' specifications. The Genetics Computer Group software package (University of Wisconsin, Madison) was used for DNA sequence analysis.

RESULTS AND DISCUSSION

Fnr and ArcA requirements for *tdc* expression. To examine the effects of *fnr* and *arcA* gene products on the anaerobic expression of the *tdc* operon, we constructed individual single-copy λ*tdc-lacZ* transcriptional (λYW245) and translational (λSH241) fusions in VJS1741 (*fnr*) and SCM4200 (*arcA*) and in their corresponding isogenic parents. Assay of β-galactosidase activities in these strains incubated anaerobically in TYE medium revealed a 4- to 20-fold reduction in LacZ levels from the *fnr* and *arcA* mutants relative to the *fnr*$^+$ and *arcA*$^+$ strains (Table 2). That the mutations in *fnr* and *arcA* were responsible for reduced LacZ activities were confirmed by complete restoration of *tdc* expression by complementing the mutant strains with intact *fnr* (data not shown) and by *arcA* gene (Table 3, experiment II). Furthermore, two independent isolates of *fnr* strains (QC2003 and SCM4105) and a different *arcA* mutant (QC2085) also showed 5- to 10-fold decrease in *tdc* expression compared with their isogenic parents. These results indicate that Fnr and ArcA proteins are essential for *tdc* transcription in vivo.

Because *tdcR* gene product is required for high-level expression of *tdcABC* (10, 33), it is possible to argue that lack of Fnr or ArcA could somehow lower the level of TdcR in the cell, thus reducing *tdc* expression. However, the following observations ruled out this possibility: (i) no significant difference in LacZ expression from the *tdcR-lacZ* fusion was found in *fnr*$^+$ and *fnr* strains, and (ii) multicopy *tdcA*$^+$ was unable to restore *tdc* expression in *fnr* and *arcA* mutants. We have previously shown (10) that excess TdcA can bypass the requirement for TdcR in *tdcR*-deficient cells; if mutation in *fnr* or *arcA* reduced the TdcR level, we would have found high *tdc* expression in VJS1741 (*fnr*) and SCM4200 (*arcA*) transformed with a high-copy-number plasmid containing *tdcA*$^+$.

Independent action of Fnr and ArcA. Recently, Compan and Touati (2) reported that anaerobic activation of *arcA* transcription in *E. coli* requires Fnr. This observation raised the important question of whether Fnr affects *tdc* expression directly by influencing the *tdc* promoter (*tdcP*), similar to that seen with many Fnr-regulated genes, or, alternatively, acts indirectly via transcriptional activation of *arcA*. To distinguish between these two possibilities, we cloned a promoterless *arcA*$^+$ coding region into expression vector pCL1921, yielding pSC1925 (see Materials and Methods), to drive *arcA* transcription from an Fnr-independent promoter. When this plasmid was introduced into an *fnr* mutant containing a λ*tdcAB-lacZ* fusion, no stimulation of LacZ activity was detected from the lysogen (Table 3, experiment I). If the only role of Fnr in *tdc* expression were to activate *arcA* transcription, we would have seen LacZ activity in the *fnr* mutant harboring pSC1925. Thus, lack of *tdc* expression in *fnr arcA*$^+$ cells implies an independent function of Fnr. Control experiments included in Table 3 show that ArcA protein was indeed expressed from the multicopy pSC1925 plasmid in the presence (experiment II) and absence (experiment III) of Fnr: ArcA from pSC1925 restored *tdc* expression in the *fnr*$^+$ *arcA* strain and reduced malate dehydrogenase activity (encoded by *mdh*) in the *fnr arcA* double mutant. Iuchi and Lin (13) have shown that Mdh expression is elevated in an *arcA* mutant during anaerobic incubation of *E. coli*. We conclude from these experiments that the effect of Fnr on *tdc* is independent of ArcA.

TABLE 2. Fnr and ArcA requirements for anaerobic *tdc* expression

Strain	Relevant genotype	β-Galactosidase activity from lysogens[a]	
		λSH241[b]	λYW245[c]
RK4353	Wild type	560	769
VJS1741	*fnr*	65 (0.12)	205 (0.26)
MC4100	Wild type	920	925
SCM4200	*arcA*	47 (0.05)	200 (0.22)

[a] Expressed in Miller units. Numbers in parentheses are relative to the wild-type value of 1.0.
[b] *tdcAB-lacZ* protein fusion.
[c] *tdcP-lacZ* operon fusion.

TABLE 3. Independent effects of Fnr and ArcA on *tdc* expression

Expt	Strain (plasmid)	Relevant genotype	Enzyme activity	
			LacZ (Miller units)	Mdh (nmol of malate oxidized/ min/mg of protein)
I	MC4100λSH241	*fnr*$^+$ *arcA*$^+$	690	220
	SCM4105λSH241	*fnr arcA*$^+$	152	209
	SCM4105λSH241 (pCL1921)	*fnr arcA*$^+$ (vector)	150	ND[a]
	SCM4105λSH241 (pSC1925)	*fnr arcA*$^+$ (*arcA*$^+$)	130	ND
II	SCM4200λSH241 (pCL1921)	*fnr*$^+$ *arcA* (vector)	100	ND
	SCM4200λSH241 (pSC1925)	*fnr*$^+$ *arcA* (*arcA*$^+$)	810	ND
III	SCM4220λSH241	*fnr arcA*	ND	920
	SCM4220λSH241 (pCL1921)	*fnr arcA* (vector)	ND	973
	SCM4220λSH241 (pSC1925)	*fnr arcA* (*arcA*$^+$)	ND	230

[a] ND, not done.

The Fnr-like site in *tdcP* is not involved in *tdc* expression. The evidence for Fnr regulation of gene expression is usually deduced from a combination of several types of experiments: a comparison of gene activity in *fnr*$^+$ and *fnr* strains, identification of a sequence in the target promoter having significant similarity with the consensus Fnr recognition site, and mutational substitutions of bases within the Fnr-like sequence in the promoter to assess the effect of Fnr protein. In a few cases, additional evidence has been obtained from footprinting experiments using wild-type and mutant Fnr proteins, which have been difficult to purify until recently because of their extreme oxygen lability (24).

The nucleotide sequence in *tdcP* around −145 shows significant similarity with the consensus Fnr-binding site with a two-base mismatch (shown in boldface letters in Fig. 1B). Because several Fnr-regulated promoters show some flexibilities in having different nucleotides in these mismatched positions (36), we inferred that Fnr might bind to *tdcP* DNA at this site to activate transcription and that mutations introduced within this 14-bp sequence would abolish *tdc* expression in an *fnr*$^+$ host. Accordingly, we made several nucleotide substitutions between −156 and −138 (shown in boldface letters in Fig. 2) by site-directed mutagenesis according to the Kunkel procedure (18) as detailed elsewhere (42). pYW77 had substitutions in three highly conserved bases within the Fnr consensus which are crucial for Fnr action, whereas pYW83 contained substitutions that created a perfectly matched Fnr consensus sequence. pYW85 and pYW86 each had five base substitutions to yield, respectively, Fnr left half-site and Fnr right half-site mutants. Some additional base substitutions were made to introduce new restriction sites for mutant screening.

Single-copy lysogens of the mutant plasmids harboring *tdcAB-lacZ* fusion were constructed in strains RK4353 (*fnr*$^+$) and VJS1741 (*fnr*). Assay of β-galactosidase activities of these Fnr site mutants in *fnr*$^+$ cells incubated anaerobically in TYE showed no significant difference in LacZ levels from that seen with the λSH241 lysogen harboring the wild-type *tdcP* sequence or that of the λYW83 lysogen containing a perfectly matched Fnr consensus site (Fig. 2). Furthermore, the mutant lysogens in *fnr* cells had drastically reduced LacZ levels, similar to that found in λSH241, indicating that a functional Fnr protein is still necessary for *tdc* expression in the Fnr site mutants.

To rule out the unlikely possibility that the Fnr half-site mutants bind Fnr, we replaced the 22-bp Fnr-like sequence with a perfectly matched CAP site (1, 36) to yield pYW84. A single-copy lysogen of λYW84 showed high LacZ levels in *fnr*$^+$ strains (Fig. 2), similar to the Fnr site mutants. Thus, complete replacement of the Fnr-like site with a CAP site did not abolish the Fnr requirement for *tdc* expression. These results clearly show that the Fnr-like sequence around −145 in *tdcP* is not involved in the Fnr-dependent expression of the *tdc* operon. This finding was not totally surprising because the Fnr-binding sites are usually found closer (between −35 and −45) to the transcription start sites of mRNA (36). A computer search of the region between the *tdcR* and *tdcA* reading frames (Fig. 1) failed to find additional sequences with similarity to the 14-bp consensus Fnr-binding site.

Fnr and ArcA affect *tdc* expression by influencing anaerobic carbon metabolism. The minimal requirement for *tdc* expression is incubation of *E. coli* cells in a mixture of four amino acids, threonine, serine, valine, and isoleucine, supplemented with fumarate and cAMP (H4 medium) in an anaerobic environment (11). Because each component in this defined mixture is absolutely essential, it was proposed that not the amino acids themselves but some metabolite(s) derived anaerobically in reactions involving an electron acceptor may function as a putative inducer for *tdc* expression. In view of this finding, it is possible to envisage potential involvement of Fnr and/or ArcA in generating an anaerobic inducer for *tdcP*, because these regulatory proteins are known to regulate a number of *E. coli* genes whose products are needed for substrate fermentation or for anaerobic respiration. For example, Fnr is required for the expression of *glpA*, *pfl*, and *frd*, encoding, respectively, the anaerobic glycerol-3-phosphate dehydrogenase, pyruvate formate-lyase, and fumarate reductase, and ArcA enhances *pfl* operon expression (4, 15, 36); in *arcA* and *fnr* strains, lack of enzymes encoded by these genes would block carbon metabolism and thus alter metabolite levels in the cell (Fig. 3).

To test whether failure to express *tdc* in *fnr* and *arcA* mutants might be attributable to the absence of one or more of these enzymes, we constructed individual single-copy lysogens of λ*tdcAB-lacZ* in *glpA*, *pfl*, and *frd* mutants (all *fnr*$^+$ and *arcA*$^+$) and their isogenic parent MC4100. Assay of β-galactosidase activities in these strains incubated anaerobically in TYE revealed greatly reduced LacZ levels in all three mutants relative to the wild-type strain (Table 4). These results indicate that interruption of carbon flow through the anaerobic pathway reactions leads to reduced *tdc* expression; they also suggest that mutations in the *fnr* and *arcA* genes appear to mimic the phenotypes of some mutants of carbon metabolism.

How does a block in anaerobic carbon metabolism decrease *tdc* transcription in vivo? We reported earlier that chromosomal TdcB expression was subject to catabolite repression by several intermediary metabolites such as glucose, gluconate,

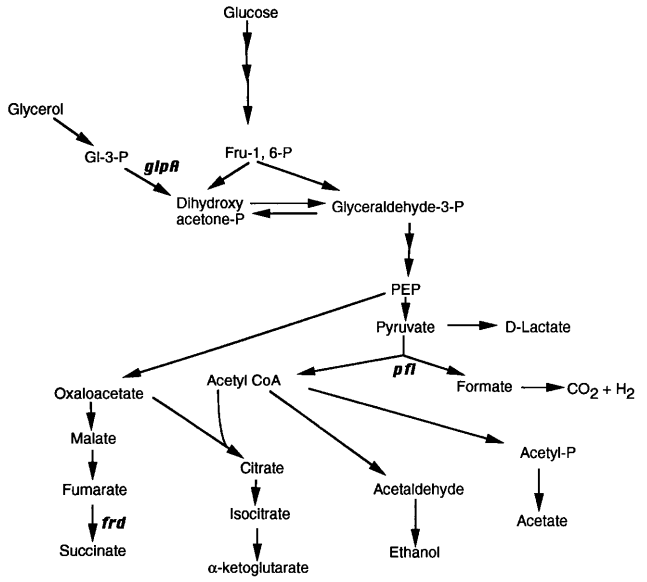

FIG. 3. Carbon flow during anaerobic growth of *E. coli*, using fermentative reactions in the absence of alternative electron acceptors. *glpA*, *pfl*, and *frd* represent the genes encoding the anaerobic glycerol-3-phosphate dehydrogenase, pyruvate-formate lyase, and fumarate reductase, respectively. Gl-3-P, glycerol-3-phosphate; Fru-1,6-P, fructose-1,6-phosphate; PEP, phosphoenolpyruvate; CoA, coenzyme A.

glycerol, and pyruvate, and significant enzyme synthesis was restored when the cultures were supplemented with cAMP (11). These experiments have now been confirmed by using a *tdcAB-lacZ* lysogen in MC4100 (wild type) incubated anaerobically in TYE: at 50 mM, glucose, pyruvate, oxaloacetate, and succinate, individually, reduced LacZ activity three- to fourfold in the absence of cAMP; addition of 4 mM cAMP completely restored LacZ expression. In view of these data, it seemed reasonable to speculate that strains harboring mutations in the *glpA*, *pfl*, and *frd* genes might accumulate some intermediary metabolites which reduce *tdc* expression by the catabolite repression mechanism and that addition of cAMP would then restore *tdc* transcription to a level similar to that seen with the wild-type strain. The data presented in Table 4 indeed show that addition of 4 mM cAMP to TYE during anaerobic incubation of the mutants almost completely restored *tdc* transcription. Furthermore, cAMP also restored the LacZ level from the λ*tdcAB-lacZ* lysogen in *fnr* and *arcA* strains. Thus, the negative effects of these mutations on *tdc* operon expression in vivo are mediated physiologically by altering the levels of some cellular metabolites which repress *tdc* transcription. It is possible to argue in this context that abnormally elevated levels of cAMP-CAP may have an epistatic effect on the activation of *tdc* transcription. However, we found no significant effect of overexpressed wild-type CAP protein (with or without added cAMP) or a CAP* mutant protein (which acts independent of cAMP) in rescuing *tdcR* and *tdcA* mutants or enhancing *tdc* expression in the wild-type strain (data not shown).

TABLE 4. Decreased *tdc* expression in various mutants and its restoration by cAMP

Strain	Relevant genotype	β-Galactosidase activity[a]	
		No cAMP	4 mM cAMP
MC4100λSH241	Wild type	1,100	1,020
SCM4250λSH241	*glpA*	57 (0.05)	1,391 (1.4)
RM201λSH241	*pfl*	289 (0.26)	846 (0.83)
DW12λSH241	*frd*	247 (0.22)	1,032 (1.0)
SCM4105λSH241	*fnr*	52 (0.05)	1,200 (1.20)
SCM4200λSH241	*arcA*	25 (0.03)	666 (0.65)

[a] Expressed in Miller units. Numbers in parentheses are relative to the wild-type value of 1.0.

Biochemical and genetic experiments performed to date clearly established that anaerobic metabolism is necessary for *tdc* operon expression in vivo; no *tdc* gene products are seen during aerobic incubation of cells in various media. In wild-type cells, a metabolite(s) derived during anaerobic reactions has a crucial role in activating *tdc* promoter. The results displayed in Table 4 suggest that the same putative inducer molecule appears to be present in cells lacking Fnr, ArcA, and the enzymes encoded by *glpA*, *pfl*, and *frd*, because all five mutants are able to express *tdc* in the presence of cAMP. The chemical identity of the inducing metabolite and how it activates *tdc* transcription remain unknown. Further investigation with mutants of anaerobic carbon metabolism and addition of fermentation end products to the growth medium to restore *tdc* expression would help in identifying the inducing metabolite.

An unusual aspect of *tdc* regulation revealed in this study was that the effects of *fnr* and *arcA* mutations on *tdc* expression are mediated strictly through physiological means: accumulation of a metabolite(s) which represses *tdc* transcription. Interestingly, an opposite physiological situation was uncovered in earlier studies on the Fnr-dependent expression of the formate-hydrogen lyase (FHL) enzyme complex. Formate, a product of pyruvate metabolism catalyzed by the *pfl* gene product, functions as an inducer of the transcriptional activator, FhlA, which then activates the genes encoding the FHL complex (23, 26). In an *fnr* mutant, reduced expression of Pfl would decrease the formate level in the cell, thus preventing Fhl expression. Thus, lack of a single regulatory gene product such as Fnr negatively affects the expression of two operons by two entirely different mechanisms. In view of these examples, it is reasonable to suggest that the roles of global regulatory proteins in specific gene transcription must be examined in the larger context of cellular metabolism.

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