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Repression of the Gene Encoding Succinate Dehydrogenase in Response to Glucose Is Mediated by the EIICB^{Glc} Protein in *Escherichia coli*[1]

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The *Escherichia coli sdhCDAB* operon encodes succinate dehydrogenase, an enzyme complex involved in the tricarboxylic acid (TCA) cycle. Expression of this operon is under complex transcriptional regulation in response to growth conditions, such as anaerobiosis and carbon sources. Typically, the expression of *sdhCDAB* is known to be subjected to "an aerobic repression" and "a glucose repression." The molecular mechanism underlying the anaerobic repression has been well documented, involving both the ArcB-ArcA two-component system and the Fnr global anaerobic regulator. However, the mechanism underlying the glucose repression is not yet clear, because the involvment of the general catabolite regulators such as CRP and CRA has been dismissed. In this study, we conducted a series of genetic analyses to identify the regulator gene(s) involved in the glucose repression of *sdh*. The results demonstrate that the EIICB^{Glc} protein (the *ptsG* gene product), a component of the major glucose transporter, acts as a crucial mediator in glucose repression. These results support the view that the EIICB^{Glc} protein functions not only as a glucose transporter, but also as a glucose-sensing signal transducer that modulates the glucose repression of the *sdhCDAB* operon.

Key words: *Escherichia coli*, glucose repression, *ptsG* gene, *sdhCDAB* gene, signal transduction.

Escherichia coli is a facultative microorganism (anaerobe) that can grow on sugars either by fermentative processes, or by respiratory processes that require an exogenous electron acceptor such as oxygen (O_2) or nitrate (for a review, see Ref. *1*). It has long been known that aerobically grown *E. coli* cells contain elevated levels of many enzymes associated with aerobic metabolism. Examples of such enzymes include members of the tricarboxylic acid (TCA) cycle and the cytochrome *o* complex, the major terminal oxidase (for reveiw, see Ref. *2*). Accordingly, in *E. coli*, it has also long been known that there is a complex and global transcriptional regulatory network responsible for producing the correct response to both aerobiosis and anaerobiosis (for a reveiw, see Ref. *3*). In fact, a number of transcriptional regulators that participate in such aerobiosis and anaerobiosis have been identified (for reveiws, see Refs. *3* and *4*, and references therein). They include Fnr, CRP (cAMP receptor protein), and CRA (or FruR), as well as several two-component signal transducers (sensors and regulators) (for a review, see Ref. *5*) such as ArcB-ArcA, NarX-NarL, and TorS-TorR. Although the molecular mechnisms of such global transcriptional regulation underlying both aerobiosis and anaerobiosis have been studied intensively in *E. coli*, a comprehensive understanding is still lacking.

In this regard, one of the best characterized genes is the *sdh* operon encoding succinate dehydrogenase (SDH), a membrane bound enzyme complex that functions as a member of the TCA cycle (for a reveiw, see Ref. *6*). The SDH enzyme is thought to be an aerobic enzyme, and the levels of SDH in cells are elevated under aerobic conditions and reduced by anaerobiosis. The genes for SDH, *sdhCDAB*, map at 16.5 min on the chromosome (*7, 8*), and the *sdhC* promoter is known to be under complex transcriptional control (*9*). Several groups, including ours, adopted *sdh-lacZ* fusion genes on the chromosome to examine how *sdh* transcription is regulated by changes in environmental stimuli relevant to aerobiosis and anaerobiosis (*10-12*). The emerging scenario from such studies is as follows. Expression of *sdh-lacZ* is highest under aerobic growth conditions, and is decreased about 10-fold in the absence of oxygen. Fnr and ArcA were identified as major transcriptional regulators for this oxygen control, and each acts as a repressor of *sdh-lacZ* expression by binding directly to the *sdhC* promoter under anaerobic growth condtions. Fnr is a global anaerobic transcriptional regulator, whose function seemingly resembles the well-known global catabolite regulator, CRP (for reveiws, see Refs. *3* and *13*). In contrast, ArcA is a typical response regulator, whose function is modulated by phosphorylation mediated by the cognate anaerobic sensor histidine kinase, ArcB (for a review, see Ref. *3*). However, the scenario is

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Abbreviations: SDH, succinate dehydrogenase; TCA, tricarboxylic acid

not as simple as described above; rather, the reality is more complex as described below.

Interestingly, results from the same series of studies described above revealed that, even under aerobic growth conditions, the expression of *sdh-lacZ* varies about 10-fold depending on the type of carbon source used or the medium richness (*11, 12*). In particular, the expression of *sdh-lacZ* is remarkably suppressed during aerobic growth in medium containing glucose. This is consistent with the fact, found a number of years ago, that SDH enzyme activities are reduced markedly by the presence of glucose in the medium (*14*). Of course, it was first postulated that CRP and/or CRA may be involved in this glucose repression (*11*), because they are well-characterized global catabolite transcriptional regulators (for a reveiw, see Ref. *4*). Surprisingly, however, it was found that the glucose repression is not mainly dependent on CRP or CRA (*11*). Thus, some other unknown regulatory element(s) must act to control the expression of *sdh* at the level of transcription. In this study, this puzzling issue was addressed, and we found that the *ptsG* gene is one such genetic determinant that affects the glucose repression of *sdh*. Since the *ptsG* gene encodes a membrane-associated component ($EIICB^{Glc}$) of the major glucose transporter, the underlying mechanism of the glucose repression of *sdh* will be discussed.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media—The *E. coli* K-12 strains used in this study are all derivatives of MC4100 (F^- *ΔlacU169 araD139 rpsL relA flbB*). OG903 is a derivative of MC4100, which carries an *sdhC-lacZ* transcriptional fusion gene on the chromosome (*15, 16*). An *mlc*::Tet^r allele was further introduced into OG903 by means of P1 transduction from KK32 (a gift from H. Aiba, Nagoya University) (*17*), to yield DMC904 (*Δmlc*). Similarly, a *ptsG*::Km^r allele was also introduced into OG903 from IT1168 (a gift from H. Aiba, Nagoya University) (*18*) to yield DCB904 (*ΔptsG*). The same *ΔptsG* allele was transferred into DAC903 (*ΔarcB*), to yield DAP904 (*ΔarcB ΔptsG*). These strains were grown mainly in Luria broth (LB) buffered with potassium phosphate (pH 7.2) either with or without glucose (40 mM). M9+glycerol and M9+glucose were also used, unless otherwise noted.

Plasmids—Plasmids were isolated (pSIN003 and pSIN-025) or constructed, except for pMLC3, which carries the *mlc* gene alone. This plasmid was a gift from H. Aiba (Nagoya University) (*17*).

Genetics—*E. coli* genetics were conducted according to standard procedures described previously (*19*).

Recombinant DNA Techniques—DNA-manipulating enzymes such as restriction endonucleases, the Klenow fragment of *E. coli* DNA polymerase I, and T4 DNA ligase, were used under the conditions recommended by the suppliers (Takara Shuzo, or Toyobo). Other recombinant DNA techniques were carried out according to a standard laboratory manual (*20*). DNA was sequenced by the dideoxy chain termination method using a sequencing kit and an automated sequencer (Perkin Elmer, ABI PRISM™ 310 Genetic Analyzer).

Enzyme Assays—β-Galactosidase activity was determined by Miller's method with slight modifications (*19*). Cells were grown to the mid-logarithmic phase in an appropriate medium under standard aerobic conditions. The cells were collected and then suspended in one volume of 250 mM sodium phosphate (pH 7.1) for the accurate determination of cell density. A portion of the cell suspension was subjected to β-galactosidase assay after permeabilization with toluene. Figures show the values for triplicate cultures (note that for clarity the error bars are omitted).

RESULTS

Experimental Rationale—An *sdhC-lacZ* transcriptional fusion gene on the chromosome was first constructed to yield the *E. coli* strain named OG903, as described previously (*12*). With our particular strain, the previously-documented glucose repression of *sdh* needed to be confirmed. When this strain was grown under fully aerobic growth conditions in Luria broth (LB, a standard rich medium for *E. coli*), a significant level of *sdh-lacZ* expression was detected as β-galactosidase activity (Fig. 1, and note that the LB used in this study is buffered with potassium phosphate). Even higher levels of β-galactosidase activity were detected in cells grown in minimal medium (M9+glycerol, a standard synthetic medium for *E. coli*) (Fig. 1). When glucose (40 mM) was added to LB, or when glycerol was replaced by glucose (40 mM) in M9-based medium, the β-galactosidase activities were markedly decreased in cells grown under otherwise the same conditions as those described above (Fig. 1). This indicates that the expression of *sdhC-lacZ* is severely repressed by glucose added to the growth medium. Such glucose repression of *sdh* transcription has been well-documented previously (*11*), as descrived above (see "INTRODUCTION"). However, the underlying molecular mechanism has not yet been clarified.

To address this particular issue, we attempted to identify a gene(s) that affects this "glucose repression," provided that a multicopy gene(s) was introduced into *E. coli* by means of plasmid transformation. This was achieved as follows. OG903 carrying the *sdhC-lacZ* gene forms pail

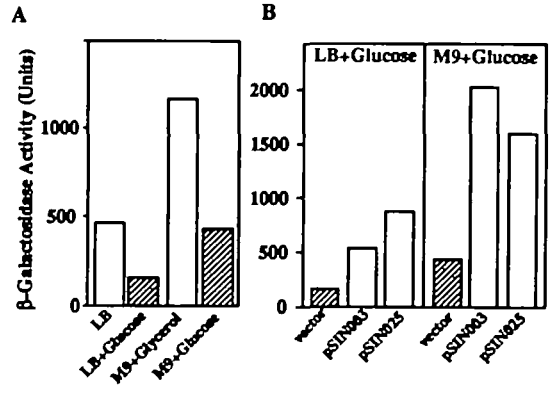

Fig. 1. **β-Galactosidase activity expressed by the *sdh-lacZ* transcriptional fusion genes.** A: *E. coli* OG903 carrying the *sdh-lacZ* transcriptional fusion gene on the chromosome was grown in the indicated medium to the mid-logarithmic phase under aerobic conditions. The cells were harvested, and then β-galactosidase activity expressed by the cells was measured. B: *E. coli* OG903 harboring each indicated plasmid were also grown in either LB+glucose or M9+glucose, and then the β-galactosidase activity was assayed under the same conditions above. For the plasmids used, see Fig. 2.

blue colonies on M9+glucose agar plates containing X-gal. We then screened an *E. coli* genomic bank constructed with an appropriately high copy-number plasmid (pACYC184), by introducing it into OG903. We looked for dark blue colonies on the plates, and a number of independent candidates were selected from among 10^5 transformants. Since we wanted to isolate a multicopy gene(s) that functions as a negative effector especially toward glucose repression, we needed to further select these candidates as follows. Candidate plasmids were isolated and re-transferred into OG903. For these transformants, β-galactosidase activities were measured in cells grown in LB with and without glucose. Selected were those plasmids that exhibited an ability to elevate β-galactosidase activity only if the cells were grown in LB-containing glucose (but not in plain LB). Finally, 9 such candidates were selected, each of which was assumed to carry a gene that functions as a muticopy (most likely negative) effector toward the glucose repression of *sdhC-lacZ*.

Isolation of Multicopy Effector Genes That Affect the Glucose Repression of sdhC-lacZ—Since each isolated plasmid contained a relatively large DNA insert (about 5 kb), we first classified them through extensive analyses involving restriction endonuclease digestion and partial DNA-sequencing. The results showed that the plasmids could be classified into two distinct groups, 4 in one and 5 in the other. In each case, the smallest clone (or DNA insert) was chosen as representative. These two plasmids were named pSIN003 and pSIN025. To demonstrate that these two plasmid clones indeed exhibit the ability to interfere with the glucose repression of *sdhC-lacZ*, the clones were transferred into OG903, and their β-galactosidase activities were measured after growth in LB+glucose (Fig. 1B). As expected, the glucose repression of *sdhC-lacZ* in LB+glucose was fully relieved, as judged by their levels of β-galactosidase activity (compare Fig. 1, A and B). The same was true for cells grown in M9+glucose (Fig. 1B). Thus, we thought that these clones might be the ones we wanted.

Identification of Multicopy Effector Genes That Affect the Glucose Repression of sdhC-lacZ—Since the entire genomic sequence of *E. coli* is now known, the structural designs of the DNA inserts, each cloned in pSIN003 and pSIN025, were easily clarified by the results of partical DNA-sequencing (Fig. 2A). As judged by their structural design, pSIN003 contains two intact genes, named *bioD* and *mlc* (*21*), whereas pSIN025 contains the *nagBACD* operon (*22*). An inspection of these genes revealed that the *mlc* gene in pSIN003 encodes a protein highly homologous to that encoded by the *nagC* gene in pSIN025 (Fig. 2B) (*21*). NagC has been characterized as a DNA-binding transcriptional repressor of the *nag* regulon, including the *nagBACD* operon that encodes genes involved in the uptake and

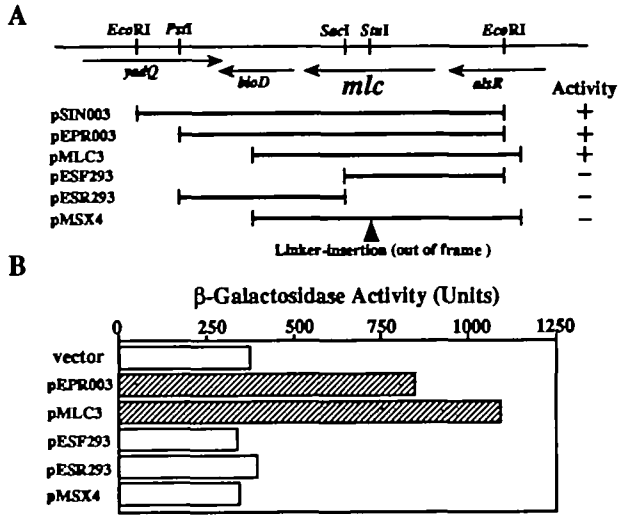

Fig. 3. **Identification of the gene responsible for the multicopy effect on glucose repression.** A: From pSIN003, three subclones (pEPR003, pESF293, pESR293) were constructed by endonuclease digestion. Plasmid pMLC3 was a gift from H. Aiba (Nagoya University) (*17*). Plasimd pMSX4 was constructed from pMLC3 by a linker-insertion (*Xho*I-8 mer) at the unique *Stu*I site. The multicopy effect of these plasmids on glucose repression was examined as described below, and the results are shown schematically (activity +, positive effect; activity −, no effect, see panel B). B: Plasmids, shown in panel A, were introduced into OG903, and then each transformant was grown in M9+glucose. β-Galactosidase activity of these transformants was measured.

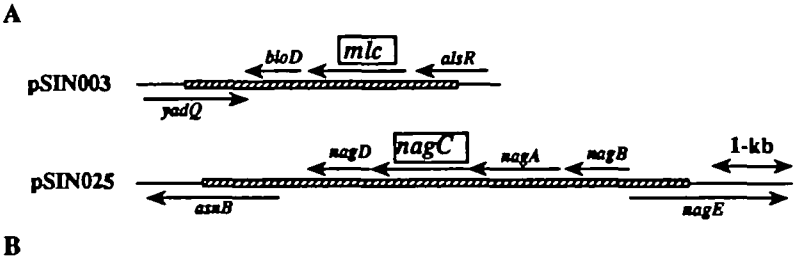

Fig. 2. **Schematic representation of the structures of the chromosomal regions encompassing the *mlc* are *nagC* genes.** A: The structures of the chromosomal regions encompassing the *mlc* and *nagC* genes are shown schematically. It was found that plasmid pSIN003, isolated in this study, contains the *mlc* region, whereas plasmid pSIN025 contains the *nagC* region. Arrows indicate the coding regions as denoted. B: The alignment of the entire amino acid sequences of the *mlc* and *nagC* gene products. Identical amino acids are highlighted (*21*).

metabolism of *N*-acetyl-glucosamine (*23-25*). The *mlc* gene was originally identified as one whose overproduction affects *E. coli* colony-size on LB+glucose agar plates (making large colonies), while its biological function has not been identified (*21*). However, during the course of this study, Mlc was reported to be a DNA-binding repressor of the *ptsG* gene encoding a major glucose transporter in *E. coli* (*17, 24, 25*). Furthermore, both Mlc and NagC were demonstrated to recognize a set of nucleotide sequences very similar to each other (*24*). These facts led us to assume that the *mlc* gene in pSIN003 and the *nagC* gene in pSIN025 are most likely the ones we wanted to identify. Thus we decided to focus our attention on the *mlc* gene.

Overexpression of the mlc Genes Results in the Relief of Glucose Repression—To confirm the above assumption that the *mlc* gene in pSIN003 is indeed responsible for the multicopy effect on glucose repression, a series of subclones was constructed from pSIN003, as shown schematically in Fig. 3A. These plasmids were transferred into OG903, and examined by measuring the β-galactosidase activities in cells grown in M9+glucose (Fig. 3B). The results indicated that the *mlc* gene alone is sufficient to relieve the glucose repression (see pMLC3 and pMSX4). By using pMLC3 carrying only the *mlc* gene, the multicopy effect on glucose repression was extensively and more quantitatively examined in cells grown in various types of medium, as summarized in Table I. Glucose repression was clearly observed in any glucose-containing medium tested (see the columns, LB+glucose, glucose, galactose+glucose, glycerol+glucose). More importantly, the multicopy *mlc* gene exhibited the ability to fully relieve glucose repression under these conditions. Moreover, the *mlc* gene had virtually no effect on the expression of *sdhC-lacZ* in cells grown in medium without glucose. Thus, we conclude that the multicopy *mlc* gene somehow specifically interfers with a mechanistic process involved in glucose repression of *sdhC-lacZ* expression.

The mlc Gene Is Not Directly Involved in the Mechanism of Glucose Repression—Since we are dealing with the multicopy effect of *mlc*, it was uncertain whether the *mlc* gene is directly involved in the presumed mechanism underlying glucose repression. A simple and direct way to address this issue is, of course, to characterize an *mlc* mutant with special reference to glucose repression. Thus, we constructed a *mlc*::Tet^r derivative of OG903, and the expression profile of *sdhC-lacZ* was examined in mutant cells grown in LB and LB+glucose (Fig. 4), as well as

TABLE I. **β-Galactosidase expressed by *E. coli* OG903 carrying the *sdh-lacZ* fusion gene on the chromosome.**

Medium	β-Galactosidase activity (units)		Ratio
	A (vector)	B (pMLC3)	(B/A)
LB	524±8	473±11	0.9
LB+glucose	116±3	426±19	3.7
M9-Based			
Glucose	299±16	917±89	3.1
Galactose	590±18	743±62	1.3
Galactose+glucose	314±18	640±18	2.0
Xylose	686±55	780±15	1.1
Glycerol	641±58	859±23	1.3
Glycerol+glucose	274±18	753±18	2.7
Succinate	1,347±128	1,330±41	1.0

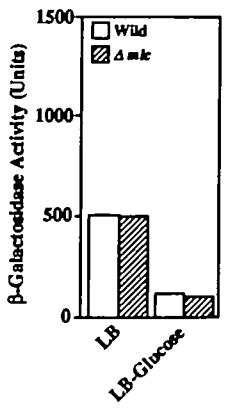

Fig. 4. **β-Galactosidase activity expressed by *sdh-lacZ* transcriptional fusion genes.** A: *E. coli* OG903 and its *mlc* deletion (*Δmlc*) derivative, each carrying the *sdh-lacZ* transcriptional fusion gene on the chromosome, were grown in the indicated medium to the mid-logarithmic phase under aerobic conditions. The cells were harvested, and then β-galactosidase activity expressed by the cells was measured.

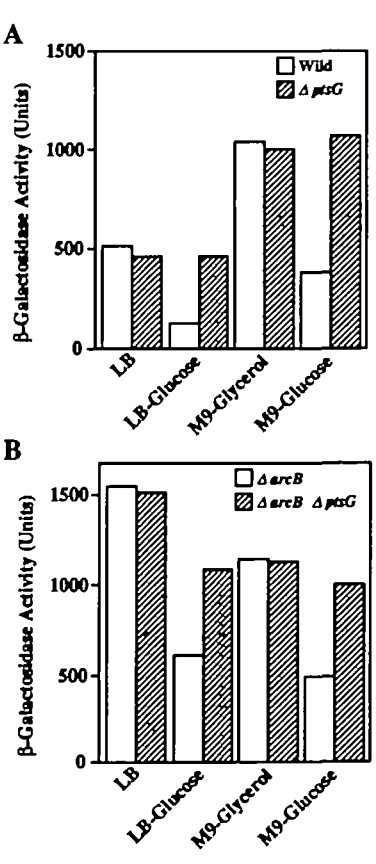

Fig. 5. **β-Galactosidase activity expressed by the *sdh-lacZ* transcriptional fusion genes.** A: *E. coli* OG903 and its *ptsG* deletion (*ΔptsG*) derivative, each carrying the *sdh-lacZ* transcriptional fusion gene on the chromosome, were grown in the indicated medium to the mid-logarithmic phase under aerobic conditions. The cells were harvested, and then β-galactosidase activity expressed by the cells was measured. B: *E. coli* DAC903 (*ΔarcB*) and its *ptsG* deletion (*ΔptsG*) derivative, each carrying the *sdh-lacZ* transcriptional fusion gene on the chromosome, were subjected to β-galactosidase assay, as described above.

M9-glycerol and M9-glucose (data not shown). The results showed that the single copy *mlc* gene on the chromosome has nothing to do with glucose repression

The ptsG Gene Is Directly Involved in the Mechanism Underlying Glucose Repression—Since the above result indicated that the *mlc* gene is not directly involved in glucose repression, we needed to envisage how the multicopy *mlc* gene is capable of interfering with glucose repression. A clear hint comes from the fact that Mlc was recently demonstrated to be a crucial DNA-binding repressor for the *ptsG* gene (*17, 24, 25*), encoding a component of the major glucose transporter [phosphotransferase system (PTS)-dependent EIIBC^{Glc}] (for a reveiw, see Ref. *26*). If Mlc is an overexpressed form of the multicopy *mlc* gene, *ptsG* gene expression would be severely repressed in cells. This plausible assumption led us to envisage that the *ptsG* gene itself might be a genetic element that controls glucose repression. This hypothetical view can be tested directly by examining a *ptsG* null derivative of OG903, with special reference to the glucose repression of *sdhC-lacZ*. The result of such a genetic analysis showed that this is indeed the case (Fig. 5A). It was demonstrated that the glucose repression is completely cancelled with a *ΔptsG* background. This is direct genetic evidence that supports the view that the *ptsG* gene is the genetic element implicated, directly or indirectly, in the underlying mechanism of the glucose repression of *sdhC-lacZ*.

Epistasis of ptsG and arcB in sdh Regulation—The above results suggest that the *ptsG* gene product functions as a mediator (or signal transducer) in the mechanism underlying the glucose repression of *sdh* under aerobic growth conditions, as will be discussed (see "DISCUSSION"). In this respect, it is well known that the ArcB sensor kinase is a crucial signal transducer involved in anaerobiosis (or anaerobic repression) of *sdh* (*3*). It was also proposed recently that this anaerobic sensor plays a role in the regulation of *sdh*, even under aerobic growth conditions (*12*). Thus, it was necessary to examine the functional interaction between the *ptsG* and *arcB* genes. Thus we conducted a simple epistatic analysis by examining the properties of a *ΔarcB ΔptsG* double-mutant in terms of glucose repression. As shown in Fig. 5B, under aerobic growth conditions, glucose repression was observed even with a *ΔarcB* background, and *ΔarcB ΔptsG* double-mutant cells exhibited an elevated level of *sdhC-lacZ* expression. These genetic results suggest that the presumed signal transducers, EIICB^{Glc} and ArcB, function in an independent (or parallel) fashion relative to each other in the regulation of *sdh*. In this regard, it should also be noted that the *ptsG* mutation has no effect on the anaerobic repression of *sdhC-lacZ* expression in cells grown in either LB or LB+ glucose (data not shown).

DISCUSSION

The mechanism of the glucose repression of *sdh CDAB* has been the subject of a long-standing debate (*11*), because the participation of both of the well-known global catabolite regulators, CRP (or cAMP) and CRA, has been dismissed (see "INTRODUCTION"). It was thus postulated that some other unidentified regulatory element(s) must be involved in the glucose regulation of *sdh* (*11*). The results of the experiments presented in this study provided new information concerning this puzzling problem. Our results suggest that the glucose repression of *sdh* involves a mechanism dependent upon the *ptsG* gene encoding the EIICB^{Glc} protein. Before discussing the mechanistic basis behind the EIICB^{Glc}-mediated glucose repression of *sdh*, the following points should be noted. First, although EIICB^{Glc} is a component of the major glucose transporter, the presumed intracellular metabolites of glucose, such as glucose-6-phosphate, should not be crucial elements by themselves for glucose repression, because glucose uptake as well as metabolism occur even in *ΔptsG* cells through redundant PTS-linked glucose transport systems in *E. coli* (*e.g.*, IICBA^{Nag}, IIABC^{Man}) (for a review, see Ref. *26*). This situation can be clearly seen in Fig. 5, in which the *ΔptsG* strain is capable of growing normally in M9-based medium containing glucose as the sole carbon sourse. Second, it is most likely that EIICB^{Glc} is not directly involved in *sdh* transcriptional repression *per se* (*e.g.*, as a DNA-binding repressor), because no DNA-binding property has been reported for EIICB^{Glc}, which is located in the cytoplasmic membrane. Therefore, EIICB^{Glc} may exert its regulatory effect on the transcriptional regulation of *sdh* through an as yet unknown unique mechanism, as will be discussed.

In general, *E. coli* need to sense the availability of nutrients in their growth environment in order to preserve energy. The regulation of *lac* operon expression during

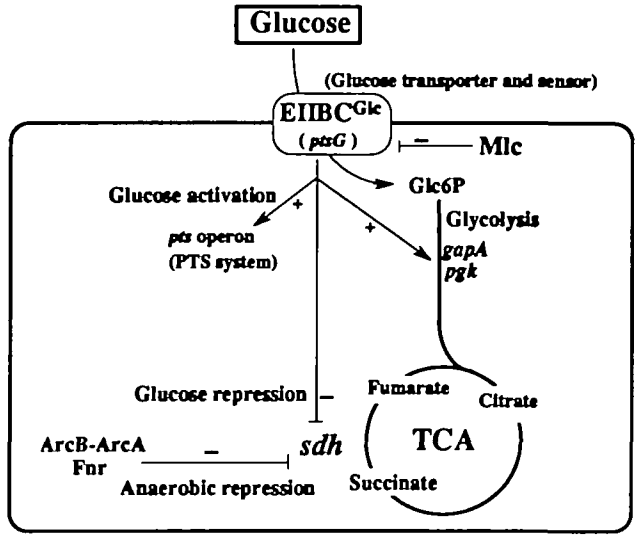

Fig. 6. **A hypothetical framework that explains the intriguing findings of this study.** The three major cellular processes relevant to glucose metabolism are shown schematically (*i.e.*, glucose transport, glycolysis, tricarboxylic acid cycle). The EIICB^{Glc} protein in the cytoplasmic membrane is the major component of the glucose transporter in *E. coli*. This transport system is driven by the phosphotransferase system (PTS), encoded by the *pts* operon (*26*). The glucose transport is coupled with the generation of glucose-6-phosphate, and the EIICB^{Glc} protein is transiently phosphorylated. *ptsG* is the structural gene for the EIICB^{Glc} protein, whose gene expression is negatively regulated by Mlc. The results from this and other studies suggest that the EIIBC^{Glc} protein functions not only as a glucose transporter, but also a glucose sensor in the medium. Through an as yet unknown signaling mechanism(s), EIICB^{Glc} may regulate a variety of genes involved in glucose metabolism in both a positive (+) and negative (−) manner. As shown in this framework, the downstream target genes involve the *pts* operon, the *gapA* gene, and the *pgk* gene, as well as the *sdhCDAB* operon. Other details are given in the text.

growth on glucose and lactose is often cited as the paradigm for such adaptation (*18*, and references therein). Curiously however, the regulation of the genes required for glucose transport and metabolism (the so-called the central pathway, including the TCA cycle) is less documented in *E. coli*. However, during the course of this study, we came across a series of interesting reports that demonstrate that glucose somehow activates the *pts* operon, which consists of the *ptsH, ptsI,* and *crr* genes (*27-29*) and encodes the histidine-containing phosphocarrier HPr protein, enzyme I (EI), and $EIIA^{Glc}$. As is well known, these are the major constituents of the PTS system (*26*). It has been proposed that this glucose-mediated activation of *pts*, which appears to be CRP-cAMP-indepedent, occurs through a signal transduction mechanism that is dependent upon the function of the *ptsG* gene product (see Fig. 6) (*27*). More recently, the expressions of both the *gapA* and *pgk* genes, which encode glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase, respectively, have also been demonstrated to be activated in a similar manner dependent on $EIICB^{Glc}$ (*30*). It may be noted that these enzymes are involved in glycolysis (Fig. 6). Thus, the expression of a set of *E. coli* genes required for glucose-uptake (*e.g., pts*) and glucose-metabolism (*e.g., gapA* and *pgk*) are coordirately regulated by the presence of glucose in the medium by an $EIICB^{Glc}$-dependent mechanism. Thus these positive effects of glucose on the transcription of *pts, gapA,* and *pgk* are similar to the findings in this study of the negative effects of glucose on *sdh* transcription (Fig. 6). Our results suggest that the $EIIBC^{Glc}$-mediated mechanism of transcriptional regulation acts not only in a positive manner, but also in a negative fashion, and also that it acts on a greater variety of target genes, which include not only glucose-transporter genes and glycolytic genes, but also genes involved in the TCA cycle. These plausible but hypothetical views are shown schematically in Fig. 6.

It is not known how $EIICB^{Glc}$ can function as a regulator (or glucose-sensing signal transducer) of the mechanism underlying the glucose repression of *sdh* (*17, 27, 30*). Reuse and Danchin proposed that glucose activation acts like a two-component system, with $EIICB^{Glc}$ acting as a sensor together with an as yet unknown transcriptional regulator (*27, 28*). In other words, glucose activation occurs through a signal transduction dependent upon the phosphorylation state of $EIICB^{Glc}$ in response to glucose as an external signal (it should be noted that $EIICB^{Glc}$ is phosphorylated through the PTS systems, see Ref. *26*). It should be emphasized that the presumed downstream regulator can be either a transcriptional activator or a repressor. This same scenario is applicable to the glucose repression of *sdh*, as characterized in this study, although the physiological meaning of the glucose repression of *sdh* is not yet clear. However, verification of this attractive but speculative view must await further experimentation. It is also worth mentioning that another component of the glucose transporter, IIA^{Glc}, also plays an interesting regulatory role, which is also modulated by the phosphorylation state. The unphosphorylated form of IIA^{Glc} acts as a negative regulator of various non-PTS sugar transporters (including the lactose permease) through direct binding (*18, 26,* and references therein). It is not known whether there is any functional linkage between the regulatory function of $IICB^{Glc}$ on *sdh*, demonstrated in this study, and that of IIA^{Glc}, as described above. This issue may be worth addressing.

In summary, in this study we succeeded in identifying the $EIICB^{Glc}$ protein as an intriguing mediator that is crucially involved in the mechanism underlying the glucose repression of the *E. coli sdhCDAB* operon. This finding should shed light on the complex mechanism by which the expression of the *sdh* operon is regulated in response to aerobiosis and anaerobiosis, and in which several regulatory elements are implicated, including the ArcB-ArcA two-components, the Fnr anaerobic global regulator, and the $EIICB^{Glc}$ protein (Fig. 6). Perhaps more impotantly, our experimental system will provide a clue to address the long-standing issue of how $EIICB^{Glc}$ funcions as the presumed glucose-sensing signal transducer.

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