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Genetic Regulation of the Glyoxylate Shunt in *Escherichia coli* K-12

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The expression of the glyoxylate shunt enzymes is required for growth of *Escherichia coli* on acetate or fatty acids as a sole carbon source. The genes for the two unique enzymes of the glyoxylate shunt, *aceA* and *aceB*, are located at 90 min on the *E. coli* K-12 genetic map. Polar mutations in the *aceB* gene eliminate *aceA* gene function, suggesting that these genes constitute an operon and the direction of transcription is from *aceB* to *aceA*. Mu *d* (Ap *lac*) fusions with the *aceA* gene have been constructed to study the regulation of the *ace* operon. Expression of the *ace* operon is under the transcriptional control of two genes: the *iclR* gene, which maps near the *ace* operon, and the *fadR* gene, which maps at 25 min, and is also involved in the regulation of the fatty acid degradation (*fad*) regulon. Merodiploid studies demonstrated that both the *iclR* and *fadR* genes regulate the glyoxylate shunt in a *trans*-dominant manner.

Growth of *Escherichia coli* on substrates such as acetate or fatty acids requires operation of the glyoxylate shunt (6). When grown on these carbon sources, cellular macromolecules must be derived from acetyl-coenzyme A (CoA). The glyoxylate shunt bypasses the two CO_2-evolving steps of the tricarboxylic acid cycle, allowing the net accumulation of carbon from acetyl-CoA. The two unique enzymes of the glyoxylate shunt, isocitrate lyase and malate synthase A, are induced in *E. coli* when acetate or fatty acids are provided as the sole carbon source (6, 21, 22). The structural genes for isocitrate lyase, *aceA*, and malate synthase A, *aceB*, map at 90 min on the revised *E. coli* K-12 linkage map (Fig. 1) (1, 2, 21). On the basis of the close genetic linkage of the *aceA* and *aceB* genes, Brice and Kornberg (2) proposed that they may form an operon. However, genetic evidence that these genes are organized in an operon has not previously been shown.

The *aceA* and *aceB* genes are coordinately regulated. The expression of these genes seems to be controlled by an adjacent regulatory gene, *iclR* (2). In addition, the expression of the glyoxylate shunt enzymes seems to be controlled by the *fadR* gene (11). The *fadR* gene maps at 25 min on the *E. coli* K-12 linkage map (Fig. 1) and is also involved in the regulation of the fatty acid degradation (*fad*) regulon (20, 21). Mutations in either the *iclR* or *fadR* gene result in elevated expression of the glyoxylate shunt enzymes under noninducing growth conditions. However, there has been no previous genetic evidence that either the *iclR* or *fadR* gene controls the expression of the glyoxylate shunt enzymes at the level of transcription. In addition, it has not been ascertained whether these genes are *cis* or *trans* acting, or whether they act in a positive or negative manner on the glyoxylate shunt.

In this paper we present evidence that the *aceA* and *aceB* genes constitute an operon under the transcriptional control of both the *iclR* and *fadR* genes.

MATERIALS AND METHODS

Bacterial strains. All strains used in the study were derivatives of *E. coli* K-12. The genotypes of these strains are shown in Table 1. Preparation of phage stocks and transductions were performed as previously described (19). Mutants for the *aceA* gene were unable to grow on acetate as a sole carbon source. The *glc* gene encodes a second malate synthase which can functionally replace the *aceB* gene product, malate synthase A (21). Thus, *aceB glc*$^+$ mutants will grow on acetate as a sole carbon source, whereas *aceB glc* mutants will not. For this reason, *aceB* mutations were selected in strains bearing a *glc* mutation. The *iclR* mutants were identified by the ability of this mutation to suppress *ppc* (phosphoenolpyruvate carboxylase) or *pps* (phosphoenolpyruvate synthase) mutations (6). Constitutive levels of glyoxylate shunt enzymes in *iclR* mutants were confirmed by in vitro enzyme assays. Contrary to previous reports (25), mutants selected for the ability to use valerate as a sole carbon source did not show constitutive levels of glyoxylate shunt enzymes. In addition, Vinopal and Fraenkel (23) have shown that the growth phenotype of mutants defective for fructose-6-phosphate kinase (*pfkA*) can be suppressed by a second mutation in the *iclR* gene. Of 10 independent *pfkA* revertants capable of growth on dextrose, none was constitutive for the glyoxylate

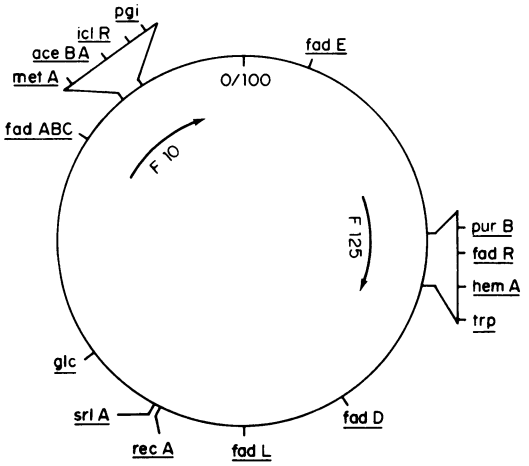

FIG. 1. Genetic linkage map of *E. coli* K-12 showing relevant markers and the episomes F10 and F125. The 25- and 90-min regions are enlarged to indicate the relative positions of markers. Adapted from the revised linkage map of Bachmann and Low (1).

shunt enzymes (unpublished data). However, it is conceivable that *iclR* mutations that suppress the *pfkA* mutation may be rarer than true revertants and other indirect suppressor mutations (23). The *fadR* mutants were selected as previously described (19).

Transposon insertions near the *aceA*, *aceB*, and *iclR* genes were constructed as previously described (11). Strains bearing transposon insertions within the *aceA* and *aceB* genes were obtained by localized mutagenesis of a *metA* mutant by transduction to Met^+ Tc^r with a P1 *vir* phage stock grown on a pool of K-12 colonies with random insertions of Tn*10* (11). Strains bearing insertions of Tn*10* were isolated after ampicillin enrichment with acetate as the sole carbon source (15). Surviving cells were plated on media containing 50 mM acetate and 10 mM succinate, and small colonies were checked for inability to grow on acetate as a sole carbon source. Such isolates were characterized genetically and biochemically. The Tn*10* insertion in strain KAT-1 eliminated isocitrate lyase activity. In addition, strain KAT-1 yielded Ace^+ recombinants when crossed wtih SM1021 (*aceB*), but not K8-5m (*aceA*). Thus KAT-1 was classified as an *aceA*::Tn*10* mutant. The Tn*10* insertion in strain KBT-1 eliminated both malate synthase and isocitrate lyase activity. Strain KBT-1 yielded Ace^+ recombinants when crossed with K8-5m, but not SM1012. Thus, KBT-1 is believed to have a Tn*10* insertion in *aceB* which is polar to the *aceA* gene.

Operon fusions of Mu *d* (Ap *lac*) with the *aceA* gene were constructed as described by Casadaban and Cohen (3). Strain TL1 was infected with Mu *d* (Ap *lac*), plated on MacConkey agar containing 25 μg of ampicillin per ml, and incubated at 30°C. The Lac^+ Ap^r colonies were replica plated onto oleate, acetate, and succinate media. Colonies unable to grow on acetate or fatty acids as a sole carbon source, but capable of growth on succinate, were genetically characterized as *aceA* mutants, and the genotypes of the mutants were confirmed enzymatically.

To construct derivatives defective in homologous recombination (*recA*), cells were infected with P1 *vir* grown on NK5304 (*srlA*::Tn*10* *recA*), and tetracycline resistant (Tc^r) transductants were selected. The *srlA*::Tn*10* and *recA* markers are about 50% cotransducible. The *recA* cotransductants were identified by their sensitivity to nitrofurantoin (2 μg/ml) (14). Tc^s derivatives of these strains were selected on fusaric acid plates as described by Maloy and Nunn (12).

Matings were performed for 2 h at 37°C as described by Miller (15). The mating mixtures were plated on minimal medium lacking methionine with streptomycin for counterselection against the donor. Mutant F-prime factors were obtained by recombination in $recA^+$ strains as described by Miller (15). Most of the merodiploids constructed were somewhat unstable when grown in rich broth, giving rise to ca. 5 to 20% monoploid segregants after overnight growth. These merodiploids gave rise to less than 0.5% monoploid segregants after overnight growth in minimal medium lacking methionine. When it was difficult to obtain spontaneous monoploid segregants, merodiploids were cured of their episome with acridine orange by the method of Miller (15).

Media and growth conditions. Bacteria were routinely incubated in a Gyrotory water bath shaker (New Brunswick Scientific Co.) at 37°C. For most genetic procedures, cells were grown on Luria broth (15). When minimal medium was required, the bacteria were usually grown on medium E (24). Carbon sources and supplements were sterilized separately and added to the culture medium before inoculation. All organic acids were neutralized with potassium hydroxide before sterilization. Acetate was added at 50 mM (final concentration). Fatty acids were provided at 5 mM in the presence of Brij 58 (5 mg/ml). All other carbon sources were provided at 25 mM. When necessary, amino acid supplements were added at a final concentration of 0.01%. Tetracycline was added at a final concentration of 20 μg/ml and ampicillin was added at a final concentration of 25 μg/ml. Cell growth was monitored at 540 nm on a Klett-Summerson colorimeter.

Enzyme assays. Crude extracts were prepared by disrupting exponential-phase cells in a French press as previously described (11). Isocitrate lyase and malate synthase were assayed as described by Maloy et al. (11). β-Galactosidase activity was assayed as described by Miller (15). Protein was determined by a microbiuret procedure (15), with bovine serum albumin as a standard.

Materials. Most reagents used in this study were as described (11). Nitrofurantoin, fusaric acid, chlortetracycline HCl, tetracycline, and ampicillin were obtained from Sigma Chemical Co., St. Louis, Mo. Tryptone, yeast extract, agar, and MacConkey agar were obtained from Difco Laboratories. All other chemicals were of reagent grade.

RESULTS

Fine-structure mapping of the *aceA*, *aceB*, and *iclR* genes. The genetic linkage of the *aceA*, *aceB*, and *iclR* genes has been previously studied by Brice and Kornberg (2) and Vanderwinkel

TABLE 1. Bacterial strains used

Strain	Genotype	Source
K-12	Prototrophic	J. Lederberg via CGSC[a]
RS3010	*fadR*	R. Simons et al. (19)
RS3040	*fadR*::Tn*10*	R. Simons et al. (19)
SM6034	*iclR zja*::Tn*10*[b]	S. Maloy et al. (11)
SM6046	*aceA iclR zja*::Tn*10*	This work
SM6001	*metA zja*::Tn*10*	S. Maloy et al. (11)
SM6009	*aceA1 zja*::Tn*10*	S. Maloy et al. (11)
SM6042	*iclR7 zja*::Tn*10 fadR*	S. Maloy and W. Nunn (13)
NK5304	*srlA*::Tn*10 recA ilv-318 thi-1 thr-3 relA*	N. Kleckner strain (20)
K8-5m	*met-24 aceA3 iclR13 galK2 lac*	H. Komberg strain (23)
SM1021	*aceB6 glc-1 thi-1 relA1 lacZ43*	S. Maloy et al. (11)
SM1023	*glc-1 thi-1 relA1 lacZ43*	This work
TL1	Δ*lac U169 strA*	G. Walker
SMUD1	TL1 *aceA*::Mu *d* (Ap *lac*)	This work
SMUD2	TL1 *iclR aceA*::Mu *d* (Ap *lac*)	This work
SMUD3	SMUD1 *fadR*::Tn*10*	This work
SMUD4	SMUD2 *fadR*::Tn*10*	This work
KAT-1	*aceA*::Tn*10*	This work
KAT-11	*aceA*::Tn*10 glc*	This work
KBT-1	*aceB*::Tn*10 glc*	This work
M11-1-S	*purB fadR*	R. Simons et al. (20)
M2-5-S	*purB fadR trp*	R. Simons et al. (20)
M11-1	F125/*purB trp*	R. Simons et al. (20)
M2-1	F125/*purB fadR trp*	R. Simons et al. (20)
M47-1	F125 *fadR*/*purB trp*	R. Simons et al. (20)
M23-1	F125 *fadR*/*purB fadR trp*	R. Simons et al. (20)
JM683	*fda-1 relA1 tonA22* DE32 DE33 Δ(*aceA-pgi*)	M. Jones-Mortimer strain via CGSC
JC1552	*argG6 metB1 his-1 leu-6 trp-31 mtl-2 xyl-7 malA1 gal-6 lacY1 str-104 tonA2 tsx-1 supE44*	J. Clark via CGSC
MM-1	JC1552 *metB*$^+$ *metA recA1*	This work
MM-2	MM-1 *iclR*	This work
MM-3	MM-1 *aceA*	This work
MM-4	MM-1 *aceA iclR*	This work
MM-5	KFL10/MM-1	This work
MM-6	KFL10/MM-3	This work
MM-7	KFL10/MM-4	This work
MM-8	KFL10 *aceA*/MM-1	This work
MM-9	KFL10 *aceA*/MM-2	This work
MM-10	KFL10 *iclR*/MM-3	This work
MM-11	KFL10 *iclR*/MM-2	This work
MM-12	KFL10 *aceA iclR*/MM-1	This work
MM-13	KFL10 *aceA iclR*/MM-2	This work

[a] CGSC strains obtained from B. Bachmann, Coli Genetic Stock Center, Yale University, New Haven, Conn.
[b] Transposon insertions are designated as previously described (13). When an insertion is not within a known gene, it is given a three-letter symbol starting with z, and the second and third letters indicate the approximate map location in minutes (e.g., *zaf* corresponds to 5 min, and *zbb* corresponds to 11 min).

and DeVlieghere (21). However, the work of Vinopal and Fraenkel (23) suggested that the reported linkage of the *iclR* gene was incorrect. Therefore, we first sought to determine the fine-structure map of the *aceA*, *aceB*, and *iclR* genes. These results are shown in Fig. 2. The *aceA* and *aceB* genes showed very close genetic linkage, in agreement with previous studies (2, 21). In addition, when the *iclR* mutation was mapped with respect to the *aceA* or *aceB* mutations, it showed close linkage with these genes. However, genetic linkage between these mutations, determined independently with respect to outside markers, suggested that the *iclR* gene was about 0.4 min away from the *aceA* and *aceB* genes (1). Similar results were also obtained by Vinopal and Fraenkel (23). These workers suggested that this discrepancy could be due to bias for co-inheritance of the *iclR* mutation and the *ace*$^+$ genotype since constitutive expression of the glyoxylate shunt increases the growth rate on acetate plates. These studies confirmed the

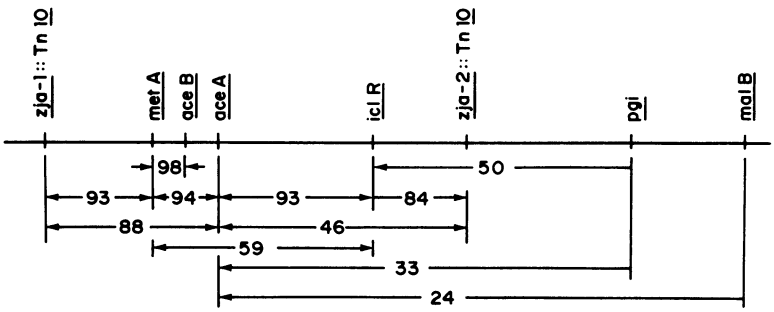

FIG. 2. Genetic linkage of the *aceA*, *aceB*, and *iclR* genes with respect to nearby markers. Values represent P1 cotransduction frequencies as percentages. When only a single arrowhead is shown, the direction of the arrowhead indicates the selected marker. In all other cases the values represent the average frequency of separate genetic crosses selecting for each marker. Not drawn exactly to scale.

gene order *metA aceB aceA iclR* as shown in Fig. 2.

Insertion mutagenesis of the *aceA* and *aceB* genes. To characterize the genetic regulation of the glyoxylate shunt genes, we first needed to find out whether these genes comprise an operon. We determined this by constructing transposon insertion mutations in the *aceA* and *aceB* genes. The transposon Tn*10* is strongly polar, preventing expression of genes downstream in the same transcriptional unit (5). When *aceA*::Tn*10* insertions were constructed, the expression of isocitrate lyase was completely eliminated; however, malate synthase was expressed in a normal fashion (Table 2). The *glc* gene encodes a second malate synthase activity which can substitute for the *aceB* gene product; therefore, *aceB* mutants were examined in a *glc* background. When *aceB*::Tn*10* insertions were constructed in a *glc* background, both malate synthase and isocitrate lyase activities were eliminated (Table 2). These data suggest that the *aceA* and *aceB* genes lie in an operon, and the direction of transcription is from *aceB* to *aceA*.

Mu *d* (Ap *lac*) fusions with the *ace* operon. The activities of the glyoxylate shunt enzymes are elevated in both *iclR* and *fadR* mutants (Table 3). However, there was no genetic evidence whether the *iclR* or *fadR* genes regulated *ace* expression at the level of transcription or at some later step. To answer this question, we constructed operon fusions of the *aceA* gene with Mu *d* (Ap *lac*). These fusions place the expression of the *lac* operon under the control of the promoter and putative operator regions of the *ace* operon (3). Since *aceA*::Mu *d* (Ap *lac*) fusions lack isocitrate lyase, these strains cannot grow on acetate as a sole carbon source. Nevertheless, the malate synthase activity in strains bearing *aceA*::Mu *d* (Ap *lac*) fusions was expressed coordinately with the β-galactosidase activity (Table 4). This finding agrees with the

TABLE 2. Polar insertion mutations in the *aceA* and *aceB* genes

Strain	Relevant genotype	Growth conditions	Sp act (nmol/min per mg of protein)	
			Isocitrate lyase	Malate synthase
K-12	*aceA*$^+$ *aceB*$^+$	Succinate	18	103
		Acetate	244	410
		Succinate + acetate	70	186
SM1023	*aceA*$^+$ *aceB*$^+$ *glc*	Succinate	22	32
		Acetate	237	96
		Succinate + acetate	75	51
KAT-1	*aceA*::Tn*10* *aceB*$^+$	Succinate	<0.5	102
		Acetate	—[a]	—
		Succinate + acetate	<0.5	249
KAT-11	*aceA*::Tn*10* *aceB*$^+$ *glc*	Succinate	<0.5	69
		Acetate	—	—
		Succinate + acetate	<0.5	198
KBT-1	*aceA*$^+$ *aceB*::Tn*10* *glc*	Succinate	<0.5	0.8
		Acetate	—	—
		Succinate + acetate	<0.5	1.0

[a] Dashes indicate no growth on this carbon source.

TABLE 3. Specific activities of glyoxylate shunt enzymes in *iclR* and *fadR* mutants

Strain	Relevant genotype	Sp act (nmol/min per mg of protein)					
		Isocitrate lyase			Malate synthase		
		Succinate	Acetate	Succinate + acetate	Succinate	Acetate	Succinate + acetate
K-12	Prototrophic	21	244	70	103	410	186
SM6034	*iclR*	209	320	276	267	594	330
RS3040	*fadR*::Tn*10*	172	272	204	371	566	389
SM6042	*iclR fadR*::Tn*10*	283	396	386	396	592	402

polarity of transposon insertions in the *ace* genes.

The relative expression of β-galactosidase in the *aceA*::Mu *d* (Ap *lac*) fusion strains paralleled the activities of isocitrate lyase and malate synthase expressed in corresponding *aceA*$^+$ strains under similar growth conditions (Tables 3 and 4). When the *aceA*::Mu *d* (Ap *lac*) fusion was present in a *fadR*$^+$ *iclR*$^+$ background, the activity of the *lacZ* gene product, β-galactosidase, was low on medium containing succinate as a sole carbon source, but was induced when grown in the presence of acetate (Table 4). The *iclR aceA*::Mu *d* (Ap *lac*) strain grown on succinate expressed levels of β-galactosidase equivalent to that of the *iclR*$^+$ *fadR*$^+$ *aceA*::Mu *d* (Ap *lac*) strain grown in the presence of acetate. These data indicate that induction of the *ace* operon by the *iclR* gene occurs at the level of transcription.

When the *aceA*::Mu *d* (Ap *lac*) fusion was present in a *fadR* background, the activity of β-galactosidase was elevated during growth on succinate, but further doubled after growth in the presence of acetate (Table 4). The β-galactosidase activity expressed by the *aceA*::Mu *d* (Ap *lac*) fusion in a *fadR iclR* double mutant was considerably higher than that of either the *fadR* or *iclR* strain. In addition, the β-galactosidase activity of the *fadR iclR aceA*::Mu *d* (Ap *lac*) strain was equivalent after growth on succinate or succinate plus acetate (Table 4). These data are consistent with a role of the *fadR* gene in the transcriptional control of the *ace* operon. Furthermore, the increased expression of the *ace* operon in the *fadR* strain after growth on acetate (18) and the greater expression of the *ace* operon in the *fadR iclR* double mutant suggest that the *iclR* and *fadR* genes may independently affect the expression of the *ace* operon.

Construction of strains merodiploid for the *iclR* gene. As shown in Fig. 2, the *iclR* gene maps near the *ace* operon at 90 min on the *E. coli* chromosome (1) and is closely linked to the *metA* gene. To examine dominance between *iclR* alleles, we constructed a series of strains merodiploid for the genes in the 90-min region. Several F-primes previously isolated and characterized by Low (9) reportedly carry chromosomal genes from this region. Three F-prime factors, KFL10, KFL11, and KFL12, were shown to complement *metA* and *aceA* mutations. The F-prime KFL12 allowed only slow growth of a host strain bearing a *metA* mutation and was prone to rapid segregation. The F-primes KFL11 and KFL10 both allowed rapid growth of host strains and segregated less readily under selective growth conditions. Since the F-prime in KFL11 was reported to be over twice the size of that in KFL10, all further studies were performed with KFL10. When inserted into strain JM683, which is deleted for the region

TABLE 4. Mu *d* (Ap *lac*) operon fusions with the *aceA* gene

Strain	Relevant genotype	Growth conditions	β-Galactosidase activity (U)[a]	Sp act (nmol/min per mg of protein)	
				Isocitrate lyase	Malate synthase
TL1	*iclR*$^+$ *fadR*$^+$ Δ*lacZ*	Succinate	<1	33	125
		Succinate + acetate	<1	151	317
		Acetate	<1	270	584
SMUD-1	*aceA*::Mu *d*(Ap *lac*) *iclR*$^+$	Succinate	20	<0.5	87
	fadR$^+$ Δ*lacZ*	Succinate + acetate	310	<0.5	368
SMUD-2	*aceA*::Mu *d*(Ap *lac*) *iclR*	Succinate	300	<0.5	273
	fadR$^+$ Δ*lacZ*	Succinate + acetate	310	<0.5	377
SMUD-3	*aceA*::Mu *d* (Ap *lac*) *iclR*$^+$	Succinate	200	<0.5	341
	fadR Δ*lacZ*	Succinate + acetate	440	<0.5	429
SMUD-4	*aceA*::Mu *d*(Ap *lac*) *iclR*	Succinate	650	<0.5	514
	fadR Δ*lacZ*	Succinate + acetate	700	<0.5	582

[a] β-Galactosidase activity expressed in units as defined by Miller (15).

containing the *ace* operon, KFL10 allowed expression of the *ace* operon in an inducible manner (data not shown). The latter results suggest that this episome carried the *aceA*$^+$, *aceB*$^+$, and *iclR*$^+$ genes. The absence of the *iclR*$^+$ gene in strain JM683 was later confirmed with an F-prime bearing the genes *metA*$^+$ *aceA*$^+$ *aceB*$^+$ *iclR*; this strain expressed the *aceA* and *aceB* gene products constitutively. When cured of the episome, strain JM683 reverted to the Ace$^-$ phenotype.

The manner of construction of the merodiploids used in the *iclR* dominance tests is shown in Table 5. In all strains, the host chromosome harbored an auxotrophic mutation in the *metA* gene which was complemented by the respective wild-type allele on the F-prime. This arrangement facilitated strain construction and prevented segregation of the episome when the merodiploids were grown in the absence of methionine. The host strains were all *recA* derivatives of JC1552 which differed only in markers related to the merodiploid analysis (Table 5).

The merodiploid strains constructed were all *recA* and therefore defective in homologous recombination between the F-prime and the main chromosome. To prove the diploidy of the *iclR* gene in these stable merodiploids, we examined the phenotypes of monoploid segregant strains obtained by acridine orange treatment. Segregants were recognized by an auxotrophic requirement for methionine as well as an inability to transfer markers in further matings. All segregants were found to be phenotypically identical to the monoploid host strain. In addition, each merodiploid was shown to be able to transfer episomal markers in matings with appropriate *recA* recipients (data not shown). This evidence indicates that the merodiploids were, in fact, diploid for the *iclR* gene.

Expression of the *ace* operon in strains merodiploid for the *iclR* locus. In wild-type *E. coli*, the glyoxylate shunt enzymes are induced only during growth in the presence of acetate. In contrast, the glyoxylate shunt enzymes are expressed constitutively in *iclR* mutants. Thus, we sought to determine whether the *iclR* gene regulates the *ace* operon in a *cis*- or *trans*-dominant manner. The data in Table 6 indicate that, whenever at least one copy of the *iclR*$^+$ gene was present, whether on the F-prime or the chromosome, the expression of the *aceA* gene was inducible by growth in the presence of acetate. This was true whether the *iclR*$^+$ gene was *cis* or *trans* to the *aceA*$^+$ allele. Only strains with no copies of the *iclR*$^+$ allele expressed the *aceA* gene constitutively. This finding implies that the *iclR*$^+$ allele is *trans*-dominant to the *iclR* allele.

Expression of the *ace* operon in strains merodiploid for the *fadR* locus. The distant map location of the *fadR* gene, as well as its known role in the control of the *fad* regulon by producing a negatively acting regulatory protein, suggested that the transcriptional control of the *ace* operon by the *fadR* gene occurred in a *trans*-dominant manner. However, to confirm this, we studied the effects of mutants merodiploid for the *fadR* locus on the expression of the *ace* operon. The construction and characterization of *fadR* merodiploids have been previously described (20). As shown in Table 7, whenever a single copy of the *fadR*$^+$ allele was present, whether on the episome or the chromosome, the glyoxylate shunt enzymes were regulated normally (i.e., induced by growth on acetate). Only when there were no *fadR*$^+$ alleles present (M2-5-S and M23-1) was the expression of the glyoxylate shunt elevated under noninducing growth conditions. This is consistent with a *trans*-dominant behavior of the *fadR*$^+$ allele.

TABLE 5. Construction of merodiploids used for testing dominance of the *iclR* locus

Strain	Donor	Recipient	Selected markers	Relevant genotype of merodiploid[a]
MM-1				0/*aceA*$^+$ *iclR*$^+$
MM-2				0/*iclR*
MM-3				0/*aceA*
MM-4				0/*aceA iclR*
MM-5	KFL10	MM-1	Met$^+$ Str^r	F'*aceA*$^+$ *iclR*$^+$/*aceA*$^+$ *iclR*$^+$
MM-6	KFL10	MM-3	Ace$^+$ Met$^+$ Str$^+$	F'*aceA*$^+$ *iclR*$^+$/*aceA iclR*$^+$
MM-7	KFL10	MM-4	Ace$^+$ Met$^+$ Str^r	F'*aceA*$^+$ *iclR*$^+$/*aceA iclR*
MM-8	SM6009	MM-1	Met$^+$ Str^r	F'*aceA iclR*$^+$/*aceA*$^+$ *iclR*$^+$
MM-9	SM6009	MM-2	Met$^+$ Str^r	F'*aceA iclR*$^+$/*aceA*$^+$ *iclR*
MM-10	SM6034	MM-3	Ace$^+$ Met$^+$ Str^r	F'*aceA*$^+$ *iclR*/*aceA iclR*$^+$
MM-11	SM6034	MM-2	Met$^+$ Str^r	F'*aceA*$^+$ *iclR*/*aceA*$^+$ *iclR*
MM-12	K8-5M	MM-1	Met$^+$ Str$^+$	F'*aceA iclR*/*aceA*$^+$ *iclR*$^+$
MM-13	K8-5M	MM-2	Met$^+$ Str^r	F'*aceA iclR*/*aceA*$^+$ *iclR*

[a] Genotype indicated by episomal alleles/chromosomal alleles. A zero indicates that no episome was present.

TABLE 6. Specific activity of isocitrate lyase in strains merodiploid for the *iclR* gene

Strain[a]	Relevant genotype	Isocitrate lyase activity (nmol/min per mg of protein)		
		Succinate	Succinate + acetate	Acetate
MM-1	0/*aceA*$^+$ *iclR*$^+$	21	98	316
MM-2	0/*aceA*$^+$ *iclR*	206	299	355
MM-4	0/*aceA iclR*	<0.5	<0.5	<0.5
MM-5	F'*aceA*$^+$ *iclR*$^+$/*aceA*$^+$ *iclR*$^+$	31	50	114
MM-6	F'*aceA*$^+$ *iclR*$^+$/*aceA iclR*$^+$	18	45	206
MM-7	F'*aceA*$^+$ *iclR*$^+$/*aceA iclR*	52	79	305
MM-8	F'*aceA iclR*$^+$/*aceA*$^+$ *iclR*$^+$	3	31	209
MM-9	F'*aceA iclR*$^+$/*aceA*$^+$ *iclR*	19	69	276
MM-10	F'*aceA*$^+$ *iclR*/*aceA iclR*$^+$	23	74	298
MM-11	F'*aceA*$^+$ *iclR*/*aceA*$^+$ *iclR*	292	326	422
MM-12	F'*aceA iclR*/*aceA*$^+$ *iclR*$^+$	17	81	310
MM-13	F'*aceA iclR*/*aceA*$^+$ *iclR*	269	285	387

[a] Construction of *iclR* merodiploids is shown in Table 4.

DISCUSSION

The genes for the glyoxylate shunt enzymes, *aceA* and *aceB*, map at 90 min on the *E. coli* K-12 chromosome (Fig. 2). Since the *aceA* and *aceB* genes are very closely linked genetically and their expression is coordinately controlled, it has been proposed that they may form an operon (2). Polar transposon insertions in the *aceB* gene eliminate expression of both the *aceB* and *aceA* genes (Table 2), indicating that these genes are transcribed as a single, polycistronic message (4, 5). Thus, the *aceA* and *aceB* genes comprise an operon, and the direction of transcription is from the *aceB* gene to the *aceA* gene.

Expression of the *ace* operon is under the transcriptional control of two genes, *iclR* and *fadR* (Tables 3 and 4). The *iclR* gene maps near the *ace* operon in the 90-min region (Fig. 2), and the *fadR* gene maps at 25 min (19). The *cis*/*trans* tests for both the *iclR* and *fadR* genes indicate that the wild-type alleles are *trans*-dominant to the mutant alleles (Tables 6 and 7). This is most simply interpreted as the production of diffusible, negatively acting regulatory elements or repressors by both the *iclR* and *fadR* genes (4, 19). The *fadR* gene has been previously shown to act as a repressor for the *fad* regulon (19). The *fad* regulon is induced during growth on long-chain fatty acids ($\geq C_{11}$) (17, 19, 20). The glyoxylate shunt enzymes are not directly induced by acetate or acetyl-CoA (6, 11). On the basis of genetic studies, Kornberg (6) proposed that the glyoxylate shunt enzymes are derepressed by low levels of pyruvate and phosphoenolpyruvate that result from growth on acetate or fatty acids

TABLE 7. Specific activities of glyoxylate shunt enzymes in strains merodiploid for the *fadR* gene

Strain	*fadR* genotype[a]	Growth conditions	Sp act (nmol/min per mg of protein)	
			Isocitrate lyase	Malate synthase
M11-1-S	0/+	Succinate	11	42
		Acetate	95	198
		Oleate	125	253
M2-5-S	0/−	Succinate	81	111
		Acetate	106	315
		Oleate	128	320
M11-1	+/+	Succinate	7	36
		Acetate	84	183
		Oleate	83	238
M12-1	+/−	Succinate	12	45
		Acetate	64	205
		Oleate	121	284
M47-1	−/+	Succinate	18	52
		Acetate	60	210
		Oleate	146	277
M23-1	−/−	Succinate	62	125
		Acetate	95	329
		Oleate	122	336

[a] *fadR* genotype shown as episomal allele/chromosomal allele. A zero indicates that no episome was present.

as a sole carbon source. However, more thorough biochemical studies have shown that this cannot fully account for the regulation of the glyoxylate shunt enzymes (7, 10). Thus, the intracellular signal for induction of the glyoxylate shunt during growth on acetate remains to be elucidated.

Both *iclR* and *fadR* mutants expressed elevated levels of *ace* enzymes during growth on the noninducing carbon source succinate (Table 3 and 4). In this case, expression of the *ace* operon was even greater in *iclR fadR* double mutants (Tables 3 and 4). Although several models could be proposed from these data, the simplest interpretation of these results is that the *iclR* and *fadR* gene products act independently to cause the partial repression of the *ace* operon; acting together, they cause the full repression of the *ace* operon.

Although several regulatory systems have been shown to be controlled by multiple positive effectors (4), no well-characterized bacterial operons have been shown to be controlled by multiple repressor proteins. However, in bacteriophage lambda, the binding of two negatively acting regulatory proteins, cI repressor and Cro protein, to the same control region determines the infective pathway utilized (8). The evolution of dual repressor control of the *ace* operon may have conferred a metabolic advantage to cells required to grow on acetate or fatty acids as a carbon source. During growth on acetate, a substantial portion of the intracellular acetyl-CoA must be fully oxidized by the complete tricarboxylic acid cycle to generate metabolic energy. On the other hand, during growth on fatty acids a considerable amount of energy is generated from the breakdown of fatty acids to acetyl-CoA. Therefore, less acetyl-CoA is needed to pass through the complete tricarboxylic acid cycle for energy production, and a greater amount of the acetyl-CoA can be shunted through the glyoxylate bypass for anabolic purposes. A dual repressor control mechanism for the *ace* operon may allow different levels of expression of the glyoxylate shunt enzymes under these conditions.

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