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Regulation of Fatty Acid Degradation in *Escherichia coli*: *fadR* Superrepressor Mutants Are Unable to Utilize Fatty Acids as the Sole Carbon Source

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Localized mutagenesis of the *fadR* region of the *Escherichia coli* chromosome resulted in the isolation of two classes of *fadR* regulatory mutants. The first class was constitutive for the fatty acid degradative enzymes and presumably defective for *fadR* function. The second class was rarer and resulted in the inability to utilize fatty acids as a sole carbon source (Fad$^-$). These *fadR* superrepressor mutants [*fadR*(S)] had greatly reduced levels of the β-oxidative enzymes required for growth on fatty acids. The *fadR*(S) mutants reverted to Fad$^+$ at a high frequency (10^{-5}), and the resulting Fad$^+$ revertants were constitutive for expression of the *fad* enzymes (*fadR*). Merodiploid analysis showed the *fadR*(S) allele to be dominant to both *fadR*$^+$ and *fadR* alleles.

Escherichia coli can utilize long-chain fatty acids (C_{12} to C_{18}) as sole carbon and energy sources (16, 17, 21). Long-chain fatty acids such as oleic acid ($C_{18:1}$) induce the fatty acid degradative enzymes required for β-oxidation (8). The *fad* enzymes are encoded in four unlinked operons which are coordinately regulated (1, 8, 13, 16). In the absence of inducer, transcription of the *fad* operons is repressed by the product of the *fadR* gene, most likely a diffusible repressor protein (19, 20). The *fad* enzymes can also utilize medium-chain fatty acids (C_6 to C_{11}) as substrates. However, wild-type *E. coli* does not grow on medium-chain fatty acids as the sole carbon source because they do not induce the *fad* regulon (16, 21). Mutants able to grow on medium-chain fatty acids such as decanoate (C_{10}) arise at high frequency (10^{-5}) and are the result of mutations in the *fadR* gene (16, 19, 21). These *fadR* mutants are constitutive for *fad* regulon expression, leading Overath et al. to propose that *fadR* encodes a diffusible repressor protein (16).

Since the initial isolation of *fadR* mutants (16), more recent studies have been published which support the model that *fadR* encodes a diffusible repressor protein: the *fadR* gene has been precisely mapped to 25.7 min on the *E. coli* chromosome and is unlinked to all the structural genes of the *fad* regulon (1, 19); insertions of transposons Tn*10* and Tn*5* into the *fadR* gene have been isolated and result in constitutive expression of the *fad* regulon (19); temperature-sensitive *fadR* mutants have been isolated which are inducible (FadR$^+$) at 30°C and constitutive (FadR$^-$) at elevated temperatures (19); F-prime complementation experiments demonstrated that *fadR*$^+$ is dominant to *fadR* in *trans* (20); and finally, the *fadR* gene has been cloned and found to encode a 29,000-dalton protein required for functional FadR activity (5).

In this study we report a novel class of *fadR* mutants that are unable to grow on fatty acids (Fad$^-$). Following hydroxylamine mutagenesis, these *fadR* superrepressor [*fadR*(S)] mutants arose less frequently than *fadR* mutants. The *fadR*(S) mutants exhibited low, uninducible levels of the *fad* enzymes. Revertants of the *fadR*(S) mutants able to grow on oleic acid also acquired a *fadR* phenotype (ability to grow on decanoate). In merodiploid studies, the *fadR*(S) allele was dominant to both *fadR* and *fadR*$^+$ alleles. The isolation of *fadR*(S) mutants strongly supports the model that the *fadR* gene encodes a classical diffusible repressor protein which exerts negative control on the *fad* regulon.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study were derived from *E. coli* K-12 and are listed in Table 1.

Media. The E medium of Vogel and Bonner (23) was used as the minimal medium. M9 minimal medium (11) was used to grow cell cultures used in enzyme assays. Luria-Bertani (LB) medium, containing (in grams per liter) Bacto-tryptone (Difco Laboratories), 10; yeast extract (Difco), 5; and NaCl, 5; was used as rich medium (4). Agar (Difco) was added to a final concentration of 1.5% for solid medium. Fatty acids (Sigma Chemical Co.) were added at 5 mM in the presence of Brij-58 (Sigma, 5 mg/ml); other carbon sources were added to 0.2%. Auxotrophic requirements were added as suggested by Davis et al. (4). Tetracycline hydrochloride (Sigma) was used at 20 μg/ml in rich medium or 10 μg/ml in minimal medium. Kanamycin sulfate (Sigma) was used at 40 μg/ml.

Transductional methods. The bacteriophage P1 *vir* was used for all transductional crosses. Transductants were purified by two successive single-colony isolations on non-selective medium. Phage lysates and transductional crosses were prepared essentially as described by Silhavy et al. (18) with alterations. For phage lysate preparations, a 0.6-ml portion of an overnight culture was diluted into 10 ml of fresh LB medium and incubated with shaking at 37°C. After 1 h, 0.1 ml of 0.5 M $CaCl_2$ was added, and the culture was returned to the incubator. After 5 min more, 0.1 ml of a P1 *vir* lysate (ca. 10^9 PFU/ml) was added, and the culture was left at room temperature for 5 min without shaking. The culture was then returned to the 37°C shaking incubator. After 2 to 3 h, lysis was complete (optical density less than 30 Klett units); the debris was removed by centrifugation,

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TABLE 1. List of strains[a]

Strain	Sex	Genotype	Source or reference
GE999	F^-	Δ*recA araD139* Δ*lacU169 rpsL150 relA1 flbB5301 ptsF25 deoC1*	G. Weinstock
H680	F^-	*purB51 trp-45 his-68 tyrA2 thi-1 lacY1 gal-6 malA1 xyl-7 mH-2 rpsL125 tonA2 tsx-70 supE44* λ^-	P. de Haan via CGSC[b]
JK268	F^-	*purB58 trpA62 trpE61 dadR1 tna-5* λ^-	J. Kuhn via CGSC
K-12	F^+	Prototroph	J. Lederberg via CGSC
RS3010	F^+	*fadR1*	15
RS5051 (W3)	F′	F′125/H680 *his*$^+$ *nalA13 fadR17*::Tn*5 srlA300*::Tn*10 recA56*	16
RS5116 (M47)	F′	F′125/H680 *tyr*$^+$ *srlA300*::Tn*10 recA56*	16
RS5117 (M48)	F′	F′125/H680 *tyr*$^+$ *srlA300*::Tn*10 recA56*	16
RS5124 (M63)	F′	F′125/H680 *tyr*$^+$ *srlA300*::Tn*10 recA56 fadR17*::Tn*5*	16
SASX41B	Hfr	*hemA41 metB1 relA1*	A. Sasarman via CGSC
TH161	F^-	Prototroph	
TH181	F^-	*zcf-2039*::Tn*10*	
TH182	F^-	*zcf-2039*::Tn*10 fadR250*(S)	
TH183	F^-	*zcf-2039*::Tn*10 fadR251*(S)	
TH184	F^-	*zcf-2039*::Tn*10 fadR252*	
TH185	F^-	*zcf-2039*::Tn*10 fadR253*	
TH186	F^-	*zcf-2039*::Tn*10 fadR254*	
TH400	F^-	*srlA300*::Tn*10*	
TH936	F^-	H680 *tyr*$^+$	
TH937	F^-	H680 *tyr*$^+$ *zcf-2039*::Tn*10*	
TH938	F^-	H680 *tyr*$^+$ *zcf-2039*::Tn*10 fadR250*(S)	
TH939	F^-	H680 *tyr*$^+$ *zcf-2039*::Tn*10 fadR251*(S)	
TH940	F^-	H680 *tyr*$^+$ *zcf-2039*::Tn*10 fadR252*	
TH941	F^-	Δ*srlA300*::Tn*10* (Tc^s)	
TH942	F^-	Δ*srlA300*::Tn*10 purB51 zcf-2039*::Tn*10*	
TH943	F^-	Δ*srlA300*::Tn*10 purB51 zcf-2039*::Tn*10 fadR250*(S)	
TH944	F^-	Δ*srlA300*::Tn*10 purB51 zcf-2039*::Tn*10 fadR251*(S)	
TH945	F^-	Δ*srlA300*::Tn*10 purB51 zcf-2039*::Tn*10 fadR252*	
TH956	F^-	Δ*recA purB51 zcf-2039*::Tn*10*	
TH957	F^-	Δ*recA purB51 zcf-2039*::Tn*10 fadR250*(S)	
TH958	F^-	Δ*recA purB51 zcf-2039*::Tn*10 fadR251*(S)	
TH959	F^-	Δ*recA purB51 zcf-2039*::Tn*10 fadR252*	
TH960	F′	F′125/TH956	
TH961	F′	F′125/TH957	
TH962	F′	F′125/TH958	
TH963	F′	F′125/TH959	
TH964	F′	F′125 *fadR23*/TH956	
TH965	F′	F′125 *fadR23*/TH957	
TH966	F′	F′125 *fadR23*/TH958	
TH967	F′	F′125 *fadR23*/TH959	
TH968	F′	F′125 *fadR24*/TH956	
TH969	F′	F′125 *fadR24*/TH957	
TH970	F′	F′125 *fadR24*/TH958	
TH971	F′	F′125 *fadR24*/TH959	
TH972	F′	F′125 *fadR1*/TH956	
TH973	F′	F′125 *fadR1*/TH957	
TH974	F′	F′125 *fadR1*/TH958	
TH975	F′	F′125 *fadR1*/TH959	

[a] Unless indicated otherwise, all strains were constructed during the course of this work.
[b] CGSC, Coli Genetic Stock Center.

0.1 ml of 1 M $MgCl_2$ was added, and the lysate was stored at 4°C over $CHCl_3$.

Conjugational methods. F^- recipient strains were grown overnight in LB medium. F-prime donor strains were grown overnight in selective medium. Plate matings were performed as described by Miller (11). Exconjugants were purified by two successive single-colony isolations on selective medium. Haploid segregants were isolated as spontaneous segregants.

Transposon mutagenesis. Transposon Tn*10* mutants were isolated as described previously (7, 19). P1 *vir* phage was grown on pools of 10,000 random Tn*10* insertion mutants. These pooled phage stocks were used to transduce cultures of strains JK268 (Table 1; *trp purB*) and SASX41B (*hemA*) to tetracycline resistance (Tet^r) and either *purB*$^+$ or *hemA*$^+$, respectively. This selects for Tet^r transductants which had coinherited the *purB*$^+$ allele or the *hemA*$^+$ allele with the Tn*10* insertion element. Linkage between these localized

Tn*10* insertions and the *purB*, *hemA*, *fadR*, and *trp* genes was determined by cotransductional analysis. For insertions near *fadR*, the order with respect to nearby genes was determined by three-factor transductional crosses. Localized insertions of Tn*5* were isolated in an analogous fashion, selecting for kanamycin resistance.

Hydroxylamine mutagenesis. Hydroxylamine mutagenesis of bacteriophage P1 *vir* was done essentially as described by Hong and Ames (6).

Biochemical procedures. In vitro activities of the β-oxidative enzymes were measured on French press whole-cell extracts. The cell extract and assay procedures were performed as described (8). Protein concentrations were determined with a Coomassie dye-binding assay (3) with commercial reagents (Bio-Rad Laboratories), against a bovine serum albumin (Sigma) standard.

Indicator plates. A modification of the 2,3,5-triphenyl tetrazolium chloride (TTC; Sigma) indicator medium developed by Bochner and Savageau (2) was used to distinguish between *fadR*$^+$ and *fadR* mutants and between *fadR*$^{+/-}$ and *fadR*(S) (Fad$^-$) mutants. This medium distinguishes carbon source utilizers from nonutilizers on the basis of their ability to reduce the TTC indicator. To detect *fadR* mutants, plates contained potassium decanoate (5 mM), Brij-58 (5 mg/ml), TTC (0.0025%), protease peptone (0.2%), and agar (1.5%; Difco) in minimal E medium (19). Colonies able to metabolize decanoate (*fadR*) were red in this medium, while colonies which could only metabolize the peptone (*fadR*$^+$) were white. To detect *fadR*(S) mutants (Fad$^-$), plates contained potassium oleate (10 mM), Brij-58 (10 mg/ml), TTC (0.005%), protease peptone (0.2%), and agar (1.5%) in minimal E medium. Colonies able to metabolize oleate (Fad$^+$) were red, while colonies unable to metabolize oleate (Fad$^-$) were white.

RESULTS

Isolation and characterization of transposon insertions in the *fadR* region. To facilitate the isolation and characterization of noninducible *fadR* mutants, we isolated Tn*10* (and Tn*5*) insertions tightly linked to the *fadR* gene. The procedure was as follows. P1 *vir* phage was grown on pools of random Tn*10* or Tn*5* insertion mutants (19). The insertions linked to *fadR* were initially isolated by transducing a *purB* mutant (JK268) to *purB*$^+$ or a *hemA* mutant (SASX41B) to *hemA*$^+$ with P1 phage stocks grown on the insertion pools. Those prototrophic transductants for either the *purB* or *hemA* markers which had coinherited either Tet^r from Tn*10* or Kan^r from Tn*5* were kept as putative insertions linked to the *fadR* gene. The positions of the Tn*5* and Tn*10* insertions relative to other genetic markers in the *fadR* region were obtained by three-factor transductional cross mapping (data not shown). These insertions are summarized in Fig. 1. One of the Tn*10* insertions, *zcf-2039*::Tn*10*, was found to be 98% cotransducible with the *fadR* locus, between *purB* and *fadR*, and was chosen for use in localized mutagenesis of the *fadR* region.

Isolation of *fadR* and *fadR*(S) mutants by localized mutagenesis. The results of all previous work on the *fadR* gene are consistent with the model that the *fadR* gene product is a diffusible, inducible repressor protein which negatively regulates the *fad* regulon. This model predicts the isolation of a class of *fadR* mutants [*fadR*(S)] unable to recognize the inducer, analogous to the noninducible alleles of the LacI repressor (22). Such *fadR*(S) mutations would map to the *fadR* locus, manifest noninducible levels of the β-oxidative enzymes (Fad$^-$), and be at least partially dominant to both *fadR*$^+$ and *fadR* alleles.

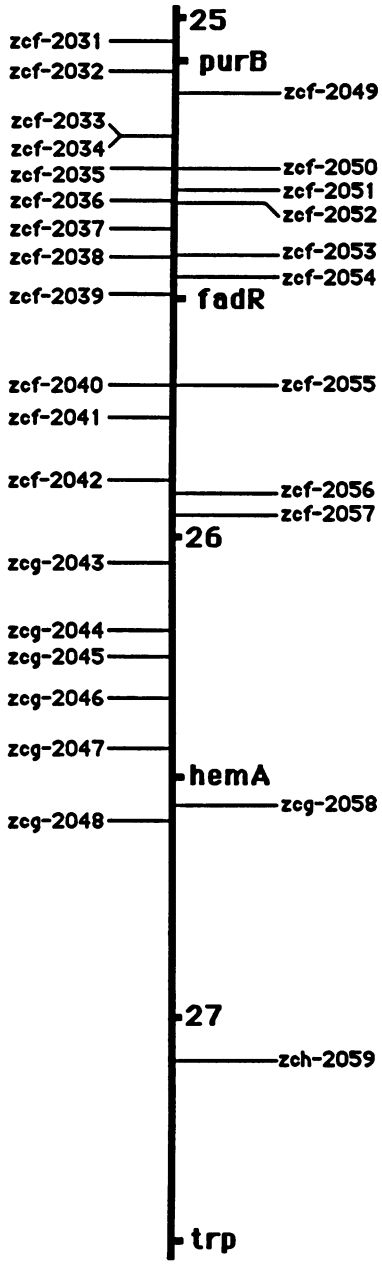

FIG. 1. Map of Tn*10* (*zcf-2031* to *zcg-2048*) and Tn*5* (*zcf-2049* to *zch-2059*) insertions near the *fadR* gene. The order of insertions with respect to nearby markers was determined by three-factor-cross analysis (data not shown). Genetic distances (shown in minutes) were estimated from P1 cotransduction frequencies (1).

To facilitate the isolation of Fad$^-$ mutants in the FadR protein, we developed a modified version of the carbon source utilization indicator medium developed by Bochner and Savageau (2) (see Materials and Methods). This indicator medium distinguishes carbon source utilizers from nonutilizers on the basis of their ability to reduce the dye TTC. These indicator plates have two carbon sources, protease peptone and fatty acid. Cells able to utilize only protease

TABLE 2. Growth properties of *fadR* mutants in minimal E medium with different carbon sources at 37°C

Strain	Relevant genotype	Growth on:			
		Dextrose	Acetate	Decanoate	Oleate
TH181	*fadR*$^+$	+	+	−	+
TH182	*fadR250*(S)	+	+	−	−
TH183	*fadR251*(S)	+	+	−	−[a]
TH184	*fadR252*	+	+	+	+
TH185	*fadR253*	+	+	+	+
TH186	*fadR254*	+	+	+	+

[a] Slight growth was observed with this strain after 2 days of incubation at 37°C.

peptone as a sole carbon and energy source manifest low reductive capacity and form white colonies. When cells are able to utilize the better fatty acid carbon source, their reductive capacity increases and they are able to reduce the TTC indicator and form red colonies. We modified the original recipe to improve the indication of utilization of the medium-chain fatty acid decanoate (C_{10}) (19) or the long-chain fatty acid oleate ($C_{18:1}$). The plates readily distinguish the relevant Fad phenotypes: *fadR*$^+$ colonies are red on TTC-oleate plates, but white on TTC-decanoate plates (decanoate is unable to induce the *fad* genes); *fadR* colonies (*fad* gene constitutive) are red on both plates; Fad$^-$ colonies [including putative *fadR*(S) mutants] are white on both.

To obtain Fad$^-$ mutations in the *fadR* region, P1 phage grown on strain TH181 (*fadR*$^+$ *zcf-2039*::Tn*10*) was mutagenized with hydroxylamine to 0.2% phage survival by the procedure of Hong and Ames (6). This mutagenized phage stock was then used to transduce wild-type strain TH161 to Tet^r on either TTC-decanoate or TTC-oleate indicator plates. On TTC-decanoate indicator plates, red colonies appeared at a frequency of about 1% of the total Tet^r transductants. Ten of these transductants were purified and shown to be *fadR*: each had a Dec$^+$ phenotype and was >97% cotransducible with *zcf-2039*::Tn*10*. From ~30,000 Tet^r transductants on TTC-oleate plates, ~200 white colonies were picked. Of these 200, only 3 remained white when retested on the TTC-oleate indicator plates. One of the three was Ole$^-$ on solid medium but Ole$^+$ in liquid medium. This unusual mutation was not linked to the Tn*10* insertion and was not characterized further. The other two mutants were Ole$^-$ in both liquid and solid media, and their Fad$^-$ mutations were ~98% linked to *zcf-2039*::Tn*10*. These two putative *fadR*(S) mutants (*fadR250* and *fadR251*) were characterized further.

Initial characterization of the *fadR*(S) mutants. The growth characteristics of isogenic *fadR*$^+$, *fadR*, and *fadR*(S) strains are shown in Table 2. While the data shown are for growth on solid medium, similar results were obtained for either solid or liquid medium. All strains grew normally with dextrose or acetate as the sole carbon source. The *fadR* mutants were able to grow on decanoate, indicating constitutive expression of the *fad* regulon (16, 19, 21), and *fadR*$^+$ or *fadR* mutants utilized oleate as a sole carbon source. In contrast, the *fadR250*(S) and *fadR251*(S) alleles showed little or no growth on oleate or decanoate, a novel phenotype for mutations in the *fadR* gene. The *fadR251*(S) allele showed very slight growth on oleate after 48 h, whereas *fadR250*(S) showed none. It is likely that these are two distinct *fadR*(S) alleles. Both of the *fadR*(S) alleles gave rise to Ole$^+$ derivatives when selected on oleate as the sole carbon source. We examined 10 independent Ole$^+$ derivatives of each allele and found all to be Dec$^+$, consistent with a total loss of *fadR* function in these derivatives.

Biochemical studies. To show that the *fadR*(S) mutants were defective in induction of the *fad* enzymes, we measured the acyl coenzyme A (CoA) dehydrogenase, hydroxylacyl-CoA dehydrogenase, and thiolase enzyme activities in crude extracts of *fadR*$^+$, *fadR* and *fadR*(S) mutant strains (Table 3). The *fadR*$^+$ strain had normal basal levels of the enzyme activities when grown on acetate. These activities were induced when the strain was grown on a combination of acetate and oleate as carbon sources. The *fadR* mutant had constitutive levels of enzyme activities. However, the *fadR*(S) mutants had uninducible levels of these activities that were ca. twofold lower than the uninduced levels of the *fadR*$^+$ strain. This may be due to tighter binding of FadR(S) to the *fad* operators or because the presence of endogenous inducer does not affect the bound FadR(S) repressor.

Dominance studies. Merodiploid strains carrying various combinations of episomal and chromosomal *fadR* alleles were constructed and their growth properties were characterized. For these constructions, we used previously characterized F'125 (9) episomes carrying *fadR*$^+$ or *fadR* alleles (20). The results of these dominance studies are shown in Table 4. The FadR$^+$ phenotype of the F'125 *fadR*$^+$/*fadR* and the F'125 *fadR*/*fadR*$^+$ merodiploids demonstrated that *fadR*$^+$ was dominant to *fadR*, as shown previously (20): they grew on oleate but not on decanoate as a sole carbon source. As expected, the *fadR*$^+$/*fadR*$^+$ and *fadR*/*fadR* merodiploids were phenotypically FadR$^+$ (Ole$^+$ Dec$^-$) and FadR (Ole$^+$ Dec$^+$), respectively. In contrast, *fadR*(S)/*fadR*$^+$ and *fadR*(S)/*fadR* merodiploids were unable to grow on either oleate or decanoate. The *fadR*$^+$/*fadR*(S) and the *fadR*/*fadR*(S) mero-

TABLE 3. Enzyme assays

Strain	Relevant genotype	Carbon source[a]	Sp act (nmol/min per mg of protein)		
			Palmitoyl-CoA dehydrogenase	β-Hydroxybutyryl-CoA dehydrogenase	Acetoacetyl-CoA thiolase
TH181	*fadR*$^+$	Ace	1.1 ± 0.1	41 ± 1	3.1 ± 0.7
		Ace + ole	3.8 ± 0.1	160 ± 30	31 ± 1
RS3010	*fadR1*	Ace	4.6 ± 0.5	240 ± 70	70 ± 1
		Ace + ole	5.5 ± 0.4	360 ± 10	190 ± 10
TH182	*fadR250*(S)	Ace	0.64 ± 0.05	22 ± 2	1.9 ± 0.1
		Ace + ole	0.83 ± 0.02	18 ± 3	5.5 ± 0.8
TH183	*fadR251*(S)	Ace	0.52 ± 0.04	31 ± 10	2.9 ± 0.2
		Ace + ole	0.34 ± 0.07	13 ± 6	1.7 ± 0.2

[a] Cells were grown in medium M9 supplemented with carbon sources at the following concentrations: potassium acetate (ace), 80 mM; potassium oleate (ole), 5 mM. All cultures contained Brij 58 (5 mg/ml).

TABLE 4. Dominance studies

Strain	Relevant genotype[a]	Growth on:		
		Dextrose	Decanoate	Oleate
TH960	*fadR*$^+$/*fadR*$^+$	+	−	+
TH961	*fadR*$^+$/*fadR250*(S)	+	−	−
TH962	*fadR*$^+$/*fadR251*(S)	+	−	−[b]
TH963	*fadR*$^+$/*fadR252*	+	−	+
TH972	*fadR1*/*fadR*$^+$	+	−	+
TH973	*fadR1*/*fadR250*(S)	+	−	−
TH974	*fadR1*/*fadR251*(S)	+	−	−[b]
TH975	*fadR1*/*fadR252*	+	+	+

[a] For *fadR1*, identical results were obtained with the *fadR23* or *fadR24* allele on the F′ strains TH964 through TH971.

[b] Slight growth was observed with this strain after 2 days of incubation at 37°C.

diploids showed the growth phenotypes of the individual *fadR*(S) alleles. The dominance of the noninducible *fadR*(S) allele to both *fadR*$^+$ and *fadR* confirmed that *fadR* encodes a diffusible repressor molecule.

DISCUSSION

The results of this study bear directly on the question of how the FadR protein regulates expression of the *fad* regulon. All previous studies are consistent with the model that the FadR protein is a repressor protein: the *fadR* gene is genetically unlinked to any known *fad* structural gene and encodes a 29,000-dalton protein (5); *fadR* insertion mutants are constitutive (19); *fadR*(Ts) mutants are constitutive at the nonpermissive temperature (19); and *fadR*$^+$ is dominant to *fadR* (20). Presumably, the FadR protein contains at least two functional domains: a DNA-binding domain that recognizes the multiple operators of the *fad* regulon, and an allosteric domain or site that recognizes the *fad* regulon inducers, presumably long-chain fatty acids (of which oleate is typical). It is likely that at least some of the recessive *fadR* mutations isolated to date have defects in their DNA-binding domains, although this has not been examined.

In this paper we report the isolation of a new class of *fadR* mutants, termed *fadR*(S), by in vitro hydroxylamine mutagenesis of a Tn*10*-linked *fadR* gene. These *fadR*(S) mutants had the phenotypes expected for mutations that would abolish the FadR repressor's allosteric site but not its DNA-binding domain: inability to utilize medium- or long-chain fatty acids as a sole carbon source (Fad$^-$), noninducibility of the activities of the *fad* regulon, and genetic dominance to both *fadR*$^+$ (inducible) and *fadR* (constitutive) alleles. These are the strongest genetic results to date that argue a direct interaction between the FadR protein and the inducer molecules, although they do not rule out a less direct induction mechanism. Further examination of both the inducer recognition and DNA-binding activities of the FadR protein must now await biochemical analysis.

Although these results imply that the FadR protein is a "classical" diffusible repressor protein, it is important to note that the FadR protein affects intermediary metabolism beyond simple repression of the *fad* regulon. The FadR protein is a corepressor of the *aceAB* operon, which encodes the structural genes for the glyoxylate shunt pathway required for the utilization of acetate (the product of fatty acid oxidation) as a sole carbon source (10). In addition, *fadR* mutations have pleiotropic effects on both fatty acid biosynthesis and short-chain fatty acid utilization (12, 14). Further characterization of the effects of the *fadR*(S) mutations on these different aspects of fatty acid metabolism may provide a better understanding of the mechanism by which this repressor protein is involved in the many aspects of fatty acid metabolism in *E. coli*.

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