

## Cloning and Characterization of a Gene (*fadR*) Involved in Regulation of Fatty Acid Metabolism in *Escherichia coli*

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The regulatory gene *fadR* has been previously characterized by classical genetic means as a diffusible protein which exerts negative control over fatty acid degradation and acetate metabolism. *fadR* has also been implicated in the regulation of unsaturated fatty acid biosynthesis. To facilitate the identification of the product of the *fadR* gene and to study the mechanism by which this multifunctional regulatory gene exerts its control, we cloned a segment of DNA containing the *fadR* gene in the phage vector  $\lambda$ L47. Subsequent subcloning of a segment of the chromosomal DNA from the  $\lambda$ *fadR*<sup>+</sup> phage into various plasmid vectors resulted in the isolation of the *fadR* gene on a 1.3-kilobase-pair *Hind*III-*Eco*RV fragment. *fadR* strains harboring the cloned *fadR*<sup>+</sup> gene showed inducible levels of fatty acid oxidation and crotonase (enoyl-coenzyme A-hydratase, *fadB*) activity. The cloned gene exerted transcriptional control over  $\beta$ -galactosidase synthesis in an *fadR* strain that had a  $\lambda\Phi$ (*fadE-lacZ*<sup>+</sup>) operon fusion. An *fadR* mutation in *fabA*(Ts) strains prevents growth at permissive temperatures without unsaturated fatty acid supplementation (Nunn et al., J. Bacteriol. 154:554-560, 1983). Plasmids carrying the *fadR*<sup>+</sup> gene suppress this unsaturated fatty acid auxotrophy in *fadR fabA*(Ts) strains at the permissive condition. Maxicell analysis identified a 29,000-dalton protein encoded by the 1.3-kilobase fragment which appeared to be associated with functional *fadR* gene activity.

The *fadR*<sup>+</sup> gene of *Escherichia coli* is a multifunctional regulatory gene mapping at 25.5 min (26) which appears to exert negative control over the fatty acid-degradative (*fad*) regulon (21, 26, 27) and the acetate (*ace*) operon (13, 15). The *fadR*<sup>+</sup> gene is also required for maximal expression of unsaturated fatty acid (UFA) biosynthesis (*fab*) (20). The product of the *fadR*<sup>+</sup> gene is believed to be a diffusible protein which exerts control over fatty acid degradation by decreasing the transcription of the *fad* structural genes (6, 24). These genes map at no fewer than four distinct loci on the *E. coli* chromosome (10, 21, 29) and encode at least five enzyme activities involved in the transport, acylation, and  $\beta$ -oxidation of medium-chain (C6 to C10) and long-chain (C12 to C18) fatty acids. Long-chain fatty acids can induce the *fad* enzymes, whereas medium-chain fatty acids cannot. Therefore, wild-type *E. coli* (*fadR*<sup>+</sup>) can utilize long-chain fatty acids such as oleate (C18:1) but not medium-chain fatty acids such as decanoate (C10<sup>-</sup> phenotype) as a sole carbon and energy source. Strains which are mutant in *fadR* have constitutive levels of the *fad* enzymes and can use decanoate as a sole carbon and energy source (C10<sup>+</sup> phenotype).

In addition to the *fad* enzymes, the expression of the glyoxylate shunt enzymes is also required for the growth of *E. coli* on acetate or fatty acids as a sole carbon source. In wild-type *E. coli* repression of the *ace* operon is under the control of two genes, *fadR* and *iclR* (13, 15). The studies of Maloy and Nunn (16) suggest that both the *iclR* and *fadR* genes regulate the glyoxylate shunt in a transdominant and synergistic manner at the level of transcription.

*fadR* has also been suggested to play a role in the regulation of UFA biosynthesis (*fab*) (20). *fadR* mutants synthesize significantly less UFAs than do wild-type strains. In addition, *fadR fad*<sup>-</sup> mutants synthesize significantly less UFAs than their *fadR*<sup>+</sup> *fad*<sup>-</sup> parents. The latter results suggest that the low levels of UFAs in *fadR* strains are not due to their constitutive levels of *fad* enzymes. Thus, a

functional *fadR* gene is required for *E. coli* to optimally synthesize UFAs. The mechanism by which *fadR* exerts this effect remains undefined at this time.

In the present work, we describe the cloning and characterization of the *fadR* gene and the identification of the *fadR* gene product by maxicell analysis. The cloned *fadR* gene will facilitate detailed studies of the mechanism(s) by which this multifunctional protein exerts control over the *fad*, *ace*, and *fab* structural genes.

### MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** The *E. coli* bacterial strains and plasmids used in this study are listed in Tables 1 and 2, respectively.

The bacteria were routinely grown in either LB broth (19) or TB broth (1) and incubated at 37°C in a New Brunswick gyratory water bath shaker. For induction of the *fad* enzymes or  $\lambda\Phi$ (*fadE-lacZ*) fusions, cells were grown in TB broth supplemented with 5 mM oleate in 0.5% Brij 58. Noninducing medium was TB broth supplemented with 0.5% Brij 58. Antibiotics were added to maintain plasmid selection as necessary. Final concentrations were 100  $\mu$ g of ampicillin (Ap) per ml, 10  $\mu$ g of tetracycline (Tc) per ml, and 45  $\mu$ g of kanomycin (Km) per ml. Bacterial growth was monitored at 540 nm in a Klett-Summerson colorimeter. Solid minimal medium E (19) supplemented with 5 mM oleate or 5 mM decanoate was used to screen the *fadR* phenotype.

The spontaneous *fadR* strains LS6734 and LS1085 were obtained by plating LE392 and C600, respectively, on minimal medium containing decanoate. Strain LS6927 was made *fadR* by transduction of LS6926, using  $\Phi$ P<sub>1</sub>vir grown on LS5381 (*fadR::Tn10*) with primary selection for tetracycline resistance. The deleted *fadR* strain LS6925 was generated by spontaneous excision of the Tn10 transposon in LS5381 as described by Maloy and Nunn (14). The  $\lambda\Phi$ (*fadE-lacZ*) fusion strain of Clark (6) was stabilized with  $\lambda$ p1(209) as described by Komeda and Iino (11) to generate strain

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TABLE 1. Strain list

Strain	Genotype-phenotype	Reference or source
LS6924	Prototrophic <i>srlA::Tn10 recA</i>	This study
LS6925	$\Delta$ <i>fadR srlA::Tn10 recA</i>	This study
LS6926	$\lambda\Phi$ ( <i>fadE-lacZ</i> <sup>+</sup> )	This study
LS6927	$\lambda\Phi$ ( <i>fadE-lacZ</i> <sup>+</sup> ) <i>fadR::Tn10</i>	This study
LS1085	<i>leu thr thi lacY tonA supE srlA::Tn10 recA fadR</i>	This study
DC531	$\Phi$ ( <i>fadE-lacZ</i> <sup>+</sup> ) of MC410	Clark (6)
LS1001	Hfr <i>thi relA</i> $\lambda$ cI857 Tc <sup>r</sup> <i>fadR</i> <sup>+</sup>	This study
LS5381	<i>fadR::Tn10</i>	(26)
LS6592	<i>fadR::Tn10 fabA2</i>	(20)
LS5484	<i>zcf::Tn10</i> <sup>a</sup>	Simons et al. (26)
LS6734	<i>fadR met</i> $\lambda$ cI857	This study

<sup>a</sup> Transposon insertions are designated as previously described (26). 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 (i.e., *zcf* corresponds to 25 min) (5).

LS6926. Strains were made *recA* by transduction with  $\Phi$ P1<sub>vir</sub> grown on NK5304 (*srlA::Tn10 recA*).

**Isolation and manipulation of DNA.** Chromosomal *E. coli* DNA was prepared by the method of Marmur (18). Lambda DNA was prepared essentially as described by Maniatis et al. (17). Large-scale (1 liter or more) isolation and purification of plasmid DNA was by the cleared lysate-polyethylene glycol precipitation method of Humphreys et al. (8). Supercoiled plasmid DNA was further purified by centrifugation in a cesium chloride density gradient containing ethidium bromide (23). Plasmid DNA from small cultures (10 to 15 ml) was isolated by the method of Ish-Horawicz and Burke (9).

For preparation of the DNA library used to select the *fadR*<sup>+</sup> clone, *E. coli* chromosomal DNA was isolated from strain LS5484 and digested with 0.4 U of the restriction endonuclease *Sau3A* per  $\mu$ g of DNA at 37°C for 15 min. Approximately 200  $\mu$ g of this partially restricted DNA was loaded onto a 10 to 30% continuous sucrose gradient to size fractionate the DNA, essentially as described by Maniatis et al. (17). Fragments 10 kilobases (kb) or larger were pooled, precipitated with ethanol, suspended in 10 mM Tris-1 mM EDTA (TE) (pH 8.0), dialyzed for 3 h with three changes against TE, centrifuged at 100,000  $\times$  *g* for 30 min, and ethanol precipitated to concentrate the sample. This size-fractionated chromosomal DNA was ligated to *Bam*HI-restricted  $\lambda$ L47 DNA at a final concentration of 200  $\mu$ g/ml. This ligation mixture was packaged in vitro and amplified in the P2 lysogen LG106 to give a high-titer phage stock (10<sup>10</sup> PFU/ml) representing 10<sup>5</sup> separate packagings. A sample of this amplified bank was used to transduce strain LS6734 to Tc<sup>r</sup>. Phages were prepared from lysogens which proved to carry *fadR*<sup>+</sup> (C10<sup>-</sup> phenotype) as well as Tc<sup>r</sup>, using a 5-min heat shock at 42°C. Recombinant Tc<sup>r</sup> *fadR*<sup>+</sup> phage was prepared from the resulting mixture of helper ( $\lambda$ cI857) and recombinant phage by lytic growth on LS6734 in LB solid medium containing tetracycline and on the P2 lysogen LG106.

The *fadR*<sup>+</sup> gene was subcloned from DNA prepared from  $\lambda$  Tc<sup>r</sup> *fadR*<sup>+</sup> by partial *Sau3A* restriction (as described above) and ligation to *Bam*HI-restricted plasmid pDF41 DNA (3). The resulting plasmid, pDF<sub>fadR</sub>, proved to be phenotypically C10<sup>-</sup> and Tc<sup>s</sup>. The plasmids pAC<sub>fadR</sub>, pAC<sub>fadR3</sub>, and pAC<sub>fadR1</sub> are *fadR*<sup>+</sup> subclones of pDF<sub>fadR</sub> prepared in the multicopy vector pACYC177 (4) by ligation of restriction endonuclease-generated fragments (listed in

Fig. 1) essentially as described by Spratt et al. (28). Deleted *fadR* subclones pACHinc1, pACHinc5, and pACES1 were similarly prepared.

*E. coli* cells were transformed essentially as described by Dagert and Ehrlich (7). Initial selection on solid medium was for a plasmid marker (*trpE* for pDF41 and Km<sup>r</sup> or Ap<sup>r</sup> for pACYC177), followed by replica plating to minimal medium containing oleate or decanoate to determine the *fadR* phenotype.

The *fadR fabA*(Ts) strain LS6592 was not able to survive the CaCl<sub>2</sub> transformation procedure. Therefore, the plasmids pAC<sub>fadR3</sub> and pACYC177 were introduced by generalized transduction with  $\Phi$ P1<sub>vir</sub> grown on strain LS6925 carrying pAC<sub>fadR3</sub> or pACYC177. Transductants were selected by growth on solid medium E containing 40 mM glycerol, 100  $\mu$ M oleate, and 100  $\mu$ g of ampicillin per ml at 30°C.

**Biochemical procedures.** Measurement of the  $\beta$ -oxidation of [1-<sup>14</sup>C]oleate was performed with whole cells as previously described (26). A sample of the same cells was ruptured in a French press and centrifuged at 30,000  $\times$  *g*, and the supernatant was assayed for crotonase activity essentially as described by Binstock and Schulz (2).  $\beta$ -Galactosidase activity was assayed as described by Clark (6). Before each of these procedures, cells were rinsed once with M9 medium (19) containing 0.5% Brij 58 and twice with M9 alone to remove all fatty acid and detergent.

**Maxicells and electrophoresis of proteins.** The procedure described by Sancar et al. (25) for labeling plasmid-encoded proteins with [<sup>35</sup>S]methionine was used as previously detailed (28).

**Chemicals.** Antibiotics and other chemicals were obtained from Sigma Chemical Co. The various restriction endonucleases and bacteriophage T4 DNA ligase were obtained from Bethesda Research Laboratories. Acrylamide, bis-acrylamide, *N,N,N',N'*-tetramethylethylenediamine, ammonium persulfate, and CsCl were of ultra-pure quality and were obtained from Bethesda Research Laboratories. All other chemicals were of reagent grade and were obtained from standard suppliers.

## RESULTS

**Cloning of *fadR* from the *E. coli* chromosome.** Since there is no direct selection for clones containing *fadR*<sup>+</sup>, an indirect selection procedure was employed which involved cloning a transposon that was adjacent to the *fadR* locus. Strain LS5484 carries the Tc<sup>r</sup> transposon Tn10 near *fadR*<sup>+</sup> (26). A recombinant DNA library was prepared from chromosomal DNA extracted from this strain in the vector  $\lambda$ L47 (12) as described above. The resultant phage pool was used to

TABLE 2. Plasmid list

Plasmid	Relevant phenotype <sup>a</sup>	Reference or source
pACYC177	Ap <sup>r</sup> Km <sup>r</sup>	(4)
pAC <sub>fadR</sub>	Km <sup>r</sup> C10 <sup>-</sup>	This study
pAC <sub>fadR3</sub>	Ap <sup>r</sup> Km <sup>r</sup> C10 <sup>-</sup>	This study
pAC <sub>fadR1</sub>	Ap <sup>r</sup> C10 <sup>-</sup>	This study
pACHinc1	Km <sup>r</sup> C10 <sup>+</sup>	This study
pACHinc5	Km <sup>r</sup> C10 <sup>+</sup>	This study
pDF <sub>fadR</sub>	TrpE <sup>+</sup> C10 <sup>-</sup>	This study
pDF41	TrpE <sup>+</sup>	D. Helinski (3)
pACES1	Ap <sup>r</sup> C10 <sup>+</sup>	This study

<sup>a</sup> Ap<sup>r</sup>, Ampicillin resistant, Tc<sup>r</sup>, tetracycline resistant; Km<sup>r</sup>, kanamycin resistant.

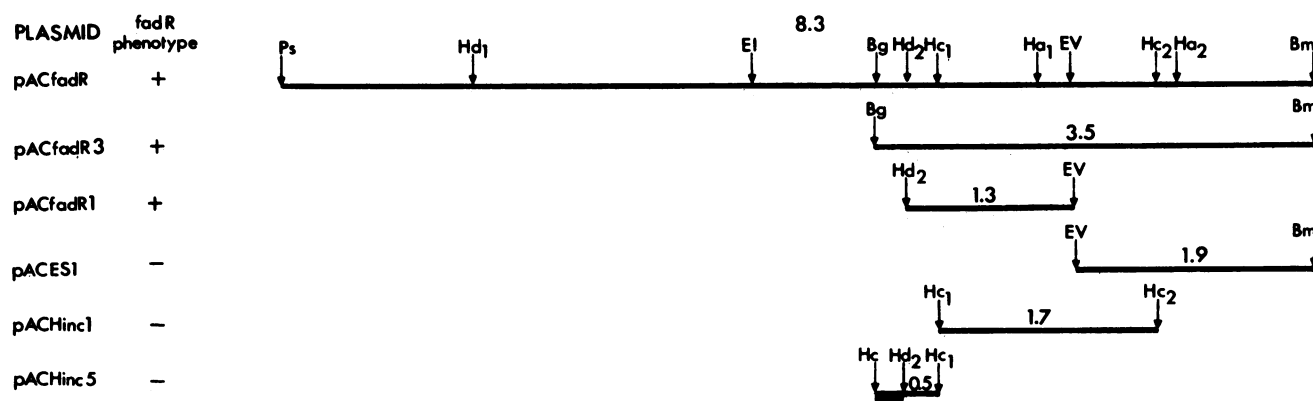


FIG. 1. Physical maps of recombinant plasmids containing portions of the *fadR* gene inserted into the plasmid vector pACYC177. Abbreviations: Ps, *Pst*I; Hd, *Hind*III; EI, *Eco*RI; Bg, *Bgl*II; Hc, *Hinc*II; EV, *Eco*RV; Ha, *Hae*II; and Bm, *Bam*HI. Numbers above the DNA fragment indicate the insert size in kb pairs. The heavy line in pACHinc5 indicates a portion of the insert derived from the vector.

select Tc<sup>r</sup> lysogens of an *fadR* derivative of the strain LE392, termed LER, using the prophage  $\lambda$ cI857 to provide homology for integration and the *cI* repressor for maintenance of lysogeny. These Tc<sup>r</sup> double lysogens were subsequently tested for the presence of *fadR*<sup>+</sup> by replica plating to minimal medium containing the medium-chain fatty acid decanoate as sole carbon and energy source. *fadR*<sup>+</sup> strains cannot utilize decanoate as a sole carbon and energy source (C10<sup>-</sup> phenotype), whereas *fadR* strains can (C10<sup>+</sup> phenotype) due to their constitutive levels of the *fad* structural gene products (21, 26, 27). Approximately 90% of the Tc<sup>r</sup> lysogens of LER were also C10<sup>-</sup> and presumed to contain a functional *fadR* gene. This was confirmed when it was determined that phage lysates prepared from one of the Tc<sup>r</sup> C10<sup>-</sup> lysogens (LS1001,  $\lambda$  Tc<sup>r</sup> *fadR*<sup>+</sup>) transduced several independently isolated *fadR* strains to both Tc<sup>r</sup> and C10<sup>-</sup>. The presence of the *fadR*<sup>+</sup> gene was further substantiated by determining whether these Tc<sup>r</sup> C10<sup>-</sup> lysogens were inducible for [<sup>14</sup>C]oleate oxidation. In all cases, the Tc<sup>r</sup> C10<sup>-</sup> lysogens of the *fadR* strains were inducible for  $\beta$ -oxidation (data not shown).

The *fadR*<sup>+</sup> gene was subcloned from  $\lambda$  Tc<sup>r</sup> *fadR*<sup>+</sup> to the single-copy plasmid vector pDF41 (courtesy of D. Helinski, University of California, San Diego). The resulting plasmid pDFfadR contained a 9.4-kb insert which carried the *fadR*<sup>+</sup> gene but not the Tc<sup>r</sup> gene. All further subcloning experiments followed the C10<sup>-</sup> phenotype. An 8.3-kb insert con-

taining *fadR*<sup>+</sup> was removed by restriction of pDFfadR with *Pst*I and *Bam*HI and inserted into the plasmid vector pACYC177 which had been restricted with the same enzymes. *fadR* strains harboring the 8.3-kb fragment inserted into pACYC177, termed pACfadR, were phenotypically C10<sup>-</sup>. This demonstrated that the *fadR*<sup>+</sup> gene could be stably maintained within the cell in a multicopy plasmid. The plasmid pACfadR was reduced by 5.0 kb by the deletion of a *Bgl*II-*Bam*HI segment (pACfadR3) (Table 2 and Fig. 1). Subsequent subcloning experiments localized the entire *fadR*<sup>+</sup> gene to a 1.3-kb *Hind*III-*Eco*RV fragment (pACfadR1). The gene contains at least two essential sites: one *Hinc*II site 0.5 kb from the *Hind*III end and one *Hae*II site 0.4 kb from the *Eco*RV end.

**$\beta$ -Oxidation in strains harboring plasmids containing the *fadR* gene.** In wild-type *E. coli*, the enzymes which catalyze fatty acid degradation are repressed by the *trans*-acting product of the *fadR* gene (21, 26, 27). High levels of the *fad* enzymes can be induced by growth in the presence of long-chain fatty acids (e.g., oleate, C18:1). Constitutive levels of the enzymes are found in *fadR* mutants (21, 27). The  $\beta$ -oxidation of [<sup>14</sup>C]oleate was compared under inducing and noninducing conditions in a  $\Delta$ *fadR recA* strain harboring the vector pACYC177 or the clone pACfadR3. The  $\Delta$ *fadR recA* strain LS6925 harboring the vector pACYC177 showed high rates of [<sup>14</sup>C]oleate oxidation under inducing or noninducing conditions, whereas the clone pACfadR3 had low levels of [<sup>14</sup>C]oleate oxidation when grown under noninducing conditions and high levels when grown under inducing conditions (24-fold induction) (Table 3). As expected, the *fadR*<sup>+</sup> *recA* strain LS6924 showed inducible levels of [<sup>14</sup>C]oleate oxidation whether harboring the vector or the clone (16- and 45-fold induction, respectively). To confirm that the regulation of the levels of  $\beta$ -oxidation was due to regulation of the *fad* enzymes, one enzyme activity, crotonase (encoded within *fadAB* [28]), was measured in extracts prepared from the same cell cultures used to assay  $\beta$ -oxidation. As expected, these data (Table 3) showed high levels of crotonase under noninducing conditions only in the  $\Delta$ *fadR recA* strain with the vector pACYC177.

**Transcriptional control of the *fadE* gene by the cloned *fadR* gene.** To assess whether the cloned *fadR* gene controls the *fad* structural genes at the level of transcription, one of the Mu d(Ap<sup>r</sup> *lacZ*) fusion strains of Clark, DC531  $\Phi$ (*fadE-lacZ*<sup>+</sup>), was stabilized with  $\lambda$ p1(209) as described by Komeda and Iino (11). In the stabilized strain  $\lambda\Phi$ (*fadE-lacZ*<sup>+</sup>),  $\beta$ -

TABLE 3. Oxidation of oleate and crotonase activity of *E. coli* strains containing an *fadR* plasmid

Plasmid <sup>a</sup>	Strain (genotype)	Growth condition <sup>b</sup>	Rate of release of [ <sup>14</sup> C]CO <sub>2</sub>	Crotonase
pACYC177	LS6924 ( <i>srl::Tn10 recA</i> )	Uninduced	1.0 <sup>c</sup>	127
		Induced	16.2	974
	LS6925 ( $\Delta$ <i>fadR srl::Tn10 recA</i> )	Uninduced	18.1	1,202
pACfadR3	LS6924 ( <i>srl::Tn10 recA</i> )	Induced	14.2	2,154
		Uninduced	0.2	35
	LS6925 ( $\Delta$ <i>fadR srl::Tn10 recA</i> )	Induced	9.1	685
pACHinc5	LS6924 ( <i>srl::Tn10 recA</i> )	Uninduced	0.39	37
		Induced	9.2	750

<sup>a</sup> See text and Fig. 1 for construction of hybrid plasmids.

<sup>b</sup> Uninduced, TB medium with 0.5% Brij 58 and kanamycin; induced, TB medium with 5 mM oleate, 0.5% Brij 58, and kanamycin.

<sup>c</sup> Values are in nanomoles per minute per milligram of protein. Results are the average of two experiments. Each sample was assayed in triplicate.

TABLE 4. Transcriptional control of  $\lambda\Phi(fadE-lacZ^+)$  by different *fadR* plasmids

Plasmid <sup>a</sup>	Strain	<i>fadR</i> genotype of $\lambda\Phi(fadE-lacZ^+)$	$\beta$ -Galactosidase activity <sup>b</sup> with the following growth conditions <sup>c</sup> :		
			TB	TB lac	TB ole
pACYC177	LS6926	Wild type	173	82	743
	LS6927	<i>fadR::Tn10</i>	932	287	837
pACfadR	LS6926	Wild type	32	24	877
	LS6927	<i>fadR::Tn10</i>	24	22	150
pACfadR3	LS6926	Wild type	31	17	396
	LS6927	<i>fadR::Tn10</i>	24	17	412
pACfadR1	LS6926	Wild type	56	42	1,089
	LS6927	<i>fadR::Tn10</i>	67	42	843
pACHinc1	LS6926	Wild type	91	44	611
	LS6927	<i>fadR::Tn10</i>	1,198	359	1,222
pACHinc5	LS6926	Wild type	164	82	1,096
	LS6927	<i>fadR::Tn10</i>	1,855	498	1,430

<sup>a</sup> See text and Fig. 1 for construction of hybrid plasmids.

<sup>b</sup> Values are in nanomoles per minute per milligram of protein.

<sup>c</sup> TB is TB medium supplemented with 0.5% Brij 58; TB lac is TB medium supplemented with 0.5% Brij 58 and 0.4% lactose; TB ole is TB medium supplemented with 0.5% Brij 58 and 5 mM oleate. All were supplemented with ampicillin or kanamycin.

galactosidase synthesis is under the control of the putative promoter-operator region of *fadE*, the gene encoding acyl-coenzyme A dehydrogenase (21).  $\beta$ -Galactosidase activity is therefore inducible by long-chain fatty acids. When an *fadR* mutation was introduced into this strain [ $\lambda\Phi(fadE-lacZ^+)$  *fadR::Tn10*],  $\beta$ -galactosidase activity was constitutive. Fusion strains harboring the plasmids pACfadR, pACfadR3, or pACfadR1 all showed induction of  $\beta$ -galactosidase when grown in the presence of the inducing substrate oleate whether the chromosomal genotype was *fadR*<sup>+</sup> or *fadR::Tn10* (Table 4). The *fadR* fusion strain LS6927 [ $\lambda\Phi(fadE-lacZ^+)$  *fadR::Tn10*] had constitutive levels of  $\beta$ -galactosidase when harboring the vector pACYC177 or deleted *fadR* subclone pACHinc1 or pACHinc5. This demonstrates that the *fadR*<sup>+</sup> gene lies between the *Hind*III and *Eco*RV restriction sites of the original pACfadR subclone and contains at least one essential *Hinc*II site (Fig. 1).

In all cases,  $\beta$ -galactosidase levels were repressed at least twofold in cells grown in noninducing medium (TB) (Table 4) containing 0.2% lactose. This result was not unexpected since the *fad* enzymes are subject to catabolite repression by glucose (6, 22, 26, 29).

**Implication of the cloned *fadR* gene in control of UFA biosynthesis.** *fadR* mutants synthesize significantly less UFAs than *fadR*<sup>+</sup> strains (20). This characteristic is phenotypically asymptomatic unless the *fadR* strain also carries a lesion in *fabA*, the structural gene for  $\beta$ -hydroxydecanoyl-thioester dehydrase. Unlike *fadR*<sup>+</sup> *fabA*(Ts) mutants, *fadR* *fabA*(Ts) strains synthesize insufficient UFAs to support their growth at low temperatures and, therefore, must be supplemented with UFAs at both low and high temperatures (20). The low levels of UFAs in the *fadR* *fabA*(Ts) strains are not due to their constitutive level of *fad* enzymes, because *fad*<sup>-</sup> derivatives of these strains also do not synthesize sufficient UFAs to support their growth at both low and high temperatures

(20). Although the control mechanism(s) is unknown, it is clear that a functional *fadR* gene is required for maximal expression of UFA biosynthesis in *E. coli* (20). As additional evidence that the plasmid pACfadR3 contains a functional *fadR* gene, we determined whether this plasmid would allow *fadR* *fabA*(Ts) strains to grow at low temperatures in the absence of UFAs. When the plasmid pACfadR3 was introduced by generalized transduction (see above) into an *fadR* *fabA*(Ts) strain, resultant transductants carrying the plasmid antibiotic resistance marker no longer required fatty acid supplementation at low temperatures (30°C) but only the restrictive temperature for *fabA*(Ts) (42°C). In contrast,  $\Phi$ P<sub>1</sub>vir grown on cells carrying the vector alone transduced the antibiotic resistance marker to *fadR* *fabA*(Ts) strains, but transductants failed to survive at any temperature without fatty acid supplementation. These results confirm that pACfadR3 carries a functional *fadR*<sup>+</sup> gene.

**Identification of the *fadR* gene product.** The maxicell procedure of Sancar et al. (25) was used to detect plasmid-encoded proteins from *fadR*<sup>+</sup> subclones pACfadR3 and pACfadR1 and deleted *fadR* subclones pACHinc1 and pACHinc5. A 29-kilodalton polypeptide was only present in the *fadR*<sup>+</sup> plasmids and is presumed to be the *fadR* gene product (Fig. 2).

## DISCUSSION

We identified a clone containing the *fadR*<sup>+</sup> gene of *E. coli* by using the primary selection for Tc<sup>r</sup> encoded by trans-

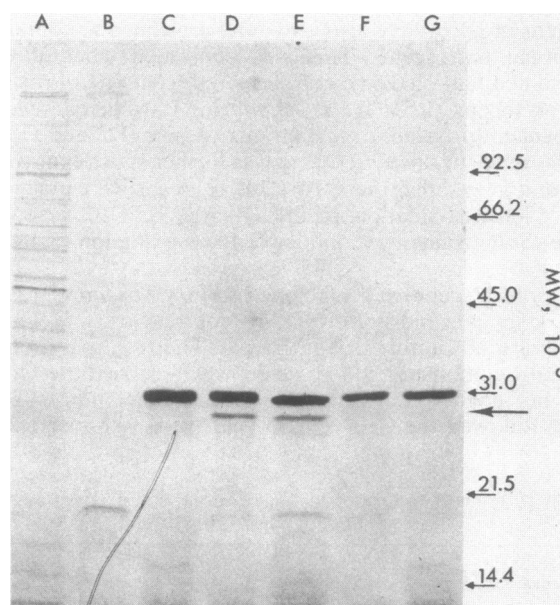


FIG. 2. Autoradiogram of [<sup>35</sup>S]methionine-labeled plasmid-encoded proteins in maxicells. Maxicells were prepared and plasmid-encoded proteins were analyzed on a 12% sodium dodecyl sulfate-polyacrylamide gel. Lanes: A, unirradiated control cells without plasmid; B, irradiated control cells without plasmid; C, pACYC177; D, pACfadR3; E, pACfadR1; F, pACHinc1; and G, pACHinc5. A total of 20,000 cpm was loaded in each sample lane. The strain used for maxicell analysis was LS1085. Short arrows indicate the positions of protein standards (numbers are in kilodaltons). The long arrow denotes the position of the *fadR* protein; note the absence of this protein in lanes C, F, and G. The 31-kilodalton protein is encoded by the antibiotic resistance gene of the plasmid and is therefore present in all lanes.

poson Tn10 inserted near the wild-type gene since a convenient, direct selection scheme for *fadR*<sup>+</sup> was not available. Once identified in a  $\lambda$ L47 recombinant phage, the *fadR*<sup>+</sup> gene was subcloned first to a single-copy plasmid (pDF41) and subsequently to a multicopy plasmid (pACYC177). As these subclones did not carry Tc<sup>r</sup>, the presence of the *fadR*<sup>+</sup> gene was determined initially by restoration of the C10<sup>-</sup> phenotype to *fadR* strains. The presence of the *fadR*<sup>+</sup> gene on these plasmids has been confirmed by studies showing that *fadR recA* strains harboring the *fadR*<sup>+</sup> plasmid pACfadR3 show inducible levels of oleate oxidation and the *fad* enzyme crotonase. Noninduced and induced levels of  $\beta$ -oxidation and crotonase are lower (at least twofold) in strains harboring the plasmid pACfadR3 than in strains harboring the vector pACYC177. This may reflect the increased copy number of the cloned gene as compared with the chromosomal gene. In addition, *fadR fabA*(Ts) strains harboring the *fadR*<sup>+</sup> clone grow at low temperatures without UFA supplementation. The latter results confirm that a functional *fadR*<sup>+</sup> gene is required for *fadR fabA*(Ts) strains to synthesize sufficient UFA to grow at permissive temperatures.

These studies confirm and extend the work of Overath et al. (21, 22) and Simons et al. (26, 27), demonstrating that the product of the *fadR*<sup>+</sup> gene is a *trans*-acting repressor protein of the *fad* regulon. Control of the *fad* structural gene by the cloned *fadR*<sup>+</sup> gene appears to be at the level of transcription (6, 24). This was confirmed by comparing  $\beta$ -galactosidase levels in a  $\lambda\Phi$ (*fadE-lacZ*<sup>+</sup>) *fadR::Tn10* strain harboring the *fadR*<sup>+</sup> plasmids pACfadR, pACfadR3, and pACfadR1 (fully inducible  $\beta$ -galactosidase activity) with the vector and deleted *fadR* plasmids pACHinc1, pACHinc5, and pACES1 (constitutive  $\beta$ -galactosidase activity).

Simons et al. (27) have shown by complementation tests that only one polypeptide is encoded by the *fadR* gene. We believe that the 29-kilodalton protein identified by the maxicell procedure is the *fadR* gene product because (i) it is the only protein synthesized by the 1.3-kb *Hind*III-*Eco*RV insert (Fig. 2), and (ii) functional *fadR* gene control correlates with the presence of this protein. This preliminary characterization of the *fadR* clone may now be further exploited to study (i) the mechanism of induction of the *fad* regulon, (ii) the interaction of the *fadR* gene product with the *fad* structural genes, and (iii) the role of this multifunctional regulator in acetate metabolism and UFA biosynthesis. It will be interesting to determine whether the cloned *fadR* gene exerts control over the *fab* structural genes and whether this control is also at the level of transcription.

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