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Regulation of Fatty Acid Transport in *Escherichia coli*: Analysis by Operon Fusion

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The regulation of the *fadL* gene, which encodes a long-chain fatty acid (LCFA) transport component, was examined by constructing a strain of *Escherichia coli* K-12 that bears a Φ(*fadL-lacZ*$^+$) operon fusion plus a wild-type *fadL* gene. This merodiploid strain expressed LCFA transport and β-galactosidase activity coordinately under noninducing, inducing, and catabolite-repressing conditions. Merodiploid strains which carried a defective *fadR* gene expressed LCFA transport and β-galactosidase activity constitutively. These results suggest that expression of the *fadL* gene is regulated by the *fadR* gene and is inducible at the level of transcription by LCFA.

Wild-type *Escherichia coli* can utilize exogenous long-chain fatty acids (LCFA; C_{11} to C_{18}) as the sole carbon and energy source (4, 9, 10, 14). Growth in medium containing LCFA coordinately induces the synthesis of at least five fatty acid degradative (*fad*) enzymes (4, 9–11, 14). Medium-chain fatty acids (MCFA; C_7 to C_{10}) can serve as substrates for the *fad* enzymes but cannot induce the synthesis of the *fad* enzymes (10, 11, 14). Therefore, only fatty acids longer than decanoic acid (C_{10}) may be used as the sole carbon source by wild-type (*fadR*$^+$) strains. Strains that constitutively synthesize the *fad* enzymes may be selected for by growth on C_{10} as the sole carbon source (10). These mutants have been termed *fadR* by Overath et al. (10) and are able to grow on MCFA and LCFA (10, 12–14). The regulatory gene (*fadR*) and the *fad* enzyme structural genes (*fadABC*, *fadD*, and *fadE*) constitute a regulon, designated the *fad* regulon (10, 12, 13). At present, all available evidence suggests that the product(s) of the *fadR* gene negatively controls the expression of the *fad* regulon (3, 10, 12–14). Through the fusion of the *lac* operon to the *fadABC* and *fadE* genes, Clark has presented evidence which suggests that the *fadR* gene product regulates the *fad* genes at the level of transcription (3).

The fatty acid transport system involves two loci: (i) *fadD*, which codes for acyl coenzyme A synthetase (10), and (ii) *fadL*, which codes for a membrane component(s) required for the transport of LCFA (7–9). The *fadL* gene, located at 50 min on the revised *E. coli* K-12 linkage map (1), is believed to be a member of the *fad* regulon. *fadR*$^+$ strains carrying a defect in the *fadL* locus cannot grow on fatty acids of a chain length of C_4 or greater and are unable to be induced for *fad* enzyme synthesis (6, 8, 9). However, *fadR fadL* strains can utilize MCFA (C_7 to C_{10}) but not LCFA (C_{11} to C_{18}) as the sole carbon and energy source (6, 8, 9). *fadR fad*$^-$ (i.e., *fadABC*, *fadD*, and *fadE*) strains are incapable of growing on fatty acids of a chain length of C_4 or greater, whereas *fadR fadL* strains can grow on MCFA (C_7 to C_{10}). This growth behavior of *fadR fadL* strains (C_{10}^+, $C_{18:1}^-$) will be referred to as the FadL phenotype.

To determine whether the *fadL* gene is regulated in a manner similar to the *fadABC* and *fadE* genes (3), *fadL-lac* operon fusions have been constructed. These fusions place the expression of the *lac* structural genes (i.e., β-galactosidase) under the control of the promoter and putative operator of the *fadL* gene. The synthesis of *lac* gene products would then correlate to the synthesis of the *fadL* gene product. Since the *fadR*$^+$ Φ(*fadL-lacZ*$^+$) strains that were constructed were incapable of transporting LCFA and were, therefore, uninducible, *fadR*$^+$ Φ(*fadL-lacZ*$^+$) fusion strains bearing a functional *fadL*$^+$ gene have also been constructed. These merodiploid strains have two promoter-operator regions of the *fadL* locus: in one copy, the control region is connected to the *fadL* structural gene in the native state, and in the other copy, the control region is fused to the structural gene *lacZ*$^+$. The normal *lac* operon is deleted in these mutants. The level of expression of the *fadL* control site can therefore be monitored by the activity of β-galactosidase in the cell extracts of *fadR*$^+$ strains grown in the presence and absence of the inducers (LCFA) of the *fad* regulon. In this paper, we report some properties of these operon fusion mutants.

Bacterial strains, media, and growth condi-

tions. All bacterial strains listed in Table 1 are derivatives of *E. coli* K-12. General techniques for bacterial and phage construction were essentially done as described by Miller (7). A spontaneous *fadR* (C_{10}^+) mutant of MC4100, designated MC4100R, was obtained by plating MC4100 on C_{10} as the sole carbon source. A *fadR*::Tn*5* derivative of MC4100, designated MC4100DK, was obtained by infecting MC4100 with phage P1 *vir* grown on LS5394 and selecting for the C_{10}^+ Kan^r colonies.

Operon fusions of Mu *d*(Ap^r *lac*) with the *fadL* gene were constructed with the aid of the genetic fusion techniques developed by Casadaban and Cohen (2). The fusions were selected in strain MC4100DK after infection with Mu *d*(Ap^r *lac*) which was prepared by heat induction of the double lysogen MAL103. After the phenotypic expression of the ampicillin resistance was allowed for as described by Clark (3), the Mu *d*(Ap^r *lac*)-infected MC4100DK cells were plated on minimal medium (9) containing 5 mM decanoate, 30 μg of ampicillin per ml, and the LCFA analog 11-bromoundecanoate (0.5 mM 11Br-C_{11}) and incubated at 30°C. The resulting Ap^r 11Br-C_{11}^r colonies were screened for the Lac^+ phenotype by replica plating onto MacConkey agar and minimal medium containing lactose as the sole carbon source. The Lac^+ Ap^r 11Br-C_{11}^r colonies were screened for the $FadL^-$ phenotype by replica plating onto oleate ($C_{18:1}$), acetate (C_2), and decanoate (C_{10}) media. Colonies exhibiting the $FadL^-$ phenotype were genetically characterized as *fadL* mutants, and their genotypes were confirmed biochemically by testing their ability to transport the LCFA $C_{18:1}$. One of these *fadR fadL*::Mu *d*(Ap^r *lac*) lysogens, LS6587, was used for further strain constructions. The fusion in LS6587 was stabilized with λpl(209) as described by Komeda and Iino (5). Figure 1 summarizes the sequence of genetic events leading to the construction of the stabilized strain LS6644 (Fig. 1c) and strain LS6659, which carries both a wild-type $fadL^+$ gene and the Φ(*fadL-lacZ*$^+$) operon fusion (Fig. 1). Through aberrant excision, a low-frequency lysate of λ Φ(*fadL-lacZ*$^+$) was obtained by UV induction of prophage λpl(209) in strain LS6644. Since only λ Φ(*fadL-lacZ*$^+$), and not λpl(209), was expected to form Lac^+ lysogens on minimal medium containing lactose as the sole carbon source, the lysate was used to transduce MC4100R to Lac^+. The Lac^+ λ^r phenotype in LS6659 was mapped at 50 min. A $fadR^+$ derivative of strain LS6659 was obtained by transduction of LS6659 to tetracycline resistance (Tc^r) with phage P1 *vir* grown on strain LS5483 (*zcf*::Tn*10*). Since the Tn*10* insertion in LS5483 is 95% cotransducible with the $fadR^+$ gene, Tc^r transductants were screened for their ability to utilize the MCFA C_{10} as the sole carbon source. A C_{10}^- Tc^r isolate, strain LS6665, was thus obtained.

Bacteria were routinely incubated in minimal medium E (9) supplemented with 15 μM thiamine. Carbon sources were added as follows: 25

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source
K-12	Prototrophic	CGSC[a]
LS5946	F^+ *zfa*::Tn*10*[b] (near *fadL*)	C. Ginsburgh
LS5483	*zcf*::Tn*10*	R. Simons
LS5394	F^- *fadR*::Tn*5 trp purB*	R. Simons
MAL103	*araD139 ΔlacU169 rpsL thi fla ara*::Mu *d*(Ap^r *lac*) (Mu *cts*)	M. Casadaban
MC4100	F^- *araD139 ΔlacU169 rpsL thi*	M. Casadaban
MC4100DK	F^- *fadR*::Tn*5* of MC4100	P1(LS5394) × MC4100
MC4100R	F^- *fadR* of MC4100	This work
MC4100Lac	F^- *lac*$^+$ of MC4100	P1(K-12) × MC4100
MC4100DCR	F^- *zfa*::Tn*10 fadL* of MC4100R	This work
LS6587	*fadL*::Mu *d*(Ap^r *lac*) of MC4100DK	This work
LS6644	F^- λ Φ(*fadL-lacZ*$^+$) of LS6587[c]	This work
LS6640	F^- $fadR^+$ of LS6644	This work
LS6659	λ Φ(*fadL-lacZ*) of MC4100R	λ Φ(*fadL-lacZ*) × MC4100R
LS6665	F^- *zcf*::Tn*10* $fadR^+$ of LS6659	P1(LS5483) × LS6659

[a] CGSC, Obtained from B. Bachmann, Coli Genetic Stock Center, Yale University, New Haven, Conn.

[b] Transposon insertions are designated as previously described (12). 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., *zaf* corresponds to 5 min, and *zbb* corresponds to 11 min).

[c] LS6644 is derived from the *fadL*::Mu *d*(Ap^r *lac*) strain LS6587, in which λp1(209) has been lysogenized and the intervening Mu has been deleted (Fig. 1c). The procedure for stabilizing the Mu *d*(Ap^r *lac*) fusion with λp1(209) was carried out as described by Komeda and Iino (5).

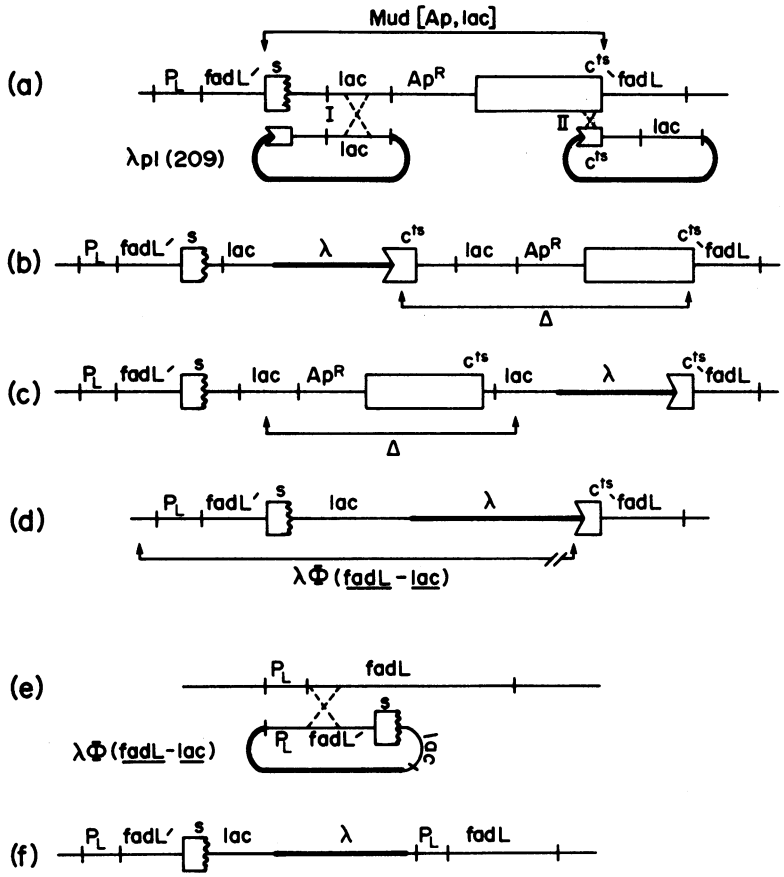

FIG. 1. Isolation of λ phage carrying the *fadL-lac* fusion and construction of the *fadL*$^+$/Φ(*fadL-lacZ*$^+$) merodiploid strains. (a) Infection of a *fadL*::Mu *d*(Ap^r *lac*) strain (LS6587) with λp1(209) phage, which integrated using *lac* homology (I) or Mu homology (II). (b) λp1(209) lysogen integrated via *lac* homology. (c) λp1(209) lysogen integrated via Mu homology. (d) Result of heat induction and subsequent excision of Mu *d*(Ap^r *lac*) from either (b) or (c). This λ Φ(*fadL-lacZ*$^+$) deletion mutant was Ap^s Lac$^+$ Ole$^-$. A λ *fadL-lacZ*$^+$ fusion phage was isolated by UV induction of (d). The *fadL*$^+$/Φ(*fadL-lacZ*$^+$) merodiploid strain was constructed by infecting the UV lysate of (d) with MC4100R (e) and isolating the Lac$^+$ lysogens (f). (This figure was partially taken from the work of Komeda and Iino [5]).

mM D-glucose, 25 mM lactose, 50 mM acetate, 5 mM decanoate, or 5 mM oleate. The fatty acids decanoate and oleate were solubilized with Brij 58 at a final concentration of 5 mg/ml. Ampicillin and kanamycin were used at 30 μg/ml, and tetracycline was used at 20 μg/ml.

Transport of the LCFA was measured as described by Maloy et al. (6) with an initial concentration of 250 μM [1-^{14}C]oleate (3.0 μCi/μmol). The following modifications were used. Triplicate 0.25-ml samples were removed at 0.25 and 2.25 min and rapidly pipetted onto Sartorius membrane filters (type SM11307, 0.2-μm pore size) covered with 2.5 ml of M9 minimal medium (6) supplemented with 5 mM oleate and 5 mg of Brij 58 per ml (M9-Brij-$C_{18:1}$). The filtered cells were then washed twice with 2.5 ml of M9-Brij.

β-Galactosidase assay. β-Galactosidase activity was routinely assayed as described by Clark (3). Before the assay, all fatty acid and detergent were removed from the cells by washing them twice with medium E.

Isolation of *fadL-lac* operon fusions. Until recently, it was difficult to isolate strains with *fadL-lac* operon fusions because such strains were phenotypically FadL$^-$, and no direct selection technique was available for discriminating *fadL* mutants from *fad* mutants (i.e., *fadD*, *fadABC*, and *fadE*). However, by using the effects of the fatty acid analog 11Br-C_{11} on *fadR fadL*$^+$ and *fadR fadL* strains, an enrichment procedure for isolating *fadL* mutants was developed. 11Br-C_{11} enters by the *fadL* transport system and appears to be bacteriocidal to *fadR*

fadL$^+$, but not *fadR fadL*, strains growing on C_{10} as the sole carbon source. Therefore, the *fadR* Δ*lac* strain MC4100DK was infected with Mu *d*(Ap^r *lac*) and plated onto 5 mM C_{10} minimal medium supplemented with 0.5 mM 11Br-C_{11} and ampicillin. Approximately 2.5% of the 11Br-C_{11}^r Ap^r colonies were defective in the *fadL* gene, and 25% of these colonies were Lac$^+$. One of these strains, designated LS6587, was used in subsequent studies. The putative insertion mutation in this strain was verified by transducing it to Tc^r with phage P1 *vir* grown on strain LS5946 (*zfa*::Tn*10*). A total of 94% of the Tc^r colonies coinherited *fadL*$^+$, of which 99% were Ap^s Lac$^-$ and 1% were Ap^r Lac$^+$ or Ap^r Lac$^-$. These results suggest that LS6587 is a *fadL*::Mu *d*(Ap^r *lac*) monolysogen. The low frequency *fadL*$^+$ transductants which remained Ap^r Lac$^+$ or Ap^r Lac$^-$ appear to be multi-Mu *d* lysogens that contain Mu *d* insertions elsewhere in the genome. To avoid working with a strain which has Mu *d* insertions elsewhere, the Mu *d*(Ap^r *lac*) fusion strain LS6587 was stabilized with λp1(209) as described above. The resulting stabilized strain was designated LS6644. The λ Φ (*fadL-lacZ*$^+$) operon fusion in the stabilized isolate was always removed when this strain was transduced to fadL$^+$ (data not shown).

When a *fadR*$^+$ derivative of LS6644, designated LS6640, was constructed, we observed that this *fadL* strain lost, as expected, its ability to grow on C_{10}. In addition, strain LS6640, unlike LS6644, grew very poorly on lactose as the sole carbon source. These results suggest that expression of the *fadL* gene is repressed in *fadR*$^+$, but not *fadR*, strains. To confirm this suggestion, we compared β-galactosidase activity in strains LS6644 and LS6640. β-Galactosidase activity in the *fadR* strain was fourfold greater than β-galactosidase activity in the *fadR*$^+$ strain LS6640 (Table 2). These data indicate that a functional *fadR* gene represses the *fadL* gene at the level of transcription.

When fusion strains LS6640 and LS6644 were cultured in acetate or acetate and oleate minimal medium, their $C_{18:1}$ transport rates were identical to that of MC4100DCR (<0.05 nmol/min per mg of protein). Since these fusion strains were incapable of transporting the LCFA oleate, we were unable to determine whether LCFA induces the levels of β-galactosidase. To determine this, *fadR*$^+$ and *fadR* strains bearing both a

TABLE 2. Activity levels of β-galactosidase and LCFA transport in λ Φ(*fadL-lacZ*$^+$) fusion strains

Strain	Relevant genotype	Growth conditions	β-Galactosidase activity (U)[b]	$C_{18:1}$ transport activity[c]
LS6665	*fadR*$^+$ *fadL*$^+$/λ Φ(*fadL-lacZ*$^+$)	D-Glucose	9.1	0.31
		Acetate	17.5	0.75
		Acetate + oleate	40.5	1.96
		Oleate	107	7.21
		Acetate + lactose	24.3	ND[d]
LS6659	*fadR fadL*$^+$/λ Φ(*fadL-lacZ*$^+$)	D-Glucose	4.5	0.37
		Acetate	58.8	5.66
		Acetate + oleate	41.6	5.65
		Oleate	94.8	7.21
		Acetate + lactose	35.0	ND
LS6640	λ Φ(*fadL-lacZ*$^+$)	D-Glucose	6.7	<0.05
		Acetate	10.7	<0.05
		Acetate + oleate	8.2	<0.05
LS6644	*fadR* λ Φ(*fadL-lacZ*$^+$)	D-Glucose	8.4	<0.05
		Acetate	48.0	<0.05
		Acetate + oleate	49.2	<0.05
MC4100Lac	*lac*$^+$ *fadL*$^+$	Oleate	3.2	<0.05
		Acetate + lactose	403	
MC4100DCR	*fadL*	Acetate	ND	<0.05
		Acetate + oleate	ND	<0.05

[a] Grown at 37°C to 2.5 × 10^8 cells per ml in minimal medium containing the indicated carbon sources. β-Galactosidase and transport activities were performed as described in the text.

[b] β-Galactosidase activity is expressed in units as defined by Miller (7).

[c] nmoles per minute per milligram of protein.

[d] ND, Not determined.

wild-type *fadL*$^+$ gene and a λ Φ(*fadL-lacZ*$^+$) operon fusion were constructed as described above. The *fadR*$^+$ *fadL*$^+$/Φ(*fadL-lacZ*$^+$) fusion strain, designated LS6665, grows on $C_{18:1}$ but not C_{10}. Like *fadR*$^+$/Φ*fadL-lacZ*$^+$) fusion strain LS6640, strain LS6665 grows very poorly on lactose. As expected, the *fadR fadL*$^+$/Φ(*fadL-lacZ*$^+$) operon fusion strain, designated LS6659, grows on C_{10}, $C_{18:1}$, and lactose. The presence of the *fadL*$^+$ gene in these strains enabled us to correlate LCFA transport activity with β-galactosidase activity. LCFA transport and β-galactosidase activity in the *fadR*$^+$ *fadL*$^+$/Φ(*fadL-lacZ*$^+$) operon fusion strain were expressed coordinately under noninducing, inducing, and catabolite-repressing growth conditions (i.e., growth on glucose) (Table 2). Under the same conditions, the *fadR fadL*$^+$/Φ(*fadL-lacZ*$^+$) operon fusion strain had constitutive levels of LCFA transport and β-galactosidase activity (Table 2). The presence of lactose in the growth media had no effect on the expression of β-galactosidase activity in either LS6665 or LS6659 (Table 2).

In summary, the findings in this paper suggest that (i) expression of the *lac* operon in the Φ(*fadL-lacZ*$^+$) operon fusion strains is under the control of the promoter and putative operator regions of the *fadL* gene, (ii) expression of the *fadL* gene is regulated by the *fadR* gene product(s), (iii) expression of the *fadL* gene is induced at the level of transcription by LCFA, and (iv) expression of the *fadL* gene is reduced under catabolite-repressing growth conditions. These findings are similar to those shown by Clark with *lac* operon fusions of the *fadABC* and *fadE* genes (3). Therefore, it appears that the *fadR* gene exerts negative control on the *fad* regulon (*fadL*, *fadE*, and *fadABC*) at the level of transcription. Studies to assess whether the *fadR* gene exerts the same transcriptional control of the *fadD* gene have, to date, been thwarted by our inability to obtain *fadD*::Mu *d* fusions. The difficulty in obtaining *fad* insertion mutations may ultimately be overcome when a procedure that enriches specifically for *fadD* mutants is developed.

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LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu− *lac* bacteriophage: in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. U.S.A. **76**:4530–4533.
3. **Clark, D.** 1981. Regulation of fatty acid degradation in *Escherichia coli*: analysis by operon fusion. J. Bacteriol. **148**:521–526.
4. **Klein, K., R. Steinberg, B. Fiethen, and P. Overath.** 1971. Fatty acid degradation in *Escherichia coli*. An inducible system for the uptake of fatty acids and further characterization of old-mutants. Eur. J. Biochem. **19**:442–450.
5. **Komeda, Y., and T. Iino.** 1979. Regulation of expression of the flagellin gene (*hag*) in *Escherichia coli* K-12: analysis of *hag-lac* gene fusions. J. Bacteriol. **139**:721–729.
6. **Maloy, S. R., C. L. Ginsburgh, R. W. Simons, and W. D. Nunn.** 1981. Transport of long and medium chain fatty acids by *Escherichia coli*. J. Biol. Chem. **256**:3735–3742.
7. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Nunn, W. D., and R. W. Simons.** 1978. Transport of long chain fatty acids by *Escherichia coli*: mapping and characterization of mutants in the *fadL* gene. Proc. Natl. Acad. Sci. U.S.A. **75**:3377–3381.
9. **Nunn, W. D., R. W. Simons, P. A. Egan, and S. R. Maloy.** 1979. Kinetics of the utilization of medium and long chain fatty acids by a mutant of *Escherichia coli* defective in *fadL* gene. J. Biol. Chem. **254**:9130–9134.
10. **Overath, P., G. Pauli, and H. U. Schairer.** 1969. Fatty acid degradation in *Escherichia coli*. An inducible acyl-CoA synthetase, the mapping of old-mutations and the isolation of regulatory mutants. Eur. J. Biochem. **7**:559–574.
11. **Overath, P., E. Raufuss, W. Stoffel, and W. Ecker.** 1967. The induction of the enzymes of fatty acid degradation in *Escherichia coli*. Biochem. Biophys. Res. Commun. **29**:28–33.
12. **Simons, R. W., P. A. Egan, H. T. Chute, and W. D. Nunn.** 1980. Regulation of fatty acid degradation in *Escherichia coli*: isolation and characterization of strains bearing insertion and temperature-sensitive mutations in gene *fadR*. J. Bacteriol. **142**:621–632.
13. **Simons, R. W., K. T. Hughes, and W. D. Nunn.** 1980. Regulation of fatty acid degradation in *Escherichia coli*: dominance studies with strains merodiploid in gene *fadR*. J. Bacteriol. **143**:726–730.
14. **Weeks, G., M. Shapiro, R. O. Burns, and S. J. Wakil.** 1969. Control of fatty acid metabolism. I. Induction of the enzymes of fatty acid oxidation in *Escherichia coli*. J. Bacteriol. **97**:827–836.