JOURNAL OF BACTERIOLOGY, Nov. 1981, p. 521-526
0021-9193/81/110521-06$02.00/0

Vol. 148, No. 2

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

DAVID CLARK†

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received 9 March 1981/Accepted 29 June 1981

Fusion of the *lacZ* gene coding for β-galactosidase to the *fadA,B* and *fadE* operons was accomplished by using the phage Mu *d* (Ap^r *lac*). In such fusion strains, β-galactosidase was induced by long-chain fatty acids and repressed by glucose, as is the normal pattern of control for the enzymes of the *fad* regulon. The level of induction seen was approximately 10-fold for both the *fadA* and *fadE* operons. These results demonstrate that the previously observed regulation of both the *fadA* and *fadE* operons is at the transcriptional level. When an insertion mutation in the *fadR* (repressor) gene was introduced into the fusion strains, β-galactosidase was produced constitutively. A series of fatty acids of different chain lengths were tested as inducers. Acids of chain lengths of 10 carbon atoms or less failed to induce, those of 12 carbon atoms induced partly, and those of 14 or more carbon atoms induced fully. Imidazole was found to counteract the glucose repression of the *fadA* operon as recently demonstrated for the *ara* operon.

Wild-type *Escherichia coli* K-12 can use fatty acids with 12 or more carbon atoms as the sole carbon source (18, 23). Growth on fatty acids requires coordinate synthesis of the β-oxidation enzymes and a transport system for fatty acids. At least five separate operons (1) under the control of the *fadR* repressor are involved (13, 14, 17, 23). The components of the β-oxidation multienzyme complex are coded for by the *fadA,B,C* operon (9, 17) and the acyl-coenzyme A (CoA) dehydrogenase is coded by the *fadE* gene (9; W. D. Nunn, unpublished data). The *aceA,B* operon specifies the enzymes of the glyoxylate cycle which are necessary for metabolism of the acetyl-CoA resulting from β-oxidation. The *aceA,B* operon is both under *fad* control (13) and induced by growth on acetate (12). The fatty acid transport system involves two loci of which *fadD* codes for acyl-CoA synthetase (8, 17) and *fadL* codes for the membrane component(s) necessary for the transport of long-chain fatty acids (14). Although acids shorter than 12 carbon atoms cannot serve as a carbon source for wild-type strains, they can be used by *fadR* mutants (17, 23) which are constitutive for the whole *fad-aceA,B* regulon due to the loss of a repressor protein (21, 22). The shorter acids are thus perfectly good substrates for β-oxidation but are incapable of inducing the requisite operons.

I have used the Mu *d* (Ap^r *lac*) phage of Casadaban and Cohen (3) to generate fusions of β-galactosidase to several operons involved in fatty acid degradation. Here I describe fusions to the operons *fadA,B,C* and *fadE* which are responsible for the degradation of fatty acids after the coupled transportation-activation process has converted external fatty acid to internal acyl-CoA (9, 17).

MATERIALS AND METHODS

Bacterial strains and media. All bacteria were strains of *E. coli* K-12 and are listed in Table 1. The phage Mu *d*1 (Ap^r *lac*) has been described by Casadaban and Cohen (3). Most of the media used have been described before (4). Fatty acids were used at 0.1% (wt/vol) for carbon sources and at 2 mM for the induction experiments. Fatty acids were neutralized with KOH and solubilized by the addition of four times their mass of Brij 58. Other carbon sources were used at 0.4%. Amino acid supplements were added at 50 μg/ml when appropriate.

Genetic methods. Transductions used P1 *vir* as previously described (4). Insertions of Tn*10* next to genes of interest were obtained by the method previously used to isolate insertions near the *acd* gene (5). Genetic fusions were selected in strain MC4100 by using the phage Mu *d*1 (Ap^r *lac*) prepared by heat induction of the double lysogen MAL103. Adsorption of the phage was carried out at a multiplicity of infection of approximately 1 in rich broth containing 10 mM $MgSO_4$ and 5 mM $CaCl_2$ for 20 min at 30°C. After a 1:10 dilution into rich broth, the culture was grown for 30 min at 30°C to allow expression of ampicillin resistance. All further growth of putative fusion strains was at 33°C unless otherwise specified. Cells were then plated onto lactose-MacConkey agar plates

† Present address: Department of Microbiology, Southern Illinois University, Carbondale, IL 62901.

TABLE 1. *Bacterial strains*

Strain	Relevant markers	Source
MAL103	Mu *cts d1* (Ap^r *lac*), Mu *cts* Δ(*pro-lac*)*XIII*, *rpsL*	M. Casabadan
MC4100	*araD139* Δ (*lacIOPZYA*)*U169 rpsL thiA*	M. Casabadan
Ymel	*mel-1 supF58*	P. Overath
K1	*fadABC5* of Ymel	P. Overath
K19	*fadE62* of Ymel	P. Overath
K27	*fadD88* of Ymel	P. Overath
Old3003	*fadD3003* of Ymel	F. Hill and D. Angelmaier
Old3008	*fadL3008* of Ymel	F. Hill and D. Angelmaier
RW11	*fadR13*::Tn*10*	W. Nunn
AK4	*fadL*::Tn*10*	Laboratory collection
DC369	*zdj*::Tn*10* (near *fadD*) *fadR16 atoC12*	See text
DC407	*zag*::Tn*10* (near *fadE*) *proA metD*	See text
NK5847	*zig*::Tn*10* (near *fadA*) *rpsL thiA lac gal xyl*	N. Kleckner
DC529	Φ(*fadABC-lacZ*) of MC4100	See text
DC530	Φ(*fadABC-lacZ*) of MC4100	See text
DC531	Φ(*fadE-lacZ*) of MC4100	See text
DC532	Φ(*fadE-lacZ*) of MC4100	See text
DC549	*fadR*::Tn*10* of DC529	P1 (RW11) × DC529
DC550	*fadR*::Tn*10* of DC530	P1 (RW11) × DC530
DC551	*fadR*::Tn*10* of DC531	P1 (RW11) × DC531
DC552	*fadR*::Tn*10* of DC532	P1 (RW11) × DC532
DC519	Φ(*lysA-lacZ*) of MC4100	See text

containing 50 μg of ampicillin per ml. Ampicillin-resistant Lac$^+$ colonies were picked onto minimal plates with either oleate or glucose as the carbon source. Mutants unable to use oleate but able to grow on glucose were kept for further investigation. Since the *fad* regulon is subject to catabolite repression, even weakly *lac*-positive colonies were included in the screening procedure. The *lysA* fusion strain DC519 Φ(*lysA-lacZ*) was found as a lysine auxotroph among those colonies unable to grow on unsupplemented minimal medium.

Assay of β-galactosidase. Cultures were grown in minimal medium E to approximately 5×10^8 cells per ml. Each assay tube contained 1.0 ml of medium E, 0.1 ml of 0.1% sodium dodecyl sulfate, and 0.1 ml of $CHCl_3$. A 0.1-ml sample of cells was added, and the assay tube was blended vigorously on a Vortex mixer. After the addition of 0.2 ml of 4-mg/ml *O*-nitrophenylgalactoside, the assays were incubated at 37°C for 15 to 45 min. The assays were stopped with 1.0 ml of 1 M Na_2CO_3 and were read at 420 nm after cells and $CHCl_3$ were sedimented by centrifugation. Preliminary experiments indicated that the fatty acids and detergent used in this study had no significant effect on β-galactosidase activity, at least for assay times of less than 1 h. Overath et al. (18) claimed that Brij 35 causes a low level of induction of the *fad* enzymes. My samples of Brij 58 showed no such effect during control experiments. It is probable that detergents manufactured today are purer than those of a dozen years ago or possible that degradation of detergents to inducing material might occur on prolonged storage. Furthermore, the acyl chain lengths of Brij 35 and Brij 58 are different, and chain length is an important factor affecting induction (see below).

RESULTS

Isolation of *fad* operon fusions. Approximately 5,000 fusion strains, isolated as described above, were screened for their ability to use oleic acid as the sole carbon source. A total of 30 mutants unable to use oleate were further examined, and 10 were also unable to use one or more of succinate, malate, or lactate, indicating lesions in the Krebs cycle or the ATP-generating pathways. Six mutants were able to grow on all carbon sources tested except acetate and oleate. Most of these mutants carried mutations in the *aceA,B* operon (unpublished data). The other 14 mutants could grow on acetate and all other carbon sources except oleate and thus carried *fad* mutations.

The separate *fad* loci involved were identified by two methods. First, each putative *fad* fusion was transduced with P1 *vir* grown on the known *fad* mutants K1 (*fadA,B,C*), K27 (*fadD*), Old3003 (*fadD*), K19 (*fadE*), Old3008 (*fadL*), and AK4 (*fadL*), and *fad*$^+$ transductants were selected. Crosses between mutants at the same locus gave no or very few *fad*$^+$ recombinants (<100 per 10^9 P1 plaque-forming units). Crosses between strains carrying mutations in distinct loci gave frequent recombinants (>5,000 per 10^9 P1 plaque-forming units). Second, insertions of the transposon Tn*10* closely cotransducible with each known *fad* locus were isolated by the same method as for insertions near the *acd* gene (5).

P1 *vir* lysates grown on strains carrying such Tn*10* insertions were used to infect each *fad* fusion strain, and tetracycline-resistant recombinants were selected. Cotransduction of each Tn*10* insert with the various *fad* mutations (and the closely linked ampicillin resistance carried on the Mu *d*1 fusion phage) was examined.

Since *fadD* and *fadL* mutants cannot transport fatty acids (9, 14, 17), it is impossible to study the presumed induction of *fadD* or *fadL* gene fusions by fatty acids until the appropriate partial diploids of the type *fadD*$^+$/Φ(*fadD-lacZ*) have been constructed. Therefore, in this paper only fusions to the *fadA,B,C* and *fadE* operons are considered.

Regulation of the *fadA,B,C* and *fadE* operons. Of the putative fusions to the *fadA,B,C* and *fadE* operons, approximately half showed induction of β-galactosidase by oleic acid. The rest are presumably either double lysogens containing a Mu helper phage inserted in the *fad* gene plus a Mu *d* (Ap^r *lac*) phage elsewhere or Mu *d*1 (Ap^r *lac*) phage in the wrong orientation for control from the *fadA* or *fadE* promoter. Table 2 shows the levels of β-galactosidase observed in representative fusion strains grown under different conditions. Induction by oleate results in an approximately 10-fold increase in β-galactosidase over the levels seen in acetate-grown cells. Catabolite repression by glucose results in little detectable enzyme, irrespective of the presence or absence of an inducer. In contrast to glucose, α-methylglucoside showed a negligible effect in repressing β-galactoside levels in the *fad* fusion strains. The presence of Casamino Acids (0.1%) depressed both the basal level of enzyme and the extent of induction by two- to threefold for cells grown on acetate as the carbon source (data not given). Table 2 also contains data for strain DC519, which contains a fusion of *lacZ* to *lysA*. This fusion shows no significant effect of oleate on β-galactosidase induction, nor is the level of β-galactosidase repressed by glucose. Thus, the effects seen with *fad-lacZ* fusions are specific for β-galactosidase under *fad* control.

Figure 1 shows the relative inducing efficiencies of fatty acids of different chain lengths. Acids of less than 12 carbon atoms failed to induce. Dodecanoic and tridecanoic acids induced partly, and acids of 14 or more carbon atoms induced fully. These results correlate well with the growth properties of *E. coli* upon these fatty acids. (The poor induction observed with palmitic acid is probably due to its low solubility. At 2 mM, palmitate was present as an emulsion, not as a true solution.)

Several derivatives of myristic acid (14:0) were also tested. 12-Methylmyristic acid induced as well as myristic acid, and 3-hydroxymyristic acid induced feebly (induction ratio, 1.6), whereas 2-bromomyristic and 2-*trans*-tetradecenoic acids failed completely to induce. These results suggest a requirement for an unmodified —CH_2CH_2COOH structure at the carboxyl end. Since such a structure is also needed for β-oxidation, this makes good physiological sense.

Effect of cyclic AMP and imidazole. When 5 mM cyclic AMP was added to cultures grown on acetate plus glucose plus oleate, the β-galactosidase level was restored to that of cultures

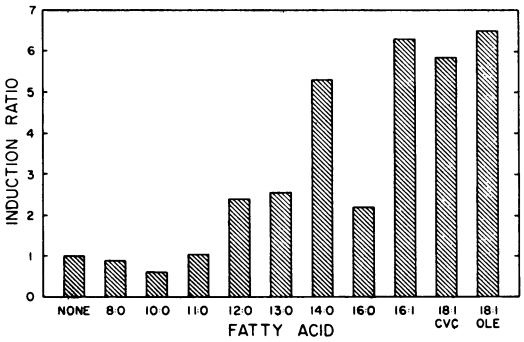

FIG. 1. *Effect of chain length upon induction. Strain DC530 Φ(fadA-lacZ) was grown in minimal medium with acetate as the carbon source and with various fatty acids present at 2 mM. The induction ratio is the β-galactosidase activity, relative to the control culture containing no fatty acid. Fatty acids are designated by n:k, where n is the number of carbon atoms and k is the number of double bonds. OLE, Oleate; CVC, cis-vaccenate; 16:1, palmitelaidate.*

TABLE 2. *Induction of fad operons*

Strain	β-Galactosidase activity[a]				
	Acetate	Acetate + oleate	Acetate + oleate + glucose	Glucose	Succinate
DC529 Φ(*fadA-lacZ*)	49	584	5.6	6.4	12.9
DC530 Φ(*fadA-lacZ*)	59	373	25	25	28
DC531 Φ(*fadE-lacZ*)	24	409	12.0	8.4	13.8
DC532 Φ(*fadE-lacZ*)	29	349	12.9	9.3	11.8
DC519 Φ(*lysA-lacZ*)	320	364	267	467	391

[a] β-Galactosidase as micromoles of *O*-nitrophenylgalactoside hydrolyzed per minute per 10^9 cells.

TABLE 3. *Cyclic AMP and imidazole*

Medium	Addition	Expression of *fadA* operon[a] (%)
Acetate	None	6.9
Acetate + oleate	None	100
Acetate + oleate + glucose	None	2.1
Acetate + oleate + glucose	Cyclic AMP (5 mM)	7.1
Acetate + oleate + glucose	Imidazole (20 mM)	12.1
Acetate + oleate + glucose	Imidazole (50 mM)	19.8

[a] The β-galactosidase level of strain DC530 Φ(*fadA-lacZ*) is expressed relative to cells grown in acetate plus oleate (i.e., full induction = 100%).

grown on acetate without glucose or oleate (Table 3). Thus, although cyclic AMP abolished the glucose repression of basal enzyme levels in non-induced cells, it had no effect on the glucose repression of induction by oleate.

Recently, imidazole, indoleacetic acid, and related compounds have been shown to replace cyclic AMP and to partly counteract the glucose repression of the arabinose operon, but not to affect several other catabolite-sensitive operons (10, 11). Strain DC530 Φ(*fadA-lacZ*) was grown in the presence of the inducer, oleate, and glucose plus various concentrations of imidazole. A 10-fold derepression of the *fadA* operon was seen in the presence of 50 mM imidazole (Table 3). When a culture of DC530 growing on acetate was diluted into medium containing oleate, glucose, and imidazole, derepression of β-galactosidase was observed only after two generations. A similar transient repression by glucose, followed by imidazole-mediated derepression, was also observed by Kline et al. (reference 10, Fig. 8). Indoleacetic acid was also found to derepress the *fad-lacZ* fusions at concentrations around 2 to 3 mM (data not shown).

Effect of mutation at the *fadR* locus. A Tn*10* insertion in the *fadR* (repressor) gene was introduced into each of the *fad-lacZ* fusion strains by P1-mediated transduction. The β-galactosidase of all fusion strains containing *fadR*::Tn*10* was expressed constitutively at a high level (Table 4). The constitutive enzyme levels in *fadR*::Tn*10* strains were equivalent to the highest levels seen when *fadR*$^+$ strains were induced with fatty acids. Furthermore, the enzyme expression in *fadR*::Tn*10* cells was much more resistant to catabolite repression by glucose. This difference could be seen on suitable indicator media. Thus, on lactose-MacConkey agar, Φ(*fad-lacZ*) *fadR*$^+$ colonies are pale pink, whereas the *fadR*::Tn*10* derivatives are deep red. Presumably, a substantial part of the glucose effect for *fadR*$^+$ cells derives from inducer exclusion (20).

Fusion strains DC530 and DC531 were treated with the mutagen ethyl methane sulfonate under conditions described previously (4), and colonies which were deep red on lactose-MacConkey agar were purified. Somewhat less than 20% of such isolates were found to carry a mutation at the *fadR* locus, as demonstrated by cotransduction with a Tn*10* insertion known to be 60% cotransducible with *fadR*. (The other mutations have not yet been analyzed but are presumably operator or promoter alterations at the individual *fad* loci.)

A variety of possible control substances were tried against the *fadR*::Tn*10* Φ(*fad-lacZ*) fusion strains. α-Methyl glucoside (10 mM) completely failed to repress β-galactosidase in succinate-grown cells, suggesting that glucose repression in the *fad* system may not be phosphotransferase mediated (19) but requires a metabolizable glucose derivative. Isopentenyladenosine (10 μg/ml) and furazolidone (0.1 μg/ml) (a nitrofuran), which are claimed to repress certain catabolite-repressible operons (6, 7), failed to show any effect on the *fad* system, even though the concentrations used were higher than those used by others (data not shown). Cyclic AMP added to glucose-grown cells caused only a very small (25%) increase in β-galactosidase levels (data not shown).

DISCUSSION

Previous work has shown that *E. coli* contains an inducible *fad* regulon specifying both the β-oxidation enzymes and a fatty acid transport system (9, 14, 17, 18, 23). This system has been shown to consist of several discrete operons which are conjointly induced by fatty acids with chain lengths of greater than 11 carbon atoms and which are subject to catabolite repression.

TABLE 4. *Effect of fadR mutation*

Strain	β-Galactosidase activity[a]			
	Acetate	Acetate + oleate	Glucose	Succinate
DC549 Φ(*fadA-lacZ*) *fadR*::Tn*10*	576	544	43	307
DC550 Φ(*fadA-lacZ*) *fadR*::Tn*10*	456	453	40	298
DC551 Φ(*fadE-lacZ*) *fadR*::Tn*10*	493	460	56	411
DC552 Φ(*fadE-lacZ*) *fadR*::Tn*10*	409	436	54	422

[a] β-Galactosidase as micromoles of *O*-nitrophenyl-galactoside hydrolyzed per minute per 10^9 cells.

The present work confirms and extends these findings and demonstrates that induction is probably at the transcriptional level. The inducer of the *fad* regulon is thought to be acyl-CoA, since *fadD* mutants which lack acyl-CoA synthetase are also defective in induction (8, 16). The finding that the β-galactosidase of *fadE-lacZ* fusion strains can be induced by fatty acids indicates that the conversion of acyl-CoA to enoyl-CoA by the acyl-CoA dehydrogenase, the product of the *fadE* gene (9; W. D. Nunn, unpublished data), is not necessary for induction and supports the theory that acyl-CoA is the inducer. Direct proof of this requires the construction and analysis of diploid fusion strains of the type *fadD*$^+$/Φ(*fadD-lacZ*).

There is considerable disagreement over the extent of induction of the β-oxidation enzymes (8, 15, 16, 18, 21, 23). Induction ratios vary from 4-fold (8) to 100-fold (16), and it has even been suggested that different components of the β-oxidation multienzyme complex, which have all been mapped at the same locus and which are all absent in the *fadA,B,C5* polar mutant (9, 17), are induced to extents differing by orders of magnitude (16). Closer examination of the published data shows a reasonable agreement on the fully induced enzyme levels but a substantial fluctuation in the estimation of the low basal levels. The probable reasons for these discrepancies are the technical difficulties involved in assaying the β-oxidation enzymes. These include the need to synthesize and purify complex substrates of uncertain stability, the insolubility of many fatty acid derivatives, complicated assay mixtures, and a multienzyme complex whose components differ in stability (2, 16). Clearly, replacement of such enzymes by β-galactosidase obviates these problems. My estimates of a 10- to 15-fold induction agree reasonably well with estimates from the laboratory of Weeks et al. (23), with later work of Overath et al. (17), and with the recent data of Simons et al. (21).

Previous work indicated that the *fad* regulon was subject to catabolite repression (18, 21, 23), and I have confirmed that this is due to transcriptional control by using operon fusions. The *fad* system appears to be very catabolite sensitive, since even succinate and casein hydrolysate, usually considered to be incapable of catabolite repression, depressed the level of *fad-lacZ* expression by two- to threefold, relative to growth on acetate. Strains constitutive for the *fad* regulon (*fadR* mutants) were much more resistant to catabolite repression than were *fadR*$^+$ strains. This suggests that a substantial part of the catabolite repression is due to inducer exclusion. Furthermore, the lack of effect of the non-metabolizable glucose analog α-methylglucoside indicates that phosphotransferase system-mediated effects are not sufficient to repress the *fad* regulon and that some metabolism of glucose is required.

The recent finding that the *ara* operon may be derepressed in the presence of glucose by imidazole derivatives (9, 10) prompted me to test the *fad* fusions. Up to 10-fold derepression was seen with imidazole. Although several catabolite-sensitive operons have been tested, only *ara* and *fad* have responded to imidazole so far. This intriguing phenomenon clearly deserves further investigation.

ACKNOWLEDGMENT

This work was supported by Public Health Service research grant AI 15650 from the National Institutes of Health to J. E. Cronan, Jr.

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. **Binstock, J. F., A. Pramanik, and H. Schultz.** 1977. Isolation of a multienzyme complex of fatty acid oxidation from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:492-495.
3. **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.
4. **Clark, D., and J. E. Cronan, Jr.** 1980. *Escherichia coli* mutants with altered control of alcohol dehydrogenase and nitrate reductase. J. Bacteriol. **141**:177-183.
5. **Clark, D., and J. E. Cronan, Jr.** 1980. Acetaldehyde coenzyme A dehydrogenase of *Escherichia coli*. J. Bacteriol. **144**:179-184.
6. **Coppola, S., A. Zoina, and P. Marino.** 1976. Interactions of N^6-(Δ^2 isopentenyl) adenosine with cyclic AMP on the regulation of growth and β-galactosidase synthesis in *Escherichia coli*. J. Gen. Microbiol. **94**:436-438.
7. **Hemlich, P., and M. Schweiger.** 1976. Nitrofurans, a group of synthetic antibiotics with a new mode of action. Discrimination of specific mRNA classes. Proc. Natl. Acad. Sci. U.S.A. **73**:3386-3390.
8. **Hill, F. F., and D. Angelmaier.** 1972. Specific enrichment of mutants of *Escherichia coli* with an altered acyl-CoA synthetase by tritium suicide. Mol. Gen. Genet. **117**:143-152.
9. **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.
10. **Kline, E. L., V. A. Bankaitis, C. S. Brown, and D. C. Montefiori.** 1980. Metabolite gene regulation: imidazole and imidazole derivatives which circumvent cyclic adenosine 3',5'-monophosphate in induction of the *Escherichia coli* L-arabinose operon. J. Bacteriol. **141**: 770-778.
11. **Kline, E. L., C. S. Brown, V. Bankaitis, D. C. Montefiori, and K. Crain.** 1980. Metabolite gene regulation of the L-arabinose operon in *Escherichia coli* with IAA and other indole derivatives. Proc. Natl. Acad. Sci. U.S.A. **77**:1768-1772.
12. **Kornberg, H. L.** 1966. The role and control of the glyoxylate cycle in *Escherichia coli*. Biochem. J. **99**:1-11.
13. **Maloy, S. R., M. Bohlander, and W. D. Nunn.** 1980. Elevated levels of glyoxylate shunt enzymes in *Escherichia coli* strains constitutive for fatty acid degrada-

tion. J. Bacteriol. **143**:720–725.

14. **Nunn, W. D., and R. W. Simons.** 1978. Transport of long chain fatty by *Escherichia coli*: mapping and characterization of mutants in the *fadL* gene. Proc. Natl. Acad. Sci. U.S.A. **75**:3377–3381.
15. **O'Brien, W. J., and F. E. Frerman.** 1973. A mutant of *Escherichia coli* with altered inducer specificity for the *fad* regulon. Biochem. Biophys. Res. Commun. **54**:697–703.
16. **O'Brien, W. J., and F. E. Frerman.** 1977. Evidence for a complex of three beta-oxidation enzymes in *Escherichia coli*: induction and localization. J. Bacteriol. **132**: 532–540.
17. **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.
18. **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.
19. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate: sugar phosphotransferase systems: structural, functional, and evolutionary interrelationships. Bacteriol. Rev. **41**:856–871.
20. **Saier, M. H., Jr., and S. Roseman.** 1976. Inducer exclusion and the regulation of the melibiose, maltose, glycerol and lactose transport systems by the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6606–6615.
21. **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.
22. **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.
23. **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.