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In Vivo Evidence that Acyl Coenzyme A Regulates DNA Binding by the *Escherichia coli* FadR Global Transcription Factor

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In vitro experiments point to fatty acyl coenzymes A (acyl-CoAs) rather than unesterified fatty acids as the small-molecule ligands regulating DNA binding by the FadR protein of *Escherichia coli*. To provide an in vivo test of this specificity, unesterified fatty acids were generated within the cellular cytosol. These fatty acids were found to be efficient modulators of FadR action only when the acids could be converted to acyl-CoAs.

The *Escherichia coli* FadR protein is a transcription factor that plays a dual role in fatty acid metabolism; this protein functions both as a repressor of the β-oxidation (*fad*) pathway and as an activator of the *fabA* gene, which is involved in unsaturated fatty acid biosynthesis (3, 9, 10, 16, 17). The role of FadR in fatty acid synthesis is to decrease the activity of the synthetic pathway when exogenous fatty acids are available for membrane lipid synthesis (10, 17), whereas in the *fad* regulon it functions as a classical transcriptional repressor (3, 9, 12, 19, 28). Recently, FadR has been shown to activate transcription of *iclR*, which encodes a repressor of the glyoxylate operon (14). Thus, FadR regulates both the conversion of fatty acids to acetyl coenzyme A (acetyl-CoA) and the utilization of acetyl-CoA (3, 9, 21). The different actions of FadR on transcription are readily explained by the positions of the operator sites to which the protein binds (17). When FadR acts as a repressor, the binding sites are within the promoter region, either overlapping the −10 or −35 region or lying just downstream of the −10 sequence (3, 17), whereas when FadR acts as an activator (12, 14, 17), the binding sites are immediately upstream of the promoter −35 region.

Addition of long-chain ($>C_{12}$) fatty acids to *E. coli* cultures results in induction of the enzymes involved in fatty acid degradation (3, 9, 12, 19, 29), the glyoxylate shunt (14, 21), and repression of unsaturated fatty acid synthesis (16, 17). These events are explained by the release of the FadR protein from all operator sites upon addition of fatty acids to the medium. Previously, we and others have argued that in vitro data indicate that the regulatory ligand causing release is fatty acyl-CoA synthesized from exogenous fatty acids by acyl-CoA synthetase rather than free (unesterified) fatty acids (FFAs). Long-chain acyl-CoAs release FadR from operator DNA in vitro (12, 17, 28), whereas long- or short-chain FFAs and short-chain acyl-CoAs are inactive. Acyl-CoA-mediated dissociation of FadR from operator DNA is reversed upon hydrolysis of the acyl-CoA (17), indicating that dissociation is not due to the detergent properties of long-chain acyl-CoAs. Moreover, a *fadR* mutant deficient in both induction of the Fad regulon and repression of unsaturated-fatty acid synthesis encodes a FadR protein that is defective in the release of operator DNA in the presence of long-chain acyl-CoAs in vitro and binds acyl-CoAs poorly (28).

Consistent with the in vitro results, mutant strains (*fadD*) that lack acyl-CoA synthetase activity fail to induce the β-oxidation pathway upon addition of long-chain fatty acids (19, 26). However, these experiments do not provide strong support for the in vitro experiments because *fadD* strains are also severely defective in the transport of fatty acids (3, 9, 26) (the synthetase activity plays an essential role in the major long-chain fatty acid transport system). Since long-chain fatty acids are unable to enter *fadD* mutant cells, the possibility that the FadR regulatory ligand is FFA (rather than fatty acyl-CoA esters) has not been tested in vivo. In this study, this question was approached via generation of FFAs within the cellular cytosol through diversion of acyl chains from the lipid biosynthetic pathway. This source of fatty acids was found to induce the Fad regulon efficiently, and this mode of induction required a functional *fadD* (acyl-CoA synthetase) gene.

Overexpression of an *E. coli* thioesterase (5, 6) was the method used to produce intracellular FFAs in the *E. coli* cytosol. *E. coli* thioesterase I (encoded by the *tesA* gene) is normally located in the cellular periplasm (4), but upon overproduction a portion of the enzyme remains in the cytosol (4, 5) (presumably due to titration of the export apparatus) and produces FFAs via cleavage of the thioester bonds of acylated species of acyl carrier protein (ACP), the intermediates of fatty acid synthesis and transfer (10). Deletion of the DNA sequence encoding the TesA leader peptide traps the thioesterase in the cytosol and results in a very high level of FFA production (6). The plasmid used contains the DNA sequence encoding the mature form of thioesterase I under the control of the *E. coli araBAD* promoter (6). Transcription of the mutant *tesA* gene gives a cytosolic protein product that (following action by the cellular methionine aminopeptidase) is identical to the mature form found in the periplasm. The plasmids used in this study, pHC122 (6) and its parental vector, pBAD22 (15), were described previously. In experiments utilizing an ampicillin-resistant Mud1 Φ(*fadBA-lacZ*) fusion (8), a kanamycin resistance determinant was introduced into plasmid pHC122 by ligation of the 1.2-kbp *Sal*I fragment of pUC4K (Pharmacia) into the *Sal*I site of the polylinker (located immediately downstream of the *′tesA* gene) to give plasmid pCY323. A derivative of pBAD22 with the resistance determinant inserted into the same site was also made and was called pCY322. In both plasmids, the kanamycin resistance gene had the same orientation as the *araBAD* promoter.

The first indication that endogenously generated FFAs could induce the *fad* regulon came from the growth behavior of strains containing the ′TesA-producing plasmid pHC122 on the short-chain fatty acids decanoate and octanoate. Wild-type *E. coli* strains are normally unable to grow on these acids as sole carbon sources (26, 29). Long-chain fatty acids induce the

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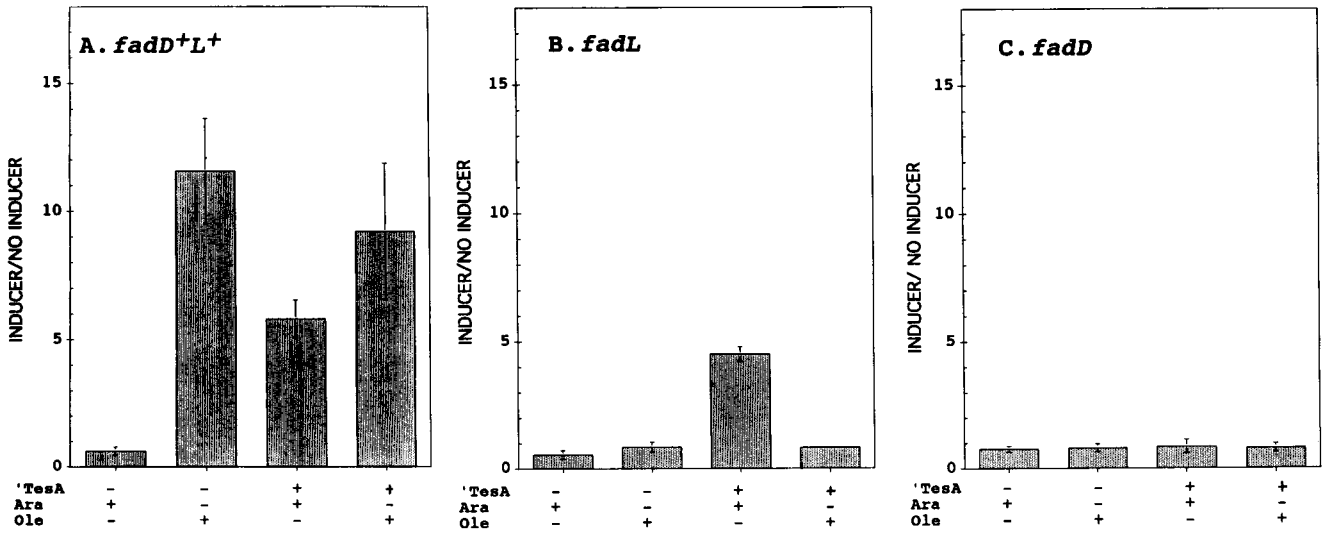

FIG. 1. Induction of the *fadBA* operon by endogenously generated fatty acids. The levels of β-galactosidase encoded by the *fadBA-lacZ* fusion are shown relative to those of the parallel control cultures grown in medium lacking either arabinose or oleate. As shown below the abscissa of each of the panels, the two left-hand bars denote the strain lacking ′TesA whereas the two right-hand bars denote the stain encoding ′TesA under control of the *araBAD* promoter. In each pair of bars, the left-hand bar denotes the culture supplemented with arabinose (Ara), the inducer of ′TesA synthesis, whereas the right-hand bar denotes the culture supplemented with oleate (Ole). The data given are the means of four to six experiments performed with independently derived cultures, and the error bars are the standard deviations from the mean (the right-hand bar of panel B lacks error bars because the standard deviation was below the graphing program cutoff value). The assays were done on log-phase cultures grown at 30°C in RB medium (1% tryptone, 0.1% yeast extract, 0.5% NaCl) containing 50 mg each of sodium ampicillin and kanamycin sulfate per ml plus 0.4% Brij 58 detergent. This medium was supplemented with either arabinose (0.02% final concentration) or potassium oleate (5 mM final concentration) as shown for at least three generations before harvest for β-galactosidase assay. β-Galactosidase was assayed as described previously (17, 23) except that the cells were harvested by centrifugation and resuspended in assay buffer before lysis. Under these conditions, strain AB264 gave a value of 4,500 Miller units (23) when fully induced with 1 mM isopropylthiogalactoside whereas strain CY1115 gave a value of 2,100 Miller units when fully induced with oleate. The strains used were as follows: panel A, strains CY1115 and CY1116 (which carried the wild-type alleles of *fadL* and *fadD*); panel B, the *fadL*::Tn*10* strains CY1117 and CY1118; and panel C, the *fadD88* strains CY1119 and CY1120. The even-numbered strains carried the vector plasmid pCY322, whereas the odd-numbered strains carried the ′TesA-encoding plasmid pCY323. All strains carried the Mud1 Φ(*fadBA-lacZ*) fusion.

β-oxidation enzymes, and although decanoate and octanoate can be degraded by the same enzymes, these shorter homologs fail to induce the pathway (26, 29). Selection for mutants that utilize decanoate or octanoate by plating wild-type cells on minimal medium containing the fatty acid as the sole carbon source results in *fadR* mutants (26, 29). It was reasoned that endogenous FFA synthesis should provide ligands that would induce the *fad* regulon and allow growth of a wild-type strain on decanoate or octanoate. The host strain used was strain AB264 (from the Coli Genetic Stock Center), a derivative of *E. coli* K-12(λ) F^+ which cannot utilize arabinose due to insertion of phage Mu into the *araBAD* operon. This strain was transformed with either the ′TesA-encoding plasmid pHC122 or the vector pBAD22, and the resulting strains (CY1096 and CY1097, respectively) were plated on decanoate minimal medium containing various concentrations of arabinose. Strain CY1097, which expressed the cytosol-trapped thioesterase, formed small colonies following several days of incubation at 37°C on decanoate plates containing low concentrations of arabinose (0.2 to 2 ng/ml) but failed to grow on decanoate plates containing 20 ng of arabinose per ml. Strain CY1096 carrying the vector plasmid pBAD22 failed to form colonies under these conditions. The concentrations of arabinose that permitted growth of strain CY1097 on decanoate were chosen on the basis of the data of Guzman and coworkers (15) in order to give low-level induction of ′TesA since higher levels of ′TesA were expected to inhibit β-oxidation via cleavage of the thioester-linked acyl-CoA intermediates. Indeed, higher concentrations of arabinose blocked growth of strain CY1097 on either oleate, a fatty acid that readily induces the *fad* regulon, or decanoate. It should be noted that these studies had to be performed on solid media since spontaneously arising *fadR* mutants overgrew liquid cultures. On solid media, the large *fadR* mutant colonies could be clearly distinguished from the slower-growing colonies resulting from endogenous induction. Growth on octanoate followed the same pattern, but as expected (26), the endogenously induced colonies grew more slowly. An attempt was made to avoid appearance of *fadR* colonies by introduction of a wild-type *fadR* gene on a multicopy plasmid (derived from pCD101 [11]) compatible with the ′TesA-producing plasmid. However, the presence of the *fadR* plasmid completely abolished the growth on decanoate or octanoate seen in the presence of low concentrations of arabinose, presumably by increasing *fad* operator occupancy (11) and thus raising the threshold level of FFA required for induction.

Direct evidence of induction of the *fad* regulon by endogenously generated FFA was obtained by use of the Φ(*fadBA-lacZ*) transcriptional fusion of Clark (8). This fusion had previously been transduced into the *ara-leu* deletion strain MC1061 to give strain MH180 (17), and the latter strain was used in this work to avoid the arabinose sensitivity of the original fusion strain. Strain MH180 was transformed with the vector plasmid pCY322 and the ′TesA-producing plasmid pCY323 to give strains CY1115 and CY1116, respectively. Growth of both strains in the presence of 5 mM oleic acid gave the large increase in β-galactosidase levels expected from prior work (8, 17) (Fig. 1A). However, only the ′TesA-producing strain, CY1116, produced high levels of β-galactosidase upon induction with arabinose (Fig. 1A). Therefore, production of FFAs by thioesterase-mediated cleavage of lipid synthetic intermediates was sufficient to induce expression of the *fadBA* operon. The levels of induction seen under these conditions appeared lower than those observed upon induction with exo-

geneous fatty acid. However, addition of arabinose to the control strain, CY1115, gave a 30 to 40% decrease in both the basal-expression and oleate-induced levels relative to cultures lacking arabinose. This effect of arabinose was observed with different lots of arabinose and is not understood but seems likely to be due to weak catabolite repression of *fadBA* expression (8, 27) by contaminants present in the arabinose. If it is assumed that the endogenously induced β-galactosidase levels were similarly decreased, then the levels of induction by endogenous and exogenous fatty acids were comparable.

Prior work in this laboratory (6) led to the unexpected finding that much of the FFA produced upon ′TesA expression is found in the culture medium rather than within the cells (which remain intact). This raised the possibility of a trivial explanation for the increased *fadBA* transcription seen upon thioesterase induction (Fig. 1A), that induction of this strain by arabinose proceeds by recapture of the FFA from the medium rather than through generation of FFA in the cellular cytoplasm. That is, induction might be the result of an indirect (via thioesterase overexpression) supplementation of the medium with fatty acids. This was shown not to be the case by introduction of a *fadL* null mutation via transduction of strains CY1115 and CY1116 to tetracycline resistance with a P1*vir* phage lysate grown on strain LS5283 (*fadL771*::Tn*10*; from W. Nunn) to give strains CY1117 and CY1118, respectively. Strains encoding defective FadL proteins lack an outer membrane protein required for uptake of long-chain FFAs and concomitant induction of the *fad* regulon (3, 9, 22, 25). Addition of oleate failed to induce either strain, indicating that long-chain fatty acid uptake was effectively blocked. However, addition of arabinose gave increased levels of β-galactosidase in the ′TesA-producing strain CY1118 (Fig. 1B). Therefore, the increase in *fadBA* expression seen upon FFA production is primarily due to intracellular FFAs rather than FFAs imported from the medium.

The results shown in Fig. 1A and B indicate that the FFA produced within the cell induces the *fad* regulon efficiently and that either the fatty acid itself or a derived metabolite, such as fatty acyl-CoA, could be the regulatory ligand. If the ligand is fatty acyl-CoA, then *fad* induction by endogenously generated FFA should be blocked by inactivation of acyl-CoA synthetase. Therefore, strains CY1115 and CY1116 were transduced to tetracycline resistance with a phage P1*vir* lysate grown on strain LS5537 (*fadD88 zea*::Tn*10*; from W. Nunn) to give strains CY1119 and CY1120, respectively. The *fadD88* mutation has been reported to be a deletion (18) and results in a complete lack of acyl-CoA synthetase as measured either by in vitro assay (2, 18, 19) or by participation in a coupled protein acylation system in vivo (20). (In reference 20, the allele was called *fadD27*, which is an unassigned allele number. Reference to the literature cited therein indicated that the allele was *fadD88*.) Cotransduction of the *fadD88* lesion with the linked Tn*10* element could not be scored by growth on oleate since the Mud1 insertion in *fadBA* blocked β-oxidation. Thus, the *fadD* marker was scored by failure of oleate supplementation to increase the basal level of β-galactosidase measured in vitro. The presence of the *fadD88* mutation in these strains was confirmed by P1 transductional backcrosses to the wild-type strain MG1655 (from the Coli Genetic Stock Center) with selection for tetracycline resistance. Transductants unable to grow on medium with oleate as the sole carbon source were found at the expected frequency (46%). (Similar backcrosses giving the expected 100% cotransduction of tetracycline resistance and lack of growth on oleate confirmed the existence of the *fadL771*::Tn*10* lesion in strains CY1117 and CY1118.) Strains CY1119 and CY1120 were completely defective in induction of *fadBA*::*lacZ* expression by fatty acids provided by either external supplementation or thioesterase action (Fig. 1C). These data therefore provide strong in vivo evidence that acyl-CoA, rather than fatty acid, is the ligand that regulates FadR action.

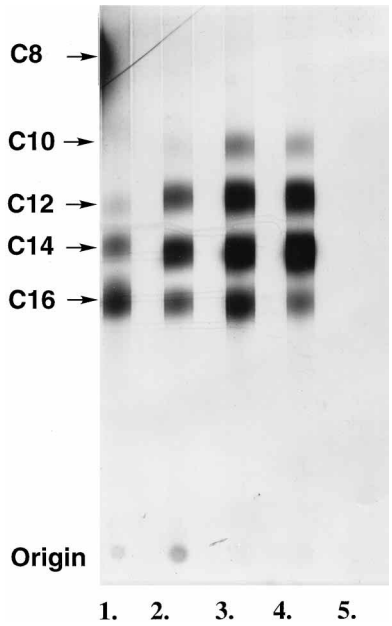

FIG. 2. Autoradiogram of the FFA species found in the culture media. The strains were grown at 30°C in the presence of arabinose and labeled with [^{14}C]acetate as described previously (6). The cells were then removed by centrifugation, and equal volumes of the media were extracted with chloroform-methanol (6). The resulting chloroform extracts were passed through a small (1-g) column of acid-washed Florasil (7) to purify the FFA fractions, which were then chromatographed on a Whatman C18 reversed-phase thin-layer chromatography plate developed in acetonitrile-acetic acid-acetone (7:1:1, by volume) followed by autoradiography. All strains carried the ′TesA-encoding plasmid except that in lane 5. Lane 1 (for comparison with the FFA fractions), fatty acids released from the cellular phospholipids of strain CY1116 by saponification (7); lane 2, FFA fraction from CY1116 (which carries the wild-type alleles of *fadL* and *fadD*); lane 3, FFA fraction from the *fadL*::Tn*10* strain CY1118; lane 4, FFA fraction from the *fadD* strain CY1120; and lane 5, FFA fraction from the *fadD* strain CY1119 (no FFA were formed due to the lack of ′TesA). The migration positions of commercial 1-^{14}C-saturated fatty acids and the origin are marked (the exposure at the top of lane 1 is due to radiation from the octanoic acid standard chromatographed in the neighboring lane). Note that in this system each of the unsaturated fatty acids cochromatographs with the saturated fatty acid that is two carbons shorter. The lower degree of labeling of strain CY1116 was due to the greater cell density in the culture (when corrected to a culture optical density at 600 nm of 1.0, the FFA counts per minute varied from 30,200 to 38,000 in the media of the three ′TesA-producing strains whereas the medium of strain CY1119 had <1% of this amount).

The somewhat lower ′TesA-engendered induction of the *fadL*::Tn*10* strain, CY1118, compared to that of the strain (CY1116) carrying the wild-type *fadL* gene suggested that a part of the induction seen in the latter strain might be due to uptake of long-chain FFAs from the medium. This in turn suggested that the *fadL* null mutation did not block the exit of FFAs to the medium. Thus, it seemed that the FadL outer membrane protein might be required only for fatty acid uptake from the medium and not for the reverse of this process. To directly test this notion, the lipids of strain CY1116 and its *fadL* derivative, CY1118, were labeled by growth in the presence of [^{14}C]acetate and the media were assayed for FFA. The amounts of FFA in the media were essentially the same for the two strains (Fig. 2), and thus the FadL protein was not required for the exit of FFAs from the cells. Another test was provided by examining the chain lengths of the medium FFA

fractions (Fig. 2). In fatty acid transport, FadL is required only for uptake of long-chain (C_{12} or greater) fatty acids whereas shorter-chain fatty acids enter the cell by an unknown mechanism that does not depend on FadL (3, 22, 25). Hence, if fatty acid exit was dependent on FadL, then the acids in the media of *fadL* strains should consist predominantly of short-chain species. As shown in Fig. 2, the chain lengths of the FFA fractions of the two strains were essentially identical, indicating that the FadL protein plays no essential role in the fatty acid exit process. [^{14}C]acetate labeling studies of strain AB264 and a *fadL771*::Tn*10* transductant of AB264 also showed similar amounts and compositions of FFA in the culture media (data not shown). The FFA content and chain length distribution (Fig. 2) in the medium of the *fadD* strain CY1120 were very similar to those of the strain with a wild-type *fadD* gene (CY1116), indicating that acyl-CoA synthetase is not required for fatty acid exit and that the failure to induce *fadBA-lacZ* expression in this strain (Fig. 1C) was not due to a lack of FFA production.

The experiments reported provide strong in vivo evidence to support the in vitro data indicating that acyl-CoA is the small-molecule ligand that regulates DNA binding by FadR. It remains possible that acyl-CoA is converted to another molecule which is the true inducer, but this seems unlikely. It also seems unlikely that the *fadD*-encoded acyl-CoA synthetase can synthesize a molecule other than acyl-CoA, since the enzyme seems very specific in vitro (18, 26) and can be functionally replaced in *E. coli* fatty acid transport by an acyl-CoA synthetase from the yeast *Saccharomyces cerevisiae* (20). There exists the possibility that acyl-ACP is the regulatory ligand, but this possibility can be eliminated because acyl-ACPs cannot be synthesized from exogenously supplied fatty acids (10).

The data of Fig. 2 indicate that the FadL protein is not required for exit of FFAs from the cytosol. The mechanism of fatty acid exit is unclear. One possibility is that these molecules are pumped from the cytosol by one of the several *E. coli* exporters involved in efflux of hydrophobic molecules and many antibiotics (13, 24). These systems are inducible and consist of an inner membrane pump that is thought to be coupled to an outer membrane channel by a periplasmic protein. The outer membrane channel can be induced together with the pump, or an existing channel can be used by the pump (13, 24). If FFA exit is mediated by an efflux pump, then FadL does not provide a channel required for exit of FFAs through the outer membrane.

Long-chain acyl-CoAs are readily cleaved by TesA (1), and thus it might be expected that overproduction of this thioesterase might inhibit rather than induce the *fad* regulon. However, this is not the case (Fig. 1). This apparent contradiction can be explained by the differences in the relative affinities of FadR and TesA for acyl-CoAs. Long-chain acyl-CoA concentrations of 5 to 10 nM are sufficient to release FadR from operator DNA (28), whereas the Michaelis-Menten constants for cleavage of these substrates by TesA are about 1,000-fold higher (1). Moreover, the enzymes of the β-oxidation cycle require acyl-CoA substrate concentrations of >30 μM for optimal activity (30), and thus it seems that the high affinity of FadR for acyl-CoA is an attribute that allows induction of the *fad* regulon in the presence of acyl-CoA-metabolizing enzymes.

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REFERENCES

1. **Barnes, E. M., Jr., and S. J. Wakil.** 1968. Studies on the mechanism of fatty acid synthesis. XIX. Preparation and properties of palmitoyl thioesterase. J. Biol. Chem. **243:**2955–2962.
2. **Black, P. N., C. C. DiRusso, A. K. Metzger, and T. L. Heimert.** 1992. Cloning, sequencing and expression of the *fadD* gene of *Escherichia coli* encoding acyl coenzyme A synthetase. J. Biol. Chem. **267:**25513–25520.
3. **Black, P. N., and C. C. DiRusso.** 1994. Molecular and biochemical analyses of fatty acid transport, metabolism, and gene regulation in *Escherichia coli*. Biochim. Biophys. Acta **1210:**123–145.
4. **Cho, H., and J. E. Cronan, Jr.** 1993. *Escherichia coli* thioesterase I: molecular cloning and sequencing of the structural gene and identification as a periplasmic enzyme. J. Biol. Chem. **268:**9238–9245.
5. **Cho, H., and J. E. Cronan, Jr.** 1994. "Protease I" of *Escherichia coli* functions as a thioesterase in vivo. J. Bacteriol. **176:**1793–1795.
6. **Cho, H., and J. E. Cronan, Jr.** 1995. Defective export of a periplasmic enzyme disrupts regulation of bacterial fatty acid synthesis. J. Biol. Chem. **270:**4216–4219.
7. **Christie, W. W.** 1982. Lipid analysis, 2nd ed. Pergamon Press, Oxford, United Kingdom.
8. **Clark, D.** 1981. Regulation of fatty acid degradation in *Escherichia coli*: analysis by operon fusion. J. Bacteriol. **148:**521–526.
9. **Clark, D. P., and J. E. Cronan, Jr.** 1996. Two-carbon compounds and fatty acids as carbon sources, p. 343–357. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Linn, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
10. **Cronan, J. E., Jr., and C. O. Rock.** 1996. Biosynthesis of membrane lipids, p. 612–636. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Linn, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
11. **DiRusso, C. C., and W. D. Nunn.** 1985. Cloning and characterization of a gene (*fadR*) involved in regulation of fatty acid metabolism in *Escherichia coli*. J. Bacteriol. **161:**583–588.
12. **DiRusso, C. C., T. L. Heimert, and A. K. Metzger.** 1992. Characterization of FadR, a global transcriptional regulator of fatty acid metabolism in *Escherichia coli*: interaction with the *fadB* promoter is prevented by long-chain fatty acyl-CoAs. J. Biol. Chem. **267:**8685–8691.
13. **George, A. M.** 1996. Multidrug resistance in enteric and other gram-negative bacteria. FEMS Microbiol. Lett. **139:**1–10.
14. **Gui, L., A. Sunnarborg, and D. C. LaPorte.** 1996. Regulated expression of a repressor protein: FadR activates *iclR*. J. Bacteriol. **178:**4704–4709.
15. **Guzman, L.-M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
16. **Henry, M. F., and J. E. Cronan, Jr.** 1991. *Escherichia coli* transcription factor that both activates fatty acid synthesis and represses fatty acid degradation. J. Mol. Biol. **222:**843–849.
17. **Henry, M. F., and J. E. Cronan, Jr.** 1992. A new mechanism of transcriptional regulation — release of an activator triggered by small molecule binding. Cell **70:**671–679.
18. **Kameda, K., and W. D. Nunn.** 1981. Purification and characterization of acyl coenzyme A synthetase from *Escherichia coli*. J. Biol. Chem. **256:**5702–5707.
19. **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.
20. **Knoll, L. J., and J. I. Gordon.** 1993. Use of strains containing *fad* mutations plus a triple plasmid system to study import of myristate, its activation by *Saccharomyces cerevisiae* acyl-CoA synthetase and its utilization by *S. cerevisiae* myristoyl-CoA-protein N-myristoyltransferase. J. Biol. Chem. **268:**4281–4290.
21. **Maloy, S. R., and W. D. Nunn.** 1981. Role of gene *fadR* in *Escherichia coli* acetate metabolism. J. Bacteriol. **148:**83–90.
22. **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.
23. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Nikaido, H.** 1994. Prevention of drug access to bacterial targets — permeability barriers and active efflux. Science **264:**382–388.
25. **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 the *fadL* gene. J. Biol. Chem. **254:**9130–9134.
26. **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.
27. **Pauli, G., R. Ehring, and P. Overath.** 1974. Fatty acid degradation in *Escherichia coli*: requirement of cyclic adenosine monophosphate and cyclic aden-

osine monophosphate receptor protein for enzyme synthesis. J. Bacteriol. **117:**1178–1183.

28. **Raman, N., and C. C. DiRusso.** 1995. Analysis of acyl coenzyme A binding to the transcription factor FadR and identification of amino acid residues in the carboxyl terminus required for ligand binding. J. Biol. Chem. **270:**1092–1097.
29. **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.
30. **Yang, S.-Y., J. Li, X.-Y. He, S. D. Cosloy, and H. Schulz.** 1988. Evidence that the *fadB* gene of the *fadAB* operon of *Escherichia coli* encodes 3-hydroxyacyl-coenzyme A (CoA) epimerase, Δ^3-*cis*-Δ^3-*trans*-enoyl-CoA isomerase, and enoyl-CoA hydratase in addition to 3-hydroxyacyl-CoA dehydrogenase. J. Bacteriol. **170:**2543–2548.