JOURNAL OF BACTERIOLOGY, May 1983, p. 554–560
0021-9193/83/050554-07$02.00/0

Vol. 154, No. 2

Role for *fadR* in Unsaturated Fatty Acid Biosynthesis in *Escherichia coli*

WILLIAM D. NUNN,[1]* KELLEEN GIFFIN,[1] DAVID CLARK,[2]† AND JOHN E. CRONAN, JR.[2]

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717,[1] and Department of Microbiology, University of Illinois, Urbana, Illinois 61801[2]

Received 29 November 1982/Accepted 3 February 1983

Escherichia coli K-12 mutants constitutive for the synthesis of the enzymes of fatty acid degradation (*fad*) synthesize significantly less unsaturated fatty acid (UFA) than do wild-type (*fadR*$^+$) strains. The constitutive *fadR* mutants synthesize less UFA than do *fadR*$^+$ strains both in vivo and in vitro. The inability of *fadR* strains to synthesize UFAs at rates comparable to those of *fadR*$^+$ strains is phenotypically asymptomatic unless the *fadR* strain also carries a lesion in *fabA*, the structural gene for β-hydroxydecanoyl-thioester dehydrase. Unlike *fadR*$^+$ *fabA*(Ts) mutants, *fadR fabA*(Ts) strains synthesize insufficient UFA to support their growth even at low temperatures and, therefore, must be supplemented with UFA at both low and high temperatures. The low levels of UFA in *fadR* strains are not due to the constitutive level of fatty acid-degrading enzymes in these strains. These results suggest that a functional *fadR* gene is required for the maximal expression of UFA biosynthesis in *E. coli*.

Unsaturated fatty acids (UFAs) comprise about one-half of the fatty acid content of wild-type *Escherichia coli* and are primarily found esterified in the second position of the *sn*-glycerol 3-phosphate backbone of the membrane phospholipids. Palmitoleic acid (Δ9 $C_{16:1}$) and *cis*-vaccenic acid (Δ11 $C_{18:1}$) are the major UFAs in this organism, whereas palmitic acid ($C_{16:0}$) is the primary saturated fatty acid. Cronan and Gelmann (3) have shown that a minimum of 15 to 20% of the total fatty acid content in the phospholipids of *E. coli* must be UFA for the cell to grow at 35°C. When the UFA content falls below 15%, growth ceases and the cells lyse (3).

To synthesize the UFA palmitoleic acid, *E. coli* requires an enzyme which introduces the double bond of unsaturates, β-hydroxydecanoyl-thioester dehydrase (the product of the *fabA* gene), and the fatty acid synthetase system, which specifically utilizes the chain-elongating enzyme encoded for by the *fabB* gene, the β-ketoacyl-acyl carrier protein (ACP) synthase I (2, 5, 7). Palmitoleic acid is converted to *cis*-vaccenic acid by the fatty acid synthetase system, which specifically utilizes the chain-elongating enzyme encoded for by the *fabF* gene, the β-ketoacyl-ACP synthase II (6–8).

Wild-type *E. coli* K-12 oxidizes fatty acids by cyclic β-oxidation and thiolytic cleavage to acetyl coenzyme A (acetyl-CoA) which is further metabolized via the tricarboxylic acid cycle and the glyoxylate shunt (15, 17, 19). The syntheses of at least five fatty acid degradation (*fad*) enzymes are coordinately induced when long-chain fatty acids (C_{12} to C_{18}) are present in the growth medium (11, 18, 22). The genes coding for the *fad* enzymes are located at several sites on the chromosome and comprise a regulon. The expression of the *fad* regulon is under negative control by the *fadR* gene (17, 19, 20), which maps at 25.5 min on the revised *E. coli* K-12 linkage map (1, 19). The *fad* enzymes are inducible in wild-type (*fadR*$^+$) strains and constitutive in *fadR* strains (17, 19, 20). The *fadR* gene also appears to negatively control the expression of the *aceAB* operon (12, 14, 15).

In this paper, we present studies which suggest that the *fadR* gene may play a role in the regulation of UFA biosynthesis. We have found that *fadR* mutants synthesize substantially less UFA than *fadR*$^+$ strains. Further studies suggest that a functional *fadR* gene is required for maximal UFA synthesis, particularly that of Δ11 $C_{18:1}$ in *E. coli*.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The strains used in this study were all derivatives of *E. coli* K-12 (Table 1). Preparation of phage stocks and transductions were performed as previously described (19). Strains bearing the *fabA2* lesion have a temperature-sensitive defect in β-hydroxydecanoyl-thioester dehydrase (4, 5), the enzyme which catalyzes the formation of the double-bond moiety of UFA. Strains LS6483

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

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source or reference
K-12	Prototrophic	CGSC[a]
RS3010	*fadR*	(19)
RS3040	*fadR*::Tn*10*	(19)
RS3069	*fadR*::Tn*5*	(19, 20)
UC1098	*fabA2 fabF*	(4)
UC1098DT	*fadR*::Tn*10 fabA2 fabF*	This work
LS5182	*pyrD his trp rpsL*	(1)
LS6483	*fabA2*	This work
LS6494	*fadR zcb*::Tn*10 fabA2*	This work
LS6495	*zcb*::Tn*10 fabA2*	This work
LS6496	*fadR*::Tn*5 fabA2*	This work
LS6502	*zcb*::Tn*10 pyrD*	This work
LS6592	*fadR*::Tn*10 fabA2*	This work
LS6593	Prototrophic	This work
LS6594	*fadR*	This work
LS6596	*fadR*::Tn*5*	This work
LS7070	*fadR*	(13)
LS7071	*fadR fadL*	(13)
LS7072	*fadR fadD zea*::Tn*10*	(13)
LS7075	*fadR fadABC*::Tn*10*	(13)
LS7076	*fadR fadE zaf*::Tn*10*	(13)

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

[b] Transposon insertions are designated as previously described (10, 14). When an insertion is not within a known gene, it is given a three-letter symbol starting with *z*. 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).

(*fabA2*) and LS6495 (*zcb*::Tn*10 fabA2*) were obtained by transducing the *zcb*::Tn*10 pyrD*$^-$ K-12 derivative (strain LS6502) to *pyrD*$^+$ with phage P1 *vir* grown on strain UC1098 (*fabA2 fabF*). Strains UC1098DT and LS6592, which carry Tn*10* insertions in their *fadR* gene, were obtained by transducing strains UC1098 and LS6483 with P1 *vir* phage grown on strain RS3040 (*fadR*::Tn*10*) to tetracycline resistance in minimal medium containing tetracycline and the UFA oleate as the sole carbon source. Strain LS6496, which carries a Tn*5* insertion in *fadR*, was obtained by transducing strain LS6483 with P1 *vir* phage grown on RS3069 (fadR::Tn*5*) to kanamycin resistance (Kn^r) in minimal medium containing kanamycin and oleate. Strain LS6494 was obtained by transducing strain RS3010 with P1 *vir* phage grown on strain LS6495 (*zcb*::Tn*10 fabA2*) to tetracyline resistance in minimal medium containing tetracycline and oleate. Strains LS6593 and LS6594 are *fabA*$^+$ transductants of strains LS6483 and LS6496 which were obtained by transducing strains LS6483 and LS6496 with P1 *vir* phage grown on strain K-12. Strain LS6502 was constructed by transducing strain K-12 to tetracycline resistance with phage P1 *vir* grown on a *zcb*::Tn*10* derivative of LS5182. Insertions of Tn*10* near the *pyrD* genes were obtained by the method previously used to isolate insertions near the *aceA* and *aceB* genes (12).

Bacteria were routinely incubated in gyratory water bath shakers at 30°C in minimal medium E (16) supplemented with 15 μM thiamine and 50 mM glycerol. When other carbon sources were used, they were added as follows: 25 mM D-glucose and 50 mM acetate. The *fadR fabA2* strains were routinely grown in the same medium supplemented with 100 μM oleate. Tetracycline was used at 20 μg/ml, and kanamycin was used at 30 μg/ml.

Lipid analysis. Phospholipids were extracted from whole cells by the method of Gelmann and Cronan (8). Fatty acids from either phospholipid or fatty acid synthetase reaction mixtures were converted to methyl esters as described by Gelmann and Cronan (8).

Radioactive fatty acid methyl esters were analyzed by chromatography on silver nitrate-impregnated Silica Gel G thin-layer plates (9). These plates were obtained by dipping Silica Gel G thin-layer plates (250 μm thick) into a solution of 15% (wt/vol) $AgNO_3$ in acetonitrile. After evaporation of the solvent, the plates were activated at 100°C for at least 2 h. Plates prepared in this manner and developed twice in toluene at −22°C gave highly reproducible separations of palmitoleic, *cis*-vaccenic, and saturated fatty acids similar to those reported previously (8).

Nonradioactive fatty acid methyl esters were resolved by gas-liquid chromatography on columns (1.8 m by 4 mm) of 10% Apiezon L on 60/80 Chromosorb W. A Varian model 3700 instrument equipped with dual-flame ionization detectors was used. This instrument was run at 220°C, with a carrier gas flow of 45 ml/min. The fatty acid methyl esters were identified as described by Gelmann and Cronan (8). Phospholipids were separated on commercial Silica Gel G plates (250 μm) as described previously (8, 13).

Fatty acid synthetase. Fatty acid synthetase was measured in vitro as described by Gelmann and Cronan (8). Crude extracts for these assays were prepared by disrupting mid-exponential-phase cells in a French press as previously described (12). The studies presented in this paper were performed with cells which had been grown in minimal medium E supplemented with thiamine, 50 mM glycerol, and 100 μM oleate.

Materials. Radioactive chemicals were obtained from New England Nuclear Corp., Boston, Mass. Thin-layer chromatographic plates were obtained from Analtech, Newark, Del.

RESULTS

UFA Synthesis in *fadR*$^+$ *fabA2* and *fadR fabA2* strains. We first became aware that the *fadR* gene played a role in UFA biosynthesis during attempts to construct *fadR* derivatives of *fadR*$^+$ *fabA2* mutants. The *fadR*$^+$ *fabA2* mutants (UC1098 and LS6483) used for these genetic manipulations were unable to grow at temperatures above 32°C unless supplemented with an UFA (Table 2). The requirement for an UFA at temperatures above 32°C is due to a lesion in the *fabA* gene which results in the formation of a β-hydroxydecanoyl-thioester dehydrase activity of greatly increased thermolability (3, 9). The *fadR*$^+$ *fabA2* mutants grew normally at temperatures below 32°C without UFA supplementation (Table 2). We were unable to construct *fadR*

TABLE 2. Growth behavior of *fadR*$^+$ *fabA2* and *fadR fabA2* strains

Strain	Genotype	Growth[a]					
		30°C, fatty acid supplement			42°C, fatty acid supplement		
		None	$C_{16:1}$	$C_{18:1}$	None	$C_{16:1}$	$C_{18:1}$
UC1098	*fadR*$^+$ *fabA2 fabF*	+	+	+	−	+	+
UC1098DT	*fadR*::Tn*10 fabA2 fabF*	−	+	+	−	+	+
LS6483	*fadR*$^+$ *fabA2*	+	+	+	−	+	+
LS6494	*fadR zcb*::Tn*10 fabA2*	−	+	+	−	+	+
LS6496	*fadR*::Tn*5 fabA2*	−	+	+	−	+	+
LS6592	*fadR*::Tn*10 fabA2*	−	+	+	−	+	+
K-12	*fadR*$^+$ *fabA*$^+$	+	+	+	+	+	+
RS3010	*fadR fabA*$^+$	+	+	+	+	+	+
RS3040	*fadR*::Tn*10 fabA*$^+$	+	+	+	+	+	+

[a] Cultures were grown in medium EB_1 (16) supplemented with 50 mM glycerol. Unsaturated fatty acids ($C_{16:1}$ or $C_{18:1}$, 100 μM) were supplemented in the growth medium. *fadR*$^+$ transductants of LS6494, LS6496, and LS6592 behaved like *fadR*$^+$ *fabA2* strain LS6483 (data not shown).

fabA2 strains at any temperature (20 to 42°C), without supplementing the selection media with UFA. Our growth studies with the *fadR fabA2* strains (UC1098DT, LS6494, LS6496, and LS6592) indicated that these strains required an UFA (either palmitoleic, oleic, or linoleic acid) to grow at 30 and 42°C. Supplementation of *fadR fabA2* strains with the saturated fatty acid palmitic acid did not permit growth (data not shown). Since the growth studies suggested that *fadR fabA2* strains do not synthesize sufficient UFA to sustain their growth at any temperature, we measured UFA biosynthesis in *fadR fabA2* strains to confirm our suspicions. Total fatty acid synthesis was measured by [^{14}C]acetate incorporation at 30°C in a minimal medium containing glycerol as the sole carbon source. The strains had been pregrown to the log phase in a minimal medium containing glycerol and supplemented with the UFA oleate, before being switched to a minimal medium containing only glycerol. When fatty acid synthesis was measured in *fadR*$^+$ *fabA2* strains, at least 40% of the total fatty acids synthesized were UFA (Table 3). In contrast, only about 20% of the total fatty acids synthesized in *fadR fabA2* strains were UFA (Table 3). Cronan and Gelmann (3) have shown that a minimum of 15 to 20% of the total fatty acid content of *E. coli* must be UFA at 35°C or growth ceases and the cells lyse. Since it is known that these organisms synthesize more UFA at lower temperatures (3), the low rate of UFA synthesis (Table 3) may explain why *fadR fabA2* strains are incapable of growth at 30°C without UFA supplementation. Interestingly, in studies with *fabF* strains that are defective in their ability to convert *cis*-palmitoleic acid to *cis*-vaccenic acid, only 4.2% of the total fatty acids in strain UC1098DT (*fadR fabA2 fabF*) were palmitoleic acid, whereas 39.6% of the total fatty acids in strain UC1098 (*fadR*$^+$ *fabA2 fabF*) were palmitoleic acid. Although the reason for the drastic decrease in UFA content in strain UC1098DT is unclear, the results suggest that control of UFA biosynthesis in *fadR* strains

TABLE 3. Fatty acid composition of *fadR*$^+$ *fabA2* and *fadR fabA2* strains

Strain[a]	Relative fatty acid composition			
	UFA (%)			SFA[b] (%)
	$C_{16:1}$	$C_{18:1}$	Total	
UC1098	39.6	1.9	41.5	58.5
UC1098DT	4.2	0.4	4.6	95.4
LS6483	23.5	15.5	39.0	61.0
LS6496	14.7	9.0	23.7	76.3
LS6592	13.0	6.9	19.9	80.1
K12	29.9	28.4	58.3	41.7
RS3010	26.4	12.4	38.8	61.2
RS3040	27.9	13.6	41.5	58.5

[a] The above strains were grown at 30°C to 2.5 × 10^8 cells per ml in minimal medium supplemented with 50 mM glycerol and 100 μM $C_{18:1}$. The strains were harvested by centrifugation, washed once, and suspended in the same medium minus $C_{18:1}$. One-milliliter samples were removed from each culture and added to test tubes containing 5 μCi of [^{14}C]acetate (57 μCi/μmol). After 30 min, incorporation was terminated by the addition of 6 ml of $CHCl_3$-CH_3OH (1:2, vol/vol). The [^{14}C]acetate-labeled lipids were extracted, processed, and analyzed for their fatty acid composition as described in the text. The rates of fatty acid synthesis in these strains were all ca. 3 nmol/min per mg of protein. *fabA*$^+$ transductants and revertants of LS6496 and LS6592 have fatty acid compositions similar to those of the *fadR fabA*$^+$ strains RS3010 and RS3040 (data not shown). *fabA*$^+$ transductants and revertants of LS6483 have the same fatty acid composition as strain K-12 (data not shown).

[b] Saturated fatty acids.

TABLE 4. Fatty acid composition of *fadR fad* strains

Strain[a]	Genotype	Relative fatty acid composition			
		UFA (%)			SFA[b] (%)
		$C_{16:1}$	$C_{18:1}$	Total	
K-12	*fadR*$^+$ *fabA*$^+$	23.5	31.0	54.5	45.5
LS7070	*fadR fabA*$^+$	16.1	17.9	34.0	66.0
LS7071	*fadR fabA*$^+$ *fadL*	16.8	7.0	23.8	76.2
LS7072	*fadR fabA*$^+$ *zea*::Tn*10 fadD*	22.4	11.9	34.3	65.7
LS7075	*fadR fabA*$^+$ *fadABC*::Tn*10*	17.0	12.8	29.8	70.2
LS7076	*fadR fabA*$^+$ *fadE zaf*::Tn*10*	22.6	16.9	39.5	60.5

[a] Grown at 30°C to 2.5 × 10^8 cells per ml in minimal medium supplemented with 50 mM glycerol. The lipids were extracted, processed, and analyzed for their fatty acid composition by gas liquid chromatography as described by Gelmann and Cronan (8). The *fadR*$^+$ derivatives of each of the above *fad* strains had a fatty acid composition comparable to that of strain K-12 (data not shown).

[b] Saturated fatty acids.

is exerted before the step catalyzed by the *fabF* gene product. The *fabF* gene product β-ketoacyl-ACP synthase II catalyzes the condensation of palmitoleoly-ACP with malonyl-ACP (5, 7).

The above studies prompted us to compare the UFA content in the *fabA*$^+$ parents of the *fadR*$^+$ *fabA2* and *fadR fabA2* strains. The *fadR fabA*$^+$ strains RS3010 and RS3040 synthesized at least 30% less UFA than the isogenic *fadR*$^+$ *fabA*$^+$ strain K-12 (Table 3). The Δ11 $C_{18:1}$ content in the *fadR fabA*$^+$ strains was at least 54% less than that in the *fadR*$^+$ *fabA*$^+$ strain (Table 3), whereas a lesser effect was seen on the Δ9 $C_{16:1}$ content. Comparable results were obtained with *fabA*$^+$ transductants (i.e., LS6593 and LS6594) of *fadR*$^+$ *fabA2* and *fadR fabA2* strains (data not shown). In other studies, *fadR* strains synthesized significantly less UFA than *fadR*$^+$ strains at 25 and 42°C (data not shown).

Since it was conceivable that the low UFA content in *fadR fabA*$^+$ and *fadR fabA2* strains was due to the preferential degradation of UFA by the constitutive level of *fad* enzymes in these strains, the UFA composition in *fad* derivatives of *fadR*$^+$ and *fadR* strains were compared. Several observations from these studies suggest that fatty acid degradation is not responsible for the low UFA content of *fadR* strains. (i) *fadR fabA2 fad* (either *fadABC* or *fadE*) strains, like their *fadR fabA2* parent (Table 2), required UFA to grow at both 30 and 42°C (data not shown). (ii) The UFA contents of the *fadR fabA2 fad* strains, like their *fadR fabA2* parent, were less than 20% at 30°C (data not shown). (iii) *fadR fabA*$^+$ *fad* strains, like their *fadR fabA*$^+$ parents, synthesized at least 30% less UFA than did the *fadR*$^+$ *fabA*$^+$ strain (Table 4). The content of both UFAs ($C_{16:1}$ and $C_{18:1}$) decreased in *fadR* strains, although the effect on Δ11 $C_{18:1}$ was more severe (data not shown). In the control experiments for the latter studies, there was no difference in the UFA content between *fadR*$^+$ *fabA*$^+$ and *fadR*$^+$ *fabA*$^+$ *fad* strains (data not shown). When the UFA composition was compared in *fadR*$^+$ and *fadR* strains under conditions of severe catabolite repression (i.e., growth in a medium containing D-glucose) and mild catabolite repression (i.e., growth in tryptone broth or minimal medium containing acetate), the *fadR* strains synthesized significantly less UFA than did *fadR*$^+$ strains (Table 5). Overall, the latter results and the studies with the *fad* derivatives of *fadR*$^+$ and *fadR* strains indicate that UFA are not preferentially degraded in *fadR* strains and that the *fad* enzymes are not responsible for altering the UFA content in these strains.

In vitro UFA synthetase activity in *fadR*$^+$ and *fadR* strains. Fatty acid synthetase activity in *fadR*$^+$ and *fadR* strains was determined in vitro. The *fadR* strains synthesized less UFA than did *fadR*$^+$ strains (Table 6).

Phospholipid synthesis in *fadR*$^+$ and *fadR* strains. Vanderwinkel et al. (21) have shown that *fadR* strains, grown on rich medium at 37°C, synthesize more cardiolipin and less phosphatidylglycerol than do *fadR*$^+$ strains. Our *fadR* strains contained slightly greater amounts of the acidic phospholipids phosphatidylglycerol and cardiolipin, but the phosphatidylglycerol/cardiolipin ratio seemed to vary with the strain examined (Table 7). The latter results are similar to those obtained by Vanderwinkel et al. (21). Therefore, the synthesis of UFA was reduced and the phospholipid composition was altered in *fadR* strains. The phospholipid compositions of *fadR*$^+$ *fabA2* strains were examined and compared with those of *fadR fabA*$^+$ strains to determine whether strains that synthesize less UFA have altered phospholipid compositions. *fadR*$^+$ *fabA2* strains synthesized less phosphatidylethanolamine and more cardiolipin than did *fadR*$^+$ *fabA*$^+$ strains (Table 7). Although the phospho-

TABLE 5. Fatty acid composition in *fadR*$^+$ *fabA*$^+$ and *fadR fabA*$^+$ strains grown on different carbon sources

Strain	Genotype	Carbon source[a]	Relative fatty acid composition			
			UFA (%)			SFA[c] (%)
			$C_{16:1}$	$C_{18:1}$	Total	
K-12	*fadR*$^+$ *fabA*$^+$	D-Glucose	23.7	45.4	69.1	30.9
		Tryptone broth	30.8	32.5	63.3	36.7
		Acetate[b]	28.5	29.6	58.1	41.9
RS3040	*fadR*::Tn*10* *fabA*$^+$	D-Glucose	25.3	28.2	53.5	46.5
		Tryptone broth	23.5	22.5	46.0	54.0
		Acetate[b]	22.2	19.5	41.6	58.4

[a] Grown at 30°C to 2.5 × 10^8 cells per ml in tryptone broth or minimal medium containing the carbon source 25 mM D-glucose. Upon reaching 2.5 × 10^8 cells per ml, 1-ml samples were removed and added to test tubes containing 5 μCi of [^{14}C]acetate (57 μCi/μmol). After 30 min, incorporation was terminated as described in Table 3, footnote *a*. The [^{14}C]acetate-labeled lipids were extracted, processed, and analyzed for their fatty acid composition as described in the text. Results comparable to those in this table were obtained when the bulk fatty acid composition of these strains, grown under the same conditions, was determined by gas-liquid chromatography (data not shown).

[b] The cells were pregrown on minimal medium containing acetate as the sole carbon source. The cell lipids were labeled with [^{14}C]acetate in minimal medium containing succinate as the sole carbon source.

[c] Saturated fatty acids.

lipid composition of *fadR*$^+$ *fabA2* strains was not altered in the same manner as *fadR fabA*$^+$ strains, these findings indicate it is possible that *E. coli* alters its phospholipid composition when it is incapable of synthesizing UFA optimally.

DISCUSSION

The studies presented in this paper suggest that a functional *fadR* gene is required for normal UFA biosynthesis in *E. coli*. The exact mechanism(s) by which the *fadR* gene affects UFA biosynthesis is unclear. The effect on UFA biosynthesis seen in *fadR* strains is not prevented by the loss of the *fabF* gene product β-ketoacyl-ACP synthase II because the synthesis of UFA in *fadR* strains carrying the *fabF* defect is significantly less than in *fadR*$^+$ strains carrying the *fabF* defect (Table 3). The lower UFA content in *fadR* strains is not a consequence of the preferential degradation of UFA by the constitutive levels of *fad* enzymes in these strains because the *fadR* and *fadR fad* strains both synthesize less UFA than do *fadR*$^+$ and *fadR*$^+$ *fad* strains (Table 4; W. D. Nunn and K. Giffin, unpublished data) and the *fadR* strains grown under conditions which severely repress the synthesis of *fad* enzymes (Table 5) synthesize significantly less UFA than *fadR*$^+$ strains grown under the same conditions.

At present it is unclear whether the *fadR* gene product affects UFA synthesis at the level of gene expression or enzyme activity (or both). Preliminary studies show that *fadR* strains seem to have lower levels of β-hydroxydecanoyl-

TABLE 6. In vitro fatty acid synthesis in *fadR*$^+$ and *fadR* strains[a]

Strain	Genotype	Relative fatty acid composition			
		UFA (%)			SFA[b] (%)
		$C_{16:1}$	$C_{18:1}$	Total	
LS6483	*fadR*$^+$ *fabA2*	7.6	14.8	22.4	77.6
LS6494	*fadR zcb*::Tn*10* *fabA2*	4.6	4.6	9.2	90.8
LS6592	*fadR*::Tn*10* *fabA2*	6.0	4.8	10.8	89.2
K-12	*fadR*$^+$ *fabA*$^+$	53.0	12.3	65.3	34.7
RS3040	*fadR*::Tn*10* *fabA*$^+$	32.5	5.2	37.7	61.8

[a] Fatty acid synthetase was measured in vitro by the method of Gelmann and Cronan (8). The cell extracts were prepared by harvesting cultures during exponential growth at 30°C in minimal medium containing 50 mM glycerol and 100 μM $C_{18:1}$. The cells were washed once with 10 mM potassium phosphate (pH 7.0), suspended in the same buffer plus β-mercaptoethanol (10 mM), and disrupted by passage through a French pressure cell. This extract was centrifuged at 50,000 × *g* for 30 min, and the supernatant fluid was used as the enzyme source. The rates of total fatty acid synthesis in all of these strains were all approximately equal (3 nmol/min per mg of protein).

[b] Saturated fatty acids.

TABLE 7. Fatty acid and phospholipid composition in *fadR*$^+$ and *fadR* strains

Strain[a]	Genotype	UFA composition (%)			PL[b] composition (%)		
		$C_{16:1}$	$C_{18:1}$	Total	PE	PG	CL
K-12	*fadR*$^+$ *fabA*$^+$	28.7	36.4	65.1	76.6	19.5	3.9
RS3010	*fadR fabA*$^+$	27.8	20.5	48.3	77.6	15.9	6.5
RS3040	*fadR*::Tn*10 fabA*$^+$	29.9	22.2	52.1	71.2	18.8	9.8
LS6483	*fadR*$^+$ *fabA2*	24.5	19.5	44.0	73.1	19.0	7.1
UC1098	*fadR*$^+$ *fabA2 fabF*	39.7	2.0	41.7	74.7	19.2	6.1

[a] Grown at 30°C in minimal medium containing 50 mM glycerol as the sole carbon source. When the cultures reached 2.5×10^8 cells per ml, 1-ml portions were removed, in duplicate, and treated as described in Table 3, footnote *a*. The rates of phospholipid synthesis in the *fadR* and *fadR*$^+$ strains were ca. 3 nmol/min per mg of protein. In studies in which the bulk fatty acid composition of these strains, grown under the same conditions, was determined by gas-liquid chromatography, the results were comparable to those in this table (data not shown).

[b] Phospholipids.

thioester dehydrase (the *fabA* gene product) activity than do *fadR*$^+$ strains and that *fadR* strains are unusually sensitive to the specific dehydrase inhibitor 3-decenoyl-*N*-acetylcysteamine (D. Clark and J. E. Cronan, unpublished data). These findings are consistent with the lower rates of UFA synthesis in *fadR* strains observed in vivo (Table 3) and in vitro (Table 6). However, other studies suggest that the decreased β-hydroxydecanoyl-thioester dehydrase level of *fadR* strains is not solely responsible for the UFA deficiency in these strains. For instance, *fadR* strains that overproduce the dehydrase, owing to a putative promoter mutation in the *fabA* gene, synthesize less UFA than *fadR*$^+$ strains carrying the same putative *fabA* promotor mutation (D. Clark, D. deMendoza, and J. E. Cronan, manuscript in preparation). More studies will have to be performed to ascertain the nature of the putative promotor mutations and to reconcile the results of Clark et al. with the findings presented in this paper. Current emphasis is now being focused on the effect of a *fadR* mutation on *fabA* and *fabB* mRNA synthesis.

ACKNOWLEDGMENTS

We thank Mary Polacco for help with some early experiments. Barbara Bachmann generously supplied many of the bacterial strains used in this study. We are grateful to Charles L. Ginsburgh, Dee Dee O'Brien, Lauren Sallus, and Sharon K. Spratt for helpful discussions and comments on the manuscript.

This work was supported by Public Health Service grant GM22466 (to W.D.N.) from the National Institute for General Medical Sciences and Public Health Service grant AI 15650 (to J.E.C., Jr.) from the National Institutes of Health. W.D.N. is an Established Investigator of the American Heart Association.

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. **Cronan, J. E., C. H. Birge, and P. R. Vagelos.** 1969. Evidence for two genes specifically involved in unsaturated fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. **100**:601–604.
3. **Cronan, J. E., and E. P. Gelmann.** 1973. An estimate of the minimum amount of unsaturated fatty acid required for growth of *Escherichia coli*. J. Biol. Chem. **248**:1188–1195.
4. **Cronan, J. E., D. F. Silbert, and D. L. Wulff.** 1972. Mapping of the *fabA* locus for unsaturated fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. **112**:206–211.
5. **D'Agnolo, G., I. S. Rosenfeld, and P. R. Vagelos.** 1975. β-Ketoacyl-acyl carrier protein synthetase. J. Biol. Chem. **250**:5283–5288.
6. **Garwin, J. L., A. L. Klages, and J. E. Cronan, Jr.** 1980. β-Ketoacyl-acyl carrier protein synthase II of *Escherichia coli*: evidence for function in the thermal regulation of fatty acid synthesis. J. Biol. Chem. **255**:3263–3265.
7. **Garwin, J. L., A. L. Klages, and J. E. Cronan, Jr.** 1980. Structural enzymatic, and genetic studies of β-ketoacyl-acyl carrier protein synthases I and II of *Escherichia coli*. J. Biol. Chem. **255**:11949–11956.
8. **Gelmann, E. P., and J. E. Cronan, Jr.** 1972. Mutant of *Escherichia coli* deficient in the synthesis of *cis*-vaccenic acid. J. Bacteriol. **112**:381–387.
9. **Ingram, L. O.** 1982. Regulation of fatty acid composition in *Escherichia coli*: a proposed common mechanism for changes induced by ethanol, chaotropic agents, and a reduction of growth temperature. J. Bacteriol. **149**:166–172.
10. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. New methods in bacterial genetics. J. Mol. Biol. **116**:125–159.
11. **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.
12. **Maloy, S. R., M. Bohlander, and W. D. Nunn.** 1980. Elevated levels of glyoxylate shunt enzymes in *Escherichia coli* strains constitutive for fatty acid degradation. J. Bacteriol. **143**:720–725.
13. **Maloy, S. R., C. L. Ginsburgh, R. W. Simons, and W. D. Nunn.** 1981. Transport of long chain and medium chain fatty acids by *Escherichia coli*. J. Biol. Chem. **256**:3735–3742.
14. **Maloy, S. R., and W. D. Nunn.** 1981. Role of gene *fadR* in *Escherichia coli* acetate metabolism. J. Bacteriol. **148**:83–90.
15. **Maloy, S. R., and W. D. Nunn.** 1982. Genetic regulation of the glyoxylate shunt in *Escherichia coli* K-12. J. Bacteriol. **149**:173–180.
16. **Nunn, W. D., and B. E. Tropp.** 1972. Effects of phenethyl alcohol on phospholipid metabolism in *Escherichia coli*. J. Bacteriol. **109**:162–168.

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. **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.
20. **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.
21. **Vanderwinkel, E., M. De Vlieghere, M. Fontaine, D. Charles, F. Denamur, D. Vandevoorde, and D. DeKegel.** 1976. Septation deficiency and phospholipid perturbation in *Escherichia coli* genetically constitutive for the beta oxidation pathway. J. Bacteriol. **127**:1389–1399.
22. **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.