Genetic and Biochemical Characterization of a Mutation (*fatA*) That Allows *trans* Unsaturated Fatty Acids To Replace the Essential *cis* Unsaturated Fatty Acids of *Escherichia coli*

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Unsaturated fatty acid auxotrophs of *Escherichia coli* are able to use only unsaturated fatty acids of the *cis* configuration as the required growth supplement. A mutation in the *fatA* gene allows such auxotrophs to utilize unsaturated fatty acids with a *trans* double bond as well as fatty acids having a *cis* double bond. The *fatA* gene was mapped to min 69 near *argG*, and the allele studied (*fatA1*) was found to be dominant over the wild-type gene. *fatA1* mutant strains grew at similar rates when supplemented with elaidate (*trans*-9-octadecenoate) or oleate (*cis*-9-octadecenoate). The *fat*⁺ strain, however, lysed when supplemented with the *trans* fatty acid. Physiological characterization of the *fatA* mutant strain was undertaken. The mutation appeared not to be involved with long-chain fatty acid transport. Introduction of lesions in known fatty acid transport genes abolished *trans* fatty acid utilization in the *fatA* mutant strain. Also, growth characteristics of the *fat*⁺ and the *fatA1* mutant strains on elaidate as the sole carbon source were identical, which indicated comparable rate of fatty acid accumulation. The mutation appeared to be involved with recognition of the *trans* configuration after uptake into the cell. The levels of *trans* fatty acid incorporation into the phospholipids of the *fat*⁺ and the *fatA* strains differed considerably, with the mutant incorporating much higher levels. No significant accumulation of elaidate into nonphospholipid cellular components was observed. The *fatA* mutation did not appear to be involved with the cellular metabolic state, as cyclic AMP had no effect on the ability of the strains to utilize *trans* fatty acids.

Escherichia coli unsaturated fatty acid auxotrophs have been very useful in elucidating the dependence of cell physiology on the physical state of the membrane phospholipids (for review, see reference 8). Such auxotrophs, which map to either of two genes, *fabA* (encoding β -hydroxydecanoyl thioester dehydrase) or *fabB* (encoding 3-ketoacyl-acyl carrier protein synthase), grow when supplemented with any of a surprisingly wide range of *cis* unsaturated fatty acids (9). These strains are also able to grow on unsaturated fatty acids having a double bond in the unnatural *trans* configuration but require a second mutational lesion for growth on *trans* fatty acids (14, 17, 28, 29, 31). Strains carrying this second lesion in addition to the original lesion in unsaturated fatty acid synthesis grow well when supplemented with either *cis* or *trans* unsaturated fatty acids but fail to grow, and ultimately lyse, when supplemented with saturated fatty acids or when left unsupplemented. These strains have been previously termed elaidate variants (13, 38, 39), since they grow when supplemented with elaidic acid (*trans*-9-octadecenoic acid), the *trans* isomer of oleic acid (*cis*-9-octadecenoic acid).

Our interest in the elaidate variant mutational lesion stemmed from two aspects. First, much of the work on the interrelationships of cell physiology and the phospholipid order-disorder phase transition has used such strains (8, 14, 28, 31). Elaidate-containing phospholipids have much higher phase transition temperatures than do phospholipids containing either oleate or the unsaturated fatty acids normally found in *E. coli*, palmitoleic acid and *cis*-vaccenic acid (28). Thus, use of these strains allows physiological comparisons

over a wide range of transition temperatures. The dependence of the large literature of lipid physical properties on this mutation of unknown character was disturbing. Second, we are interested in the metabolic reactions involved in completion of fatty acyl chains and their incorporation into phospholipid, and it seemed possible that the elaidate variant lesion might involve one of these steps.

In this paper, we show an elaidate variant to be a discrete, genetically dominant, mutational lesion. We have mapped this lesion (called *fatA*) to min 69 of the *E. coli* genetic map and shown that the basis for the phenotype displayed by *fatA* strains is not due to altered transport of *trans* fatty acids but rather to events occurring after uptake of these acids into phospholipids.

MATERIALS AND METHODS

Media, growth conditions, and bacterial strains. The strains of *Escherichia coli* K-12 used in this study are listed in Table 1. RB medium, containing 10 g of tryptone, 5 g of NaCl, and 1 g of yeast extract, was used for rich medium. Minimal medium was E salts (23) supplemented with 0.4% glucose and 0.01% L-amino acids. The potassium salts of oleate (*cis*-9-octadecenoate) or elaidate (*trans*-9-octadecenoate) to be used either as the sole carbon source or as an unsaturated fatty acid supplement were added at 0.1% in the presence of 1% Brij 58 detergent. Antibiotics were added to the following final concentrations (in micrograms per milliliter): tetracycline hydrochloride, 16; sodium ampicillin, 50; chloramphenicol, 50; and kanamycin sulfate, 40. Carbon sources, antibiotics, and supplements were all obtained from Sigma Chemical Co., St. Louis, Mo. Solid media contained 1.5% Bacto-Agar (Difco Laboratories, Detroit, Mich.). Culture densities were monitored with a Klett-Summerson photo-

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TABLE 1. Strains of *E. coli* K-12

Strain	Relevant genotype ^a	Reference or source
CY50	<i>fabA2</i>	6
LCD9	CY50 <i>fatA1</i>	This study
LCD10	LCD9 <i>zha-6::Tn10 fat</i> ⁺	36 ^b ; this study
LCD11	LCD9 <i>zha-6::Tn10</i>	36; this study
LCD14	LCD9 <i>recA</i>	This study
LCD28	AB312 <i>zha-6::Tn10</i>	21; this study
LCD75	LCD9 <i>zgi-203::Tn10 fat</i> ⁺	CGSG ^c ; this study
LCD76	LCD9 <i>zgi-203::Tn10</i>	CGSG; this study
LCD95	LCD9 <i>zea::Tn10 fadD</i>	22; this study
LCD98	LCD9 <i>fadL::Tn10</i>	5; this study
LCD158	CY50 <i>fadR::Tn10</i>	34; this study
LCD159	LCD9 <i>fadR::Tn10</i>	34; this study
LCD183	LCD9 <i>fab</i> ⁺	This study
LCD189	LCD183 <i>fabB15</i>	15; this study
LCD229	LCD183 <i>fabB20</i>	36; this study

^a Genetic nomenclature is that of Bachmann (1).

^b Source of a particular allele or *Tn10* used in construction of the strain shown. Strains were constructed by P1 transduction.

^c CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

electric colorimeter (Klett Manufacturing Co., New York, N.Y.) equipped with a green filter.

Genetic techniques. All strains were constructed by standard genetic techniques. Generalized transducing phage P1 *vir* was propagated in liquid culture and used for all transductions as described by Silhavy et al. (33). Conjugational matings were performed in liquid culture and then plated on selective media as described by Miller (23).

Preparation and analysis of cellular phospholipids. Total phospholipids were extracted from cell suspensions by the procedure of Bligh and Dyer (2). Phospholipid acyl groups were converted to methyl esters by transesterification in sodium methoxide-methanol (32). Free fatty acids were esterified with HCl-methanol (7). The methyl esters were separated by gas-liquid chromatography, using a 20-ft (ca. 610-cm) column (1/8-in. [ca. 0.32-cm] diameter) of 15% OV-275 on Chromosorb PAW-DMCS (100/120 mesh; Supelco, Inc., Bellefonte, Pa.) at 198°C or on silica gel G plates (Analtech, Inc., Newark, Del.) impregnated with 20% silver nitrate. Both methods resolve *cis* and *trans* fatty acid esters. Phospholipid species were separated by thin-layer chromatography on silica gel G plates predeveloped in acetone and then developed in CHCl₃-methanol-H₂O (65:25:4 by volume). Phospholipid species were visualized with iodine or by autoradiography. The appropriate areas of silica gel were then scraped from the plates and either eluted for further analysis or suspended in PCS scintillation solution (Amersham Corp., Arlington Heights, Ill.) for quantitation of radioactivity. Phospholipase A₂ (Sigma) was used to release acyl groups from position 2 of the phospholipids (3), and the resulting fatty acids were methylated and analyzed by gas chromatography.

Bound lipids (lipoprotein and lipid A) were separated from the phospholipids by exhaustively extracting a cell pellet from a 5-ml culture with CHCl₃-methanol-H₂O (1:2:0.8 by volume; 24). Murein lipoprotein was separated from the lipid A by boiling the residue in 4% sodium dodecyl sulfate for 2 h. The insoluble lipoprotein was pelleted by centrifugation at 171,000 × *g* for 2 h. The pellet was washed once with boiling 4% sodium dodecyl sulfate. Both the sodium dodecyl sulfate supernatant fraction containing lipid A and the lipoprotein pellet were counted in PCS scintillation solution.

Radiolabeled compounds. [1-¹⁴C]oleate was purchased from Amersham and used as the starting material for the

synthesis of [1-¹⁴C]elaidate by isomerization with HNO₂ (20). The elaidate was purified from the unreacted oleate on silver nitrate silica gel plates, and its purity was assayed after conversion of a sample to the methyl ester.

RESULTS

Selection for elaidate variants. Strains carrying a temperature-sensitive mutation in the *fabA* locus require unsaturated fatty acid supplementation at 42°C, the nonpermissive temperature (10), whereas growth at 30°C proceeds normally without supplementation. Elaidate variants were isolated spontaneously. A culture of strain CY50 (*fabA*) in stationary phase was plated on medium supplemented with elaidate (*trans*-9-octadecanoate) at 42°C. Colonies able to utilize elaidate as the required unsaturated fatty acid supplement appeared at a frequency of approximately 5×10^{-8} . Purified isolates were retested for both the original *fabA* lesion and the elaidate-utilizing phenotype, and one isolate, strain LCD9, was chosen for further study.

We have chosen the mnemonic *fat* (for fatty acid utilization) for the phenotype exhibited by this mutant. The gene defined by this mutation will hereafter be referred to as *fatA*.

Genetic mapping of *fatA*. The approximate map location of the *fatA* gene was determined by using three Hfr strains, HfrH, KL96, and KL14, whose points of origin roughly divide the *E. coli* chromosome into thirds. A *Tn9* insertion closely linked to *fatA* was isolated by random mutagenesis of a *fat*⁺ strain, pooling of the chloramphenicol-resistant "hops," and transduction into the *fatA* strain, screening for colonies which had lost the mutant phenotype (18). The *fatA* mutation was moved into each of the three Hfr strains by phage P1 transduction, using selection for the chloramphenicol resistance of *Tn9*. The resulting Hfr strains were then mated with a *fabA gyrA* strain and plated in the presence of nalidixic acid and chloramphenicol. The exconjugates were scored for the *fatA* phenotype on elaidate plates at 42°C. Only the mating in which the Hfr derived from strain KL14 was the donor gave rise to *fatA* exconjugates, which indicated that the gene was located between approximately min 65 and min 100 on the chromosome.

Further time-of-entry experiments suggested that the gene was located near the point of origin of strain KL14. Since the *Tn9*-containing strains were lost upon storage, other strains were sought for mapping purposes. Three *Tn10* insertions in the min-65 to -75 region (37) were tested for linkage to *fatA*. One of the three insertions (*zha-6::Tn10*), which lies near min 70, was found to cotransduce with a frequency of approximately 35% with the *fatA* gene, whereas the other two insertions (*zhc-9::Tn10* and *zhc-12::Tn10*), which lie closer to min 72, were unlinked. These results indicated that *fatA* was located close to min 69. Since *argG* is located in this region, linkage of this gene to *zha-6::Tn10* was tested. *argG* was found to be cotransduced with this *Tn10* insertion at a frequency of approximately 15 to 25%, which compares well with the cotransduction frequency reported by Vallari and Rock (37). A three-factor cross among the three markers gave the clockwise gene order *argG-fatA-zha-6::Tn10* (Table 2). Genetic mapping with a second *Tn10* (*zgi-203::Tn10*) confirmed this gene order (Fig. 1).

The *fatA1* allele is dominant over *fat*⁺. The *fatA* phenotype could be the result of either loss of function present in the wild type or acquisition of a new function. To decide between these possibilities, we constructed merodiploids of the *fatA* region containing both a mutant and a wild-type gene and scored the growth phenotype of these strains on

TABLE 2. Mapping of the *fatA* locus by three-factor crosses by P1 transduction

Expt. no. ^a	No. tested	Recombinant type	No. of each type
1	395	<i>argG fatA</i>	1
		<i>argG fat</i> ⁺	94
		<i>arg</i> ⁺ <i>fatA</i>	273
		<i>arg</i> ⁺ <i>fat</i> ⁺	27
2	479	<i>argG fatA</i>	9
		<i>argG fat</i> ⁺	360
		<i>arg</i> ⁺ <i>fatA</i>	61
		<i>arg</i> ⁺ <i>fat</i> ⁺	49

^a In experiment 1, LCD121 (*zha-6::Tn10 argG*) was the donor; in experiment 2, LCD61 (*zgi-203::Tn10 argG*) was the donor. In both cases, LCD9 (*fatA*) was the recipient and the selected marker was tetracycline resistance.

elaidate. Strain LCD14 (*fabA fatA1 recA*) was used as the recipient in a mating with the Hfr strain LCD28, which had the point of origin of strain AB312 and carried the *Tn10* linked to *fatA*. The point of origin of strain AB312 and the progeny strain LCD28 lies near min 66, with the *argG* gene being transferred early (21). Interrupted matings with selection for tetracycline resistance resulted in the recipient acquiring F' factors carrying the *fatA* wild-type region. The matings were interrupted and plated at various times to allow transfer of the *fatA* region without transfer of the *fabA* region. Each exconjugate scored was first tested to ensure that the wild-type *fabA* gene had not been transferred. Since *recA* does not lie between the point of origin of transfer and the *fabA* gene, the wild-type allele of this gene could not be

transferred. When the auxotrophic isolates were tested for the *fatA* phenotype, all were found to grow on elaidate, which indicated that the mutant allele was dominant over the wild type. The resulting F'-containing strain was mated into both the original recipient and a *fat*⁺ strain. The resulting exconjugates supported the original observation; that is, the *fatA*/F' *fat*⁺ strain was phenotypically *fatA*, whereas the *fat*⁺/F' *fat*⁺ strain was phenotypically *fat*⁺. The genetic dominance suggested that the nature of the *fatA1* mutation was the acquisition of a function that the cell had previously lacked rather than loss of a function.

The *fatA1* mutation is not gene or allele specific. Strains exhibiting unsaturated fatty acid auxotrophy due to a mutation in the *fabB* gene were able to grow on elaidic acid when the strains also carried the *fatA1* lesion (data not shown). In these experiments, the *fabA* lesion was removed by transduction to fatty acid prototrophy (strain LCD183), and either of two temperature-sensitive *fabB* lesions (*fabB15* or *fabB20*) was introduced by cotransduction, with selection for a *Tn10* insertion closely linked to *fabB*. The resulting strains (LCD 189 and LCD229, respectively) were able to utilize elaidate as the unsaturated fatty acid required for growth at 42°C.

Physiological characterization of *fatA* strains. The ability of *fatA* strains to utilize *trans* fatty acids was not specific to elaidate but extended to other *trans* fatty acids. The isogenic strains CY50 (*fat*⁺) and LCD9 (*fatA*) were tested for utilization of two *trans* fatty acids, palmitelaidate (*trans*-9-hexadecenoic acid) and *trans*-vacenate (*trans*-11-octadecenoic acid) at the restrictive temperature. The parent strain, CY50, was unable to use any *trans* fatty acid; only *cis* fatty acids allowed growth, whereas the *fatA* mutant, LCD9, was able to utilize elaidate, *trans*-vacenate, or palmitelaidate to support growth (data not shown).

To further characterize the *fatA* mutation, growth of the isogenic pair of strains, CY50 and LCD9, at the restrictive temperature in the presence or absence of unsaturated fatty acid supplementation was compared. After a 50-fold dilution into medium supplemented with oleate, both strains reached saturation by 4 h (Fig. 2). However, without supplementation, growth was arrested after 1 h and cell lysis began. When the *fatA* strain was supplemented with elaidate, growth was essentially identical to that found with oleate supplementation (Fig. 2). The *fat*⁺ parent strain, however, continued to grow past 1 h with elaidate supplementation, but growth was arrested by 2 h and cell lysis ensued.

The growth studies were repeated in strains in which a *fadE* mutation had been introduced, causing a block in β -oxidation (19, 30). The results were identical to those in Fig. 2 (data not shown), which indicated that the difference between the variant and the nonvariant was a difference in the incorporation of elaidate into phospholipids and not due to the toxicity of (or an inability to utilize) some breakdown product of elaidate. The finding that growth of the parental *fat*⁺ strain continued for a longer period in the presence of elaidate supplementation than in the absence of the fatty acid suggested that elaidate entered the *fat*⁺ cell but that the fatty acid somehow failed to be properly utilized for lipid synthesis or function.

One possible explanation for the phenotype exhibited by the *fatA* mutant is that elaidate enters the *E. coli* cell by a system different from that utilized in transport of other long-chain fatty acids. To test this possibility, we constructed *fatA fabA* strains that also carried mutations in either of two well-characterized genes required for fatty acid transport (*fadD* or *fadL*; 25-27, 30). Although these strains

FIG. 1. Genetic map of the min 69 region. Percentages are cotransduction frequencies between markers as determined by transductional crosses (see text). In each case, tetracycline resistance was the selected marker.

FIG. 2. Cell growth in the presence or absence of unsaturated fatty acid supplementation. (A) Strain CY50 (*fat*⁺); (B) strain LCD9 (*fatA*). Symbols: Δ , no supplementation; $\blacktriangle$, plus oleate; $\blacktriangledown$, plus elaidate. Cultures were grown in rich medium at 42°C as described in the text.

grew well at 30°C, where fatty acid supplementation is unnecessary, at 42°C neither strain was able to grow when supplemented with either oleate or elaidate. It should also be noted that a mutation in the gene for the negative regulator of the *fad* regulon, *fadR* (19, 34), had no apparent effect on the phenotype of either the parent or the mutant when these strains were tested for growth on *trans* fatty acids. Thus, elaidate seemed to utilize the same transport mechanism as do other long-chain fatty acids.

To test whether the *fatA* mutation allowed higher levels of elaidate to enter the cell, we examined the growth of the mutant and parent strains on *trans* fatty acids as the sole carbon source. We inoculated minimal medium containing Brij 58 detergent and either oleate or elaidate with strains CY50 (*fat*⁺) and LCD9 (*fatA*) and monitored growth in liquid culture. We found that the presence of the *fatA* mutation provided no significant advantage for strains grown on *trans* fatty acids as the carbon source; *fat*⁺ and *fatA* strains grew at essentially the same rate on this fatty acid (data not shown). Note that the amount of fatty acid required for growth as the sole carbon source exceeded the amount required for effective supplementation of an unsaturated fatty acid auxotroph by about 20-fold.

Elaidate is incorporated into the phospholipids of *fat*⁺ and *fatA* strains. The finding that addition of elaidate to a *fat*⁺ strain resulted in a longer interval before cellular lysis at 42°C suggested that elaidate might be incorporated into the phospholipids of both *fat*⁺ and *fatA* strains. To test this possibility directly, we compared the fatty acid compositions of isogenic strains grown on oleate and elaidate. Strains LCD10 (*fat*⁺) and LCD11 (*fatA*) were grown in the presence of either fatty acid supplement for 2 h at 42°C. The phospholipids were extracted, the fatty acids were converted to their methyl esters, and the fatty acid composition was determined by gas-liquid chromatography. When either

strain was supplemented with oleate, this fatty acid made up 6 to 18% of the total fatty acid. When the *fat*⁺ strain was supplemented with elaidate, 10 to 14% of the total fatty acid was elaidate. In the *fatA* mutant, however, elaidate made up 48 to 62% of the total fatty acid. Thus, although both strains transported and incorporated the *trans* fatty acid, the mutant incorporated much more elaidate than did the *fat*⁺ strain. Incorporation of elaidate into the phospholipids was further verified by labeling of cultures with [1-¹⁴C]oleate or [1-¹⁴C]elaidate, extraction of the phospholipids, and chromatography on silica gel plates. Autoradiography revealed that all of the major phospholipid species contained labeled fatty acids in both strains, regardless of which fatty acid was supplied (Fig. 3).

Elaidate is found primarily esterified to the 2 position of the phospholipids. Other workers previously reported (14) that an elaidate variant of a *fabB* strain contained a large proportion of phospholipids with two elaidoyl groups, which suggested that the mutation allowed larger amounts of the less fluid *trans* fatty acid to be incorporated to maintain proper membrane fluidity and thus counteract the deleterious effect of its presence in the lipids. The possibility existed that the *fatA* phenotype was the result of aberrant usage of the elaidate in the phospholipids such that it would be incorporated at both fatty acyl positions. To examine this possibility, isogenic strains LCD75 (*fat*⁺) and LCD76 (*fatA*) were grown for 2 h at 42°C in either oleate or elaidate, and the phospholipids were extracted. Total cellular phospholipids were purified from any unincorporated fatty acids. The phospholipids were then treated with phospholipase A₂ to cleave the fatty acyl chain from position 2 of the phospholipid backbone, and the resulting lysophospholipids and free fatty acids were separated by thin-layer chromatography, collected, and converted to fatty acid methyl esters. The esters were analyzed by gas-liquid chromatography. In both

FIG. 3. Labeling of cellular phospholipids with ^{14}C -fatty acids. Cells were grown at 42°C in rich medium supplemented with either $[1\text{-}^{14}\text{C}]\text{oleate}$ or $[1\text{-}^{14}\text{C}]\text{elaidate}$. Phospholipids were extracted and chromatographed on silica gel G plates as described in the text. Lanes: 1, strain CY50 supplemented with oleate; 2, strain CY50 supplemented with elaidate; 3, free oleate; 4, free elaidate; 5, strain LCD9 supplemented with oleate; 6, strain LCD9 supplemented with elaidate. Abbreviations: f, solvent front; o, origin; FFA, free fatty acid; CL, cardiolipin; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

strains, oleate was found exclusively as the released fatty acid, with greater than 99% of the incorporated oleate at position 2. Elaidate was found at both positions, with the majority released from position 2. The phospholipids of the *fat*⁺ strain contained 36% of the total elaidate at position 1 and 64% at position 2, whereas the phospholipids of the *fatA* mutant strain contained 24% at position 1 and 76% at position 2. The phospholipids of the mutant contained three-fold more elaidate than did those of the wild type overall, as expected from the previous experiment. These results show that in the mutant, the *trans* fatty acid was not incorporated to the same extent at both positions, contrary to the findings reported elsewhere with an uncharacterized elaidate variant (14). However, it should be noted that the previous workers grew their cultures in the presence of elaidate for many generations, whereas our comparative studies were necessarily limited to short time periods because of lysis of the *fat*⁺ cultures.

Incorporation of elaidic acid into lipid moieties other than phospholipid. Phospholipids are by far the major cellular component containing unsaturated fatty acids. However, unsaturated fatty acids are found in the lipid moiety of lipoproteins and, under certain growth conditions, in the lipid A moiety of lipopolysaccharide. Since *trans* fatty acids have structural and physical properties intermediate between those of saturated and *cis* unsaturated fatty acids, it also seemed possible that elaidic acid might replace saturated fatty acids in the murein lipoprotein or in lipid A or both. Therefore, the *fatA* lesion might possibly be involved

in the metabolism of these nonphospholipid lipidic components. To resolve this question, we determined the extent to which radiolabeled oleate and elaidate were incorporated into these components. Cultures of strains CY50 (*fat*⁺) and LCD9 (*fatA*) were grown to early exponential phase at the permissive temperature and then transferred to 42°C concomitantly with the addition of either $[1\text{-}^{14}\text{C}]\text{oleate}$ or $[1\text{-}^{14}\text{C}]\text{elaidate}$. After a 2-h labeling period, the cells were washed free of fatty acid and detergent, and the phospholipids were exhaustively extracted. The remaining cellular components contained the bound lipid, which consisted of lipid A and murein lipoprotein. The extents to which the labeled unsaturated fatty acids were incorporated into the bound lipid were approximately the same in the two strains (Table 3). The lipoprotein fraction was also separated from lipid A, and there was no significant difference between the *fat*⁺ and *fatA* strains in the amount of incorporation (data not shown). Thus, the *fatA* mutation appeared to be involved only with the fate of the *trans* unsaturated fatty acids incorporated into phospholipids.

cAMP has no effect on *trans* fatty acid utilization. It was previously reported that the detrimental effect of *trans* fatty acids on unsaturated fatty acid auxotrophs is due not only to the lowering of membrane fluidity but also to an effect on the cellular metabolic state (35). According to this report, addition of cyclic AMP (cAMP) to the growth medium of fatty acid auxotrophs alleviated the detrimental effect that *trans* fatty acids had on growth. We tested the parent strain (CY50) and the *fatA* mutant strain (LDC9) on medium

TABLE 3. Incorporation of labeled fatty acids into lipidic cellular components

Strain	Supplement	Amt incorporated (nmol/10 ⁸ cells)		Bound/ total
		Total phospholipid	Bound lipid ^a	
CY50	[1- ¹⁴ C]oleate	2.7	0.36	0.13
LCD9	[1- ¹⁴ C]oleate	3.3	0.44	0.13
CY50	[1- ¹⁴ C]elaidate	1.6	0.45	0.28
LCD9	[1- ¹⁴ C]elaidate	2.1	0.53	0.25

^a Bound lipid consists of lipid A and murein lipoprotein.

containing elaidate and cAMP and found cAMP addition to have no effect on growth (data not shown). To verify that the cAMP was able to enter the cells, β -galactosidase assays were performed in the presence and absence of cAMP, and the expected alleviation of catabolite repression on expression of the *lacZ* gene was seen (data not shown). Thus, it appeared that the *fatA* lesion was not involved in regulation of the cellular metabolic state.

DISCUSSION

We have described a mutational lesion in a gene, designated *fatA*, which allows an unsaturated fatty acid auxotroph to utilize the *trans* isomer of long-chain unsaturated fatty acids for the synthesis of cellular phospholipids. The nature of the *fatA* mutation remains unclear, although our results limit the possible functions of the *fatA* gene product.

The simplest explanation for the inability of the *fat*⁺ strain to utilize *trans* fatty acids as an unsaturated fatty acid supplement would be a failure of these acids to enter the cell. The data presented in this report argue strongly against this possibility. The *trans* fatty acid elaidate was found incorporated at least to the extent of the *cis* isomer into the phospholipids of both the *fat*⁺ and the *fatA* mutant strains. This finding would indicate that the *fat*⁺ strain indeed transports *trans* fatty acids, which are subsequently incorporated into the phospholipids. Alternatively, it is possible that the *fatA* mutation results in an increased activity of an existing transport system specific for *trans* fatty acids; however, both the *fat*⁺ and the *fatA* mutant strains were able to utilize the *trans* fatty acid as the sole carbon source at the same rate. Since the amount of fatty acid needed as a carbon source is much greater than the amount needed for supplementation, growth on fatty acids as the sole carbon source should be more sensitive to changes in transport activity than is growth on fatty acids as a supplement. Finally, using mutations in known fatty acid transport genes, we have shown that *trans* fatty acids enter the cell by the same mechanism as do other long-chain fatty acids. Thus, increased transport of *trans* fatty acids relative to *cis* fatty acids seems an unlikely explanation for the phenotype exhibited by the *fatA* mutant.

The *fatA* gene product may be involved with the fate of the *trans* fatty acid after uptake into the cell or may allow the cell to tolerate the presence of the *trans* fatty acid in the phospholipids. We have shown that the difference between the *fat*⁺ strain and the *fatA* mutant strain lies in the incorporation of the *trans* fatty acid into the phospholipids and not into other cellular lipid components. Since the different rates of elaidate incorporation into phospholipid are observed in *fat*⁺ and *fatA1* strains immediately upon exposure of the cells to labeled elaidate, before a significant

amount of elaidate has been incorporated (data not shown), the tolerance interpretation alone is unable to account for the phenomenon. Clearly, in both strains elaidate is utilized differently than oleate, as oleate is found almost exclusively at position 2 on the phospholipid backbone, whereas one-quarter to one-third of the elaidate is found at position 1. In addition, in both strains, a variable amount of radiolabeled elaidate remained as intracellular free fatty acid (Fig. 3), whereas free oleate was not observed in these experiments. The amount of free fatty acid varied too greatly among experiments to allow us to determine the significance of this result. The most significant difference between the two strains was the amount of elaidate incorporated into the phospholipids during the labeling period, the *fatA* strain incorporating six times more elaidate than oleate, whereas the *fat*⁺ strain incorporated similar amounts of the two acids. Previous studies have shown that phospholipids containing predominantly the *trans* isomers of unsaturated fatty acids have fluidity intermediate between that of phospholipids containing *cis* unsaturated fatty acids and those comprised of saturated fatty acids (28). At a given temperature, more *trans* isomer would be required to maintain proper membrane fluidity (28, 31). Given that the transport machinery recognizes both *cis* and *trans* fatty acids, there may be an internal mechanism for differentiating the two isomers. Esfahani et al. (11, 12) suggested that in addition to the regulation of unsaturated fatty acid synthesis, there may be another level of control which minimizes variations in the fluidity of the phospholipid by regulating the fatty acid composition. The *fatA* gene, then, may be involved in a system for regulating the incorporation of the unnatural *trans* isomer into the phospholipids after uptake. Alternatively, the *fatA* gene product may be a protein that requires interaction with the membrane phospholipids for function. One such enzyme which requires lipid interaction for activation is the well-characterized pyruvate oxidase enzyme of *E. coli* (4, 16). The *fatA* gene product may be a similar protein, with the mutation affecting phospholipid recognition. Cellular metabolic processes, such as transport and respiration, are strongly dependent on the physical state of the membrane, which indicates a possible interaction between proteins involved with these processes and the cellular phospholipids. To further define the role of the *fatA* gene, we are examining the effects of temperature changes on the incorporation of elaidate into the phospholipids. We are also isolating and characterizing second-site insertion mutations which suppress the *fatA* mutant phenotype. By analyzing the functional relationships between the genes, the role of the *fatA* gene in phospholipid metabolism may be clarified.

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