JOURNAL OF BACTERIOLOGY, Apr. 1984, p. 286–295
0021-9193/84/040286-10$02.00/0

Vol. 158, No. 1

Cloning and Manipulation of the *Escherichia coli* Cyclopropane Fatty Acid Synthase Gene: Physiological Aspects of Enzyme Overproduction

DENNIS W. GROGAN AND JOHN E. CRONAN, JR.*

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

Received 27 September 1983/Accepted 10 January 1984

Like many other eubacteria, cultures of *Escherichia coli* accumulate cyclopropane fatty acids (CFAs) at a well-defined stage of growth, due to the action of the cytoplasmic enzyme CFA synthase. We report the isolation of the putative structural gene, *cfa*, for this enzyme on an *E. coli*-ColE1 chimeric plasmid by the use of an autoradiographic colony screening technique. When introduced into a variety of *E. coli* strains, this plasmid, pLC18-11, induced corresponding increases in CFA content and CFA synthase activity. Subsequent manipulation of the *cfa* locus, facilitated by the insertion of pLC18-11 into a bacteriophage lambda vector, allowed genetic and physiological studies of CFA synthase in *E. coli*. Overproduction of this enzyme via multicopy *cfa* plasmids caused abnormally high levels of CFA in membrane phospholipid but no discernable growth perturbation. Infection with phage lambda derivatives bearing *cfa* caused transient overproduction of the enzyme, although p_L-mediated expression of *cfa* could not be demonstrated in plasmids derived from such phages. CFA synthase specific activities could be raised to very high levels by using *cfa* runaway-replication plasmids. A variety of physiological factors were found to modulate the levels of CFA synthase in normal and gene-amplified cultures. These studies argue against several possible mechanisms for the temporal regulation of CFA formation.

Since the discovery of lactobacillic acid in 1950, cyclopropane fatty acids (CFAs) have been demonstrated in the membrane phospholipids of a variety of eubacteria (13). These unusual fatty acids are formed in situ by the transfer of a methyl group from *S*-adenosylmethionine to the double bond of an unsaturated fatty acid (UFA) of a phospholipid molecule (21). This unique membrane modification occurs preferentially in late exponential and early stationary phase (6, 21). In *Escherichia coli*, it is catalyzed by a soluble cytoplasmic enzyme, CFA synthase, which binds to bilayers of substrate (i.e., UFA-containing) phospholipids and apparently cyclopropanates the phospholipids of both faces of such bilayers in vitro as well as in vivo (40).

CFA synthesis in *E. coli* raises many intriguing biological questions. It is not known how CFA synthase catalyzes methyl transfer from a hydrophilic donor molecule to a sequestered, hydrophobic acceptor, how it gains access to both leaflets of a phospholipid bilayer, or how CFA-containing phospholipid molecules translocate to the outer membrane. Although explanations for the regulation of CFA synthesis in other organisms have been reported (19, 35), these do not account for the timed appearance of CFA in *E. coli*. Furthermore, no physiological role for CFA has been demonstrated in *E. coli*, nor has CFA synthesis been characterized genetically. Study of these questions has been precluded in several respects. The CFA synthase of *E. coli* is a minor and remarkable labile enzyme which has not been purified to homogeneity despite extensive efforts (40; F. R. Taylor, Ph.D. thesis, Yale University, New Haven, Conn., 1977). Furthermore, though a mutant of *E. coli* has been isolated which lacks CFA, this *cfa* lesion produces no physiological phenotype (39). The silent nature of *cfa* has in turn sharply limited the genetic techniques that can be used to manipulate and study this gene.

We have coped with these difficulties by isolating the *cfa* locus on a small segment of the *E. coli* chromosome. We reasoned that a *cfa* clone would allow the genetic manipulation of CFA synthase levels and thus facilitate study of physiological aspects of CFA formation. In addition, gene amplification would expedite the purification of CFA synthase and its subsequent characterization in vitro. We report the cloning of the *cfa* locus and its expression from various genetic constructions. With the aid of these constructions we also characterize relationships between CFA synthase levels and gross physiology in *E. coli*.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study, all derivatives of *E. coli* K-12, are listed in Table 1. All strains were λ^- unless otherwise indicated.

Cultures were routinely grown in T broth (10 g of tryptone, 5 g of NaCl, 1 mmol of $MgSO_4$, and 0.5 mg of thiamine-hydrochloride per liter) or R broth (10 g of tryptone, 5 g of NaCl, and 1 g of yeast extract per liter) at 37°C (with the exception of λ*c*I857 lysogens, which were grown at 30°C) or in medium M9 (26) supplemented with appropriate amino acids or nucleotides or both at 0.1 mg/ml. Cultures to be infected with λ derivatives were grown in broth supplemented with 0.2% maltose. For determining the time course of fatty acid composition, cultures were grown in M9 containing 0.4% glucose, 0.03% adenine, 0.03% guanosine, and 0.02% each of the L-isomers of tryptophan, lysine, threonine, proline, leucine, and methionine. Turbidity was measured in a Klett-Summerson colorimeter (green filter), using diluted samples of those cultures exceeding 110 Klett units. Under these conditions, one Klett unit corresponds to 2×10^6 to 3×10^6 cells per ml.

Genetic techniques. Host strains were constructed by standard techniques for conjugation in liquid medium and for P1-mediated transduction (26). Manipulations of bacteriophage λ derivatives essentially followed the procedures of Davis et al. (9). Plasmids were introduced into strains by

* Corresponding author.

TABLE 1. Bacterial and phage strains

Designation	Relevant genotype	Source or derivation
AB1157	F⁻ *thr*-1 *leu*-6 *proA2 his*-4 *argE3 str*^{r}	CGSC[a] (1)
C600	F⁻ *thr*⁻ *leu*⁻ *thi*⁻ *lacY supE*	D. Wulff (1)
CY178	F⁻ *glpT glpK14 plsB26*	Laboratory collection
DC371	F⁻ *gshA2 srlC*::Tn*10 thr*-1 *leu*-6 *proA2 his*-4	D. P. Clark
DG2	Hfr (KL16), *ptsI*::(λ*c*I857 *red*3) *thi*⁻	W. Epstein
FT1	F⁻ *proC32 purE42 lysA23 trpE38 metE70 metB1 str*^{r}	Laboratory collection (39)
FT17	F⁻ *cfa*⁻; other markers as in FT1	Laboratory collection (39)
GI5	F⁻ *recA srlC*::Tn*10*; other markers as in FT1	This work[b]
JA200	F⁺ *recA* Δ*trpE5 thr*⁻ *leu*⁻	CGSC
JC5491	Hfr (KL16) *ilv*-318 *thr*-300 *recB21 recC22 relA*	CGSC
K12ΔH1Δtrp	F⁻ Δ*trpEA2* Δ(*bio-uvrB*) (λN7N53*c*I857ΔH1[c])	Bernard (3)
MC4100	F⁺ *araD139* Δ*lacU119 thi*⁻ *str*^{r}	M. Casadaban (4)
MO	F⁺ (?) of HfrH *thi*⁻	W. Reznikoff
NK6659	F⁻ *recA srlC*::Tn*10*	N. Kleckner
NS428	F⁻ *recA* (λ*A*11 *b2 red*3 *c*I857*S7*)	N. Sternberg
NS433	F⁻ (λ*E*4 *b2 red*3 *c*I857 *S7*); other markers as in NS428	N. Sternberg
λ*c*I857	*c*I857	Laboratory collection
λ*c*I857S7	*c*I857 *S7*	Laboratory collection
λL47.1	*sbhI*^{0} *chiA*131 Δ(*srI*1-2) *imm*^{434} *cI*⁻ *srI4*^{0} *nin5 shn6*^{0} *srI5*^{0}	W. J. Brammar
λGI1	Δ(*int-ral*)::pLC18-11; other markers as in λL47.1	15
λGI2	*S7 nin*⁺ *shn6*⁺ *srI5*⁺, other markers as in λGI1	15
λGI8	imm λ*c*I857 *S*⁺; other markers as in λGI2	This work[d]
λGI13	Δ(*srI*1-2):: pBR322 *S7*; other markers as in λGI8	This work[d]
λGI16	pLC18-11 in reverse orientation, *S*⁺; other markers as in λGI8	This work[d]
λGI24Δ11	pLC18-11Δ(*sbg-sbh*)Δ11; other markers as in λGI2	This work[d]

[a] *E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, Conn.

[b] Strain FT1 was transduced to Tet^r by P1 (NK6659) and scored for *recA* by testing UV sensitivity.

[c] ΔH1 deletes λ genes *cro-R-A-J-b2*.

[d] See Fig. 1 and the text.

transformation of $CaCl_2$-treated cells with purified plasmid DNA, or occasionally by P1-mediated transduction or conjugal transfer from a plasmid-bearing donor. Recombinant clones containing the plasmid were selected by growth on R agar plates containing 50 μg of sodium ampicillin per ml or crude colicin E1. Colicin was prepared from JF390 cells by extraction with 1 M NaCl after induction of cultures with mitomycin C (12). Large quantities of plasmid DNA were purified from concentrated cell suspension by ethidium bromide-cesium chloride gradients (9) or by alkaline lysis (24). Phage DNA was purified by repeated phenol extraction of concentrated phage suspensions. Methods of restriction endonuclease digestion and analysis and cloning procedures were essentially those of Lynn et al. (23).

Construction of *cfa* phage and plasmids. The *cfa* insert of λGI2 was reversed with respect to λ promoters by excision and reinsertion at the flanking *Bam*HI sites to yield λGI16. Thermoinducible *cfa* phage were derived from λGI2 by a genetic cross with λ*c*I857 and selecting *spi imm*λ progeny. Excess DNA flanking *cfa* in λGI2 was reduced in two steps: (i) excision in vitro of the right-hand *Bgl*II-*Bam*HI segment of the *cfa* insert, and (ii) deletion of sequences to the left of *cfa* by EDTA selection (15). The resulting phage, λGI24Δ11, provided a 7.5-kilobase (kb) *Hin*dIII fragment bearing *cfa* "downstream" from p_L. This fragment was inserted into pBR322 to yield a plasmid, pGI4, which required a λ-immune host and overproduced CFA synthase.

This requirement for λ immunity was exploited in the construction of the Δp_L plasmid pGI6 and $\Delta p_L\Delta cfa$ plasmid pGI5. The inability of ColEI-type plasmids to replicate in *polA* hosts was similarly used to select a fusion hybrid of pGI6 and the replication-runaway plasmid pKN402 (42). A *cfa*$^+$ deletion of this hybrid served as the source of the small *cfa* plasmid pGI13 and a plasmid with the insert in reverse orientation, pGI14.

Scoring of *cfa* by colony autoradiography. The CFA screening method was based on the method used by Taylor and Cronan to isolate *cfa* mutants (39) and for genetic mapping of the *cfa* locus (Taylor, Ph.D. thesis). Clones to be screened for CFA production were spotted onto large plates containing R agar with appropriate selective agents and incubated for 12 h at 37°C, and the resulting patches of cells were adsorbed to sheets of Whatman no. 1 filter paper. The sheets were wetted for 10 min with a few drops of medium E (44) containing 0.4% succinate and the protein synthesis inhibitors chloramphenicol, L-valine, and L-serine hydroxamate at 0.1 mg/ml each. The sheets were blotted by laying them face up on paper towels and repeating the process twice. Finally, the sheets were soaked in the same medium containing L-[*methyl*-^{14}C]methionine at 5 to 10 μg/ml and 1 μCi/ml and incubated at 33.5°C for 2 h. The colonies were fixed by soaking for 10 min in 10% trichloroacetic acid, then soaking for 10 min in boiling 5% trichloroacetic acid, followed by three water washes (15 min each). After drying, the sheets were clamped to a sheet of X-ray film between two sheets of glass and exposed for 2 to 5 days. The resulting autoradiograms were compared with the original filter sheets stained with Coomassie brilliant blue (31) to identify colonies with abnormal [*methyl*-^{14}C]methionine incorporation.

Fatty acid analysis. The strains to be analyzed were grown to saturation at 37°C in 2 ml of R broth (with the appropriate selective agents for plasmid maintenance), then concentrated and resuspended to a final volume of 0.8 ml. Fatty acid methyl esters of membrane phospholipids were prepared and analyzed by gas chromatography as previously described (7). Fatty acid compositions were calculated as percentages of total by weight, using triangulation of plotted peaks or electronic integration (Hewlett-Packard 3390A reporting integrator).

Enzyme assays. Cell-free extracts were made by two methods, each performed at 0 to 4°C. For small cultures or sequential samples of a culture, the cell pellet from a 30- to 50-ml sample was suspended and frozen in 0.7 to 1.0 ml of cold lysis buffer containing 2 mM EDTA, 0.5 mg of lysozyme per ml, and 0.05 mg of pancreatic DNase per ml in 50 mM potassium phosphate (pH 7.5). The suspensions were then thawed, sonicated intermittently for 20 to 50 s, and centri-

fuged at 12,000 × *g* to remove unbroken cells and large debris. For larger quantities of cells, a cell pellet was suspended in a small volume of 50 mM potassium phosphate and passed through a French pressure cell at 14,000 to 16,000 lb/in^2, then freed of large debris by centrifugation at 12,000 × *g* for 15 to 20 min.

The CFA synthase activity in crude cell-free extracts was assayed at 37°C as described by Taylor and Cronan (40). The filter disks were counted in PCS scintillation fluid (Amersham Corp.), and units of enzyme activity were derived from total counts per minute incorporated, specific radioactivity of [*methyl*-^{3}H]*S*-adenosylmethionine present in the assay, and time of incubation (normally 30 to 50 min). The β-lactamase assay mixture contained 100 μM 6-β-furylacrylamidopenicillanic acid (Calbiochem), 0.02% gelatin, and 0.5 mM dithioerythritol in 20 mM potassium phosphate (pH 6.5). Cell extract diluted 1:10 (2 μl) was added to 1.0 ml of this mixture and incubated in a 1-cm quartz cuvette at 27°C, and absorbance at 330 nm was recorded as a function of time. Under these conditions, the net change in absorbance upon hydrolysis is 4,500 M^{-1}, and the apparent β-lactamase activity is 30 to 40% of that determined by the iodometric titration assay of Perret (30), using 7 mM ampicillin as substrate. As defined here, one unit of cyclopropane fatty acid synthase forms 1 pmol of CFA per min at 37°C, and one unit of β-lactamase degrades 1 μmol of 6-β-furylacrylamidopenicillanic acid per min at 27°C.

RESULTS

Since the chromosomal location of *cfa* was unknown, we cloned the CFA synthase gene by screening the hybrid *E. coli*–ColE1 gene bank of Clarke and Carbon (5) for a plasmid which would complement the *cfa* lesion of *E. coli* FT17 (39). The membrane fatty acids of candidates isolated by an autoradiographic screen (see above) were then analyzed by gas-liquid chromatography. This yielded a strain, FT17(pLC18-11), with a dramatically altered fatty acid composition in which the UFA were nearly quantitatively replaced by their cyclopropane derivatives. The abundance of CFA in strain FT17(pLC18-11) resulted from overproduction of CFA synthase. Assays of cell-free extracts of this stain showed its specific activity to be 6 to 10 times higher than that of the parent strain FT1. Plasmid pLC18-11 was introduced into a variety of *E. coli* strains by using F-mediated transfer. In each case, introduction of this plasmid raised the specific activity of CFA synthase and induced a corresponding shift in the fatty acid composition of the membrane phospholipids (Table 2).

Plasmid DNA purified from strain FT1(pLC18-11) proved to be of high molecular weight (20 kb), and because the *cfa* lesion is phenotypically silent, pLC1811 possessed no selectable or conveniently scored marker aside from immunity to colicin E1 (which proved unsatisfactory as a selection for plasmid-containing cells). To facilitate physiological studies of CFA synthase expression, therefore, we first subcloned pLC18-11 into the high-capacity bacteriophage λ vector λL47.1 of Loenen and Brammar (22). This greatly expedited propagation and purification of *cfa* DNA for analysis and manipulations in vitro and enabled many λ genetic techniques to be applied toward the study of CFA synthase expression and function.

Relevant λ*cfa* constructions are described in Table 1, and their genetic and physical maps appear in Fig. 1. For details of recombinant phage construction and physical localization of *cfa*, see reference 15. The techniques available for manipulating λ derivatives also facilitated the construction of several small, selectable *cfa* plasmids. Physical maps of these plasmids and an outline of their construction appear in Fig. 2.

Growth and CFA formation of plasmid-carrying strains. In batch culture, *E. coli* and other eubacteria do not form CFA at a continuous rate; rather, there is a burst of synthesis at a specific stage of growth, generally the transition from exponential to stationary phase (6, 19, 21). We studied the effect of abnormally high levels of CFA synthase on the kinetics of CFA formation by monitoring the fatty acid composition of batch cultures bearing multicopy *cfa* plasmids. Strains JA200(pLC1-3) and JA200(pLC18-11) were grown in parallel batch cultures at 37°C (plasmid pLC1-3 carries genes for xylose utilization and served as the control). Fatty acid

TABLE 2. Effect of *cfa* plasmids on CFA synthase activity and fatty acid composition[a]

Strain	CFA synthase[b] (U per mg of protein)	Fatty acids (% by wt)[b]						% Conversion to CFA[c]	
		14:0	16:0	16:1	17Δ	18:1	19Δ	16:1	18:1
DC371	70	1.2	49.8	<0.5	20.9	11.5	16.5	99	59
DC371(pLC18-11)	770	1.6	53.9	<0.5	21.8	<0.5	22.7	100	99
AB1157	10	3.4	49.6	19.5	14.3	12.2	1.0	42	8
AB1157(pLC18-11)	870	3.5	50.0	<0.5	36.1	<0.5	10.5	100	99
GI5	20	6.7	44.0	3.3	28.2	6.8	10.9	90	62
GI5(pLC18-11)	560	3.6	63.3	<0.5	23.0	<0.5	10.0	100	99
JA200(pLC1-3)	170	3.5	50.0	2.3	30.1	5.2	8.9	93	63
JA200(pLC18-11)	930	3.7	47.1	<0.5	35.2	3.0	11.0	99	79
JC5491(λ)	12	2.2	41.9	15.4	17.6	18.7	3.5	53	16
JC5491(λ)(pGI4)[d]	82	4.7	43.9	<1.0	33.3	2.6	16.3	99	86

[a] Indicated strains were grown in R broth to stationary phase at 37°C.

[b] Crude cell-free extracts were assayed for CFA synthase activity, protein concentration, and fatty acid composition as described in the text.

[c] Percent conversion was calculated by the formula [(percent by weight of CFA)/(percent by weight of CFA + percent by weight of UFA)] × 100.

[d] See Fig. 2.

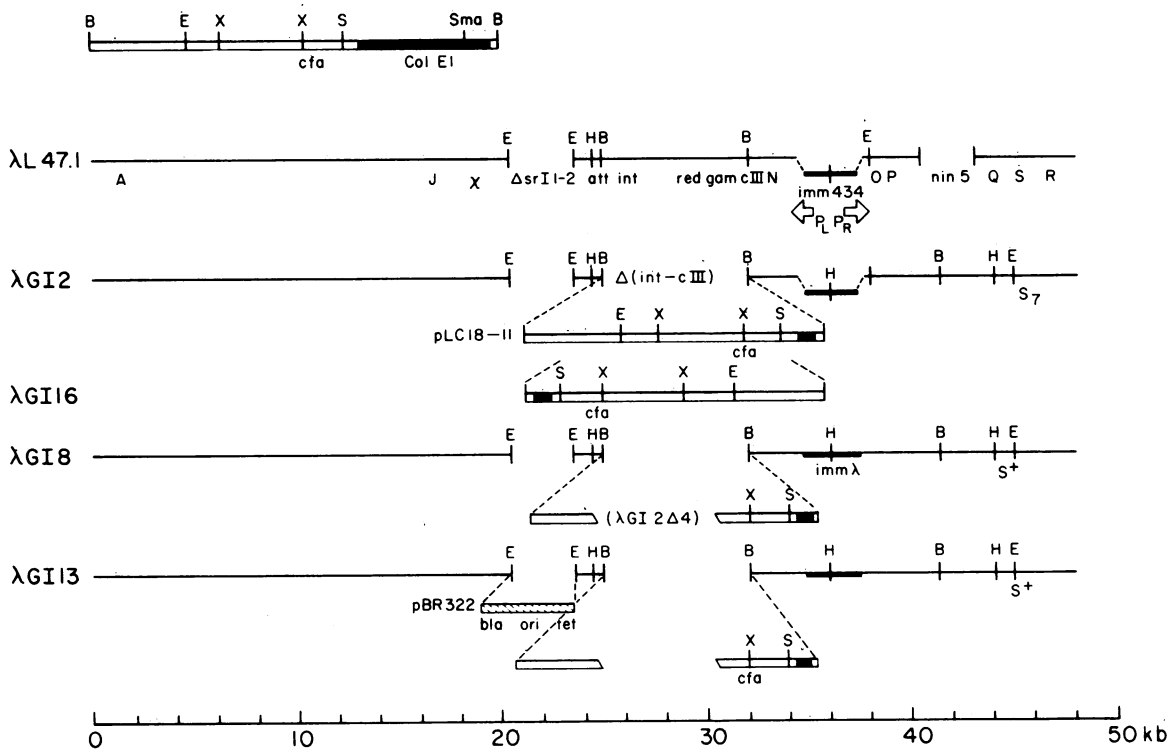

FIG. 1. Partial physical-genetic maps of pLC18-11 (top), λL47.1, and derivative *cfa* phage. Symbols: horizontal lines, λDNA; open bars, *E. coli* chromosomal DNA; solid bars, ColE1 DNA; hatched bars, pBR322 DNA. Scale at bottom indicates length in kilobase pairs (kb). Recognition sites for restriction enzymes are abbreviated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; Sma, *Sma*I; X, *Xho*I. For more complete physical-genetic maps of λ and pBR322 and explanations of gene symbols, see references 36 and 37. Open arrows indicate direction of transcription. For characterization of the λGI2Δ4 deletion, see reference 15.

analyses at several time points showed that JA200(pLC1-3) behaved typically, accumulating CFA only during the onset of stationary phase. In contrast, JA200(pLC18-11) contained high levels of CFA in all stages of growth (data not shown). It should be noted that despite the high levels of CFA present in exponential phase, this strain exhibited a reproducible, though slight, increase in cyclopropanation corresponding to the onset of stationary phase. In addition to altered kinetics of CFA accumulation, strains harboring pLC18-11 had growth rates and cell yields which were depressed approximately 30% relative to those harboring plasmid pLC1-3.

Since the pLC18-11 carrying strain had an abnormally high cyclopropane fatty acid content, the slower growth could be due to cyclopropane acyl chains exerting a bacteriostatic effect per se, a cytotoxic or energy-depleting action of CFA synthase, or perhaps some unrelated function also encoded by pLC18-11. Construction of plasmids pGI5 and pGI6 showed the latter possibility to be correct. These small isogenic plasmids contained only a fraction of the 15-kb chromosomal segment of plasmid pLC18-11; plasmid pGI6 differs from pGI5 in retaining an additional segment of 2.8 kb coding for *cfa* (Fig. 2). Both plasmids were introduced into strain JA200 by transformation to ampicillin resistance. Strains JA200(pG15) and JA200(pGI6) gave identical growth curves in defined medium at 37°C. When grown to stationary phase in R broth plus ampicillin at 37°C, these two strains gave identical viable titers (2.6×10^9 CFU/ml) and identical cell morphologies under phase-contrast microscopy. It appears, therefore, that abnormally high levels of CFA and CFA synthase gave no gross physiological perturbation and that the diminished growth of JA200(pLC18-11) resulted from an extraneous plasmid-mediated function.

The courses of CFA accumulation in strains JA200(pGI5) and JA200(pGI6) with growth (Fig. 3) were nearly identical to those of JA200(pLC1-3) and JA200(pLC18-11), respectively (data not shown). Strain JA200(pGI5) demonstrated the growth-dependent modification of membrane fatty acids typically seen in aerobic batch culture. CFAs occur only at low levels in exponential phase, but late in exponential phase cyclopropanation accelerates rapidly and appears complete by stationary phase (6, 21). However, in the *cfa* plasmid-carrying strain JA200(pGI6), high levels of CFA were present throughout exponential growth (Fig. 3). Whereas normally the palmitoleoyl group is more rapidly and completely converted to its cyclopropane derivative than is the *cis*-vaccenoyl group, both unsaturated acids were nearly quantitatively converted to CFA, given a sufficient level of gene amplification (Fig. 3D).

CFA synthase expression from phage lambda vectors. λGI1 was identified as a *cfa* phage based on DNA restriction fragments. Fatty acid analyses of the lytic debris from λGI1 and λL47.1 infections showed enrichment for CFA in λGI1-infected cultures. Assay of extracts of infected cells harvested before lysis (35 min) showed that λGI1 induced at least a 20-fold amplification of CFA synthase (data not shown). Construction of the lysis-defective (*S7*) derivative λGI2 made it possible to follow enzyme activity and CFA accumulation with time without the complication of lysis (Fig. 4). Nonsuppressing (*sup*^{0}) host cells growing exponentially at

37°C were infected with λ*c*I857 or λGI2, and CFA synthase activity and fatty acid modification were followed with time. Infection with λGI2 caused CFA synthase activity to increase rapidly after 30 min, giving a 30-fold overproduction at 2 h before declining. Accumulation of CFA in the membranes of λGI2-infected cells followed the increase in enzyme activity, and the rate of CFA formation roughly corresponded to the level of CFA synthase activity at each time point (compare Fig. 4A and 4C). In both cases, conversion of palmitoleoyl residues to CFA was more rapid and complete than conversion of *cis*-vaccenoyl residues.

Construction of λGI8 and λGI13 allowed stable lysogens of various strains to be made which carried *cfa* on a repressed prophage. Lysogens of λGI8 were recovered as temperature-sensitve *imm*λ survivors of infection by λGI8 at high multiplicity. Since λGI8 is deleted for *int*, these lysogens presumably were formed by homologous recombination at the *cfa* or *rac* loci (11). Lysogens of phasmid λGI13 were isolated as temperature-sensitive Tet^r Amp^r survivors of λGI13 infection. Both types of lysogens could be induced for lytic growth of the phage by shifting the culture from low to high temperature, which inactivates the thermolabile *c*I857 repressor and initiates transcription of early phage genes from the p_L and p_R promoters (16). In the repressed state, λGI8 and λGI13 continue to produce CFA synthase, as demonstrated in lysogens of strain FT17. Strains FT17(λGI8) and FT17(λGI13) grown to stationary phase at 30°C contained normal quantities of CFA, whereas strains FT17 and FT17(λ*c*I857) contained none. This indicates that the native *cfa* promoter (or possibly a fortuitous promotor) has been preserved in the construction of these lysogenic derivatives and functions constituitively in the absence of λ gene expression.

To determine the direction of *cfa* transcription, we constructed λ *cfa* phage bearing *cfa* in both possible orientations and compared the initial kinetics of CFA synthase production from these vectors, based on the following reasoning. During the early period of λ infection (5 to 15 min), RNA polymerase transcribes the central region of the phage genome in the leftward direction, originating at promoter p_L. This transcription is powerful but transient, due to the concomitant accumulation of *cro* repressor (16). As a result, foreign genes situated in the central region which transcribe in the leftward direction are expressed by p_L early in infection, whereas those transcribing rightward are silenced until p_L transcription subsides (18). Two lytic phage carrying *cfa* in opposite orientations, therefore, would be expected to differ in the initial kinetics of CFA synthase production. Such an effect was demonstrated when λGI2 and λGI16 were used at high multiplicity, showing that the native *cfa* promoter transcribes leftward in λGI2 (Table 3).

This result implied that the *c*I857 derivatives of λGI2 were

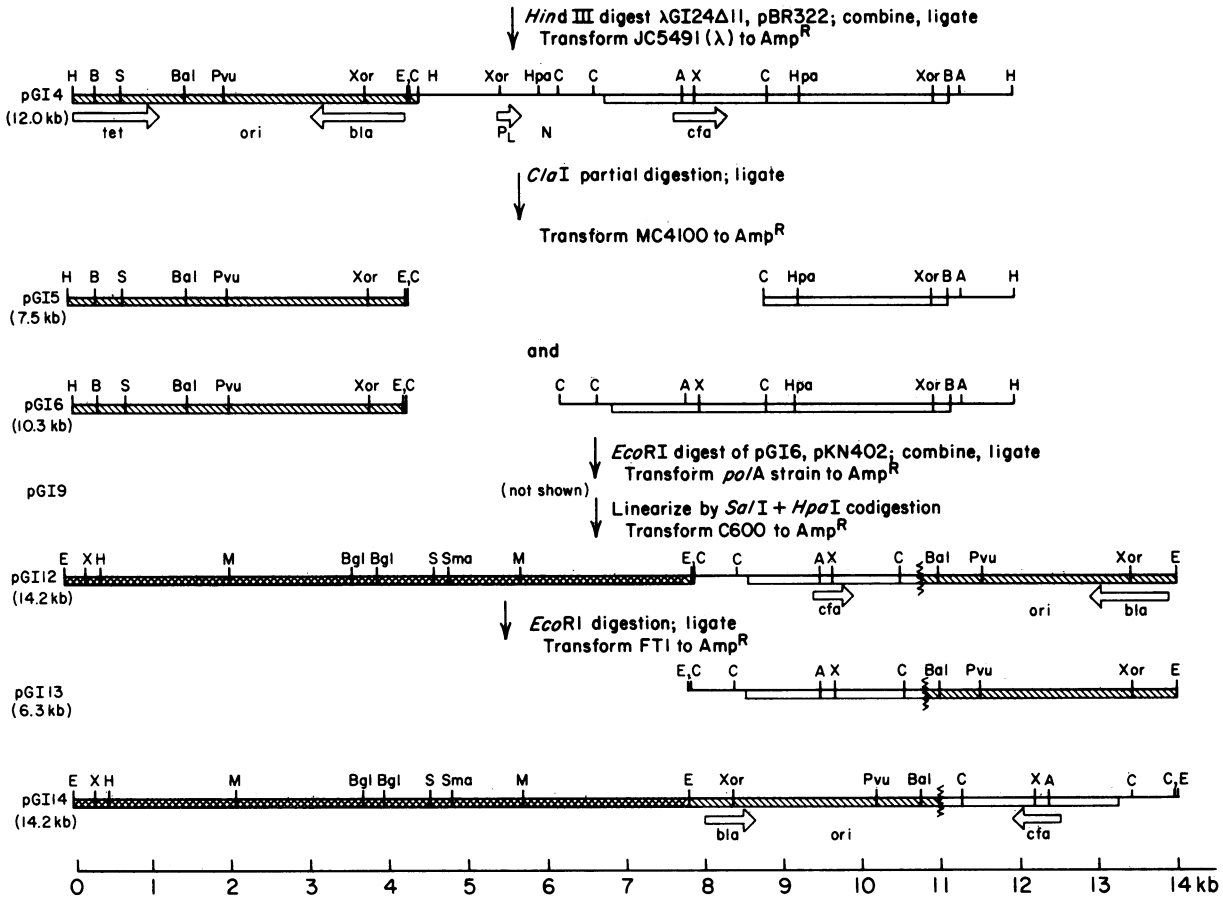

FIG. 2. Outline of construction of *cfa* plasmids (see the text). Physical loci are symbolized as follows: crosshatched bars, pKN402 DNA; A, *Ava*I; Bal, *Bal*I; Bgl, *Bgl*II; C, *Cla*I; Hpa, *Hpa*I; M, *Mlu*I; Pvu, *Pvu*II; Xor, *Xor*II. Jagged line represents approximate location of a novel joint generated by deletion (41). Maps of left-end portions of pGI12 and pGI14 were deduced from restriction analysis of purified pKN402 DNA and data of Remaut et al. (32). Other symbols are as in Fig. 1.

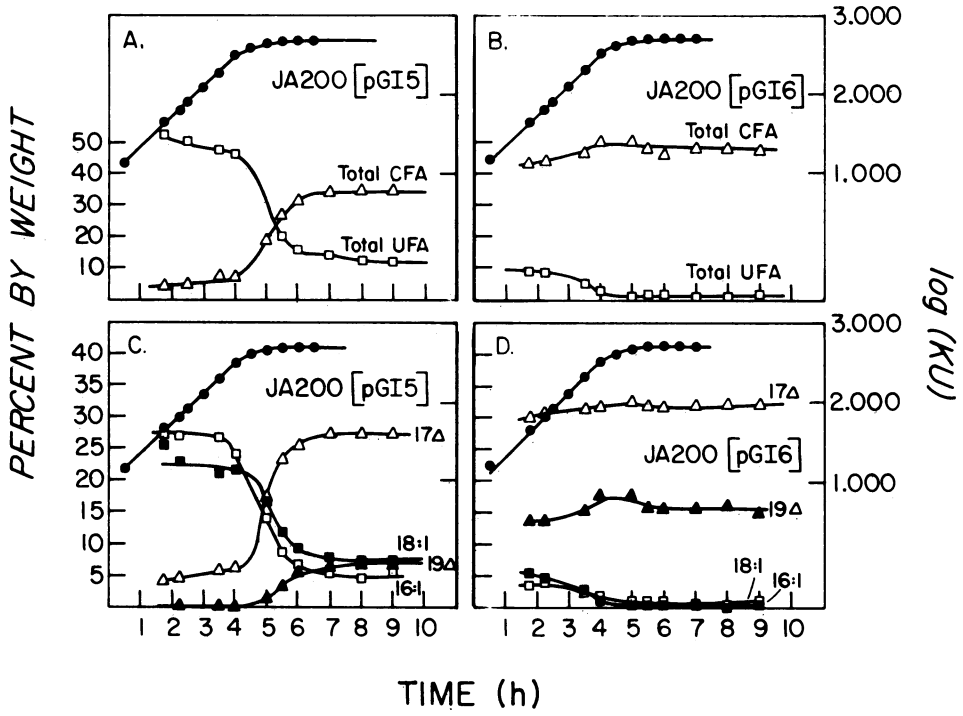

FIG. 3. Fatty acid composition of strains JA200(pGI5) and JA200(pGI6) as a function of time. Approximately 10^{10} cells were withdrawn from cultures at indicated times, chilled, harvested by centrifugation, and analyzed for fatty acid composition as described in the text. Symbols: ●, culture turbidity; □, ■, UFA; △, ▲, CFA; 16:1, palmitoleic acid; 17Δ, 9,10-methylene hexadecaomoic acid; 18:1, *cis*-vaccenic acid; 19Δ, lactobacillic acid.

well suited for transient high-level expression of CFA synthase via thermal induction of corresponding lysogens, since *cfa* was situated for transcription from p_L in all of these derivatives. The response of such lysogens to thermal induction was enigmatic, as illustrated by strain FT17(λGI13). Induction of this strain prompted a rapid decline in the specific activity of CFA synthase. Similarly, thermal induction of various chromosomal lysogens failed to elevate CFA synthase levels. The effect could not be attributed to an intrinsic instability of CFA synthase at elevated temperatures; both nonamplified and plasmid-amplified cultures routinely showed greater activity at high temperature than at low temperature.

Expression from plasmids. For several reasons, we desired a genetic system that could be conveniently propagated and give very high expression of *cfa* on demand. We therefore constructed a derivative of pBR322 containing *cfa* situated approximately 2 kb downstream from the phage λ p_L promoter. We found that thermal induction of this plasmid, pGI4, in host strain NS433, decreased, rather than increased, *cfa* expression. Constant-temperature induction of strain MC4100(λ)(pGI4) by 10 μg of mitomycin C per ml (33) caused only a 50% increase of CFA synthase levels. To insure that the λCro protein did not limit p_L-mediated transcription, plasmid pGI4 was introduced into a strain carrying a *c*I857 prophage deleted for *cro* (3). Thermal induction of this strain, K12ΔH1Δ*trp*(pGI4), gave a transient depression, rather than the elevation of CFA synthase levels (data not shown). The three strains also differed somewhat in their expression of the *bla* gene of pGI4.

Since enzyme activities declined after termperature shift of strains bearing a *cfa* plasmid, we investigated whether thermal induction of λ*c*I857 interfered with expression of CFA synthase and perhaps other plasmid-encoded genes, using plasmids pGI5 and pGI6 in a λ*c*I857 *S7* lysogen (Table 4). The data point to two conclusions: (i) heat-pulse induction of λ*c*I857 antagonized CFA synthase expression, in contrast to growth at constant temperature, and (ii) β-lactamase expression decreased with increasing size of the plasmid, and the decrease was disproportionately large compared with the size increment. This was especially evident with pGI6 and may reflect insert DNA interfering with *bla* expression or plasmid replication functions or both. Finally, we constructed *cfa* plasmids capable of establishing very high copy numbers due to a temperature-sensitive lesion in replication control. Strains bearing the "*cfa*-runaway" plasmids pGI12 or pGI14 were grown at 30 or 40°C. CFA synthase levels (and the plasmid copy number, as indicated by β-lactamase activity [43]), were much higher in the 40°C cultures. This behavior was exemplified by FT1(pGI14) (Table 5).

Physiological factors affecting CFA synthase expression. Experience with several strains of *E. coli* K-12 grown under a variety of conditions has shown that CFA synthase levels may vary to a remarkable degree. We have observed specific activities ranging from 1 to over 300 U/mg of protein in crude extracts of unamplified *cfa*$^+$ cultures. This variability did not arise from interference by other methylation reactions, as shown by assaying extracts of various amplified and unamplified strains in the normal manner followed by fractionation of the incorporated label. More than 96% of the ^{3}H-label was eluted from the filter disk with $CHCl_3$–CH_3OH,

regardless of the apparent activity of the extract. Upon saponification and acidification, essentially all of the radioactivity extracted into a nonpolar solvent, confirming incorporation into fatty acid. The method of cell disruption did not noticeably influence the yield of the enzyme, and the fatty acid composition of an extract consistently correlated with its CFA synthase activity (e.g., see Table 2). Much of the observed variations apparently resulted from differences in genetic background; strains which consistently produced little CFA gave low CFA synthase activites in vitro. Furthermore, such strains often gave poor expression from plasmid or phage clones. Conversely, strains with intrinsically high synthase levels generally performed well when genetically amplified (e.g., see Tables 2 and 5).

TABLE 3. Initial kinetics of *cfa* expression[a]

Time (min) postinfection	CFA synthase (U/mg of protein)	
	λGI2	λGI16
3	2.5	4.7
6	5.0	3.4
10	13.5	4.8
15	17.9	4.4
20	26.0	6.0

[a] Strain FT17 was grown to a density of 4×10^8 cells per ml, infected with the *cfa* phage indicated, and assayed for CFA synthase activity as described in the text.

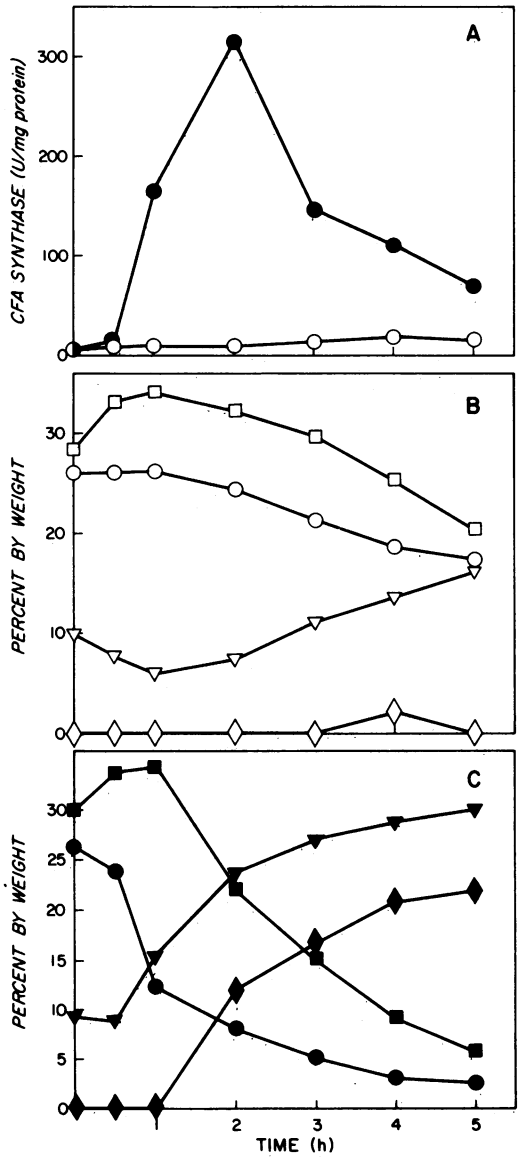

FIG. 4. Infection with λ*cfa* phage. Approximately 8×10^{10} cells of strain MO growing exponentially in T broth were concentrated, infected with indicated phage at a multiplicity of 9, then diluted to 600 ml in T broth and aerated at 37°C. Panel A shows the resulting time course of CFA synthase activity (units per milligram of protein). (B and C) Time courses of fatty acid modification symbols: ○, ●, palmitoleic acid; □, ■, *cis*-vaccenic acid; ▽, ▼, 9,10-methylene hexadecanoic acid; ◇, ◆, lactobacillic acid. Open symbols, λ*c*I857 *S7*; closed symbols, λGI2.

We have also examined certain growth parameters reported to influence CFA synthesis in *E. coli* (20, 38). For example, contrary to the results of McGarrity and Armstrong (25), strain FT1 grown in defined medium showed no increase in CFA content with increased osmotic strength (23% [0.6 M] sucrose or 23% polyethylene glycol [PEG-1000]), whereas the growth rate decreased by 43% in each case. Also, CFA synthase levels in FT1(pGI14) grown in 1% tryptone and 0.1% yeast extract showed negligible change when NaCl was added to the medium in the range of 0 to 1.4%. Enzyme activity did correlate with other culture parameters, however. Within the pH range of 5.6 to 8.3, CFA synthase expression from pGI14 was favored by acidic conditions. In general, higher CFA synthase levels resulted from growth in broth rather than in defined medium. With regard to culture age, the extractable activity generally increased during exponential growth, peaking during the onset of stationary phase and declining thereafter (cf. reference 6). Growth of strain FT1(pGI13) at 40°C yielded a 76% higher CFA synthase activity than growth at 30°C. Adding glucose to 0.4% stimulated enzyme activity an additional 40%. The degree of aeration of the culture also seemed quite relevant. Cultures usually gave maximal expression under conditions of poor aeration, as opposed to vigorous aeration or fermentative growth (Table 5). This applied to both unamplified and plasmid-amplified strains.

DISCUSSION

The hybrid plasmid isolated in this study, pLC18-11, complemented the *cfa* lesion of strain FT17 and, when introduced into various *cfa*$^+$ backgrounds, increased CFA synthase activity severalfold. Selectable plasmids containing a small portion of pLC18-11 gave *cfa* gene amplification in agreement with the apparent copy number of the respective plasmid. Amplification by phage or plasmid clones induced a corresponding increase in the CFA content of membrane fatty acids in all strains examined. The relative resistance of *cis*-vaccenoyl groups to cyclopropanation can be overcome by sufficiently elevated CFA synthase activities. Thus, fatty acid analysis of stationary-phase cultures provides a crude estimate of CFA synthase activity over a wide range. We tentatively conclude that pLC18-11 and its *cfa*$^+$ plasmid and bacteriophage derivatives carry the structural gene(s) for the cyclopropane fatty acid synthase of *E. coli*.

Increased gene dosage in a favorable genetic background has been the most effective approach to overproduction of CFA synthase to date. The relative inefficacy of p_L-*cfa* plasmids remains unexplained, since their requirement for λ

TABLE 4. Effect of λ induction on gene expression from *cfa* plasmids lacking p_L

Plasmid	CFA synthase (U/mg of protein)						β-Lactamase (U/mg of protein)					
	Growth temp[a] (°C)			Time (min) postshift[b]			Growth temp[a] (°C)			Time (min) postshift[b]		
	30	33	36	0	40	80	30	33	36	0	40	80
pBR322	6	8	18				2.83	2.97	5.32	4.14	2.51	2.38
pGI5	12	16	23	23	17	16	0.93	0.84	0.93	1.62	1.23	1.45
pGI6	166	192	246	248	220	141	0.16	0.18	0.28	0.20	0.28	0.41

[a] Strain NS428 bearing the plasmid indicated was grown at the indicated temperature in R broth to stationary phase.
[b] Exponentially growing cultures were thermally induced by shifting from 30 to 42°C for 20 min, then to 37°C.

immunity indicates that p_L is functional. Possible retroregulation (14) of *cfa* by RNase III, interference of plasmid replication by transcription through *ori*, and loss of *N* or *nutL* function were also investigated. In each case, the appropriate corrective measures produced a negligible increase in CFA synthase amplification. It should be noted that although p_L is a very powerful promoter, it has proven difficult to harness for the expression of many heterologous genes (3, 10, 27, 28). Although N protein is required to guarantee transcriptional "read-through" into distal genes, its overproduction interferes with host transcription (29). In some cases, the intervening DNA sequence must be altered in an ill-defined manner to achieve p_L-mediated expression of a structural gene. Because *cfa* has been only approximately localized and because assaying its expression is laborious, we have not attempted the detailed molecular analysis necessary to identify and correct the defect apparent in pGI4.

The physiological importance of CFA synthase has yet to be elucidated. The phase transition temperature of pure phosphatidylcholine is slightly increased by replacing UFA with CFA (34), yet liposomes made from either CFA or UFA bulk phospholipid of *E. coli* exhibit the same thermotropic phase transition behavior (8). Furthermore, Taylor and Cronan (39; Taylor, Ph.D. thesis) could not demonstrate a physiological phenotype for the *cfa* mutant FT17. All treatments, including prolonged incubation, drying on filter disks, acidic sodium lactate, amethoprim, detergents, sodium chloride, rapid freeze-thaw, and lysis of spheroplasts, affected FT17 and its parent strain FT1 in a similar manner. In attempts to approximate a colonic environment, we have grown strains FT1 and FT17 under strongly reducing conditions in a medium with and without volatile fatty acids (17) and have seen no significant differences in growth or survival. The viable titers of stationary-phase FT1 and FT17 suspensions declined similarly when treated with toxic concentrations of Ba^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Zn^{2+}, Br^-, or I^-, and when incubated for 10 days at 25°C in water under 1 atm (100 kPa) of O_2. We thus remain unable to demonstrate any obvious growth or survival defect caused by lack of CFA.

Conversely, overproduction of CFA has not proven deleterious. Plasmid-amplified cultures show that neither elevated levels of CFA synthase nor unnatural abundance of CFA in exponential phase interferes with growth. Strains FT1 and JA200 showed negligible loss of isogenic cfa^+ or cfa^- plasmids (pGI5, pGI6, pGI13, pBR322) after eight generations of growth under nonselective conditions. Under optimal conditions, *cfa* phage and cfa^+ pBR322 plasmids produced enzyme levels of 200 to 500 U/mg of protein, but higher levels can be obtained (Table 5). The gross physiology of *E. coli* thus seems indifferent to the activity of this membrane modification system. CFA synthase activity does respond to growth conditions, however. The enzyme levels in normal and plasmid-amplified strains varied with growth temperature, aeration rate, culture age, and medium pH in a manner similar to that observed for CFA in earlier studies (19, 20). The interaction of these and other factors no doubt contributes to the culture-to-culture variability observed for this enzyme.

The kinetics of CFA accumulation in batch cultures of *E. coli* is an interesting regulatory phenomenon. Production of CFA synthase de novo cannot account for the sudden appearance of CFA late in exponential phase, since the

TABLE 5. Factors affecting CFA synthase expression

Genetic background			Aeration				Gene copy no.			
							β-Lactamase (U/mg of protein)		CFA synthase (U/mg of protein)	
Strain	Unamplified	Bearing pGI4	Strain	High	Low	None	30°C	40°C	30°C	40°C
DG2	2.1	17	FT17	1.7	1.3	0.9	1.5	9.2	141	1,570
FT17(λ)	1.3	74	MC4100	1.1	5.0	2.5				
MO(λ	13	86	MC4100(λ)(pGI4)	41	88	66				
NS433	57	302								

[a] Unless otherwise indicated, all values are CFA synthase activity expressed in units per milligram of protein for cultures grown in R broth.
[b] Strains indicated were grown for 7 h at 30°C with moderate aeration.
[c] Cultures were grown overnight at 37°C under the following aeration conditions: high, 30 ml of broth in a 250-ml culture flask shaken at 240 rpm; low, 45 ml of broth in a 50-ml culture flask shaken at 240 rpm; none, 50-ml screw-cap flask filled to brim, sealed tightly, not shaken.
[d] Strain FT1(pGI14) was grown at 30°C to mid-exponential phase and diluted to yield two duplicate cultures (45 ml each). These were grown at 30°C, and one set was shifted to 40°C at a density of 10 to 20 Klett units. Cultures were harvested in late exponential phase (150 to 180 Klett units) and assayed as described in the text.

enzyme is present throughout all stages of growth. (Growth phase-specific removal of a potent inhibitor of the enzyme is more difficult to discredit, in view of the large dilutions accompanying assay of the enzyme in vitro [6, 40]). Another possible CFA regulatory mechanism involves some type of physical barrier within the cell that modulates the access of CFA synthase to membrane phospholipid and thus prevents catalysis during exponential growth. This model resembles that of regulation by intracellular inhibitor, and both are discredited to a large extent by the ease with which gene amplification abolishes the temporal lag of CFA synthesis.

This latter observation raised yet another (albeit problematic) explanation for temporal regulation. If, as a result of kinetic competition, cellular fatty acid composition were a function of the relative activities of CFA synthase and some other process such as membrane growth or cell division, then the CFA content of rapidly growing cultures could be sharply increased by either retarding growth or increasing CFA synthase activity. (The former situation is exemplified by onset of stationary phase and the latter by plasmid- or phase-amplified strains). Such a simplistic competing-rate model accordingly predicts that abrupt imposition of stasis should prompt abrupt accumulation of CFA, regardless of cell density. This was tested with a *plsB* mutant, CY178, which quickly halts phospholipid biosynthesis upon removal of glycerol supplementation (2). The fatty acid compositions of parallel cultures of exponentially growing CY178 cells were monitored after one culture was starved for glycerol. Although starvation for glycerol induced an abrupt, premature stasis, it did not prompt a corresponding increase in CFA content (data not shown).

Thus, each of several plausible models can be experimentally discounted as the sole means by which CFA synthesis is temporally regulated in *E. coli*. On the other hand, some of the available data can be interpreted as the result of these mechanisms acting to minor extents. Extractable CFA synthase activity does fluctuate somewhat as a function of culture age, for example (6), and imposition of premature stasis does promote a slight accumulation of CFA relative to an exponentially growing culture (data not shown). Although they contain high levels of CFA, even plasmid-amplified cultures typically do not achieve complete conversion until stationary phase (data not shown). It seems reasonable, therefore, to allow for the simultaneous superposition of these processes, and perhaps others, in accounting for the kinetics of CFA accumulation typically observed in *E. coli*.

Although the various *cfa* constructions described here have shed some light on the physiology of cyclopropane fatty acid synthesis in *E. coli*, their greater potential lies in facilitating study of CFA synthase in vitro. Increasing the *cfa* gene dosage has proven effective in elevating the cellular levels of this enzyme. By adjusting growth conditions and using cfa runaway plasmids, specific activities of 2,000 U/mg of protein should be reproducibly obtained. Such overproduction will greatly facilitate attempts to purify this unstable enzyme. In this way we hope to study modification of model membranes by homogeneous CFA synthase in vitro and thus to characterize the unique mode of protein-lipid interaction of this enzyme.

ACKNOWLEDGMENTS

We thank D. Aperion, B. Bachmann, W. Brammar, J. Foulds, and K. Nordstrom for supplying bacterial strains, λ phage, and plasmids. Keith Backman and Doug Berg provided helpful discussions.

This work was supported by Public Health Service grant GM 26156 from the National Institutes of Health. D.W.G. was the recipient of a Public Health Service predoctoral traineeship from the National Institutes of Health and a National Science Foundation graduate fellowship.

LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Bell, R. M.** 1974. Mutants of *Escherichia coli* defective in membrane phospholipid synthesis: macromolecular synthesis in an sn-glycerol 3-phosphate acyltransferase K_m mutant. J. Bacteriol. **117**:1065–1076.
3. **Bernard, H.-U. E. Remaut, M. V. Hershfield, H. K. Das, D. R. Helinski, C.Yanoksky, and N. Franklin.** 1979. Construction of plasmid cloning vehicles that promote gene expression from the bacteriophage lambda P_L promoter. Gene **5**:59–76.
4. **Casadaban, M. J.** 1976. Transposition and fusion of the lac genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–555.
5. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic ColE1 hybrids representative of the entire *E. coli* genome. Cell **9**:91–99.
6. **Cronan, J. E., Jr.** 1968. Phospholipid alterations during growth of *Escherichia coli*. J. Bacteriol. **95**:2054–2061.
7. **Cronan, J. E., Jr., W. D. Nunn, and J. G. Batchelor.** 1974. Studies on the cyclopropane fatty acids in *Escherichia coli*. Biochim. Biophys. Acta **348**:63–75.
8. **Cronan, J. E., Jr., R. Reed, F. R. Taylor, and M. B. Jackson.** 1979. Properties and biosynthesis of cylopropane fatty acids in *Escherichia coli*. J. Bacteriol. **138**:118–121.
9. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
10. **Derom, C., D. Gheysen, and W. Fiers.** 1982. High-level synthesis in *Escherichia coli* of the SV40 small-t antigen under the control of the bacteriophage lambda P_L promoter. Gene **17**:45–54.
11. **Evans, R., N. Seely, and P. Kuemple.** 1979. Loss of *rac* locus DNA in merozygotes of *Escherichia coli* K-12. Mol. Gen. Genet. **175**:245–250.
12. **Foulds, J., and C. Barrett.** 1973. Characterization of *Escherichia coli* mutants tolerant to bacteriocin JF246: two new classes of tolerant mutants. J. Bacteriol. **116**:885–892.
13. **Goldfine, H.** 1972. Comparative aspects of bacterial lipids. Adv. Microb. Physiol. **8**:1–58.
14. **Gottesman, M., A. Oppenheim, and D. Court.** 1982. Retroregulation: control of gene expression from sites distal to gene. Cell **29**:727–728.
15. **Grogan, D. W., and J. E. Cronan, Jr.** 1983. Use of lambda phasmids for deletion mapping of non-selectable markers cloned in plasmids. Gene **22**:75–83.
16. **Hershowitz, I., and D. Hagen.** 1980. The lysis-lysogeny decision of phage λ: explicit programming and responsiveness. Annu. Rev. Genet. **14**:399–445.
17. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1972. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg, Va.
18. **Hopkins, A. S., N. E. Murray, and W. J. Brammar.** 1976. Characterization of λ*trp*-transducing bacteriophages made *in vitro*. J. Mol. Biol. **107**:549–569.
19. **Jaques, N., and A. L. Hunt.** 1980. Studies on cyclopropane fatty acid synthesis: effect of carbon source and oxygen tension on cyclopropane fatty acid synthase activity in *Pseudomonas denifrificans*. Biochim. Biophys. Acta **619**:453–470.
20. **Knivett, V. A., and J. Cullen.** 1965. Some factors affecting cyclopropane fatty acid formation in *Escherichia coli*. Biochem. J. **96**:771–776.
21. **Law, J. H.** 1971. Biosynthesis of cyclopropane rings. Acc. Chem. Res. **4**:199–203.
22. **Loenen, W. A. M., and W. J. Brammar.** 1980. A bacteriophage lambda vector for cloning large DNA fragments made with several restriction enzymes. Gene **10**:249–259.
23. **Lynn, S. P., J. F. Gardner, and W. S. Reznikoff.** 1982. Attenua-

tion regulation in the *thr* operon of *Escherichia coli* K-12: molecular cloning and transcription of the controlling region. J. Bacteriol. **152**:363–371.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **McGarrity, J. T., and J. B. Armstrong.** 1981. The effect of temperature and other growth conditions on the fatty acid composition of *Escherichia coli*. Can. J. Biochem. **27**:835–840.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Moir, A., and W. J. Brammar.** 1976. The use of specialized transducing phages in the amplification of enzyme production. Mol. Gen. Genet. **149**:87–99.
28. **Murray, N. E., and W. S. Kelley.** 1979. Characterization of *polA* transducing phages: effective expression of the *E. coli polA* gene. Mol. Gen. Genet. **175**:77–87.
29. **Nakamura, Y., and H. Ucida.** 1983. Isolation of conditionally lethal amber mutations affecting synthesis of the *nusA* protein of *Escherichia coli*. Mol. Gen. Genet. **190**:196–203.
30. **Perret, C. J.** 1954. Iodometric assay of penicillinase. Nature (London) **174**:1012–1013.
31. **Raetz, C. R.** 1975. Isolation of *Escherichia coli* mutants defective in enzymes of membrane lipid synthesis. Proc. Natl. Acad. Sci. U.S.A. **72**:2274–2278.
32. **Remaut, E., H. Tsao, and W. Fiers.** 1983. Improved plasmid vectors with a thermoinducible expression and temperature-regulated runaway replication. Gene **22**:103–113.
33. **Roberts, J. W., and C. W. Roberts.** 1975. Proteolytic cleavage of bacteriophage lambda repressor in induction. Proc. Natl. Acad. Sci. U.S.A. **72**:147–151.
34. **Silvius, J. R., and R. N. McElhaney.** 1979. Effects of phospholipid acyl chain structure on thermotropic phase properties. 2. Phosphatidylcholines with unsaturated or cyclopropane acyl chains. Chem. Phys. Lipids **25**:125–134.
35. **Smith, D. D., Jr., and S. J. Norton.** 1980. *S*-Adenosyl methionine, cyclopropane fatty acid synthase, and the production of lactobacillic acid in *Lactobacillus plantarum*. Arch. Biochem. Biophys. **205**:564–570.
36. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. **43**:77–90.
37. **Szybalski, E. H., and W. Szybalski.** 1979. A comprehensive molecular map of bacteriophage lambda. Gene **7**:217–270.
38. **Taguchi, M., K. Izui, and H. Katsuki.** 1980. Augmentation of cyclopropane fatty acid synthesis under stringent control in *Escherichia coli*. J. Biochem. **88**:1879–1882.
39. **Taylor, F., and J. E. Cronan, Jr.** 1976. Selection and properties of *Escherichia coli* mutants defective in the synthesis of cyclopropane fatty acids. J. Bacteriol. **125**:518–523.
40. **Taylor, F. R., and J. E. Cronan, Jr.** 1979. Cyclopropane fatty acid synthase of *Escherichia coli*: stabilization, purification, and interaction with phospholipid vesicles. Biochemistry **15**:3292–3300.
41. **Thompson, R., and M. Achtman.** 1979. The control region of the sex factor DNA transfer cistrons: physical mapping by deletion analysis. Mol. Gen. Genet. **169**:49–57.
42. **Uhlin, B. E., S. Molin, P. Gustafsson, and K. Nordstrom.** 1979. Plasmids with temperature-dependent copy number for amplification of cloned genes and their products. Gene **6**:91–106.
43. **Uhlin, B. E., and K. Nordstrom.** 1977. R plasmid gene dosage effects in *Escherichia coli* K-12: copy mutants of the R plasmid R1 *drd*-19. Plasmid **1**:1–7.
44. **Vogel, H. J., and D. M. Bonner.** 1956. Acetyl-ornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.