Regulation of Fatty Acid Biosynthesis in Escherichia coli

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INTRODUCTION

The study of the membrane lipids of Escherichia coli has contributed greatly to our understanding of the synthesis and function of membrane lipids in general. In addition to the many other advantages of this bacterium, the lipid composition of E. coli is among the simplest known, consisting of only three major fatty acids and three phospholipid species. This review will concentrate on the synthesis of the fatty acid components, since the synthesis and function of the phospholipid and lipid A components have been reviewed within the last few years (122).

It should be noted that fatty acid synthesis in E. coli has provided a paradigm of predictive value for other bacteria and in plants. For example, the synthesis of polyketide antibiotics by Streptomyces species and related organisms is known to proceed by a mechanism analogous to fatty acid synthesis, and this conservation of mechanism is supported by the striking sequence similarities between genes of the polyketide pathway and those of E. coli fatty acid synthesis (63). Indeed, Sherman has shown that a mutation in a polyketide synthetic gene that encodes a putative β-ketoacyl-acyl carrier protein (ACP) synthase activity can be complemented by the E. coli gene (fabB) that encodes β-ketoacyl-ACP synthase I (134). A second example is the nod genes that determine host species specificity of the rhizobia. Sequence similarity between these nod genes and those of E. coli fatty acid synthesis suggested that the species determinants were acylated molecules, a prediction that has been confirmed (38, 86, 106, 143). The study of fatty acid synthesis in plants also owes much to the E. coli prototype. Plant fatty acid synthesis occurs in the chloroplast (essentially a symbiotic cyanobacterium) and is catalyzed by a series of enzymes similar to those of E. coli. Many plant lipid synthetic enzymes have been purified and characterized by methods based on those developed for the E. coli proteins, and the available sequences of the plant proteins are similar to those of the analogous E. coli proteins. The plant lipid field has also profited from the predictive value of the E. coli work. For example, the discovery of

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TABLE 1. Known enzymes of fatty acid biosynthesis in E. coli

| Gene | Protein or function affected | Map position | Mutant biochemical or growth phenotype |
|------|-----------------------------|--------------|---------------------------------------|
| aas  | Acyl-ACP synthetase         | 61           | Accumulation of lysophosphatidyl ethanolamine |
| acsA | Acetyl-CoA carboxylase       | 4            | Carboxyltransferase α subunit |
| acsB | Acetyl-CoA carboxylase       | 71           | BCCP subunit |
| acsC | Acetyl-CoA carboxylase       | 71           | Biotin carboxylase subunit |
| acsD | Acetyl-CoA carboxylase       | 50           | Carboxyltransferase β subunit |
| acsE | Acetyl-CoA carboxylase       | 24           | ACP structural gene |
| acsF | ACP synthetase              | 43           | Accumulation of apo-ACP |
| cfa  | Cyclopropane fatty acid synthase | 36     | Cells lack cyclopropane fatty acids |
| fabA | β-Hydroxydecanoyl ACP dehydrogenase | 22   | Unsaturated fatty acid auxotroph |
| fabB | β-Ketoacyl-ACP synthase III | 50          | Overproduction of saturated fatty acids |
| fabD | Malonyl-CoA:ACP transacylase | 24          | Unsaturated fatty acid auxotroph |
| fabE | Acetyl-CoA carboxylase       | 71           | Ts* auxotroph requiring both saturated and unsaturated fatty acids |
| fabF | β-Ketoacyl-ACP synthase II  | 24           | Now designated accB |
| fabG | β-Ketoacyl-ACP reductase     | 24           | Altered thermal regulation |
| fabH | β-Ketoacyl-ACP synthase III | 24           | |
| fabR | Transcriptional regulator of fabA | 26   | |
| fadA | UDP-Glc-Nac acyltransferase  | 69           | Utilization of trans-unsaturated fatty acids |
| orf-17| Putative dehydrase          | 4            | Accumulation of lipid A |
| pksB | sn-Glycerol-3-phosphate acyltransferase | 92  | Unknown |
| pksC | 1-Acylglycerol phosphate acyltransferase | 65 | Glycerol-3-phosphate auxotroph |
| pksX | Unknown                      | 24           | Required for PlsB* phenotype |
| tesA | Thioesterase I              | 12           | Defective enzyme activity |
| tesB | Thioesterase II             | 10           | Defective enzyme activity |

*Ts, temperature sensitive.

β-ketoacyl-ACP synthase III in E. coli led directly to the discovery of an enzyme with similar substrate specificity in plants (22).

OVERVIEW OF TYPE II FATTY ACID SYNTHASE SYSTEMS

Reactions of Fatty Acid Biosynthesis

The fatty acid synthase system in E. coli is the archetype of the type II or dissociated fatty acid synthase systems. Each of the individual reactions are carried out by separate proteins that can be purified independently of the other enzymes in the pathway and are encoded by unique genes. There are often multiple proteins that carry out the same basic chemical reaction. However, because of differences in substrate specificity, each plays a unique role in the physiological regulation of the spectrum of products produced by the pathway and hence in the biophysical properties of the membrane bilayer. One of the principal challenges of current research in this area is determining the number and function of these isozymes. The two important cofactors in fatty acid synthesis are coenzyme A (CoA) and ACP, which are involved in carrying the growing acyl chain from one enzyme to another and supplying precursors for the condensation reactions. The known enzymes of fatty acid biosynthesis and their genes are listed in Table 1.

The precursors for fatty acid biosynthesis are derived from the acetyl-CoA pool. Malonyl-CoA is made available to the enzymes of fatty acid biosynthesis by its conversion to malonyl-ACP by malonyl-CoA:ACP transacylase (Fig. 2).

There are three possible mechanisms for the initiation of fatty acid biosynthesis in E. coli (Fig. 2). First, β-ketoacyl-ACP synthase III catalyzes the condensation of acetyl-CoA with malonyl-ACP to yield acetoacetyl-ACP. In the second

![FIG. 1. Reactions catalyzed by acetyl-CoA carboxylase. The first step is the carboxylation of BCCP (the accB gene product) by biotin carboxylase (the accC gene product). The second step is the transfer of the CO₂ moiety to acetyl-CoA by transcarboxylase, a heterodimer composed of the accA and accD gene products, Pₐ inorganic phosphate. Reproduced from reference 66a with permission.](http://mmbr.asm.org/)

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pathway, the acetate moiety is first transferred from acetyl-CoA to acetyl-ACP by either acetyl-CoA:ACP transacylase or condensing enzyme III. Then the acetyl-ACP is condensed with malonyl-ACP by condensing enzyme I (or alternatively condensing enzyme II). The third pathway involves the decarboxylation of malonyl-ACP by synthase I to form acetyl-ACP, which is subsequently condensed with malonyl-ACP. The evidence for the existence of these pathways and their relative contributions to the initiation of fatty acid biosynthesis is an area of current interest and is reviewed in more detail below.

A specific dehydrase enzyme, β-hydroxydecanoyl-ACP dehydrase (the fabA gene product), first described by Bloch and coworkers (10a), catalyzes a key reaction at the point that unsaturated fatty acid biosynthesis diverges from saturated fatty acid synthesis (Fig. 4). This dehydrase catalyzes the dehydration reaction shown in Fig. 3 but is also capable

FIG. 2. Pathways for the initiation of fatty acid biosynthesis. There are three potential pathways for the formation of acetoacetyl-ACP in E. coli. First, β-ketoacyl-ACP synthase III catalyzes the condensation of acetyl-CoA with malonyl-ACP. In the second pathway the acetate moiety is transferred from acetyl-CoA to acetyl-ACP by either acetyl-CoA transacylase or β-ketoacyl-ACP synthase III and then the acetyl-ACP is condensed with malonyl-ACP by β-ketoacyl-ACP synthase I (or synthase II). The third pathway is the decarboxylation of malonyl-ACP by synthase I to form acetyl-ACP, which is subsequently condensed with malonyl-ACP. Synthase I is the only condensing enzyme required for the initiation of fatty acid biosynthesis by the third pathway. Abbreviations: ACC, acetyl-CoA carboxylase; MTA, malonyl-CoA:ACP transacylase; AT3, acetyl-CoA:ACP transacylase; KASIII, β-ketoacyl-ACP synthase I; KASIII, β-ketoacyl-ACP synthase III. Reproduced from reference 66a with permission.

FIG. 3. Elongation cycle of fatty acid biosynthesis. Each elongation cycle is initiated by the condensation of malonyl-ACP with acyl-ACP carried out by one of the β-ketoacyl-ACP synthases. The next step is the reduction of the β-ketoester by β-ketoacyl-ACP reductase. The β-β-hydroxylacyl-ACP is then dehydrated to the trans-2 unsaturated acyl-ACP, which is reduced by enoyl-reductase to generate an acyl-ACP two carbons longer than the original acyl-ACP substrate. Reproduced from reference 66a with permission.
Control of Intracellular CoA Concentration

CoA is an essential cofactor in numerous metabolic pathways including the supply of the precursors to the earliest steps of fatty acid synthesis in E. coli. CoA is synthesized by a sequence of five reactions from pantothenate (17), and intracellular CoA levels are controlled by the modulation of several key enzyme activities in the pathway (Fig. 5). E. coli synthesizes pantothenate and regulates the pool such that the intracellular pool remains small (<1 μM). The regulation proceeds by efflux of excess endogenous pantothenate from the cell (68), and thus pantothenate is one of the few metabolic intermediates excreted by wild-type E. coli cells. Pantothenate is also transported into the cell by a specific transporter, encoded by the panF gene (66, 159), which is an inner membrane protein that catalyzes the sodium-dependent uptake of pantothenate (160). The rate of pantothenate transport, however, is not a controlling factor in the scheme of CoA production (68). CoA biosynthesis is governed primarily by feedback inhibition of pantothenate kinase mediated by the concentration of intracellular nonesterified CoA and, to a lesser extent, by the total CoA acyl-thioester pool (157, 158). The phosphorylation of pantothenate by the kinase is the first biochemical reaction in the pathway. In vitro studies show that CoA kinetically competes for the ATP-binding site on the enzyme (158) and that the Ks for inhibition are within the physiological range of intracellular CoA concentrations (158). A temperature-sensitive mutant strain of E. coli with defective pantothenate kinase activity was isolated, and the pantothenate kinase structural gene (coaA) was localized to min 89.9 of the chromosome (161). Mutants which possessed a pantothenate kinase activity that was refractory to feedback inhibition by CoA were also isolated (157). Strains harboring this mutation have CoA levels that are significantly elevated compared with those in strains containing the wild-type kinase, and this mutant also overproduces both intra- and extracellular 4'-phosphopantetheine (157). The gene encoding pantothenate kinase (coaA) has been cloned and is located at kb 3532 of the E. coli physical map (140). The importance of feedback regulation of pantothenate kinase in the control of CoA content is underscored by the finding that strains containing multiple copies of the coaA gene possess 76-fold-higher specific activities of pantothenate kinase; however, there is only a 2.7-fold increase in the steady-state level of CoA (141).

Pantothenate and 4'-phosphopantetheine are the two intermediates in the CoA biosynthetic pathway detected in the highest concentrations (70). This indicates that the phosphopantetheine adenyltransferase activity, which converts 4'-phosphopantetheine to dephospho-CoA, is another rate-limiting step in addition to the pantothenate kinase activity. At both enzymatic steps, constriction of the metabolic flux is reflected by increased pool sizes of the substrates to a maximum level and, beyond that concentration, efflux of the substrates into the medium. Extracellular phosphopantetheine is largely derived from turnover of the ACP prosthetic group, and phosphopantetheine efflux from the cell is irreversible (70).

The acyl-CoA thioester pool includes acetyl, malonyl, and succinyl groups, and long-chain acyl-CoAs are virtually undetectable (72) except when cells are grown on oleate as a carbon source. In the presence of exogenous oleate, intracellular oleoyl-CoA concentrations rise to approximately 5 μM (75). This low concentration points to the rapidity of
fatty acid metabolism via β-oxidation, but the level of long-chain acyl-CoA is sufficiently high to be physiologically relevant for the transcriptional regulation of the fabA gene (see below). The relative distribution of the short-chain acyl-CoAs changes with growth on different carbon sources (158). The CoA level is intimately related to the acetate concentration within the cell. A mutant E. coli strain (an aceEF strain) lacking pyruvate dehydrogenase is unable to produce the necessary acetyl-CoA from glucose and must be grown with acetate as a supplement in the medium (85). Thus, the acetyl-CoA pool in this strain is a function of the extracellular acetate concentration, and when acetate is suddenly removed, the free CoA level rises immediately. The cells subsequently respond by rapidly degrading more than 30% of the total CoA pool and effluxing one of the degradation products, 4'-phosphopantetheine (157). Under these drastic conditions, 4'-phosphopantetheine arises from direct breakdown of CoA and not from turnover of the ACP prosthetic group. When cells are starved for pantetheine, the metabolic precursor of CoA, the succinyl-CoA pool disappears and protein synthesis is diminished (72). The diminished CoA level resulting from pantetheine starvation is adequate for the support of fatty acid biosynthesis and does not affect lipid production directly (72). However, regulation of fatty acid synthesis is coupled with the rate of protein synthesis in wild-type E. coli (92, 109, 143); therefore, the supply of succinyl-CoA, the metabolic precursor to several amino acids, may be an important factor in maintaining the balance between macromolecular and fatty acid synthesis.

Another hypothesis that warrants investigation is the possibility that the ratio of acetyl-CoA to nonesterified CoA plays a role in determining the rate of fatty acid synthesis. Experiments with mammalian fatty acid synthases suggest that this parameter may be important. Low concentrations of nonesterified CoA are required for fatty acid biosynthesis by the synthases, whereas higher concentrations of CoA inhibit the fatty acid synthase (142, 146). The same behavior is found with β-ketoacyl-ACP synthase III activity in vitro, where a trace of CoA is required for activity but higher concentrations of CoA inhibit the rate of condensation (151). These data point to a role for the acetyl-CoA/CoA ratio in controlling the initiation of fatty acid biosynthesis. However, the significance of these observations must be tested in vivo.

Central Importance of ACP

ACP is a necessary component of all the reactions of fatty acid biosynthesis, including initiation, elongation, and trans-
fer to the membrane bilayer. The fatty acid intermediates are covalently bound to ACP, and these ACP thioesters are recognized as substrates for the enzymes of the pathway. ACP is one of the most abundant proteins in *E. coli*, constituting 0.25% of the total soluble protein (~6 × 10^6 molecules per cell)). The complete amino acid sequence of the homogeneous protein (162) has provided the basis for prediction of its secondary structure (128), and high-resolution nuclear magnetic resonance spectroscopy has defined the solution structure of ACP (62, 81). ACP (molecular weight, 8,860) is composed of a preponderance of acidic residues largely grouped into three α-helices, and there is recent evidence for a short fourth helix as originally predicted (81). ACP is a rod-shaped protein, and the α-helices determine the major axis of the structure. An acyl intermediate of fatty acid biosynthesis is bound to the protein as a thioester attached to the terminal sulfhydryl of the 4'-phosphopantetheine prosthetic group. The prosthetic group sulfhydryl is the only thiol group in *E. coli* ACP. The prosthetic group is in turn attached to the protein via a phosphodiester linkage to Ser-36 of the protein. Ser-36 is located in a β-turn situated between the second and third α-helical segments of ACP. The fatty acyl chain of an acyl-ACP intermediate extends up along the second helix, making contact with residues Ile-54 and Ala-59 (128). The pocket of the protein that accommodates the fatty acid has a length corresponding approximately to a six-carbon acyl group (128), and occupation of this site by a hydrocarbon moiety stabilizes the rod-shaped protein structure (128). In contrast, when a charged thioester such as malonate is bound to the prosthetic group within the pocket, the acyl-ACP is more susceptible to hydrodynamic expansion (128), increasing the probability of exposure of the reactive thioester bond. Malonyl-ACP reacts with the numerous acyl-enzyme intermediates of all three condensing enzymes and repeatedly donates the two-carbon units that are incorporated into the growing fatty acid.

The prosthetic group of ACP undergoes metabolic turnover, and the apoprotein is functionally inactive. The [ACP]synthase enzyme (45) transfers the 4'-phosphopantetheine portion from CoA to the protein, and [ACP]phospho- diesterase (156) cleaves the prosthetic group from ACP (Fig. 5). The phosphopantetheine is recycled into CoA biosynthesis via the phosphopantetheine adenylyltransferase or is excrated into the medium (70). The CoA pool is approximately eight times larger than the ACP pool in normally growing cells (68). In addition, virtually all of the ACP is maintained in the active, holo-form in vivo (69); therefore, the supply of prosthetic groups does not limit fatty acid biosynthesis. The operation of the prosthetic group turnover cycle appears to be involved in governing the intracellular CoA concentration (71). During logarithmic growth, a significant pool of unacylated holo-ACP can be found in vivo. Inhibition of acyl-transfer, however, causes accumulation of acyl-ACPs, and the supply of unacylated ACP is no longer detectable (129). Factors that restrain either chain elongation or fatty acid transfer to the membrane bilayer may cause the supply of ACP protein to be limiting, but the size of the ACP pool must be severely depleted before an effect on fatty acid and phospholipid synthesis can be detected (69, 119). The cellular concentration of ACP protein is controlled, and massive overproduction of ACP encoded by an inducible plasmid vector is lethal to *E. coli* (79). The deleterious effects of ACP overexpression are difficult to reconcile with the already high levels of the protein normally expressed. However, much of the protein expressed in the inducible systems is apo-ACP (79), leading to the working hypothesis that apo-ACP binds to and competitively inhibits the enzymes of fatty acid biosynthesis. The results of experiments testing the effects of apo-ACP may explain why the overproduction of this protein is lethal. However, ACP is also required for the synthesis of membrane-derived oligosaccharides (149) and tightly associates with MukB, a protein required for correct chromosome partitioning in *E. coli* (109). These surprising findings underscore the diversity of ACP functions and the interesting roles of ACP in cell physiology.

### CONTROL OF TOTAL FATTY ACID CONTENT

#### Importance of Early Steps in the Pathway

The identity of the enzyme or enzyme system that functions as the pacemaker of fatty acid biosynthesis remains one of the major unanswered questions in the field; however, it seems clear that regulation of fatty acid production occurs at an early stage in fatty acid biosynthesis. Two lines of evidence support this conclusion. First, direct measurements of the acyl-ACP pool size and composition (129) show that the pool is almost devoid of long-chain acyl-ACPs. However, these acyl-ACPs accumulate in vivo when utilization by the glycerol phosphate acyltransferase system is blocked. The second line of evidence comes from the regulation of phospholipid biosynthesis during the stringent response. Several laboratories have reported that phospholipid production decreases dramatically following the starvation of a rel*+* but not a relA strain (98, 120). Careful measurements have established that the effector of this regulation is most probably ppGpp; a nucleotide that accumulates during the stringent response (112). Furthermore, metabolic labeling experiments in *plsB* mutants blocked in phospholipid biosynthesis clearly point to an early stage in fatty acid biosynthesis as the target for ppGpp regulation (95, 113, 145). Experiments directed at elucidating ppGpp regulation in vitro have not provided clear results. However, it is worth noting that the carboxyltransferase reaction of acetyl-CoA carboxylase is inhibited by ppGpp in vitro (120), and this observation warrants further investigation in light of the potential role of acetyl-CoA carboxylase in regulating the pathway (see below).

### Structure and Function of Acetyl-CoA Carboxylase

Acetyl-CoA carboxylase catalyzes the first committed step of fatty acid (and hence lipid) synthesis (1). The overall reaction is composed of two distinct half-reactions (1, 52); (i) the ATP-dependent carboxylation of biotin with bicarbonate to form carboxybiotin, followed by (ii) transfer of the carboxy group from carboxybiotin to acetyl-CoA to form malonyl-CoA. The biotin is covalently coupled to a small (16.7-kDa) protein called biotin carboxyl carrier protein (BCCP) (147). The biotin must be coupled to BCCP for acetyl-CoA carboxylase to function, and the coupling reaction is catalyzed by a specific enzyme, biotin ligase (see below).

The two acetyl-CoA carboxylase half-reactions are catalyzed by different subunits of this enzyme, which is composed of four nonidentical subunits. Carboxylation of biotin is catalyzed by biotin carboxylase, a homodimeric enzyme composed of 55-kDa subunits which copurifies with BCCP (which also is a homodimer) (52). The enzyme that transfers the carboxy group from the biotin moiety of BCCP is the carboxyltransferase component, which is a heterotetramer.
composed of two copies of each of two dissimilar subunits, called \( \alpha \) and \( \beta \) (88). In cell extracts the overall acetyl-CoA carboxylase reaction (acetyl-CoA to malonyl-CoA) is lost and only the separate BCCP-biotin carboxylase and carboxyltransferase components are detected. The overall acetyl-CoA carboxylase reaction can be reconstituted in vitro by using high concentrations of the purified components (52, 121). This result suggests that the BCCP-biotin carboxylase and carboxyltransferase components are not tightly bound to one another in vivo. We assume that the enzyme present in vivo is composed of one copy of each component (two molecules of each of the four subunits) and has a molecular mass of ca. 280 kDa. This assumption is based on results (46) obtained with the acetyl-CoA carboxylase of *Pseudomonas citronellolis*, which can be readily isolated as a complex of about 280 kDa in the presence of high salt concentrations, whereas low-salt concentrations give two components analogous to those seen in *E. coli*. The high-salt complex contains equal amounts of the four subunits. The *P. citronellolis* subunits are similar to those of *E. coli* except for their stable association under high-salt conditions. The BCCP components of the two organisms are interchangeable in both in vitro half-reactions (46, 58) catalyzed by the carboxylase proteins of either organism. The overall *P. citronellolis* acetyl-CoA carboxylase reaction can be reconstituted with each BCCP (58). Moreover, the gene encoding the BCCP of *P. aeruginosa* complements an *E. coli* accB mutant and an open reading frame (ORF) just downstream of the BCCP gene has a deduced amino acid sequence very similar to that of *E. coli* biotin carboxylase (the accC gene product) (8). It will be of interest to determine whether the in vivo substitution of pseudomonad gene products for those of *E. coli* will allow isolation of an intact acetyl-CoA carboxylase from *E. coli* cells.

The genes encoding all four subunits of acetyl-CoA carboxylase from *E. coli* have recently been cloned and sequenced. Isolation of the BCCP gene (accB) was facilitated by the prior amino acid sequencing of a large proteolytic C-terminal fragment of the protein (147). Li and Cronan (90) isolated the BCCP gene by a reverse genetics approach, whereas two other groups came upon the BCCP coding sequence while sequencing neighboring genes (5,100). Li and Cronan (90) also showed that an ORF located just downstream of the accB gene encoded biotin carboxylase, accC, and this was independently confirmed by Kondo et al. (82). The accB and accC genes are cotranscribed and thus make up a small operon (90).

The genes encoding the \( \alpha \) and \( \beta \) subunits of the carboxyltransferase component were recently identified (88). The proteins were purified, and the N termini were determined. The N terminus of the \( \beta \) subunit matched a segment of a previously sequenced ORF called dedB (for downstream DNA) and usg (for upstream gene). Subsequent protein chemistry showed that the purified \( \beta \) subunit had been shortened by N-terminal proteolysis during purification and that the \( \beta \) subunit was encoded by the dedB/usg sequence. Moreover, a previously isolated mutant with a temperature-sensitive defect early in the fatty acid biosynthetic pathway was shown to have a lesion within the dedB/usg gene (92). The dedB/usg gene has therefore been renamed accD. The gene encoding the \( \alpha \) subunit (called accA) of the carboxyltransferase component was isolated by synthesis of an oligonucleotide probe deduced from the N-terminal sequence, and the gene maps directly downstream of the *polC* (*dnaE*) gene that encodes the catalytic subunit of the replicative DNA polymerase III (92). The accA and accD genes map at min 4.3 and 40, respectively, and thus are far removed from the accBC operon (min 72) and from one another.

It should be noted that the *E. coli* acetyl-CoA carboxylase proteins are very similar to those of biotin-dependent carboxylase enzymes of other organisms. The closest match is to the propionyl-CoA carboxylase of mammalian mitochondria (131). This mitochondrial enzyme is composed of two subunits; one can be aligned with the *E. coli* AccA and AccB proteins, whereas the other subunit matches the AccD and AccC proteins. Thus, the sequence of one subunit of the mammalian propionyl-CoA carboxylase suggests that it is a fusion of the accC and accB genes, whereas the sequence of the second subunit suggests a fusion of the accD and accA genes. Also noteworthy is a strong similarity between the accD gene and an ORF of unknown function present in all three sequenced chloroplast genomes (89). Thus, the predictive value of *E. coli* data may again prove valuable for studies of plants.

The regulation of *E. coli* acetyl-CoA carboxylase is unclear. The accB and accC genes are cotranscribed from a promoter located unusually far upstream of the accB gene (90). Although RNA polymerase initiating transcription at this promoter traverses both a region of DNA reported to attain a static curve (bent DNA) and a small ORF just upstream of accB, deletion of either of these features has no effect on accBC transcription (90). The major accA promoter lies within the coding sequence of the *polC* gene, although transcription through *polC* and perhaps other upstream genes also reads through the accA sequence (88). The significance of this transcription pattern is presently unclear. The accD gene is transcribed from a promoter located within the upstream *dedA* gene (91). Transcription of all four acc genes is under growth rate control, the rate of transcription decreasing with decreased growth rate (91). However, the situation is complex in that the accBC operon seems to be regulated by a mechanism that differs from the mechanism that regulates the accA and accD genes (91). Introduction of many copies of the accBC operon results in only modest increases (less than threefold) in accBC transcription and protein products, whereas similar experiments with the accA and accD genes give the expected overproduction of transcription and translation products. Moreover, replacement of the normal accBC promoter with a heterologous lac promoter gives the expected overproduction of gene products (90). We hypothesize that accBC transcription is regulated by a positive activator in limiting supply. We favor regulation by an activator over regulation by a repressor, since a repressor should be titrated (at least somewhat) by copy number.

The accBC genes are also implicated in regulation at diverse growth temperatures. Karow and Georgopoulos have isolated a collection of null mutants (with transposon insertions) unable to grow at elevated temperatures (76). One of these, with a mutation in the *htrB* gene, fails to grow at temperatures above 33°C. Many second-site suppressors of the *htrB* mutation which allow growth at 42°C have been isolated, and one class of these suppressors maps in the accBC genes (76). These suppressors are thought to decrease the function of BCCP and/or biotin carboxylase, since one such suppressor is an ISI insertion into the DNA segment encoding the leader mRNA. This insertion blocks transcription from the normal accBC promoter but substitutes transcription from a weak outward-reading ISI promoter.

The recent isolation of all of the *acc* genes will allow
various tests of the role of acetyl-CoA carboxylase as a pacemaker of fatty acid synthesis. All four subunits can be simultaneously overexpressed from a series of compatible plasmids and should result in the overproduction of acetyl-CoA carboxylase activity. Will these cells overproduce malonyl-CoA, malonyl-ACP, fatty acids, etc.? If malonyl-ACP is overproduced but fatty acids are not, acetyl-CoA carboxylase cannot be a rate-limiting step. If malonyl-ACP is not overproduced, this implies a deficiency of malonyl-CoA:ACP transacylase. However, this enzyme, the product of the fabD gene, can also be overexpressed (96a), and thus definitive experiments testing this hypothesis can now be designed.

It should also be noted that the synthesis of BCCP, the accB gene product, regulates the synthesis of biotin, the essential cofactor of acetyl-CoA carboxylase (28). Biotin biosynthesis is regulated by a repression mechanism that has two unusual features. First, the repressor protein (called BirA) that binds to the operator site of the biotin operon is also the ligase that covalently attaches biotin to apo-BCCP. Second, the corepressor required for BirA binding to the operator is biotinyl-AMP (the product of the first half-reaction of the ligase reaction) rather than biotin. Maximal rates of bio operon transcription (derepression) occur when the biotin supply is severely limited (e.g., biotin starvation of a bio auxotroph). Since any biotinyl-AMP synthesized is rapidly consumed in biotin formation of the acceptor protein (apo-BCCP) molecules, no significant amount of BirA-biotinyl-AMP complex accumulates. Thus, the bio operator is seldom occupied and transcription is maximal. Repression of bio operon transcription occurs when the supply of biotin is in excess of that needed to biotinolate apo-BCCP. Under these conditions, the BirA-biotinyl-AMP complex accumulates, binds to the bio operator, and represses transcription from both promoters.

The novel feature of the regulatory system is that at all physiological levels of biotin, the rate of operon transcription depends on the supply of the biotin acceptor protein, apo-BCCP. This has been demonstrated by introduction of plasmids that overproduce BCCP or another biotin acceptor protein (e.g., the 1.3S subunit of Propionibacterium shermanii transcarboxylase which acts as a gratuitous inducer) (90). Thus at a given biotin level, an increase in the level of apo-BCCP to be biotinolated decreases the level of the BirA-biotinyl-AMP repressor complex via consumption of the biotinyl-AMP corepressor. This decrease in the level of active repressor complexes results in increased transcription of the operon. Hence, the rate of biotin operon transcription is therefore sensitive not only to the intracellular concentration of biotin but also to the supply of the protein to which the biotin must be attached in order to fulfill its essential metabolic role. Accumulation of the unmodified protein increases the rate of synthesis of the small molecule needed for the posttranslational modification, a rather tidy regulatory loop.

Initiation of Fatty Acid Biosynthesis

The acetyl-CoA pool is the source of metabolic precursors for fatty acid biosynthesis. Acetyl-CoA serves as the primer for acetyl-chain formation, contributing the two carbon units at the methyl end of each fatty acid. Acetyl-CoA is a substrate for β-ketoacyl-ACP synthase III (acetoacetyl-ACP synthase) and is incorporated directly into the first four-carbon fatty acid (67, 74). Acetyl-CoA can also be converted into acetyl-ACP by a transacylase activity, and the resulting acetyl-ACP, in turn, can serve as the primer when alternative condensing enzymes such as β-ketoacyl-ACP synthase I participate in the initiation of fatty acid production. For many years, the acetyl-CoA:ACP transacylase activity in E. coli was considered to be a discrete protein (2, 168) that exhibited an in vitro catalytic specific activity at least 10 times lower than that of other steps in the pathway (67). Recently, however, the acetyl-CoA:ACP transacylase reaction was shown to be catalyzed by synthase III (152), and the acetyl-CoA:ACP transacylase activity measured previously (2, 168) may represent a partial reaction of this condensing enzyme. Whether a unique acetyl-CoA:ACP transacylase activity is present in E. coli remains to be determined, and establishing this important point is an area of intense investigation that is crucial to understanding the regulation of fatty acid initiation.

Malonyl-CoA is usually thought to be used only in elongation steps in fatty acid biosynthesis. However, both β-ketoacyl-ACP synthases I and II are capable of initiating fatty acid synthesis in the absence of acetyl-ACP primer by utilizing a side reaction involving malonyl-ACP deacetylase. This pathway can be easily demonstrated in vitro (1). Whether or not it is a contributor to initiation in vivo awaits experimental verification. However, overproduction of synthase I renders E. coli resistant to the antibiotic thiolactomycin (153; see below). Under these conditions it appears that synthase I is the only condensing enzyme required for growth, suggesting that initiation of fatty acid synthesis by malonyl-ACP deacetylase could be the major route utilized in the presence of thiolactomycin and perhaps under other physiological conditions. Experimental assessment of the contribution of the three possible pathways for initiation must be one of the next steps.

The acetyl-CoA pool is large compared with the acetyl-ACP, malonyl-CoA, and malonyl-ACP pools of E. coli (72, 129). This observation indicates that both the acetyl-CoA:ACP transacylase and acetyl-CoA carboxylase activities are rate limiting in vivo. The supply of ACP, however, does not seem to be a limiting factor in the scheme of fatty acid biosynthesis, since there is a significant pool of unacylated holo-ACP during logarithmic growth (69). In addition to unacylated ACP, there are significant pools of acetyl-ACP and malonyl-ACP (129), but acyl-ACPs of four carbons or longer are not detectable (129). This demonstrates that the first condensation reaction is also rate limiting. The discovery that acetyl-CoA:ACP transacylase activity is associated with the acetoacetyl-ACP synthase enzyme specific for the first condensation reaction in the pathway suggests that regulation of the synthase may be coordinated with regulation of acetyl-CoA carboxylase. The coordinate regulation would act to control the elongation of fatty acids in order to keep pace with the production of primer. The balance between these two enzymes also affects the chain length of fatty acids (see below). The precise role of the initiation steps in regulating fatty acid formation is not obvious, and current research is focused on defining the relationship between synthase III activity and the rate of fatty acid initiation.

REGULATION OF PHOSPHOLIPID FATTY ACID COMPOSITION

Control of Unsaturated Fatty Acid Content

Role of β-hydroxydecanoyl-ACP dehydrase (fabA). The unsaturated and saturated fatty acid biosynthetic pathways
diverge at the point at which \( \beta \)-hydroxydecanoyl-ACP dehydrase introduces a double bond into a growing fatty acid chain. The enzyme, a homodimer of 18-kDa subunits, is distinct from the \( \beta \)-hydroxyxyacyl-ACP dehydrase that participates in the elongation reactions (3). \( \beta \)-Hydroxydecanoyl-ACP dehydrase specifically catalyzes the dehydration of \( \beta \)-hydroxydecanoyl-ACP to a mixture of trans-2-decenoyl-ACP and cis-3-decenoyl-ACP (10). The reaction proceeds via the formation of trans-2-decenoyl-ACP as an enzyme-bound intermediate which can disassociate from the enzyme. If disassociation occurs, the trans-2 intermediate is reduced by an enoyl-ACP reductase and subsequently converted to saturated fatty acids. Enzyme-bound trans-2-decenoyl-ACP, however, is isomerized to cis-3-decenoyl-ACP. The double bond is preserved, and the cis-3 intermediate is elongated to the unsaturated fatty acids of \( E. coli \), palmitoleic acid and cis-vaccenic acid.

The first mutants isolated that were blocked in fatty acid biosynthesis, called \( fabA \) mutants, lacked \( \beta \)-hydroxydecanoyl-ACP dehydrase (136). These mutants are unable to synthesize unsaturated fatty acids, but they synthesize saturated fatty acids normally. It was found that, in vitro, mutant \( fabA \) enzyme forms neither cis-3- nor trans-2-decenoyl-ACP. This finding, along with the observation that saturated fatty acid synthesis continues in vivo, indicated that another dehydrase is available for saturated fatty acid synthesis. This second enzyme is able to catalyze the formation of trans-2-decenoyl-ACP but is unable to catalyze the isomerase reaction. The additional dehydrase activity is presumably the enzyme(s) responsible for the dehydration of shorter- and longer-chain saturated \( \beta \)-hydroxyacyl-ACPs.

In the absence of thermal regulation, the ratio of unsaturated to saturated fatty acids in \( E. coli \) is dependent on the levels of \( \beta \)-hydroxydecanoyl-ACP dehydrase and \( \beta \)-ketocacyl-ACP synthase I. It was shown that overproduction of the \( fabA \) gene product in vivo did not increase the amount of unsaturated fatty acids but significantly increased the amount of saturated fatty acids incorporated into membrane phospholipid (21). This indicated that, although \( \beta \)-hydroxydecanoyl-ACP dehydrase is required for the synthesis of unsaturated fatty acids, the level of enzyme activity does not limit the rate of unsaturated fatty acid synthesis. Introduction of multiple copies of the \( fabB \) gene (encoding synthase I) reversed the effect of dehydrase overproduction, resulting in wild-type fatty acid compositions (21). Thus, the step more likely to limit the rate of unsaturated fatty acid synthesis is the elongation of cis-3-decenoyl-ACP catalyzed by synthase I. The levels of expression of the \( fabA \) and \( fabB \) genes therefore appear to establish a basal ratio of unsaturated to saturated fatty acid synthesis in the absence of thermal regulation. Modulation of the fatty acid composition of membrane phospholipid in response to temperature shift is discussed below.

The nucleotide sequence of the \( fabB \) gene has been determined (31), and the deduced amino acid sequence has been confirmed by protein chemistry. The DNA sequence of the \( fabB \) gene has enabled workers to pursue a surprising finding made nearly 10 years ago (114), i.e., that the negative regulator of \( \beta \)-oxidation, FadR, has a positive role in regulating cellular levels of \( \beta \)-hydroxydecanoyl-ACP dehydrase. Recent work has elucidated a novel transcriptional regulatory mechanism of the \( fabB \) gene by the FadR protein (see below).

**Role of \( \beta \)-ketocacyl-ACP synthase I (\( fabB \)).** The original unsaturated fatty acid auxotrophs isolated were divided into two complementation groups (29). The first group of mutants deficient in \( \beta \)-hydroxydecanoyl-ACP dehydrase, were termed \( fabA \) mutants (see above). The second complementation group was shown to possess normal levels of the FabA dehydrase but still required unsaturated fatty acids for growth. In addition, these mutations (named \( fabB \)) mapped to the 50-min region of the \( E. coli \) chromosome, far from the \( fabA \) locus (min 21.5). This was the first evidence that, aside from the \( fabA \)-encoded dehydrase, there exists at least one additional enzyme available for the synthesis of unsaturated fatty acids. It was later found that the \( fabB \) gene encoded a condensing enzyme, \( \beta \)-ketocacyl-ACP synthase I (130). Further investigation revealed the presence of an additional synthase activity in \( E. coli \), \( \beta \)-ketocacyl-ACP synthase II (35).

**Synthase I and synthase II are both capable of participating in saturated and unsaturated fatty acid synthesis.** The enzymes have been shown, in vitro, to function similarly with all substrates except palmitoleoyl-ACP; palmitoleoyl-ACP is an excellent substrate for synthase II but not for synthase I (35, 49). This observation is consistent with the role of synthase II in the regulation of fatty acid composition of the membrane phospholipid in response to temperature (see below). Strains lacking synthase I, however, require unsaturated fatty acids for growth; therefore, in vivo, synthase I must catalyze a key reaction in unsaturated fatty acid synthesis that synthase II cannot. This reaction is probably the elongation of cis-3-decenoyl-ACP, although this has not been shown experimentally. This step is also thought to be the rate-limiting step in unsaturated fatty acid synthesis (see above).

**The \( fabB \) gene has been cloned (40, 78) and sequenced (78).** The deduced amino acid sequence encodes a protein of 42.6 kDa, which is consistent with the estimated monomeric molecular mass of purified synthase I (49, 78). The overpro-
duction of synthase I has resulted in two observations. First, when overproduced, the enzyme overcomes its poor ability to elongate palmitoleate, and an increased amount of cis-vaccenic acid is incorporated into phospholipid (40). The increase, however, has no effect on the temperature regulation of fatty acid composition (see below). Second, excess cellular synthase I renders E. coli resistant to the antibiotic thiolactomycin (see below). In early studies, Alberts et al. observed that synthase I catalyzes malonyl-ACP decarboxylation at high enzyme concentrations (1). It was thought that this reaction offered the cell an alternative initiation pathway for fatty acid biosynthesis; the decarboxylation of malonyl-ACP results in the production of acetyl-ACP, which can subsequently be used as a primer for chain elongation. With the isolation of thiolactomycin-resistant strains that overproduce synthase I, this hypothesis has been borne out in vivo. In the presence of the antibiotic, excess synthase I appears to allow the cell to bypass the two initiation pathways, acetyl transacylase and synthase III, by catalyzing the decarboxylation of malonyl-ACP to form the initiation primer, acetyl-ACP. Given the above observations, synthase I appears to be the only condensing enzyme in E. coli that is absolutely required for growth.

Transcriptional regulation by the fadR gene product. FadR protein was first discovered as a repressor that regulates the fatty acid degradation (fad) regulon, which includes genes of β-oxidation and fatty acid transport (118, 137, 138). Mutants having a defective fadR gene are constitutive for β-oxidation. Such fadR mutants grow on short-chain (less than C₁₀) fatty acids that fail to induce the regulon. The function of FadR in fad gene regulation is straightforward and follows the E. coli lactose repressor paradigm. In cells growing in the absence of fatty acids, FadR binds to operator sites upstream of the fad gene coding sequences and represses transcription of these genes. Exogenous fatty acids enter the cell and are converted to acyl-CoA thioesters, which bind to FadR. The FadR–acyl-CoA complex then dissociates from the operators, resulting in transcription of the fad regulon genes and hence β-oxidation. This view of FadR function in E. coli must now be expanded in light of the finding that the FadR protein acts as a positive activator in the transcription of a fatty acid synthetic gene, fabA.

The first indication of the dual role of FadR in fatty acid metabolism was the finding that introduction of a fadR mutation into a conditional (temperature-sensitive) fabA mutant strain converted the strain to a nonconditional phenotype (114). Strains additionally defective in β-oxidation did not alter the fabA phenotype, indicating that the effect on fabA was due to the fadR mutation per se rather than to induction of the β-oxidation pathway. A second indication of a role for FadR in fatty acid synthesis was the increased sensitivity of fadR strains to a specific inhibitor of the FabA enzyme (114). Subsequent analyses showed that fadR strains had decreased unsaturated fatty acid contents relative to isogenic wild-type strains, indicating that functional FadR was necessary for either fabA gene expression and/or enzyme function (114). The former hypothesis was shown to be correct by Henry and Cronan (60), who demonstrated that FadR is a positive transcriptional activator of fabA expression. The level of fabA gene expression is decreased 12-fold in a fadR null mutant. This regulation accounts for the decreased unsaturated fatty acid content of fadR strains as well as the unexpected double-mutant and inhibitor results mentioned above. (E. coli can tolerate the somewhat lowered unsaturated fatty acid content resulting from either decreased fabA gene expression or a partially defective FabA enzyme, but not the greatly lowered unsaturated fatty acid content resulting from decreased expression of a partially defective enzyme.)

In fadR null mutants the fabA gene is transcribed from two weak promoters of about equal strength, whereas in wild-type strains a 20-fold increase in transcription from the proximal promoter is seen (60). FadR binds to a 17-bp DNA sequence located in the −40 region of this promoter (61). This binding site is located at the position most often used by transcriptional activators of σ⁺⁺ promoters. The DNA sequence is similar to those found within the promoters of two fad genes, fabBA and fabD, where FadR acts as a repressor (42). How can FadR act as both a transcriptional activator of fatty acid synthesis and as a repressor of the fatty acid degradation regulon? Several other examples of activator proteins that also act as repressors (generally in autoregulation) are known (23a). The distinction between these two roles usually depends on the location of the protein-binding site relative to the transcriptional start. Most σ⁺⁺-dependent promoters have their activator sites positioned such that the bound protein overlaps position −40, whereas DNA-binding proteins act as repressors when positioned within a larger downstream region of the promoter, generally between −30 and +10 (23a). This simple scheme seems to readily explain FadR action. The binding site for fabA activation is centered at −40, whereas the fadBA- and fadD-promoter sites (where FadR represses) are centered at +9 and +20, respectively. Thus, by analogy with other systems with similar properties (23a, 124a), we expect that FadR binding to the fabA DNA would aid RNA polymerase binding or action via protein-protein interactions. Likewise, FadR binding to the fad regulon operators would hinder the binding or action of RNA polymerase. Given this latter role, it seems surprising that FadR fails to repress its own synthesis. It should be noted that very recent in vitro experiments by DiRusso et al. (42, 42a) have demonstrated that purified FadR activates the proximal fabA promoter and represses the fabBA and fabD promoters.

Transcriptional activation of fabA gene expression is inhibited by fatty acids in vivo, and this is due to decreased activity of the proximal promoter (61). Moreover, fatty acyl-CoAs inhibit the binding of FadR to the −40 region of the proximal promoter and the acyl chain lengths of the acyl-CoAs effective in FadR release from the DNA accurately reflect those of the fatty acids effective in decreasing fabA expression in vivo (61). A similar pattern was seen for the induction of the β-oxidation genes (42). Thus FadR seems to monitor the intracellular concentration of long-chain acyl-CoA molecules and coordinately regulates fatty acid synthesis and oxidation in response to the levels of these compounds.

The fadR gene has been cloned (43) and sequenced (41). The deduced amino acid sequence predicts a helix-turn-helix motif common to DNA-binding proteins. In fact, FadR has recently been assigned to a new family of bacterial regulatory proteins (57). The members of this family show similarities in sequence, molecular size, predicted secondary structure, and regulatory function. The FadR-binding site of fabBA possesses nearly perfect dyad symmetry (42), suggesting that FadR is homodimeric in structure. At present, however, dimerization of FadR in solution has not been detected. Purification by gel filtration results in a protein of 29 kDa (42), the predicted molecular mass of a FadR monomer. Negative trans-dominant mutations have recently been isolated (42), suggesting that the protein binds in a multimeric form. The inability to reconcile the biochemical
and genetic data, however, is not unprecedented. LexA, the repressor of SOS-inducible genes, dimerizes weakly in solution (150) and has been shown to bind as a monomer to its half-operator site (80). Subsequent cooperative binding of the second LexA monomer produces dimerization on the DNA. One could envision such a "dimerization" mechanism for FadR. The determination of the functional form of FadR in vivo is under active investigation.

The unraveling of this regulatory mystery has provided the first example of a regulatory protein that positively regulates the biosynthesis of a molecule and negatively regulates the catabolism of the same family of molecules. FadR regulation of the fabA gene is also the first report of a repression system mediated by positive control (Fig. 7). In the absence of fatty acyl-CoAs, FadR binding enables RNA polymerase to function in fabA transcription, whereas, in the presence of fatty acyl-CoAs, FadR is released from the DNA and fabA transcription decreases.

A plausible rationale for the physiological relevance of this regulatory system seems apparent. A major environment of \( E. coli \), the colon, can be a rich source of unsaturated fatty acids. These fatty acids may be used for phospholipid precursors as well as being an energy and carbon source. In such an environment, endogenous synthesis of unsaturated fatty acids would be unnecessary and inefficient. The presence of the fatty acyl-CoAs therefore results in decreased \( \text{fabA} \) expression and hence decreased endogenous synthesis of unsaturated fatty acids. If, instead, only saturated fatty acids are available, repression of \( \text{fabA} \) transcription is less efficient and the presence of the second FadR-independent promoter, together with residual function of the FadR-regulated promoter, allows a basal level of dehydrase to be produced. This allows synthesis, although decreased, of the unsaturated acids needed for functional phospholipids. When exogenous fatty acids are not available to the cell, FadR binds to the DNA, increasing \( \text{fabA} \) transcription and the synthesis of endogenous unsaturated fatty acids.

Temperature Modulation of Fatty Acid Composition by the \( \text{fabF} \) Gene Product

Thermal regulation of membrane fluidity seems common to all organisms. At physiological temperatures, normal cell function requires a membrane bilayer in a largely fluid state. As growth temperatures are lowered, however, the membrane undergoes a reversible change from a fluid (disordered) to a nonfluid (ordered) state. In \( E. coli \), like most organisms, the temperature at the point at which this transition occurs depends on the fatty acid composition of the membrane phospholipids. Marr and Ingrahm (97) first noted that \( E. coli \) adjusts its fatty acid composition in response to lower growth temperature by increasing the amount of cis-vaccenic acid and decreasing the amount of palmitic acid incorporated into the membrane phospholipid. The amount of palmitoleate incorporated, however, remains unchanged. Lower growth temperature results in an increase in the number of diunsaturated phospholipids in the membrane. At 37°C, palmitic acid occupies position 1 of the phospholipid backbone while palmitoleic acid is found only at position 2 (6, 30). As the growth temperature is lowered, cis-vaccenic acid competes with palmitic acid for position 1 of the newly synthesized phospholipids. This mechanism is thought to allow an organism to regulate the fluidity of its membrane to optimize membrane function at various growth temperatures.

The elucidation of the mechanism of thermal regulation in \( E. coli \) involved a number of independent observations. First, Gelmann and Cronan (50) observed that one of the original \( \text{fabA} \) mutants contained very low levels of cis-vaccenic acid. Reversion and transductional analysis showed that this second phenotype was independent of the \( \text{fabA} \) mutation. Termed cvc, the strain was not only deficient in the elongation of palmitoleoyl-ACP to cis-vaccenyl-ACP but also unable to increase the amount of cis-vaccenic acid incorporated into phospholipid upon shift to a lower growth temperature. This suggested that the elongation of palmitoleoyl-ACP played a role in thermal regulation. Genetic characterization of the cvc mutation, however, was precluded at that time because the mutant showed no growth phenotype. Following the isolation of the cvc mutant, studies by D'Agnolo et al. showed that \( \beta \)-ketoacyl-ACP synthase II, a newly discovered synthase activity in \( E. coli \), possessed a greater reactivity with palmitoleoyl-ACP than did synthase I (already known to be encoded by the \( \text{fabB} \) gene) (35). The existence of the new synthase was postulated to explain the cvc mutant phenotype isolated earlier by Gelmann and Cronan. Probably the most significant finding, however, was the observation that the increased rate of cis-vaccenic acid synthesis characteristic of thermal regulation is evident within 30 s after temperature downshift (47). This indicated that neither mRNA or protein synthesis is required for fatty acid composition adjustment; therefore, thermal regulation is exerted by a protein present at all temperatures but active only at low temperatures. Garwin et al. subsequently
showed that not only was palmitoleoyl-ACP an excellent substrate for synthase II but the effect was exacerbated at lower temperatures (49). Finally, it was demonstrated that the cvc mutants lack synthase II (48). The Cvc− phenotype and the lack of synthase II are due to a mutation in the same gene, fabF. Reversion of a fabF mutation results in restoration of synthase II activity, cis-vaccenic acid synthesis, and temperature regulation (48). Synthase II, therefore, was firmly established as playing an essential role in the thermal regulation of the fatty acid composition of E. coli.

Although it was known that fabF mutants lacked temperature control of fatty acid composition, it was still unknown whether the mere presence of cis-vaccenic acid conferred thermal regulation or whether the presence of synthase II was required for such a response. The observation that overproduction of synthase I produced an appreciable increase in the cis-vaccenic acid content of membrane phospholipids enabled this question to be addressed. A plasmid carrying the fabB gene was transformed into a fabF mutant, and the increased cis-vaccenic acid content of cells overproducing synthase I in a strain lacking synthase II was found to be independent of growth temperature (40). Therefore, the sole enzyme responsible for thermal modulation of the fatty acid composition is synthase II.

There is direct evidence, however, for a second site of temperature-dependent control which functions only in the incorporation of exogenously supplied fatty acids. Cronan (27) and Sinesky (139) found that when supplemented with saturated and unsaturated fatty acids at lower growth temperatures, E. coli preferentially incorporates unsaturated fatty acids into phospholipid. As the growth temperature is increased, saturated fatty acid incorporation increases. Cronan ruled out differential effects on transport, β-oxidation, or synthesis of endogenous acids as explanations for these results (27). This preferential incorporation of exogenously supplied unsaturated fatty acids at different growth temperatures suggests an additional but unknown thermal regulatory mechanism. It is clear from the fabF data, however, that this mechanism is not involved in temperature regulation of lipid composition in cells growing without exogenously supplied fatty acids.

Strains harboring a temperature-sensitive mutation in the fabB gene and an additional mutation in the fabF gene are incapable of synthesizing any long-chain fatty acid at the nonpermissive temperature (48). Even supplementation with oleate, an unsaturated fatty acid that allows growth of unsaturated fatty acid auxotrophs, fails to permit growth of a double fabB(Ts) fabF mutant at high temperature because of the inability of the strain to synthesize saturated fatty acids. Synthases I and II are therefore the only synthase activities present in E. coli active in the synthesis of long-chain fatty acids. Both synthases I and II may catalyze the synthesis of saturated fatty acids, but synthase I is required for the synthesis of unsaturated fatty acids (see above). In addition, both synthases play a major role in controlling fatty acid chain length in E. coli (see below).

An interesting mutation, called Vtr, causes cells to overproduce cis-vaccenic acid at all temperatures (14, 39). The Vtr mutation has been shown to be allelic to the fabF gene (155). Efforts to detect a kinetic defect in synthase II of a Vtr mutant, however, have been unsuccessful (155). It is thought that protein-protein interactions must occur between the fatty acid biosynthetic enzymes, and perhaps the defect present in the Vtr mutant may not be detected unless such interactions are present. Further investigation of the genetic nature of the Vtr mutation should provide insight into the structural aspects of synthase II important in temperature regulation.

The progress of investigation of the thermal regulatory activity of synthase II has been hampered because of unsuccessful attempts at cloning the fabF gene. The inability to clone the fabF gene was more fully understood when it was found that the gene encoding ACP, acpP, maps very close to the fabF locus (125). Since overproduction of ACP is toxic to the cell, this may have precluded cloning of the fabF gene. Insertion of an antibiotic resistance gene into the E. coli chromosome directly downstream of the acpP gene confers a fabF phenotype (i.e., low cis-vaccenic acid content and absence of the synthase II protein) (125). This insertion has been cloned, and DNA surrounding the insertion has been sequenced. The available sequence has approximately 45% amino acid identity with the fabB gene, encoding synthase I (96). Recent attempts to clone the fabF gene have been unsuccessful because of apparent DNA instability in medium- to high-copy-number vectors (96). The use of a very-low-copy-number vector, pSC101, has enabled the isolation of the region of DNA harboring the fabF gene. Characterization of the fabF gene is under active investigation.

**Regulation of Fatty Acid Chain Length**

Palmitate, palmitoleate, and cis-vaccenate make up the bulk of the fatty acids found in E. coli membranes. The β-ketoacyl-ACP synthases play a major role in controlling the chain length and production of these fatty acids. In vitro substrate specificity experiments support the view that membrane phospholipids do not contain high levels of fatty acids with chain lengths longer than 18 carbon atoms because the precursor acyl-ACPs are poor substrates for the synthases. Investigation of changes in cellular fatty acid composition when the genes encoding the condensing enzymes are either deleted or overexpressed has given the most valuable insight into the function of the synthases. Inactivation of synthase I (fabB) leads to a lack of unsaturated fatty acids and therefore a deficiency in cis-vaccenate (29). On the other hand, overexpression of synthase I leads to the overproduction of cis-vaccenate (40). Thus, the elevated activity of synthase I allows it to elongate acyl-ACPs that are not normal substrates of this enzyme. Strains with mutations in synthase II are unable to synthesize cis-vaccenate and are therefore devoid of 18-carbon fatty acids in the membrane. The role of this enzyme in temperature control of fatty acid composition is reviewed above and is the major contribution of this enzyme to fatty acid composition. Clones that greatly overexpress synthase II have not yet been isolated. Mutants severely impaired in synthase III activity are enriched in 18-carbon fatty acids, whereas the overexpression of synthase III causes a decrease in the average fatty acid chain length and the appearance of significant amounts of myristic acid in the phospholipids (152). This effect is attributed to an increased rate of fatty acid initiation, which leads to a deficiency in malonyl-CoA for the terminal elongation reactions.

The activity of the glycerolphosphate acyltransferase system is the other important component involved in regulating acyl chain length. When phospholipid synthesis is arrested at the glycerolphosphate acyltransferase step (by glycerol starvation of a plsB mutant), the fatty acids that accumulate have abnormally long chain lengths (e.g., 20 and 22 carbons) (32). Therefore, competition between the rate of elongation by the condensing enzymes, the supply of malonyl-CoA, and the utilization of acyl-ACPs by the acyltransferase appears
to be the most significant determinants of fatty acid chain length.

Cyclopropane Fatty Acid Synthesis

The synthesis of cyclopropane fatty acids (CFA) is more properly viewed as a postsynthetic modification, since the substrate fatty acids are already esterified into membrane-localized phospholipid molecules. The reaction is a methylation of these double bonds, the methylene donor being S-adenosylmethionine (SAM). Much is known about CFA synthesis, but the two most interesting questions remain. First, how does the soluble CFA synthase together with the soluble substrate, SAM, gain access to the phospholipids of the inner and outer membranes? Second, why are these acids made by a large variety of bacteria? Mutants which completely lack CFA synthase activity (as a result of null mutations in the cfa gene) exist, but they grow and survive normally under virtually all conditions (51). The only exception to this finding is that cfa mutant strains are more sensitive to freeze-thaw treatment than are isogenic cfa* strains.

Recent efforts have focused on the purification of CFA synthase and the regulation of cfa gene expression. CFA synthase has been purified to homogeneity, and the DNA sequence of the cfa gene has been found (166). Together, these data demonstrate that CFA synthase is a protein of 44 kDa encoded by the cfa gene. The only similarity of the deduced amino acid sequence of CFA synthase to those of other known proteins is a sequence conserved in other SAM-utilizing enzymes that is believed to be the SAM-binding site (166).

The regulation of CFA formation is unusual. The bulk of CFA synthesis occurs as cultures enter the stationary phase of growth. However, the level of enzyme activity assayed in vitro varies less than threefold with growth phase (26, 148). That is, the enzyme is present in log phase cells but somehow fails to function. Recently, transcriptional analyses of CFA gene transcription indicated the presence of two promoters of apparently equal strengths (165). The more upstream promoter functions throughout the growth curve, whereas the proximal promoter is active only as cultures enter stationary phase. It appears that the proximal promoter requires a special sigma factor (sigma S) encoded by the katF gene (11). Indeed, the CFA content of katF strains is very low, and transcription from the proximal promoter is absent in these strains (165).

ALTERNATE DESTINATIONS FOR FATTY ACIDS

Lipid A Biosynthesis

The only major fatty acid that is not a component of the phospholipids is β-hydroxymyristate. Rather, this fatty acid is attached by both ester and amide linkages to the saccharide residues of the lipid A portion of lipopolysaccharide (122). β-Hydroxymyristate is derived from the central fatty acid biosynthetic machinery; however, exactly how the β-hydroxymyrystoyl-ACP is channeled to lipid A biosynthesis, rather than elongated for phospholipid synthesis, is a major unanswered question. The acyltransferases that catalyze the formation of lipid A are very specific for β-hydroxymyristate (for a review, see reference 123), and one hypothesis is that these acyltransferases effectively compete with β-hydroxymyrystoyl-ACP dehydroacylase for the available acyl-ACP. Consistent with this view are the observations that inhibition of fatty acid synthesis always has a greater effect on the production of fatty acids for membrane phospholipids than on the production of β-hydroxymyristate for lipid A biosynthesis (145). The UDP-GlcNAc acyltransferase is the first committed step in lipid A biosynthesis and exclusively utilizes acyl-ACP as the acyl donor. The inability of the acyltransferases in lipid A biosynthesis to use acyl-CoA thioesters explains why the E. coli mutants defective in both unsaturated and saturated fatty acid biosynthesis have not been isolated and why lipid A cannot be radiolabeled with exogenous β-hydroxymyristate. Lauroyal-ACP and myristoyl-ACP are also specifically required for the acylation of the hydroxyl groups of β-hydroxymyristate moieties in the latter stages of lipid A biosynthesis. Thus, fatty acid biosynthesis is intimately involved in the production of lipid A. At min 4 of the genetic map is a cluster of genes involved in the synthesis of lipid A and DNA (123). The gene order in this region is orf-17-lpxA-lpxB-orf-23-dnaE-accA. This entire section of the chromosome has been sequenced, although the number of promoters and the mechanisms that regulate expression of this gene cluster are obscure. Coleman and Raetz (23) sequenced one of the unknown open reading frames (orf-17) but were unable to discern its function. A comparison between the protein sequence of FabA and the predicted amino acid sequence of orf-17 reveals a homology throughout the protein, particularly in the region surrounding the active-site histidine. These data strongly suggest that orf-17 encodes a second β-hydroxacycyl-ACP dehydrase. Recently, orf-17 was cloned, and the protein produced by this gene catalyzes the dehydration of β-hydroxymyrystoyl-ACP (124). The localization of the dehydrase in the same gene cluster as the acyltransferases raises the intriguing possibility that these two proteins work in concert to regulate the amount of β-hydroxymyristate diverted to lipid A biosynthesis.

Membrane Proteins

Another alternate destination for fatty acids is the acylation of membrane proteins. The most abundant protein is the major outer membrane lipoprotein which contains an amino-terminal diacylglycerol cysteine residue that also contains an amide-linked fatty acid on the amino terminus. In this case, the fatty acids that are transferred to the lipoprotein are not derived from the fatty acid pool. Instead they are transferred to the prolipoprotein from the phospholipid pool (53, 54, 83). The 1-position of phosphatidylethanolamine is a major source of lipoprotein fatty acids (73, 127), but it is clear that other phospholipids serve as acyl donors when phosphatidylethanolamine is absent (84). This acylation process generates lysophospholipids that are reacylated by the inner membrane 2-acyl-glycerophosphoethanolamine (GPE) acyltransferase (73). This acyltransferase can use acyl-ACPs from fatty acid biosynthesis or can convert fatty acid to acyl-ACP in the presence of ATP-Mg2+ (24). This latter reaction appears to be the preferred pathway since 2-acyl-GPE acyltransferase binds ACP with high affinity and the rate of reaction with fatty acid is much higher than with acyl-ACP. The fatty acids used in the membrane phospholipid turnover cycle may be donated directly by acyl-ACP but are most likely to arise from thioester cleavage of acyl-ACP. The mechanism of fatty acid activation by the acyltransferase/synthetase and the region of the protein required for high-affinity ACP binding are areas of active investigation.

A colony autoradiography approach was used to isolate
mutants (aas) lacking acyl-ACP synthase activity (64). Extracts from aas mutants also lack 2-acyl-GPE acyltransferase activity, and the mutant strains are incapable of acyl-CoA-independent incorporation of exogenous fatty acids into phosphatidylethanolamine. All these defects are due to a mutation in the aas locus at min 61 of the E. coli chromosome (64). The aas gene has been cloned and expresses an 81-kDa protein (75).

ACP-dependent fatty acylation has also been reported to be necessary for the activation of the E. coli non-toxic prohemolysin to the mature toxin, hemolysin (65). Activation was shown to involve the transfer of the acyl group from acyl-ACP to prohemolysin. It is postulated that the acyltransferase activity required for activation resides in HlyC, which is co-synthesized with prohemolysin and is known to be required for activation. However, the HlyC protein is not catalytic in vitro (170), and since crude extracts were used in the experiments, it is unclear whether there are additional components in the extract that are also required for prohemolysin acylation.

**Synthesis of Fatty Acid-Related Vitamins**

Two vitamins, lipoic acid and biotin, have fatty acid chain-like moieties. Lipoic acid is a C7 fatty acid (octanoic acid) with thiol groups substituted for protons on the C-6 and C-8 carbons (followed by disulfide formation between the C-6 and C-8 thiois). Biotin is a heterocyclic (C, N, S) ring connected to a C7 pentanoic fatty acid chain. Both coenzymes are active only in a protein-bound form. Each is covalently attached to a few proteins by distinct and specific ligases which join the carboxyl group of lipoic acid or biotin to the e amino groups of specific protein acceptors (36, 131). Recently, Morris and Cronan (102) have cloned and sequenced the *iplA* gene which encodes a lipoate-protein ligase. Experiments with *iplA* mutants have indicated that *E. coli* contains at least two lipoate-protein ligase isozymes. Two lipoate ligase activities have been reported by Brookfield et al. (15). However, these two activities apparently result from the single *iplA* gene product (16). In contrast, the biotin ligase is well studied, since it also functions as the repressor of the biotin biosynthetic operon (see above) (28).

There is good evidence that octanoic acid is the direct precursor of lipoic acid (4) and that the synthesis of lipoic acid proceeds by stepwise addition of single thiol groups (167). The source of octanoic acid seems almost certain to be the fatty acid synthetic pathway, and it is of interest that octanoyl-ACP is a preferred product of β-ketoacyl-ACP synthase III in vivo. Lipoic acid synthesis seems likely to involve an enzyme that removes octanoyl-ACP from the fatty acid synthetic cycle and cleaves the thioester bond, giving octanoic acid (or perhaps some sulfur-containing derivative). It seems that thioester hydrolysis of the octanoyl-ACP must occur at some stage in lipoic acid synthesis since the carboxyl-group must be free to interact with the ligease. A recent observation by Ali et al. (4) indicates that *E. coli* contains a pool of octanoic acid. These workers found that normally lipoylated proteins synthesized during lipoate starvation of a lipoic acid auxotroph became modified with octanoic acid. A similar result is seen following overexpression of protein domains that accept lipoic acid. This is consistent with the finding that octanoic acid can act as a substrate for lipoate ligase in vitro (15). Indeed, Morris et al. (103) have found that under similar starvation conditions, the lipoylated proteins are labeled with exogenous octanoic acid in vivo and that this labeling does not seem to involve the conversion of octanoic acid to a thioester. These observations suggest the possibility that lipoic acid is synthesized by modification of octanoate previously bound to lipoylated proteins. However, a direct test of this pathway gave negative results (126).

The synthesis of biotin involves the production of a C7 dicarboxylic acid, pimelic acid (44). The synthesis of this intermediate proceeds by an unknown mechanism. Several possible pathways (e.g., cleavage of the double bond of the cis-7-tetradecenoic acid intermediate of fatty acid synthesis; omega oxidation) are precluded by the requirements for oxygen in those reactions. Biotin synthesis proceeds in *E. coli* grown under strictly anaerobic conditions. The only remaining plausible pathway is that proposed in 1963 by Lezius et al. (87). These workers suggested that malonyl-CoA might function in place of acetyl-CoA in fatty acid synthesis, resulting in the fatty acid methyl group being replaced by a methoxy group. Further elongation cycles would then give pimelic acid. This proposal predated the establishment of the role of ACP in fatty acid synthesis, and the proposed condensation reactions seem equally (if not more) likely to proceed with ACP thioesters. Although this remains a plausible pathway, it may be difficult to demonstrate in vitro because of the conversion of malonyl-ACP to acetyl-ACP catalyzed by the β-ketoacyl-ACP synthases of *E. coli*. It should be noted that since the essential fatty acid synthetic protein, BCCP, is the only biotinated protein in *E. coli*, biotin is required for the synthesis of biotin.

**Thioesterases**

*E. coli* contains two well-characterized enzymes that cleave the thioester bond of acyl-CoA molecules, giving the free species of CoA and fatty acid. Both enzymes are much less active on palmitoyl-ACP than on acetyl-CoA because of the sequestration of the thioester bond by the ACP moiety. Thioesterase I is a protein of 20.5 kDa, encoded by the *tesA* gene, that cleaves acyl-CoAs of more than 12 C atoms and is unable to cleave 3-hydroxyacyl-CoA thioesters (12). The *tesA* gene maps at min 11.6 of the genetic map, and the deduced amino acid sequence of TesA has active-site residues arranged in a manner similar to those found in several mammalian thioesterases (19). The active site is also closely related to those of serine proteases, consistent with covariant labeling and inhibition of TesA by serine esterase inhibitors (7). A comparison of the *tesA* DNA sequence and that determined from the purified protein demonstrated that 26 amino acids are removed from the N terminus of the primary translation product, leading to a prediction that TesA is a periplasmic enzyme. This prediction was confirmed by the demonstration that thioesterase I can be quantitatively released from *E. coli* cells by osmotic-shock treatment (19).

In contrast, thioesterase II is a tetrameric protein of a 32-kDa subunit encoded by the *tesB* gene at min 10 of the *E. coli* linkage map (104, 107). Thioesterase II cleaves acyl-CoAs of more than six C atoms and 3-hydroxyacyl-CoAs but is unable to cleave acyl-pantetheine thioesters (12, 144). TesB lacks the active site motif found in other thioesterases and shows no sequence similarity to other known proteins (104). Iodoacetamide inhibits thioesterase II, and the modified residue is a histidine residue, thus implicating this base in cleavage of the thioester bond (104).

The physiological function of thioesterases I and II is unknown, and the presence of these enzymes in *E. coli* seems an enigma. The chromosomal copies of both *tesA* and *tesB* have been disrupted to give null mutants (19, 104).
Neither the tesA nor tesB null mutants affect cell growth (the tesA and tesB mutants were isolated by reverse genetics and a brute-force screen, respectively). A tesAB double null mutant strain also grows normally (19). This lack of a growth phenotype indicates that neither protein is essential for cell growth. However, it remains possible that the function of both enzymes can be replaced by another enzyme. Indeed, the tesB null mutant still retains about 10% of the wild-type activity, indicating the existence of a third thioesterase in E. coli (19).

The overexpression of either the TesA or TesB enzyme to levels that greatly exceed the normal level also has no effect on growth of E. coli (19, 104). A similar result is seen when several mammalian thioesterases are expressed in E. coli (105). However, when the cDNA encoding a novel thioesterase from the California bay tree is expressed in E. coli, a massive amount of lauric acid accumulates in the culture medium (164). This thioesterase is very specific for C12 acyl-ACP thioesters both in vitro and in vivo and seems to have a unique ability to gain access to the thioester bond linking the growing fatty acid chain to ACP and release the chain when it reaches 12 C atoms. Consistent with the unusual nature of this cleavage reaction, the deduced amino acid sequence of this enzyme is unrelated to that of any other known thioesterase. The striking finding of lauric acid release is of recent origin, and the characterization is incomplete. However, the difference between the results obtained with this plant thioesterase and those of E. coli implies that the bacterial thioesterases are unable to cleave acyl-ACPs in vivo. This is in agreement with the prior in vitro results (144).

The E. coli thioesterases are most active on acyl-CoA substrates in vitro, but it is difficult to believe that these enzymes cleave acyl-CoAs in vivo. Detectable quantities of acyl-CoA molecules are found in E. coli only when β-oxidation is induced (growth on a fatty acid of more than 12 C atoms as the sole C source). If the E. coli thioesterases present in such cells were active on acyl-CoAs, β-oxidation would be inhibited. However, fatty acids are a good carbon source for E. coli, better than acetate. This remains the case even when TesA or TesB activities are overexpressed more than 10-fold. Therefore, if the E. coli thioesterases cleave acyl-CoAs in vivo, the acyl-CoA intermediates involved in β-oxidation must somehow be shielded from the thioesterases. There is some evidence that these intermediates may be channeled between β-oxidation enzyme active sites (169), but acyl-CoAs are also acyl donors in the incorporation of exogenous fatty acids into the phospholipids of E. coli and regulate lipid metabolism by interaction with FadR protein (see above). It is difficult to see how acyl-CoAs could be shielded from TesA and TesB during all these interactions.

At present we seem forced to conclude that the E. coli enzymes we assay as thioesterases in vitro are not thioesterases in vivo. It seems most reasonable to propose that TesA and TesB are acyltransferases rather than thioesterases. Acyltransferase reactions often proceed through an acyl enzyme intermediate that can hydrolyze in the absence of an appropriate acyl acceptor. This hydrolysis reaction is indistinguishable from thioesterase action. Moreover, there are families of acyltransferases and thioesterases which have similar active sites. However, tesA, tesB, and tesAB null mutants synthesize all of the known fatty-acyl-CoA molecules of E. coli in a normal manner (104). In the case of TesA, a rationale for the presence of a thioesterase in the periplasm of E. coli is not obvious. The usual explanation for periplasmic hydrolytic enzymes is to allow scavenging of portions of metabolically useful molecules (116). For example, phosphorylated metabolic intermediates can be hydrolyzed by periplasmic phosphatases to products that can then be transported across the cytoplasmic membrane. However, thioesterases such as acyl-CoAs hydrolyze spontaneously in aqueous solution, especially at pH values greater than 7, and thus enzymatic hydrolysis would not appear to be needed. The instability, together with the fact that acyl-CoAs are found only as metabolic intermediates, suggests that the primary role of thioesterase I might be to hydrolyze substrates other than acyl-CoAs. Reasonable candidates for alternative physiologically relevant substrates for thioesterase I are not obvious. Thioesterase I hydrolyzes only long-acyl chain (>C12) substrates and is inactive both on shorter-chain substrates and on long-chain substrates that contain a 3-OH substituent. This narrow specificity suggests that any alternative substrate must also contain long-chain acyl groups. Oxygen esters would seem the most reasonable alternate substrates, but the long-chain acylated molecules abundant in nature (e.g., glycerides) form large micelles at very low concentrations. Such micelles should be unable to pass through the small pores of the E. coli outer membrane (108). Indeed, the primary function of the outer membrane of the enterobacteria such as E. coli is thought to be to protect the cytoplasmic membrane from surface agents such as lipid micelles (108). A possible clue to thioesterase I function may come from the photosynthetic bacterium Rhodopseudomonas sphaeroides, which has been reported to contain two thioesterases that seem very similar to TesA and TesB of E. coli (13). The lower-molecular-weight enzyme of R. sphaeroides seems to have physical properties very similar to those of E. coli thioesterase I. Moreover, this TesA-like enzyme is dioxygenfluorophosphate sensitive and has a specificity of acyl-CoA hydrolysis virtually identical to that of E. coli thioesterase I. It seems likely that this R. sphaeroides thioesterase is located in the periplasm of this gram-negative bacterium. If so, this would suggest an important role outside the enteric environment (R. sphaeroides is not an enteric organism) for periplasmic thioesterases in bacterial physiology.

ANTIBIOTIC INHIBITION OF FATTY ACID BIOSYNTHESIS

N-Decenoyl-N-Acetylcysteamine

The dehydrase enzyme that catalyzes both the dehydration and isomerization reactions is specifically and irreversibly inhibited by the acetylenic substrate analog 3-decenoyl-N-acetylcysteamine (3-decenoyl-NAC) (59). The allelic inhibitor 2,3-decadienoyl-NAC inhibits dehydrase activity even more effectively (59). 3-Decenoyl-NAC concentrations of 10 to 50 μM completely inhibit bacterial growth (77), but growth inhibition is relieved by addition of unsaturated fatty acids to the medium (24). Saturated fatty acid synthesis continues at its normal pace in the presence of 3-decenoyl-NAC and supplies necessary precursors for lipopolysaccharide production. Although the dehydrase is required for unsaturated fatty acid synthesis and is unique to the type II systems, one drawback to the use of dehydrase inhibitors as antimicrobial drugs is that they would be ineffective in physiological environments where unsaturated fatty acids are available to other microorganisms. However, this inhibitor is useful in the isolation of mutants altered in the expression of the fabA gene (21).
Cerulenin

Cerulenin, (2R)(3S)-2,3-epoxy-4-oxo-7,10-dodecadienoyl-amide, is a fungal product that is an irreversible inhibitor of \( \beta \)-ketoacyl-ACP synthase I and II activities (33, 163) and is extremely effective in blocking the growth of a broad spectrum of bacteria (117, 163). Cerulenin blocks \( \beta \)-ketoacyl-ACP synthase activity by covalent modification of the synthase active site (33), and inhibition correlates with the binding of 1 mol of cerulenin per mol of enzyme (33). \( \beta \)-Ketoacyl-ACP synthases I and II contain a fatty acyl-binding site and a malonyl-ACP-binding site (34). Incubation of \( \beta \)-ketoacyl-ACP synthases with acyl-ACP protects the enzymes from cerulenin inhibition. These data strongly support the concept that cerulenin binds to the fatty acyl-binding site of the condensing enzyme. Although cerulenin has proven to be a versatile biochemical tool (117, 163), it is not a suitable antibiotic, because it is also a potent inhibitor of the condensing enzyme reaction catalyzed by the multi-functional mammalian (type I) fatty acid synthase (163). This observation is not surprising since the type I multifunctional synthases have a fatty acyl-binding site analogous to the site on prokaryotic \( \beta \)-ketoacyl-ACP synthases I and II (Fig. 6). \( \beta \)-Ketoacyl-ACP synthase III (acetoacetyl-ACP synthase) is not inhibited by cerulenin (67), indicating that this condensing enzyme lacks the fatty acyl-binding site. Treatment of \( E. \ coli \) with cerulenin leads to the accumulation of octanoyl-ACP in vivo (74). However, butyryl-ACP is the only product that accumulates in cerulenin-treated extracts in vitro (74). This indicates that the latter condensation steps in the pathway are effectively blocked but that the initial condensation reactions are able to proceed in the presence of the antibiotic. Consistent with these observations, \( \beta \)-ketoacyl-ACP synthase III does not catalyze the condensation of long-chain acyl moieties with malonyl-ACP in vitro (74). Thus, \( \beta \)-ketoacyl-ACP synthase III retains the malonyl-ACP site present in synthases I and II but does not possess the fatty acyl-binding site characteristic of the \( \beta \)-ketoacyl-ACP synthases in both the type I and II fatty acid synthases.

Thiolactomycin

Thiolactomycin, (4S)(2E,5E)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide, is a unique antibiotic structure that inhibits type II but not type I fatty acid synthases (56, 99, 110, 111, 115, 132). The antibiotic is not toxic to mice and affords significant protection against urinary tract and intraperitoneal bacterial infections (99). An analysis of the individual enzymes of the type II fatty acid synthase shows that the \( \beta \)-ketoacyl-ACP synthase activity and acetyl-CoA:ACP transacylase activity are the only activities inhibited by thiolactomycin (110). The observations that malonyl-ACP protects the synthases from thiolactomycin inhibition and that they are competitively inhibited with respect to malonyl-ACP are consistent with the conclusion that thiolactomycin interacts with the malonyl-ACP site rather than the \( \beta \)-acetyl-ACP site on the condensing enzymes. All three condensing enzymes are inhibited by thiolactomycin both in vivo and in vitro (67). There is one report that thiolactomycin does not inhibit acetyl transacylase (94). These data indicate that the enzyme purified by these workers is not synthase III.

To investigate the mechanism of thiolactomycin action in more detail, a thiolactomycin-resistant mutant of \( E. \ coli \) (strain CDMS) was isolated and characterized (67). The \( \beta \)-ketoacyl-ACP synthase III activity in extracts from strain CDMS was refractory to thiolactomycin inhibition. However, it is not clear whether mutation of synthase III is capable of imparting high-level thiolactomycin resistance (67, 153). It is possible that this is a thiolactomycin uptake defect, since the fatty acid synthase extracted from strain CDMS is sensitive to thiolactomycin inhibition but fatty acid synthesis in the intact organism is highly resistant (154). Recently, the thiolactomycin resistance phenotype was mapped to min 57.5 of the chromosome (75). This is the same location as the \( nalB \) gene (55), and \( nalB \) mutants are also resistant to thiolactomycin. In the same region there is a multidrug resistance operon (the \( emr \) operon), which has been cloned and sequenced (93). The likely possibility that thiolactomycin resistance, \( NaLB \), and \( Emr \) phenotypes are due to mutations in the same operon is currently being tested.

Overproduction of synthase I is also a mechanism for thiolactomycin resistance. Plasmids containing the \( fabB \) gene impart thiolactomycin resistance, whereas the overproduction of synthase III does not (153). Clones that overexpress synthase II are not available, but since synthase II is not an essential condensing enzyme (50), it is unlikely that overexpression of synthase II will lead to thiolactomycin resistance. These data suggest that synthase I is the only essential condensing enzyme in \( E. \ coli \).

PERSPECTIVES

Although we understand in some detail the pathway of fatty acid synthesis and the mechanisms regulating the mixture of fatty acids produced, information on other regulatory facets of fatty acid synthesis remains scant. For example, the mechanisms regulating the rate of fatty acid synthesis are not yet known. Another mystery is how the rate of fatty acid synthesis is integrated with the cellular growth rate such that the ratio of fatty acid content to cell mass remains constant at differing growth rates (growth rate control). Growth rate control of a variety of different cellular processes (e.g., DNA replication and transcription and translation of a large number of genes) has been intensively studied, but no general mechanism has yet emerged. It seems probable that we know so little of the genes involved in global growth rate regulation because strains with mutations in these genes are very likely to be pleiotropic and hence difficult to study. However, given the present state of knowledge of lipid synthesis and the experimental tools now available, detailed studies of mutants that affect fatty acid synthesis together with other cellular processes should prove fruitful in unraveling such global control mechanisms. A clear example of the virtues of studying pleiotropic mutants is the FadR regulatory system discovered through study of an unexpected pleiotropism. We still have much to learn from \( E. \ coli \) and fatty acid synthesis is almost certainly not an exception. An object lesson is the recent detailed study of the DNA sequence of a segment of the \( E. \ coli \) genome in which the functions of only half of the putative genes either are known or can be deduced (37, 171).

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