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Lipid Biosynthesis

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INTRODUCTION

Lipids are an essential constituent of all plant cells. The vegetative cells of plants contain ~5 to 10% lipid by dry weight, and almost all of this weight is found in the membranes. Although each square centimeter of a plant leaf may contain only 0.2 mg of lipid, this amount can account for ~400 cm^2 of membrane, reflecting the fact that membrane lipids are arranged in layers just two molecules thick (5 to 8 nm). If a leaf mesophyll cell were expanded a million times, the membranes would still be less than 1 cm thick. Despite their slight dimensions, however, the lipid membranes are the major barriers that delineate the cell and its compartments, and they form the sites where many essential processes occur, including the light harvesting and electron transport reactions of photosynthesis.

Some plant cells produce much more lipid than does a leaf mesophyll cell. Lipids are the major form of carbon storage in the seeds of many plant species, constituting up to ~60% of the dry weight of such seeds. Epidermal cells produce cuticular lipids that coat the surface of plants, providing the crucial hydrophobic barrier that prevents water loss and also forming a protection against pathogens and other environmental stresses. In addition to the abundant cellular lipids, minor amounts of fatty acids are important as precursors to the hormone jasmonic acid and in acylation of certain membrane proteins.

Unlike the other major constituents of plants (proteins, carbohydrates, and nucleic acids), lipids are defined on the basis of their physical properties rather than their common chemical structure. Thus, lipids are often loosely defined as those compounds that are insoluble in water and that can be extracted from cells by nonpolar organic solvents (such as chloroform). As such, this class of compound is extremely diverse in structure and actually constitutes the products of several distinct biosynthetic pathways. The most abundant types of lipid in most cells, however, are those that derive from the fatty acid and glycerolipid biosynthetic pathway, and these lipids constitute the major subject of this article. Other recent reviews include Ohlrogge et al. (1993b), Kinney (1994), Miquel and Browse (1994), and Töpfer et al. (1995).

Other classes of lipid include many types of compounds derived from the isoprenoid pathway. Over 25,000 different isoprenoid-derived compounds have been described in the plant kingdom, making this probably the richest store of chemical structures in the biosphere. Most of these compounds are considered "secondary" metabolites because they are not found in all cells and are probably not essential to cell growth. However, the sterols, gibberellins, abscisic acid, and the phytol side chain of chlorophyll are also derived from this pathway. A recent book by Moore (1993) and articles in this issue by Bartley and Scolnick (1995) and McGarvey and Croteau (1995) provide more detailed information on some of these other lipid classes.

The fatty acid biosynthesis pathway is a primary metabolic pathway, because it is found in every cell of the plant and is essential to growth. Inhibitors of fatty acid biosynthesis are lethal to cells, and no mutations that block fatty acid synthesis have been isolated. The major fatty acids of plants (and most other organisms) have a chain length of 16 or 18 carbons and contain from one to three *cis* double bonds. Five fatty acids (18:1, 18:2, 18:3, 16:0, and in some species, 16:3) make up over 90% of the acyl chains of the structural glycerolipids of almost all plant membranes (Figure 1).

Fatty acids in cells are almost never found in the form of "free" fatty acids. Instead, their carboxyl group is esterified or otherwise modified. In membranes, almost all the fatty acids are found esterified to glycerol; this class of lipid is termed glycerolipids. Membrane glycerolipids have fatty acids attached to both the sn-1 and sn-2 positions of the glycerol backbone and a polar headgroup attached to the sn-3 position (Figure 1). The combination of nonpolar fatty acyl chains and polar headgroups leads to the amphipathic physical properties of glycerolipids, which are essential to formation of membrane bilayers. If all three positions on glycerol are esterified with fatty acids, a "triacylglycerol" structure results that is not suitable for membranes but instead constitutes the major form of lipid storage in seeds. The cuticular lipids, which are found on the surface of all terrestrial plants (von Wettstein-Knowles, 1993), contain cutin, which is a polymer of primarily 16- and 18-carbon hydroxy fatty acids cross-linked by esterification of their carboxyl groups to hydroxyl groups on neighboring acyl chains. Wax esters in the cuticular lipids are formed by esterification of fatty acids to fatty alcohols. Finally, many fatty acids are reduced to aldehydes and alcohols that remain embedded in the complex cuticular lipid matrix.

Although fatty acids are major constituents of every membrane in a cell and are also found outside cells in the cuticular

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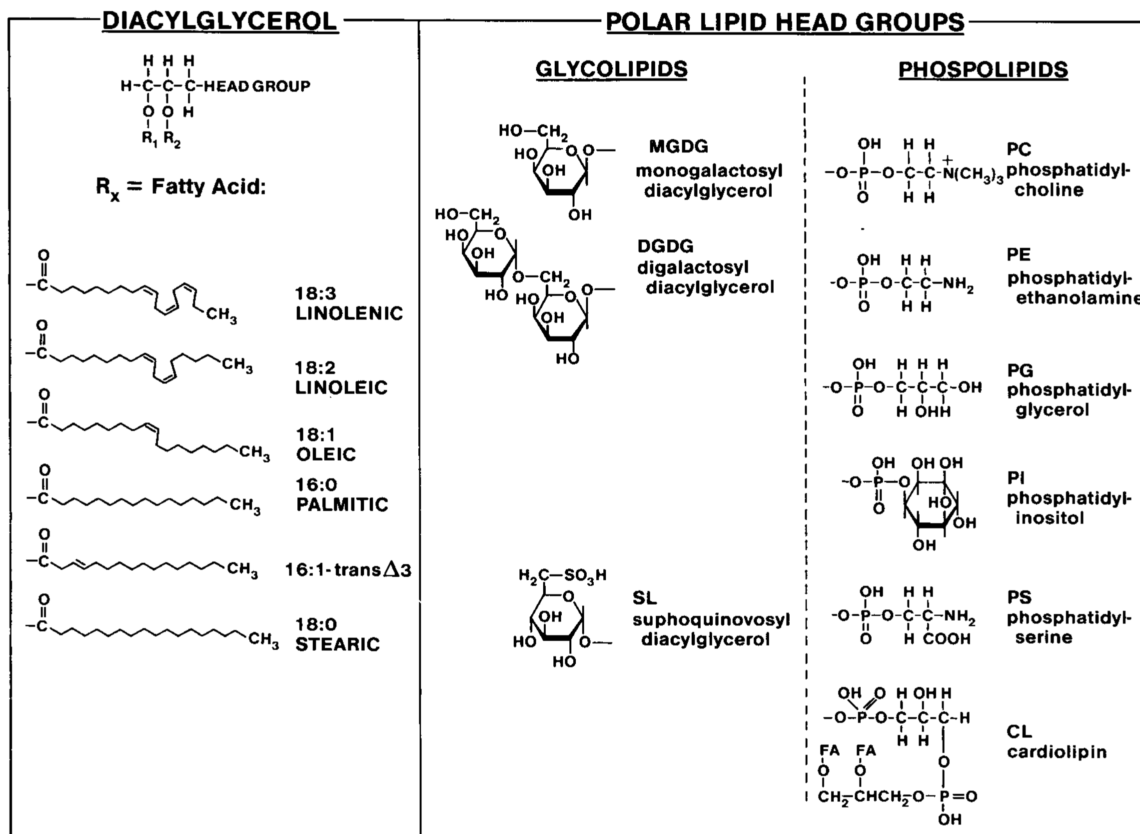

Figure 1. Structures of the Major Fatty Acids and Glycerolipids of Plant Cell Membranes.

The fatty acid and glycerolipid structures are arranged in approximate order of their abundance in plant leaves. Note that the fatty acids are referred to by the number of carbon atoms (before the colon) and the number of double bonds (after the colon).

lipids, their major site of synthesis is within the plastid. In this regard, the process of lipid biosynthesis in plants is fundamentally different from that in animals and fungi, which produce fatty acids primarily in the cytosol. The plastid localization of fatty acid synthesis means that unlike animals and fungi, plants must have mechanisms to export fatty acids from the plastid to other sites in the cell. Furthermore, there must be mechanisms by which the rest of the cell controls the production and export of fatty acids from the plastid. How the demand for fatty acids for assembly of extraplastidial lipids is communicated to the plastid is a major unknown in plant lipid metabolism.

SUBSTRATES FOR FATTY ACID SYNTHESIS

All the carbon atoms found in a fatty acid are derived from the pool of acetyl-coenzyme A (CoA) present in the plastid. The concentration of acetyl-CoA in chloroplasts is only 30 to 50 μM (Post-Beittenmiller et al., 1992), which is sufficient to supply the needs of fatty acid synthesis for only a few seconds. Nevertheless, acetyl-CoA pools remain relatively constant, even when rates of fatty acid synthesis vary greatly, as in light (when synthesis is relatively high) and dark (when synthesis is low). Thus, a system must be available that rapidly produces acetyl-CoA in the plastid for fatty acid production.

A major unresolved question in plant metabolism is how this pool of acetyl-CoA is generated. The most straightforward pathway would be through the action of plastidial pyruvate dehydrogenase (PDH) acting on pyruvate, either derived from the glycolytic pathway or perhaps produced as a side reaction of ribulose bisphosphate carboxylase activity (Andrews and Kane, 1991). However, this route has been questioned on several grounds. First, although PDH activities are generally high in nongreen plastids, PDH activity in isolated chloroplasts of some species is insufficient to account for rates of fatty acid synthesis (Lernmark and Gardeström, 1994, and references therein). In addition, chloroplasts contain an extremely active acetyl-CoA synthetase, and free acetate has been found superior to pyruvate and other substrates as a precursor of fatty acid synthesis by isolated chloroplasts (reviewed by Roughan and Slack, 1982). These considerations have led to suggestions

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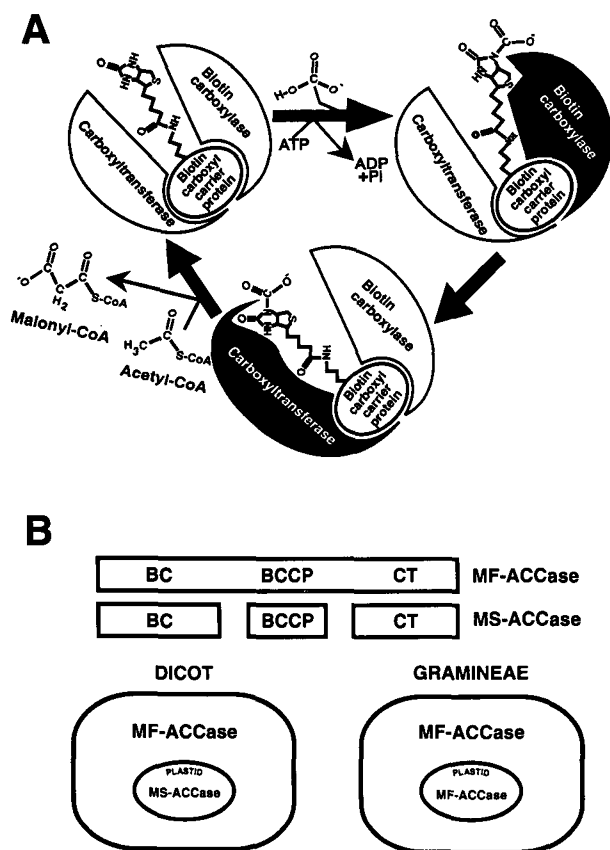

Figure 2. The Acetyl-CoA Carboxylase Reaction.

(A) ACCase has three functional regions: biotin carboxylase, which activates CO_2 by attaching it to biotin in an ATP-dependent reaction; biotin carboxyl carrier protein; and carboxyltransferase, which transfers activated CO_2 from the biotin carboxylase region to acetyl-CoA, producing malonyl-CoA.

(B) Two forms of ACCase occur in plants. A multifunctional structure (MF-ACCase) has the three functional regions shown in **(A)** encoded in a single, large (>200 kD) polypeptide. A multisubunit structure (MS-ACCase) consists of three or more subunits that form a large complex. The MF-ACCase is believed to occur in the cytosol of dicots and in both the plastid and cytosol of graminaceous plants. The MS-ACCase occurs in the plastids of most other plants, including all dicots examined so far.

of a number of alternate pathways, including production of acetyl-CoA by a mitochondrial PDH followed by transport of free acetate or acetylcarnitine to the plastid. Free acetate entering plastids is activated to acetyl-CoA by acetyl-CoA synthetase, an enzyme with 5- to 15-fold higher activity than the in vivo rate of fatty acid synthesis (Roughan and Ohlrogge, 1994). In addition, cytosolic malate and glucose-6-phosphate have been proposed as precursors of the plastid acetyl-CoA pool in oilseeds (Smith et al., 1992; Kang and Rawsthorne, 1994).

Thus, our understanding of how carbon moves from photosynthesis into acetyl-CoA is clouded by an abundance of potential pathways. Furthermore, essentially all of the suggestions on the origin of plastidial acetyl-CoA are based on in vitro analyses of enzyme activities or precursor incorporations. In no case do in vivo data address how photosynthate is metabolized to produce acetyl-CoA for fatty acid synthesis. Because of the central role of acetyl-CoA in many metabolic pathways, it is likely that more than one pathway may contribute to maintaining the acetyl-CoA pool, and which pathway is used may vary with tissue, developmental stage, light/dark conditions, and species.

STRUCTURE AND ROLE OF ACETYL-COA CARBOXYLASE

The enzyme acetyl-CoA carboxylase (ACCase) is generally considered to catalyze the first reaction of the fatty acid biosynthetic pathway—the formation of malonyl-CoA from acetyl-CoA and CO_2. This reaction actually takes place in two steps, which are catalyzed by a single enzyme complex, as shown in Figure 2A. In the first reaction, which is ATP dependent, CO_2 (from HCO_3^-) is transferred by the biotin carboxylase portion of ACCase to a nitrogen of a biotin prosthetic group attached to the ε-amino group of a lysine residue. In the second reaction, catalyzed by the carboxyltransferase, the activated CO_2 is transferred from biotin to acetyl-CoA to form malonyl-CoA.

The structure of the plant ACCase has been a subject of considerable confusion in the past, but recent evidence from several laboratories is starting to provide new insights into the organization of this complex key regulatory enzyme (Figure 2B). The confusion arose in large part because plants contain different forms of the enzyme, one of which easily loses activity during attempts to characterize it.

It is now understood that there are at least two different types of ACCase structures. In one type of organization (frequently referred to as prokaryotic), ACCase consists of several separate subunits assembled into a 700-kD complex (Sasaki et al., 1993; Alban et al., 1994). At present, we know some details about three of the subunits. The biotin carboxylase is an ~50-kD polypeptide that is nuclear encoded (Shorrosh et al., 1995). The biotin carboxyl carrier protein (BCCP) is a 34- to 38-kD protein that is almost certainly also nuclear encoded. A gene for a third subunit (~65 to 80 kD) has been identified in the plastid genome by its homology to one of the carboxyltransferase subunits of *Escherichia coli* ACCase. This is the only component of plant lipid metabolism known to be encoded in the plastid genome. Furthermore, it may be unusual among plastome-encoded proteins in that its expression does not seem highly regulated by light. Antibodies to this carboxyltransferase subunit inhibit ACCase activity and coprecipitate the BCCP subunit (Sasaki et al., 1993). This result indicates that, unlike in *E. coli*, the separate subunits associate in a complex whose components can be coprecipitated. At present, it is not clear if the three known subunits are sufficient to produce an active

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ACCase complex. Most likely, other components of the ACCase complex exist that have yet to be characterized, because even dimers of the described subunits do not add up to a 700-kD complex. The 700-kD complex remains associated during gel filtration experiments, but attempts by several groups to purify it to homogeneity have resulted in the loss of activity, presumably due to dissociation of the subunits. An intensive research effort is currently under way to characterize further the structure of this complex.

In the second type of ACCase organization, the three components of the reaction are present on a single large multifunctional polypeptide. This structure is termed eukaryotic because it is similar to that found in the cytosol of yeast and animals. Several genes and cDNA clones have been isolated for this type of ACCase from plants, animals, and fungi, all of which encode proteins with the biotin carboxylase domain at the N terminus, the BCCP domain in the middle, and the carboxyltransferase at the C terminus.

The two ACCase isozymes have several important differences in their biochemical properties. The multifunctional enzyme has a much lower K_m for acetyl-CoA than the multisubunit complex and has the ability to carboxylate propionyl-CoA at substantial rates, which the multisubunit complex does not. In addition, the multifunctional enzyme is sensitive to several important herbicides of the aryloxyphenoxy–propionic acid and cyclohexane–1,3-dione classes that have no effect on the multisubunit ACCase.

Which type of ACCase structure is present depends on its subcellular localization and the type of plant. Dicots have both types of enzyme. The prokaryotic multisubunit form is found in plastids, whereas the eukaryotic multifunctional polypeptide structure occurs outside the plastids, most likely in the cytosol. The plastidial isozyme of ACCase is involved primarily, if not exclusively, in supplying malonyl-CoA for de novo fatty acid synthesis. A second isozyme of ACCase is presumably needed in the cytosol to supply malonyl-CoA for a variety of pathways, including fatty acid elongation for cuticular lipid production and flavonoid biosynthesis. Both pathways are found primarily in leaf epidermal cells, and the epidermis is indeed the main location of the multifunctional ACCase in leaves (Alban et al., 1994). In addition, the elongation of oleic acid (C18) to erucic acid (C22) is a major malonyl-CoA–dependent pathway in some oilseeds, such as *Brassica napus.* This elongation occurs outside the plastid and presumably depends on the cytosolic ACCase isozyme. Finally, substantial concentrations of malonic acid that may derive from a cytosolic ACCase isozyme occur in the leaf and root of soybean (Stumpf and Burris, 1981). Although a cytosolic location seems most reasonable for the multifunctional ACCases that have been cloned from dicots (Shorrosh et al., 1994), such a location has yet to be demonstrated directly.

Although the evidence is still fragmentary, many monocots share with dicots the occurrence and localization of the two types of ACCase. However, the Gramineae family of plants is different in that both the plastid and cytosolic ACCase isozymes are large multifunctional polypeptides (Egli et al., 1993; Konishi and Sasaki, 1994). Coincident with this evolutionary difference, the chloroplast genomes of rice and maize have lost the gene that encodes the putative carboxyltransferase subunit of the prokaryotic-type ACCase. The difference in ACCase organization in the Gramineae has now provided an explanation for the action of the grass-specific herbicides, which inhibit only the eukaryotic form of the enzyme (Konishi and Sasaki, 1994). Although both the cytosolic and plastid eukaryotic ACCases are inhibited by these herbicides, the plastid form in the Gramineae is much more sensitive than is the cytosolic form, and the plastid fatty acid synthesis pathway is more essential to growth than are the secondary pathways dependent upon the cytosolic ACCase.

REGULATION OF FATTY ACID SYNTHESIS

In animals, yeast, *E. coli*, and plants, ACCase is a regulatory enzyme that controls, at least in part, the rate of fatty acid synthesis. Light/dark regulation of ACCase activity is responsible for the light/dark modulation of fatty acid synthesis rates of spinach leaves (Post-Beittenmiller et al., 1991, 1992). In addition, fatty acid synthesis in tobacco suspension cells is subject to feedback inhibition by lipids provided exogenously in the media, and this feedback appears to act at the level of ACCase activity (Shintani and Ohlrogge, 1995). Although the regulatory role of ACCase is well established in some tissues, several important questions remain about how flux through the fatty acid synthesis pathway is controlled.

(1) What regulates ACCase activity? Although ACCase activity may determine the rate of fatty acid synthesis, understanding the regulation of lipid metabolism requires an understanding of what factors control ACCase. In animals and fungi, ACCase is regulated by several biochemical mechanisms, including phosphorylation, activation by citrate, and feedback inhibition by acyl-CoA. None of these mechanisms has yet been shown to occur in plants; nevertheless, clearly biochemical regulation occurs. The rate of fatty acid synthesis in leaves is six-fold higher in the light than in the dark. Although part of the light/dark control in vivo is likely to arise from alterations in cofactor supply, ACCase rapidly extracted from light-incubated chloroplasts is two- to fourfold more active than that from dark-incubated chloroplasts, even when in vitro conditions and cofactors are identical (Ohlrogge et al., 1993a). At present, we have no explanation for this difference in activity.

(2) What other enzymes control the flux of fatty acid synthesis? ACCase may be only one of a number of enzymes that can be considered rate limiting. The condensing enzymes (see later discussion), in particular, 3-ketoacyl-ACP synthase III (KAS III), are also logical control points. In some metabolic pathways, control is spread over several regulatory enzymes, and the flux control coefficient of each varies with the conditions. Now that clones are available for ACCase and most of the other enzymes of fatty acid synthesis, transgenic plant experiments

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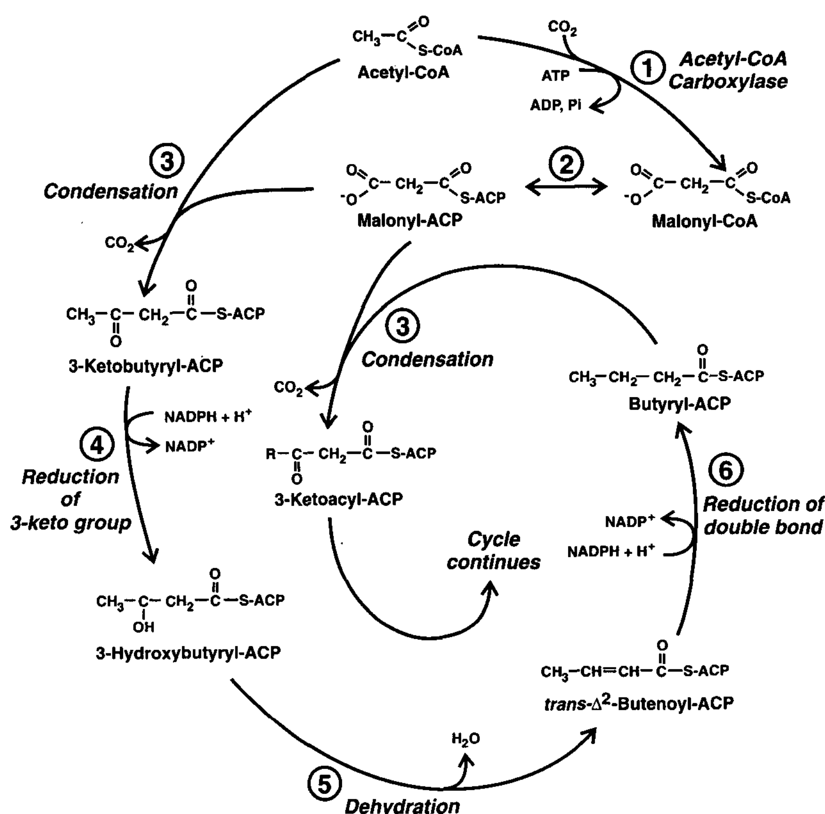

Figure 3. The Reactions of Saturated Fatty Acid Biosynthesis.

Acetyl-CoA is the basic building block of the fatty acid chain and enters the pathway both as a substrate for acetyl-CoA carboxylase (reaction 1) and as a primer for the initial condensation reaction (reaction 3). Reaction 2, catalyzed by malonyl-CoA:ACP transacylase, transfers malonyl from CoA to form malonyl-ACP, which is the carbon donor for all subsequent elongation reactions. After each condensation, the 3-ketoacyl-ACP product is reduced (reaction 4), dehydrated (reaction 5), and reduced again (reaction 6), by 3-ketoacyl-ACP reductase, 3-hydroxyacyl-ACP dehydrase, and enoyl-ACP reductase, respectively.

will provide crucial in vivo tests of the role of each enzyme in controlling flux through the pathway.

THE FATTY ACID SYNTHESIS PATHWAY

Plants are fundamentally different from other eukaryotes in the molecular organization of the enzymes of fatty acid synthesis. Overall, to produce a 16- or 18-carbon fatty acid from acetyl-CoA and malonyl-CoA, at least 30 enzymatic reactions are required. In animals, fungi, and some bacteria, all of these reactions are catalyzed by a multifunctional polypeptide complex located in the cytosol. In plants, the individual enzymes of the pathway are dissociable soluble components located in the stroma of plastids. Although the component enzymes of plant fatty acid synthesis can be separated easily in vitro, an intriguing question is whether they may be organized in vivo into a supramolecular complex.

The central carbon donor for fatty acid synthesis is the malonyl-CoA produced by ACCase. However, before entering the fatty acid synthesis pathway, the malonyl group is transferred from CoA to a protein cofactor, acyl carrier protein (ACP). From this point on, all the reactions of the pathway involve ACP until the 16- or 18-carbon product is ready for transfer to glycerolipids or export from the plastid (Figure 3). ACP is a small (9 kD) acidic protein that contains a phosphopantethein prosthetic group to which the growing acyl chain is attached as a thioester. After transfer to ACP, the malonyl-thioester enters into a series of condensation reactions with acyl-ACP (or acetyl-CoA) acceptors. These reactions result in the formation of a carbon–carbon bond and in the release of the CO_2 that was added by the ACCase reaction. Removal of this CO_2 helps to drive this reaction forward, making it essentially irreversible.

At least three separate condensing enzymes (also known as 3-ketoacyl-ACP synthases) are required to produce an 18-carbon fatty acid. The first condensation of acetyl-CoA and malonyl-ACP to form a four-carbon product is catalyzed by

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