Subcellular Biochemistry 99

J. Robin Harris
Jon Marles-Wright *Editors*

Macromolecular Protein Complexes IV

Structure and Function

Springer

Subcellular Biochemistry

Volume 99

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Preface

The *Subcellular Biochemistry* series is moving toward Volume 100! Having started as a quarterly journal in 1973, under the editorship of the late Donald Roodyn, it evolved in 1978 to a book series. The diversity of content over the past ~50 years has been considerable, whilst retaining the underlying broad aim of presenting review chapters on the diverse subcellular components, from the organelle to the molecular level. Recent years have seen a strong emphasis upon the structure and function of macromolecular complexes, resulting in several thematically related volumes.

Volume 82 (2017) was devoted to Fibrous Proteins, followed by Volume 83, Macromolecular Protein Complexes. Because of the large amount of data available at this time, and the willingness of scientists to contribute to the series, Volume 87 and Volume 88 (2018) were published, on Membrane and Virus Proteins Complexes, respectively. Subsequently, a further book (Volume 93, 2019) entitled *Macromolecular Protein Complexes II* pulled together *all aspects of the broad field* and was followed by *Macromolecular Protein Complexes III* (2021). Several earlier related books have been included in the series, generating an almost encyclopedic coverage, with some macromolecular topics already being updated.

This volume has continued this approach and now contains defined sub-sections: Soluble Protein Complexes, Membrane Protein Complexes, Fibrous Protein Complexes, and Virus Protein Complexes. Owing to the exceptionally difficult COVID-19 times faced by scientists in academia and in research institutes, a number of initially agreed interesting chapters have been lost during production, but the remaining seventeen chapters presented here have enabled us to compile a strong and interesting book.

As in previous volumes, x-ray crystallography and cryo-electron microscopy of the structural biology of protein complexes predominates, with functional considerations also apparent in most chapters. Indeed, the impact of molecular genetics and the study of dynamic/flexible states and assembly/disassembly of macromolecular structures have become increasingly important, along with benefits for the understanding of disease states.

Rather than expanding here upon some individual chapters, we simply wish to emphasize the on-going strength of this group of chapters and refer the reader to the chapter list of the book. We have been impressed by the quality and outstanding interest of the material in these chapters and the enthusiasm shown by our authors. We hope that this book, as print version and available online as the e-book and individual e-chapters, will be of use and interest to the bioscience community.

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Contents

Chapter 1
Fatty Acid Synthase: Structure, Function, and Regulation

Aybeg N. Günenc, Benjamin Graf, Holger Stark, and Ashwin Chari

Abstract Fatty acid (FA) biosynthesis plays a central role in the metabolism of living cells as building blocks of biological membranes, energy reserves of the cell, and precursors to second messenger molecules. In keeping with its central metabolic role, FA biosynthesis impacts several cellular functions and its misfunction is linked to disease, such as cancer, obesity, and non-alcoholic fatty liver disease. Cellular FA biosynthesis is conducted by fatty acid synthases (FAS). All FAS enzymes catalyze similar biosynthetic reactions, but the functional architectures adopted by these cellular catalysts can differ substantially. This variability in FAS structure amongst various organisms and the essential role played by FA biosynthetic pathways makes this metabolic route a valuable target for the development of antibiotics. Beyond cellular FA biosynthesis, the quest for renewable energy sources has piqued interest in FA biosynthetic pathway engineering to generate biofuels and fatty acid derived chemicals. For these applications, based on FA biosynthetic pathways, to succeed, detailed metabolic, functional and structural insights into FAS are required, along with an intimate knowledge into the regulation of FAS. In this review, we summarize our present knowledge about the functional, structural, and regulatory aspects of FAS.

Keywords Fatty acid biosynthesis · Fatty acid synthase · ACP · FAS structure · FAS regulation · Biofuels · Oleochemicals

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The Importance of Fatty Acids and Fatty Acid Biosynthesis

Fatty acids (FAs) are essential molecules of all living organisms and their biosynthesis is essential for the survival of all organisms. In bacteria, yeasts, and plants, the de novo biosynthesis of FAs is both necessary and sufficient for survival (Schweizer and Hofmann 2004; White et al. 2005; Chan and Vogel 2010). Metazoans, on the other hand, additionally rely upon dietary uptake of some fatty acids and make proportionally less use of de novo FA biosynthesis pathways, for example, for the biosynthesis of some cofactors (Burr and Brown 1933; Kastaniotis et al. 2017). FAs primarily act as compact sources for energy storage, especially in the form of triglycerides, occupying less space than carbohydrates of similar energy content (Nelson and Cox 2013). In almost all organisms (except Archaea) (Jain et al. 2014), cellular membranes are made of phospholipids, composed of a phosphate containing hydrophilic head group containing two FAs (Nelson and Cox 2013). A variety of fatty acids can coexist in any given single organism, e.g., different chain lengths, differing functional groups such as alkenes, isopropyl, and methyl groups. These differences cause alterations in membranes when incorporated, or provide different values of energy when metabolized (Nelson and Cox 2013). Membrane fluidity is increased by incorporating phospholipids containing unsaturated FAs in comparison to when phospholipids containing saturated FAs are inserted (Vagelos 1971; White et al. 2005; Cronan and Thomas 2009). The degradation of unsaturated FAs to mobilize energy reserves is also energetically more favorable (Nelson and Cox 2013).

In addition, FAs serve as precursors to second messenger molecules and therefore control cellular signaling, intercellular communication, and hormonal regulation (Graber et al. 1994). This is accomplished by low-density lipoprotein (LDL) and high-density lipoprotein (HDL) formation that are also crucial to ensure steroid transport throughout the bodies of higher animals (Nelson and Cox 2013). The maintenance and equilibrium of these varieties need strict and self-responsive regulation.

Consequently, FA production is vital for all living organisms. It is therefore not surprising that subtle changes in FA biosynthesis impact numerous essential cellular functions, and are thus linked to ailments like cancer, obesity, and non-alcoholic fatty liver disease (Ronnett et al. 2006; López et al. 2007; Wang and DuBois 2012). The essential requirement of de novo FA biosynthesis, and the substantial architectural differences of the biosynthetic enzymes as discussed below, make this metabolic pathway a desirable target for antibiotic discovery (Campbell and Cronan 2001; Yao and Rock 2017).

Beyond academic interest in FA biosynthetic mechanisms, strained petroleum supplies have sparked interest in these cellular processes (Kallio et al. 2014; Zhang et al. 2021; Liu et al. 2021). An active quest is afoot to define alternative, renewable energy sources to replace liquid fuels by exploiting, and modifying FA biosynthetic mechanisms. This search is not limited to fuels, but also to replace the large foundry

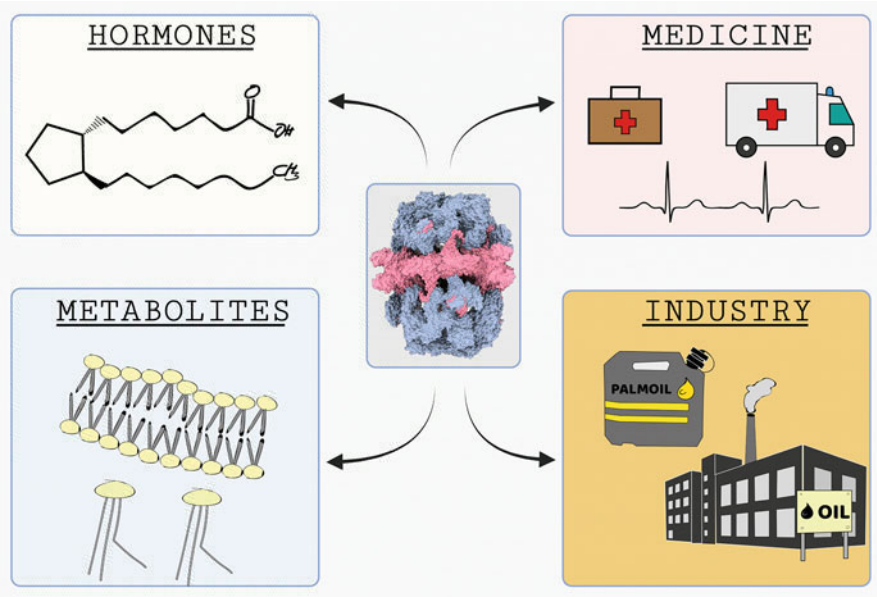

Fig. 1.1 The essential roles played by fatty acid biosynthetic mechanisms and fatty acid synthases in Biology, Medicine, and Biotechnology. Fatty acid synthases, represented by the yeast fatty acid synthase, are the cellular powerhouses which produce fatty acids (center). Fatty acids are essential building blocks of phospho- and glycolipids which form biological membranes (bottom left). They also represent precursors to second messenger molecules, which are paramount to cellular and intercellular signalling (top left). Cells need to maintain a fine homeostatic balance with regard to their fatty acid profiles, and imbalances can elicit disease (top right). Fatty acid biosynthetic mechanisms are exploited to generate fuels and precursors for chemical industry from renewable sources (bottom right)

of oleochemicals, FA-derived compounds. The essential functions of FAs in Biology, Medicine, and Biotechnology are summarized in Fig. 1.1.

Historical Background

Many different classes of lipids, defined as water-insoluble biological substances, were known and utilized since ancient times. FAs, an important component of lipids, are carboxylic acids with long aliphatic chains initially isolated from animal tissues, and first described by the French Biochemist Michel Eugène Chevreul (Chevreul 1823). At this time in the early half of the nineteenth century, Biochemistry and Organic Chemistry were considered only to be branches of analytical chemistry, since biological material was thought to be of "vital essence" that cannot be reproduced synthetically by humans. Thorough study of enzymes, investigation of plant and animal tissue extracts and secretions have now allowed Biochemistry to be

a more mechanistic subject. After implementation of Germ Theory led by systematic experiments, first by Louis Pasteur in the middle of the nineteenth century, and later, towards the end of the nineteenth century, by Robert Koch (1882), sterile technique emerged pioneered by Pasteur (1879), Joseph Lister, and Joseph Snow (Snow 1849; Pitt and Aubin 2012). Microbiology then blossomed into a scientific discipline. Biochemistry became a field closely associated with Microbiology and scientists started to use microbial cell lysates and isolate metabolites from microbial cultures to understand central pathways.

At this point, the first steps to understand the metabolism of fatty acids were started through the investigations of Feodor Lynen and Konrad Bloch. Both Lynen and Bloch were using microbial cultures (mostly yeast) and animal tissues to understand important pathways related to central metabolism (Lynen 1964; Bloch 1965). One interesting point was the metabolism of acetic acid, which was thought to have a significant role in many biological reactions (Lipmann 1954). Konrad Bloch investigated acetic acid metabolism in various animal tissues and managed to trace the building blocks of cholesterol and fatty acids to C2 units. Since these observations were derived amongst others by feeding rats with radiolabeled acetic acid (Rittenberq and Bloch 1944), a monumental prediction was made that cholesterol and fatty acids derived from "a form of activated acetic acid" (Lipmann 1954). This activated form of acetic acid was found to be acetyl-coenzyme A (acetyl-CoA) by Feodor Lynen (Lynen et al. 1951). Lynen inferred, based on the seminal studies of Fritz Lipman on pantothenic acid (Klein and Lipmann 1953a, b), that not just acetate, but Acetyl-CoA, was an important component of FA degradation cycle and tried to elucidate the cycle further (Lynen 1964). From his investigations, he speculated that FA biosynthesis is just the reversal of the FA degradation cycle (Lynen 1964). It was proven wrong by Wakil, Vagelos and colleagues, who found out that synthesis takes place in the cytoplasm, while degradation takes place in mitochondria (Gibson et al. 1958; Smith et al. 2003). Lynen, along with others, later focused on finding and characterizing these biosynthetic enzymes. Both Bloch and Lynen shared the Nobel Prize for Medicine and Physiology in 1964 for their discoveries in "Fatty Acid and Cholesterol metabolism" (Lynen 1964; Bloch 1965).

Chemical characterization of fatty acid synthase (FAS) enzymes took substantially more time. The *Escherichia coli* (*E. coli*) acyl carrier protein (ACP), AcpP, was the first FAS protein to be discovered by Wakil, Vagelos and colleagues and the complete amino acid sequence to be documented (MAJERUS et al. 1965; Pugh and Wakil 1965). Later on, catalytic components of the *E. coli* system were also discovered and characterized by a combination of genetic and biochemical approaches (Volpe and Vagelos 1973). In yeast, the FAS complex was found and characterized in the early 1960s also led by Feodor Lynen (Sumper et al. 1969; Lynen et al. 1980). Metazoan FAS has a different story: biochemical and genetic evidence suggested all necessary catalytic components to be present in a single polypeptide. Wakil and colleagues fractionated the system to homogeneity and spear-headed the biochemical characterization (Mattick et al. 1983a, b; Wong et al. 1983; Tsukamoto et al. 1983).

Biochemical Steps in FA Biosynthesis

The cellular machinery that is responsible for FA biosynthesis in the various kingdoms of life can differ substantially, as discussed below. Yet notably, the biochemical reactions leading to saturated FA biosynthesis steps, at their core, are conserved (Fig. 1.2).

A central aspect in FA biosynthesis is that no substrates, intermediates, and products diffuse freely into the individual enzyme active sites, but only as covalent adducts to the acyl carrier protein (ACP) (Chan and Vogel 2010; Crosby and Crump 2012). To be able to function in FA biosynthesis, the ACP has to be modified on a defined serine side-chain by a post-translational modification (Beld et al. 2014). The post-translational modification is achieved with the help of Coenzyme A (CoA) by a group of enzymes called phosphopantetheinyl-transferases (PPT) (Fig. 1.3). The resulting 4′-phosphopantetheine (PPant) arm of the ACP had been termed as a "swinging arm" in the late 1960s by Lynen and colleagues (Sumper et al. 1969). Notably, bacteria possess enzymes, holo-ACP hydrolases (AcpH), that are able to catalyze the inverse reaction of removing the ACP PPant arm (Thomas and Cronan 2005).

FA biosynthesis can be sub-divided into 4 temporal cycles: activation, priming, elongation, and termination and requires the co-substrates acetyl-CoA, malonyl-

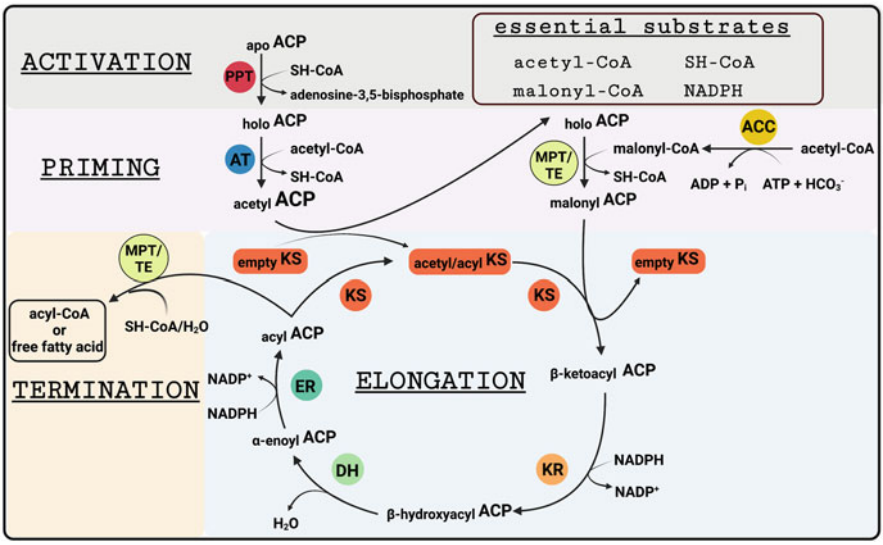

Fig. 1.2 Overview of the de novo FA biosynthesis cycle. FA biosynthesis requires CoA, acetyl-CoA, malonyl-CoA, and NADPH for functions. Malonyl-CoA is generated by the condensation of acetyl-CoA with CO_2 by the enzyme ACC. Four phases catalyzed by FAS, consisting of activation, priming, elongation, and termination, can be discerned in the FA biosynthesis cycle. A key feature is that all chemical reactions occur, when substrates and intermediates are affixed as chemical adducts to the ACP. This general FA biosynthesis cycle is used by virtually all organisms

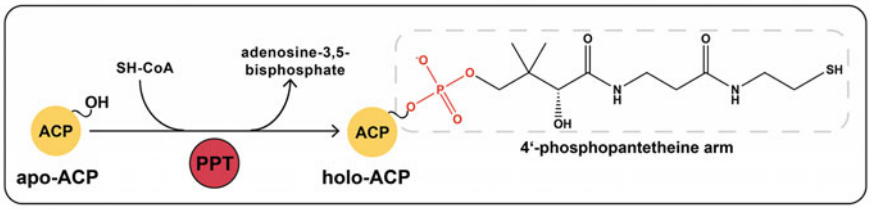

Fig. 1.3 The activation step of the FA biosynthesis cycle. The apo- acyl carrier protein (ACP) is modified on a defined serine side chain with the help of coenzyme A (CoA) by phosphopantetheinyl-transferases (PPT). This endows the ACP with a 4′-phosphopantetheine arm and a unique thiol on which substrates, intermediates, and products are attached as covalent (thioester-) adducts

CoA, and the reduced form of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) (Fig. 1.2). The starting point of a fatty acid is the "activated form of acetic-acid" discovered by Lynen, acetyl-CoA, which is produced as a result of glycolysis (Lynen 1964; Perham 2000). Acetate (or acetyl-CoA) can also be considered a fatty acid (or fatty-acyl-CoA) with a two-carbon chain length. Malonyl-CoA is not bound to metabolic flux and is produced by an acetyl-CoA carboxylase enzyme (ACC) (Cronan and Waldrop 2002; Brownsey et al. 2006). The ACC reaction uses acetyl-CoA, ATP, and carbonate and represents the first committed step in FA biosynthesis (Fig. 1.4a). ACC contains a carrier protein which is functionally analogous to the ACP in FAS, the biotin carboxyl carrier protein (BCCP). The BCCP is also post-translationally activated to perform its function, yet, instead of PPant, its swinging arm is a biotin molecule that is postranslationally attached to a conserved lysine of the BCCP (Cronan and Waldrop 2002; Brownsey et al. 2006). FA biosynthesis is not a solitary pathway, where malonyl-CoA is used, as it can be used in polyketide synthesis of secondary metabolism as well (Katz and Donadio 1993; Smith and Tsai 2007; Tsai 2018). Priming FAS occurs through ping-pong mechanisms (Beld et al. 2015; Finzel et al. 2015; Heil et al. 2019), where acetyl- and malonyl- moieties are first transferred onto active site nucleophiles prior to the thioesterification of the ACP PPant arm (Fig. 1.4b, c).

Elongation steps in fatty acid biosynthesis are fueled by malonyl-CoA (Fig. 1.5). Two different instances can be discerned: The first step in elongation is the condensation of acetate with malonate, or the initiation of the FA biosynthesis cycle. For this, the acetyl-primed ACP trans-thioesterifies the acetyl group onto an active site cysteine thiol. In a second step, either the same or another ACP is primed by a malonyl group (Fig. 1.5). The malonyl decarboxylates and the resulting acetyl-ACP anion condenses with the thioacetyl, the result is a β-ketoacyl-ACP product and carbon dioxide as a side-product (Wakil et al. 1983; Smith et al. 2003; Beld et al. 2015; Finzel et al. 2015). In the second instance, an intermediate fatty acyl-ACP trans-thioesterifies onto an active site cysteine thiol (Fig. 1.5). The malonyl-primed ACP then again decarboxylates, and the resulting acetyl-ACP anion condenses with the thioacyl group. In any case, this reaction adds stoichiometrically two carbons to

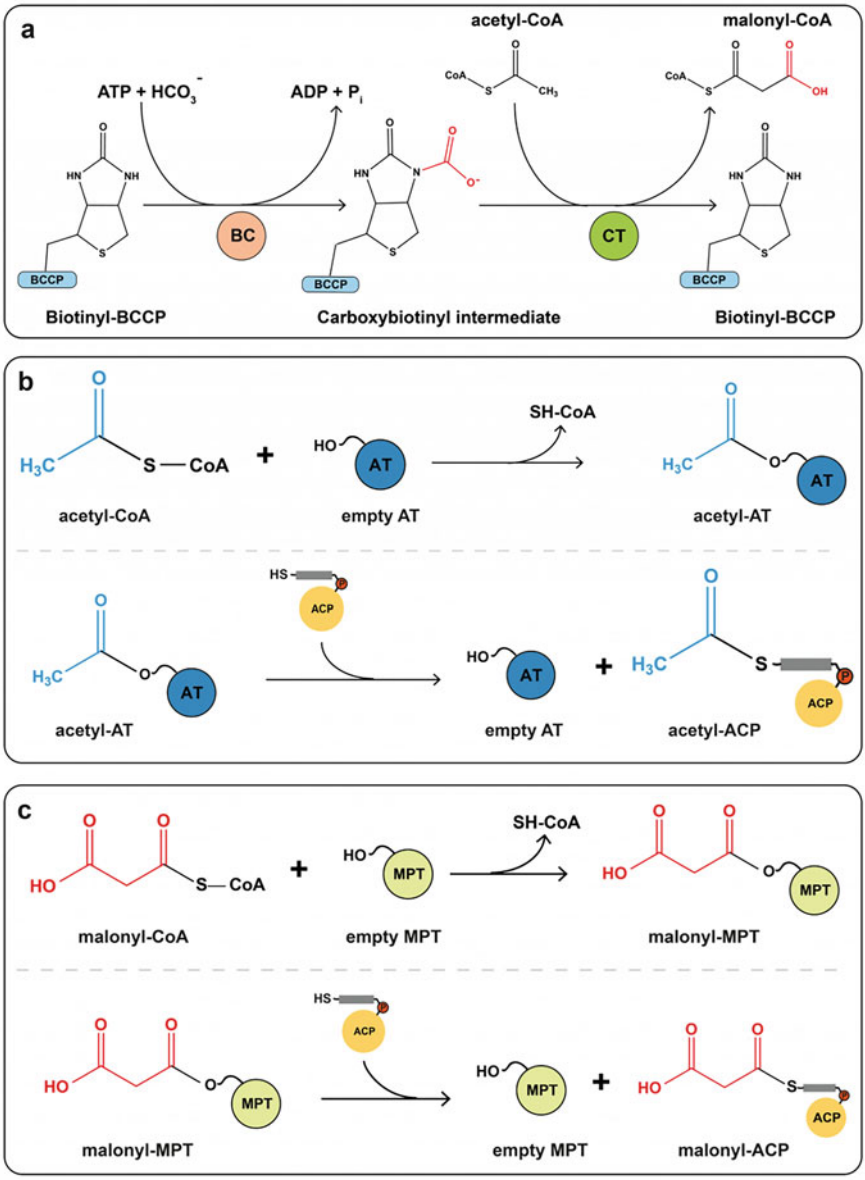

Fig. 1.4 Priming reactions in FA biosynthesis as employed in yeast FAS. (**a**) Generation of malonyl-CoA by acetyl-CoA carboxylases (ACC). The BCCP component of ACC is modified by a biotin swinging arm. Initially, the biotin carboxylase (BC) part of ACC phosphorylates carbonate to carbonyl phosphate, which is then used by BC to carboxylate biotin. The carboxyltransferase (CT) of ACC then transfers the carboxyl group to acetyl-CoA to form the malonyl-CoA co-substrate of FA biosynthesis. (**b**) Acetyl priming of FAS. The acetyl transferase (AT) of FAS uses an acetyl-CoA molecule to esterify a serine active site nucleophile by acetyl (acetyl-AT). This acetyl is then trans-esterified to the ACP PPant thiol. (**c**) Malonyl priming of FAS. The malonyl−/palmitoyl-transferase (MPT) of FAS uses a malonyl-CoA molecule to esterify a serine active site nucleophile by malonyl (malonyl-MPT). This malonyl group is then trans-esterified to the ACP PPant thiol

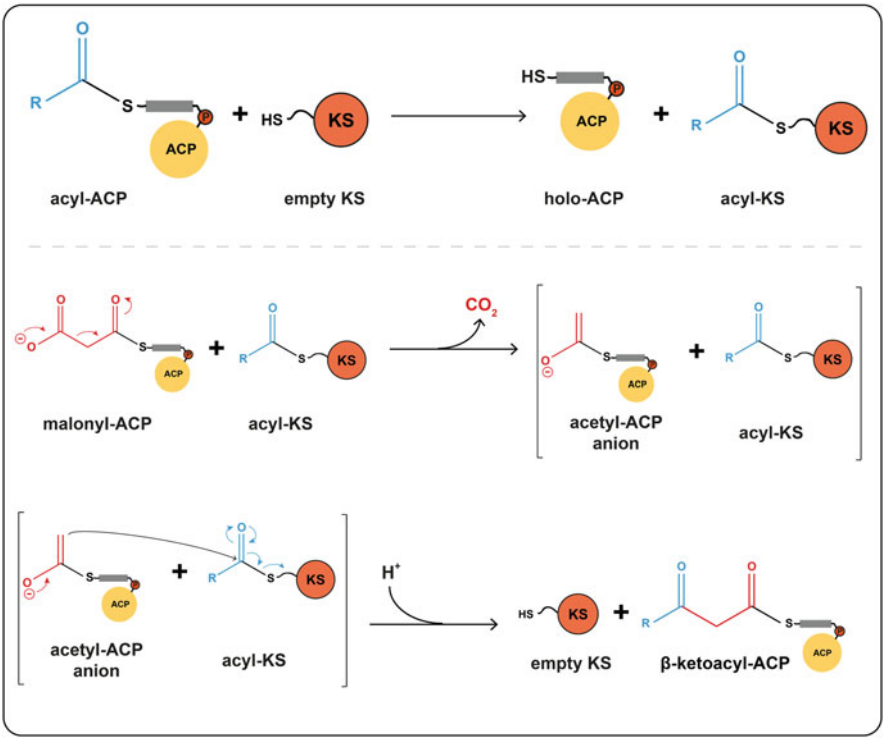

Fig. 1.5 The condensation reaction in FA biosynthesis. First, either the acetyl- or acyl-primed ACP trans-thioesterifies its acyl cargo (blue) onto an active site thiol of the empty ketosynthase (KS; top panel). Then the same or a different ACP primed with malonyl enters the acyl-KS active site. First the malonyl-primed ACP decarboxylates resulting in the formation of CO_2 as a side product and the generation of a transient acetyl-ACP anion (middle panel). The transient acetyl-ACP anion then condenses with the acetyl- or acyl-KS to regenerate the empty KS thiol and generates a two-carbon elongate β-ketoacyl-ACP

the growing fatty acid chain. Because of this process, biologically relevant fatty acids are always composed of an even-numbered carbon skeleton. The number can be odd, if the de novo synthesized fatty acid is subsequently modified with certain enzymes (Nelson and Cox 2013).

In the ensuing elongation steps (Fig. 1.6), the β-ketoacyl-ACP intermediate is then reduced in the first NADPH-consuming step to a β-hydroxyacyl-ACP. This is then followed by a dehydration reaction, where the removal of water generates an α-enoyl-ACP. Finally, in the second NADPH-consuming step, which reduces the α-enoyl-ACP to the fatty acyl-ACP in saturated form that is now elongated by a two-carbon unit. The enoyl reduction can occur by the direct transfer of reducing equivalents from NADPH to the α-enoyl-ACP, or can employ, in certain instances, flavin mononucleotide (FMN) as intermediary. In the latter cases, the reduced form

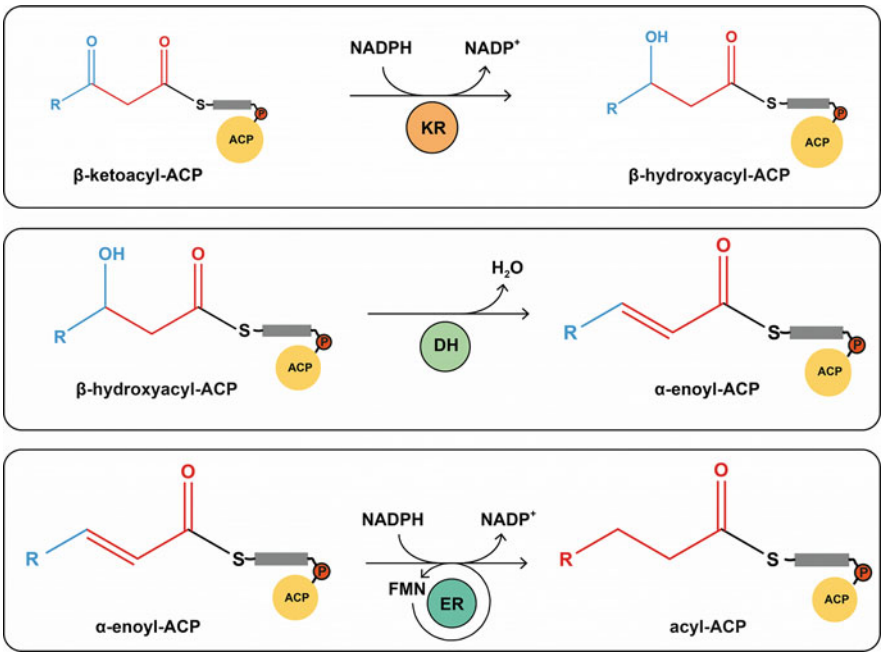

Fig. 1.6 Elongation steps in FA biosynthesis as employed in yeast FAS. The β-ketoacyl-ACP is initially reduced by NADPH in the ketoreductase (KR) to β-hydroxyacyl-ACP (top panel). Subsequent water removal from the β-hydroxyacyl-ACP by the dehydratase (DH) generates α-enoyl-ACP. The α-enoyl-ACP is then reduced by the enoyl reductase (ER) to the elongated acyl-ACP. Two versions of this reactions have evolved in nature: the one depicted here from yeast utilizes FMN as a co-factor to reduce the α-enoyl-ACP and NADPH to regenerate FMN. In other instances NADPH directly acts as a reductant for α-enoyl-ACP

of FMN ($FMNH_2$) reduce the α-enoyl-ACP and the reducing equivalents of NADPH are utilized to re-generate FMN to $FMNH_2$ (Fig. 1.6).

Malonyl-priming, condensation, and elongation cycles continue elongating the fatty acyl-ACP by sequential two-carbon units until the desired fatty acid length is obtained. The termination step also uses two distinct mechanisms and therefore generates two different products as a result of FA biosynthesis (Fig. 1.7). Some organisms transfer the fatty acyl-ACP to a free CoA molecule, resulting in acyl-CoAs as products (such as yeasts) (Tehlivets et al. 2007), others hydrolyze fatty acyl-ACP with water to generate free fatty acids (animalia) (Smith et al. 2003; Smith and Tsai 2007; Maier et al. 2010; Herbst et al. 2018). Bacterial, mitochondrial, and chloroplastic systems do not appear to release fatty acyl chains from the ACP, but rather fatty acyl-ACPs directly act as substrates in downstream reactions (Ohlrogge and Jaworski 1997; Cronan and Thomas 2009). Considering the importance of the ACP in all FAS biochemical reactions, it is justified to consider the acylated PPant arm together with ACP as the true substrate for FAS. As this consideration extends to the ACC BCCP and the biotin swinging arm, and the lipoyl domains of pyruvate

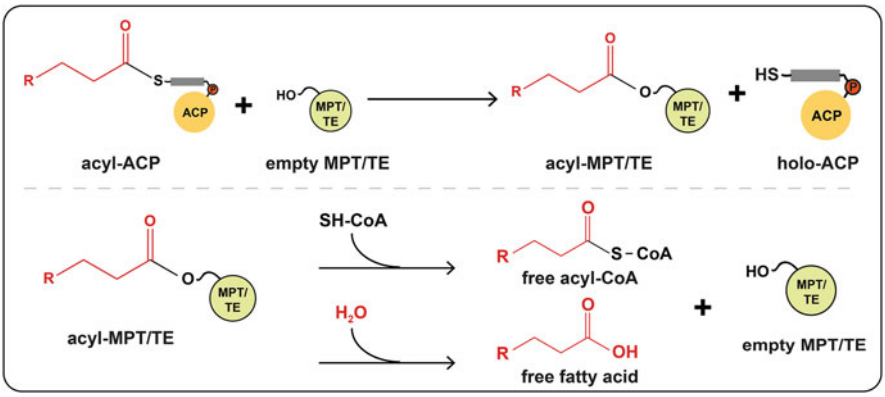

Fig. 1.7 Termination reactions in FA biosynthesis. When the fatty acyl-ACP has reached the appropriate chain length, it is transferred onto the termination enzyme, either malonyl−/palmitoyl-transferase (MPT in yeast) or thioesterase (TE in mammalia). In the respective termination enzyme, the fatty acyl-ACP thioester is hydrolyzed by an active site serine side chain generating acyl-MPT or acyl-TE and holo-ACP (top panel). At this point, the mechanisms and resulting products diverge, the acyl-MPT reacts with the thiol of a free CoA molecule to give rise to an empty MPT and a free acyl-CoA. The acyl-TE is hydrolyzed by a water molecule to generate an empty TE and a free fatty acid product (bottom panel)

dehydrogenase complexes, Perham, in the beginning of the twenty-first century, justifiably extended the swinging arm hypothesis to a swinging domain hypothesis (Perham 2000).

Type I and Type II FAS Architectures: Enzyme- and Enzymatic Domain Functions

As follows from the discussion of biochemical steps in FA biosynthesis, most reactions appear to be highly conserved. While differences are apparent, these seem to be variations on a theme rather than substantially divergent pathways. One would therefore be inclined to assume that the FA biosynthetic machinery likewise bears a striking resemblance in the various kingdoms of life. However, even from the very initial attempts to fractionate FA biosynthetic enzymes from various organisms, they appeared to differ. Some, primarily bacterial, mitochondrial, and chloroplastic systems, were strongly dependent on the addition of exogenous holo-ACP for the reconstitution of biosynthetic activity. On the other hand, some other systems appeared to be self-sufficient, only reliant on the addition of an ample supply of co-substrates for efficient function (Bloch 1969, 2006; Lynen et al. 1980; Wakil et al. 1983; Schweizer and Hofmann 2004; White et al. 2005; Smith and Tsai 2007; Cronan and Thomas 2009; Maier et al. 2010). These varying functional properties hinted at architectural and structural differences (Fig. 1.8).

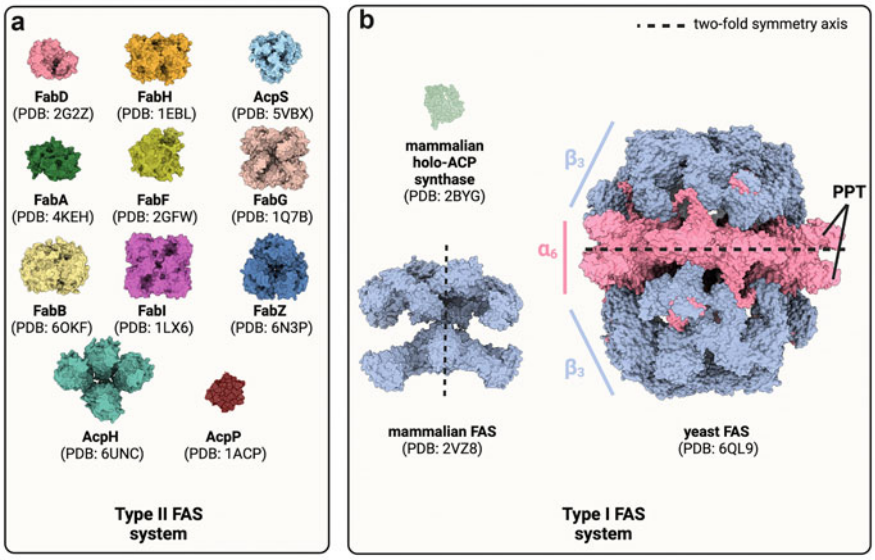

Fig. 1.8 Architectures of Type I and Type II FAS systems. (**a**) Type II systems predominate in bacteria, mitochondria, and chloroplasts. Representatively, in this figure, we show structures of the *E. coli* enzymes. The holo-ACP synthase (AcpS, PDB-ID: 5VBX) is the PPT responsible for the generation of the PPant arm in holo-ACP, the ACP (AcpP, PDB-ID: 1ACP), and the holo-ACP hydrolase (AcpH, PDB-ID: 6UNC) affect ACP function. The malonyl-CoA ACP transacylase (FabD, PDB-ID: 2G2Z), the β-ketoacyl-ACP synthase (KAS) III (FabH, PDB-ID: 1EBL), which are together responsible for the initiation of FA biosynthesis, the first priming ACP and the second performing the first condensation step. The KAS I enzyme (FabB, PDB-ID: 6OKF) is responsible for condensation steps leading to C6-C14 fatty acyl-ACPs, whereas KAS II (FabF, PDB-ID: 2GFW) generates C6-C16 fatty acyl-ACPs. The β-ketoacyl-ACP reductase (FabG, PDB-ID: 1Q7B), the two β-hydroxyacyl-ACP dehydratases (FabZ, PDB-ID: 6N3P and FabA, PDB-ID 4KEH), and the α-enoylacyl-ACP reductase (FabI, PDB-ID: 1LX6) comprise the elongation module of Type II systems. (**b**) Two different Type I FAS systems have evolved in nature. Left panel: In the mammalian system, a α_2 homodimeric X-shaped FAS (PDB-ID: 2VZ8) is found which is 540 kDa in size. ACP and TE domains are not resolved in the structure, but are present as C-terminal fusions on each of the α polypeptide chains. The mammalian PPT (holo-ACP synthase, PDB-ID: 2BYG) is separate and the only exogenous protein required for FA biosynthesis in the mammalian system. Right panel: The most integrated FA biosynthetic machinery is represented by the Type I yeast FAS. It exists as a 2-2.5 MDa homo-hexameric barrel structure, which is composed of either six copies of a single polypeptide chain $(\alpha)_6$ or two polypeptide chains $(\alpha\beta)_6$. In the *S. cerevisiae* FAS shown here (PDB-ID: 6QL9), where the building block is a αβ heterodimer, the 6 α-chains (colored in purple) form a central wheel of the structure, whereas the 6 β-chains (colored in blue) form the domes of the barrel. All enzymatic activities are present in the yeast FAS barrel, with ACP tethered as domains within the barrel, all enzyme active sites are pointing into the barrel cavity, and the PPT is located on the outside of the barrel at the central wheel

Structurally, fatty acid synthases can be divided into two different groups, called Type I and Type II systems (Fig. 1.8). In Type II systems, all enzymes needed for fatty acid synthesis are expressed as individual proteins (Schweizer and Hofmann 2004; White et al. 2005; Cronan and Thomas 2009). What is observed is that these

enzymes form multimeric structures; where, for example, the dehydratases (DH) form dimers (FabA) or hexamers (FabZ), ketosynthases (KS) form dimers, keto- and enoylreductase (KR and ER, respectively) form tetramers. These multimeric forms are also thought to be subject to allosteric regulation (Schweizer and Hofmann 2004; White et al. 2005; Cronan and Thomas 2009). The acyl carrier protein (ACP), is also a monomeric single protein. All enzymes and facilitators are able to diffuse freely in the cytoplasm. As the half-life of priming, condensation, and elongation intermediates are quite short (Bloch 1969; Lopez-Lara and Soto 2018), this hints that ACP modification needs to occur efficiently. This is facilitated *via* two main-mechanisms: First, the ACP is an abundant protein and its concentration is kept very high. In *E. coli* the ACP, AcpP, comprises 0.25% of total protein and concentrations reaching up to 0.15 mM (Jackowski and Rock 1983; van den Boom and Cronan 1989). Secondly, it is thought that ACP is able to find its interaction partners through specific interactions. Once the final fatty acyl-ACP product is attained, it is transferred to downstream reactions. Every Type II enzyme has independently evolved, and therefore many functional homologues can be found in the Type II FAS system (Ruppe et al. 2020). These are thought to influence product yield and composition. Hence, the Type II system is an apparently less efficient, but more versatile pathway that readily lends itself to produce fatty acids of greater diversity (van den Boom and Cronan 1989; White et al. 2005; Cronan and Thomas 2009).

Since each enzyme is separate in Type II systems, it makes it simpler to explain enzyme functions in these systems. In the following, we will use the *E. coli* FAS for this purpose (Fig. 1.9a). As mentioned earlier, during the entire FA biosynthetic process, all substrates, intermediates, and products are always covalent adducts to ACP, the AcpP protein in *E. coli*. The PPant arm in AcpP is affixed to Ser37, the PPT is AcpS, and *E. coli*, like some other bacteria, contains an enzyme, AcpH, that is able to catalyze the hydrolysis of the PPant arm to generate apo-AcpP (Fig. 1.8a) (Vagelos and Larrabes 1967; Thomas and Cronan 2005). The malonyl-CoA generating ACC in *E. coli* is composed of four subunits: AccA, AccB, AccC, and AccD (Cronan and Waldrop 2002). In *E. coli* FA biosynthesis, ACP is initially primed by the malonyl-CoA-ACP transacylase FabD, whereas acetyl-CoA is not used to prime ACP. The ketoacyl-ACP synthase (KAS) III protein FabH performs the first condensation reaction between malonyl-ACP and acetyl-CoA to generate acetoacetyl-CoA. Later condensation steps are performed by the KAS I enzyme FabB to generate C_6-C_{14}-β-ketoacyl-ACP, whereas the KAS II enzyme FabF yields C_6-C_{18}-β-ketoacyl-ACP (Cronan and Thomas 2009). The β-ketoacyl-ACP reductase FabG utilizes the reducing equivalents of NADPH to generate β-hydroxyacyl-ACP. The two β-hydroxyacyl-ACP dehydratases, FabA and FabZ, remove a water molecule to yield α-enoyl-ACP, where the double-bond is in trans-configuration. A unique feature of FabA is that it can isomerize the α-enoyl-decanoyl-ACP to a β-enoyl-ACP, with a cis-double bond. This β-enoyl-ACP can directly re-enter a condensation reaction with malonyl-ACP and either FabB or FabF to generate a mono-unsaturated FA (Cronan and Thomas 2009). Elongation continues with the NADPH-dependent reduction of α-enoyl-ACP by the α-enoyl-ACP reductase FabI to the two-carbon elongated which is deposited onto the active site thiol of either

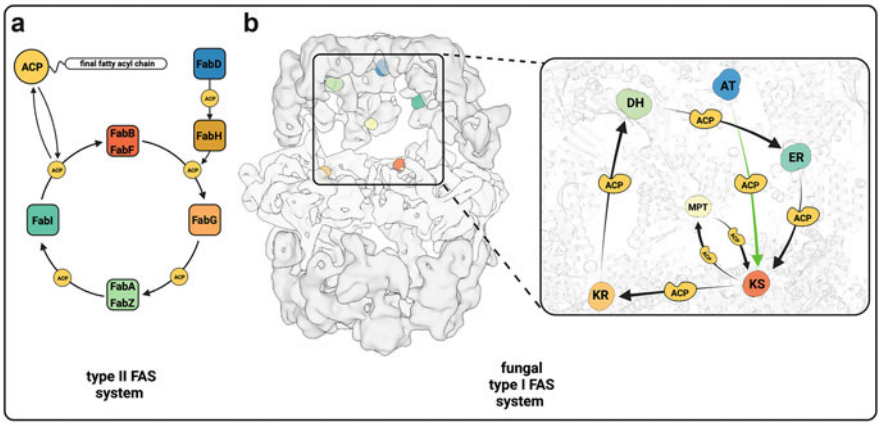

Fig. 1.9 Fatty acid biosynthesis cycles of Type I and Type II systems. (**a**) Type II systems strongly depend on the exogenous addition of holo-ACP (reflected by solid lines). An additional feature is that multiple enzymes can have seemingly redundant activities in the Type II system. This is illustrated by FabB and FabF KS enzymes, as well as FabA and FabZ DH enzymes. (**b**) In the left panel, the yeast Type I FAS barrel is shown, with active site clefts of enzymatic domains colored in the same scheme as in (**a**). The right panel indicates a zoom-in of the window depicted in the left panel. FA biosynthesis cycles in Type I FAS systems are independent and self-sufficient, requiring only addition of co-substrates for activity. Based on the spatial proximity enzyme active site clefts, elongation cycles have been proposed to run in clock-wise direction as indicated by the arrows

FabB or FabF for yet another condensation with malonyl-ACP. FabI is considered to be the last irreversible reaction of the fatty acid synthesis cycle. Thus, it is vital for the cycle to continue, as other intermediates tend to dissociate quickly. That is why FabI is considered to be a promising drug target for novel antimicrobials (Lu and Tonge 2008). Once the fatty-acyl-ACP chain has reached a suitable length (usually C_{12}-C_{16}-acyl-ACP), the mature fatty acid chain can be used for downstream pathways (Fig. 1.9a). In bacterial systems, the fatty acid stays bound to the ACP and when needed interacts with downstream partners such as those required to produce phospholipids or triglycerides (Bartholow et al. 2021). In *E. coli*, AcpP is known to have more partner proteins other than FAS enzymes, which is also an active area of research. As of today, *E. coli* AcpP is known to have ~30 interaction partners in the *E. coli* proteome (Gully et al. 2003; Gully and Bouveret 2006).

Type I systems, in contrast, are composed of large, multi-domain polypeptides. They form multimeric assemblies that contain all necessary enzymatic activities in the same macromolecular complex. The ACP is incorporated as a fusion into the polypeptide chains of these assemblies and therefore becomes an inherent domain of these FAS complexes which shunts the growing FA chain to the various enzymatic domains of the complex. Type I systems themselves come in two different flavors (Fig. 1.8b): Mammalian FAS is a homodimer, where each monomer contains all necessary domains. Its dimeric structure is found to be X-shaped and works in an open environment, as suggested by its 3.2 Å crystal structure (Maier et al. 2008).

Unfortunately, neither ACP nor the terminating thioesterase (TE) domains are resolved in the structure. However, it is well understood that the final FA product is released from the complex as free fatty acid (Smith et al. 2003; Maier et al. 2010; Herbst et al. 2018). Electron microscopic studies have shed light on the conformational flexibility of the whole enzyme and revealed that substantial conformational motion is required for FA biosynthesis to occur (Brink et al. 2004; Asturias et al. 2005; Brignole et al. 2009). Mammalian Type I FAS systems will not be the focus of this review. However, the interested reader is referred to several excellent reviews, where functional aspects of mammalian Type I FAS systems and the structural basis of their function are discussed in great detail (Smith and Tsai 2007; Maier et al. 2010; Herbst et al. 2018; Heil et al. 2019).

The yeast-like Type I FAS, on the other hand, is a hexameric barrel-like structure (Figs. 1.8b and 1.9b). In the yeast *Saccharomyces cerevisiae*, for example, all activities are present on two polypeptides and the hexameric barrel is of a $(\alpha\beta)_6$-Type (Jenni et al. 2007; Leibundgut et al. 2007; Lomakin et al. 2007; Johansson et al. 2008; Gipson et al. 2010; Joppe et al. 2020; Singh et al. 2020). In *S. cerevisiae* FAS, the six α-subunit copies form a central wheel which divides the barrel equatorially, three β-subunits on each side of this equatorial divide form the two domes of the hexameric barrel (Fig. 1.8b). Thus, the αβ-heterodimer can be considered the building block of the yeast FAS hexamer. This has profound implications for the assembly of yeast FAS, which occurs co-translationally (Shiber et al. 2018). The ACP domain resides within the barrel, where all enzymatic sites are also facing, and is tethered by two anchors to the α-subunit. The PPT responsible for activating the ACP with the PPant arm (on Ser 180 of the α-subunit) is encoded on the C-terminal end of the α-subunit and resides on the outside of the central wheel in monomeric form in the assembled particle. However, for activity, the PPT must oligomerize (Johansson et al. 2009), therefore holo-ACP formation is thought to occur during assembly in yeast (Fischer et al. 2020). All enzymatic domains in yeast FAS are arranged in a manner that the active sites' cavities are opened to the inner cavities of the dome (Fig. 1.9b).

FA biosynthesis in yeast FAS starts with the priming of ACP to acetyl-ACP at the acetyltransferase (AT) domain of the β-subunit which is located at the top of the domes (Fig. 1.9b). The acetyl-ACP then transfers the acetyl group onto the active site cysteine of the α-subunit ketosynthase (KS) domain. The ACP is then primed to malonyl-ACP by the malonyl−/palmitoyl-transferase (MPT) domain, which is formed by both α- and β-subunits and might be another explanation for the co-translational assembly of yeast FAS (Shiber et al. 2018; Fischer et al. 2020). The malonyl-ACP condenses with the acetyl-KS to form β-ketoacyl-ACP, which then proceeds to the α-subunit encoded ketoreductase (KR) domain. The KR domain reduces the β-ketoacyl-ACP to β-hydroxyacyl-ACP using the reducing equivalents of NADPH. The β-subunit encoded dehydratase (DH) domain then removes water to generate α-enoylacyl-ACP. The α-enoylacyl-ACP is subsequently reduced by the $FMNH_2$ cofactor of the β-subunit encoded enoylreductase (ER) domain to the two-carbon elongated fatty acyl-ACP, which is then transferred onto the KS active site thiol. The FMN is then regenerated to the $FMNH_2$ by NADPH and the acyl-KS

can react yet again with a malonyl-primed-ACP. The cycle of condensation, ketoreduction, dehydration, and enoylreduction is repeated 6–7 times to generate the preferred C_{16}/C_{18}-fatty-acyl products of the yeast FAS. At this stage, the acyl-CoA is transferred to the MPT, where the fatty acyl product reacts with CoA and is released as a fatty acyl-CoA molecule.

This closed environment is specific to the fungal FAS and it allows for an estimated local ACP concentration of about 1 mM (Lynen et al. 1980; Maier et al. 2010), enables a rate of 18 FA biosynthetic cycles a minute (Fichtlscherer et al. 2000), and allows for strict regulation (Lynen et al. 1980; Maier et al. 2010; Gajewski et al. 2017a, b; Herbst et al. 2018). Some other yeasts, fungi, and most notably the Corynebacteria/Mycobacteria/Nocardia (CMN) bacteria have FAS organized as α_6-Type homohexamers where the αβ-heterodimer is fused to a single α-chain (Schweizer and Hofmann 2004; Ciccarelli et al. 2013; Elad et al. 2018). Type I systems are known to be more efficient and exhibit narrower product ranges than Type II Systems.

Phylogeny of FAS Architectures

As discussed in the earlier sections, FA biosynthesis is central in the eubacterial, protistic, plant, fungal, and animal kingdoms of life (López-Lara and Geiger 2010). Archaebacteria, on the other hand, are thought to be lacking a FA biosynthesis mechanism, or at least FAs appear not to be essential for them (Lombard et al. 2012). Viewed phylogenetically (Fig. 1.10), one can conclude that Type I systems most likely evolved from the concatenation of dissociated Type II FAS enzymes to multidomain multienzyme polypeptides. Type II FAS enzymes can be observed in eubacteria, as well as organelles of prokaryotic origin (mitochondria, chloroplasts, apicoplasts). In archaea, FA biosynthesis is not a central part of their metabolic cycle since they possess membranes composed of isoprenoids, instead of phospholipids. As a consequence, for a long while, archaebacteria were thought to be devoid of FA biosynthetic enzymes and possess no FAS. Nevertheless, recent observations indicate that they do indeed utilize FAs. These are attached to either their proteins as post-translational modifications, or are found in membranes. Moreover, archaea appear to encode for FA biosynthetic enzymes that are of Type II origin, suggesting the existence of FAS in several archaeal species (Lombard et al. 2012). However, the significance of these findings is yet to be determined.

When considering the eukaryota branch, one significant event is the endosymbiosis of an unknown alpha-proteobacterium which evolved into today's mitochondria. It has been observed that mitochondria still possess an FA biosynthesis system reminiscent of the bacterial Type II FAS. This system is proposed to be involved in mitochondrial membrane remodeling but also production of certain metabolic intermediates, such as lipoic acid (Kastaniotis et al. 2017). The second endosymbiosis event that led to the incorporation of plastid-like organelles encompasses the photosynthetic eukaryotes, and other protists of the TSAR superphylum. Similar to the

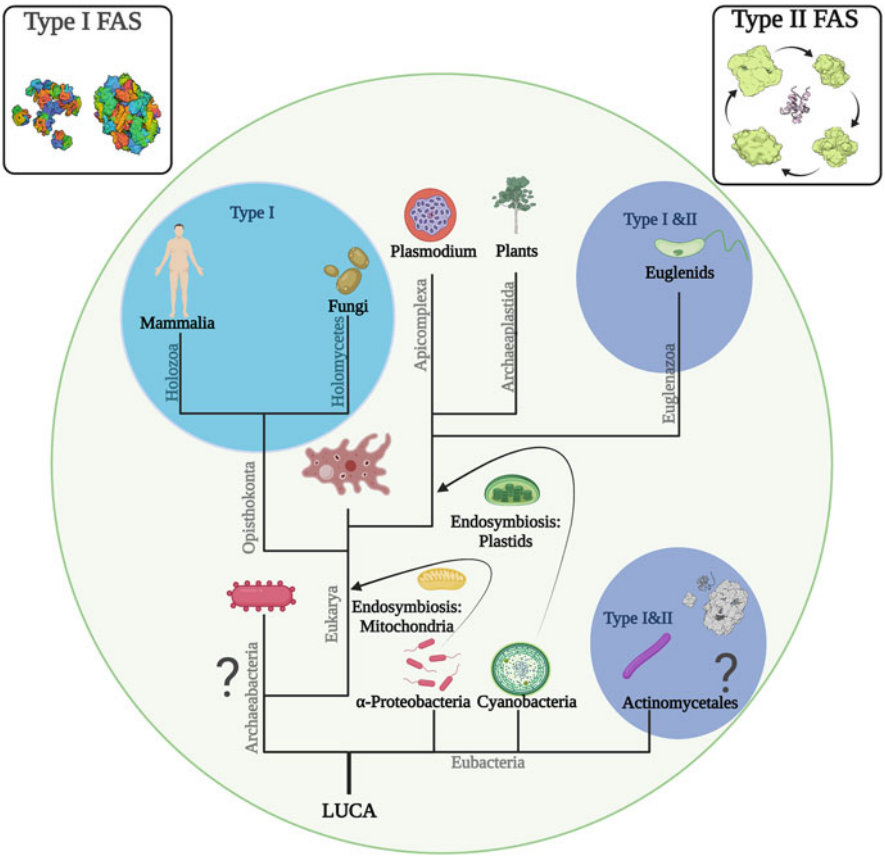

Fig. 1.10 Phylogeny of FAS architectures. Dissociated Type II enzymes appear to be the early forms of FA biosynthetic enzymes, whereas the multimeric Type I enzymes emerge later in the phylogenetic tree. Type II enzymes are widely found in all genera of life. In Archaea, FAS enzymes are not essential components of metabolism, since their membranes are mostly composed of isoprenoid variants. Yet, archaea possess Type II FAS enzymes since it has been observed to be important for certain metabolic processes. As a consequence of their endosymbiotic origin, mitochondria and plastids also possess Type II enzymes, like their prokaryotic ancestors. For plants, FA biosynthesis mainly occurs in chloroplasts. For various species within the SAR superphylum, different plastid Types exist for different metabolic processes. For example, Apicomplexan species such as Plasmodium falciparum and Toxoplasma gondii possess plastid organelles that are called apicoplasts, which are important for certain stages in their life cycle. Type I enzymes have been observed to occur within opisthokonts such as holozoa and holomycota. In certain situations, different branches have separately evolved both systems within their metabolism, because of their atypical fatty acid derivatives. The CMN bacteria possess both Type I and Type II systems to produce their very long and branched mycolic acids, aided by a polyketide synthase pks13 in mycobacterium tuberculosis. Poly-unsaturated fatty acid producing organisms can have Type I, II, and PKS-like enzymes to continue the process

situation in mitochondria, the endosymbiont also brought their own bacterial-Type Type II FAS systems which have remained present in these plastids. For plants, both carbon fixation and electron coupling *via* photolysis happen in the chloroplast, which also makes it the primary organelle where energy and carbon source surplus can occur (Ohlrogge and Jaworski 1997). This is in accordance with the fact that FA synthesis takes place in the chloroplasts of such organisms (Ohlrogge and Jaworski 1997).

Under the TSAR superphylum, a group called apicomplexans, which also includes toxoplasma and plasmodium species, are known to possess plastid organelles that are called as apicoplastids. These organelles do not perform photosynthesis, but they are responsible for important metabolic functions such as fatty acid synthesis. For plasmodium species, which is the causative agent of malaria, it is known that this fatty acid synthesis pathway is crucial for the sporozoite to merozoite transformation in the liver (Shears et al. 2015). This switch in the life cycle of plasmodium is well known to be a key event leading to the acute stages of malarial disease (Phillips et al. 2017), also being important for immune recognition (Mazier et al. 1990). For Plasmodium falciparum, it was later observed that the Type II FAS is rather more important for the sporozoite development in the midgut of mosquitos (van Schaijk et al. 2014). Consequently, the plasmodium FAS is a validated and valuable target for anti-malarial drugs.

Type I systems appear independently in several phyla. Under the phylum Unikonta, under which animal and fungal species are found, we observe the appearance of Type I systems. As mentioned earlier, the Type I architecture in animals is in an X-shaped homodimeric form, whereas in fungi, the Type I architecture is homohexameric where the building block is either a αβ-heterodimer or a single α chain. The single α chain homohexamer predominant in microbial FAS of some bacteria also is present in CMN bacteria, alongside with a Type II system (Marrakchi et al. 2014). CMN-bacteria are known to produce extremely long, dual-branched fatty acids that are termed mycolic acids, which are significant for their cell wall structures. In these organisms, the Type I system is described to exhibit a bi-modal product distribution of $C_{16/18}$-CoA and $C_{22\text{-}26}$-CoA (Marrakchi et al. 2014). The $C_{20\text{-}24}$-CoA comprise the FA branch of mycolic acids, the other mero-branch stems from the Type II system elongating C_{16}-CoA to $C_{42\text{-}62}$-CoA (Marrakchi et al. 2014). The FA branch is activated by a specialized ACC and fused with the mero branch in a specialized polyketide synthase pks13 (Marrakchi et al. 2014). This makes both Types of FAS in Mycobacterium tuberculosis targets of vital importance in antimycobacterial strategies (Bosch et al. 2021).

Regulation of FA Biosynthesis

Fatty acid synthesis is a crucial but cumbersome process. It needs a high energy-containing carbon source and ATP to synthesize its three co-substrates acetyl-CoA, malonyl-CoA, and NADPH. For the synthesis of a C_{16} FA, for example, cellular

metabolism has to produce the energy that is contained in 122 ATP molecules (Nelson and Cox 2013). As alluded to earlier, FAs in the form of triglycerides are compact sources for energy storage that occupy less physical space than the energetic equivalent in the form of carbohydrates. Therefore, when carbon sources are abundant, microorganisms tend to favor FA biosynthesis to build new membranes and store excess carbon. Metazoans more strongly rely on their diet to acquire FAs rather than de novo biosynthesis to produce their own (Burr and Brown 1933). Nevertheless, FA biosynthesis, which does not occur in all cells and tissues of a metazoan, does take place in some specialized cell types. These include adipocytes and hepatocytes that regulate blood sugar levels by taking up excess monosaccharides, and the lactating mammary gland (Lane and Moss 1971; Suburu et al. 2014).

The three co-substrates of FAS (acetyl-CoA, malonyl-CoA, and NADPH) are provided from different sources. Whenever there is a surplus of carbon sources, acetyl-CoA is obtained through glycolysis in the citric acid cycle by the pyruvate dehydrogenase complex which oxidatively decarboxylates pyruvate to produce acetyl-CoA (Perham 2000). The citric acid cycle also elevates the eponymous citrate concentrations which can be used by ATP-citrate lyase to produce acetyl-CoA (Verschueren et al. 2019; Wei et al. 2019; Granchi 2019). In low carbon flux, acetyl-CoA synthetase, alcohol dehydrogenase, and the degradation of branched-chain ketogenic amino acids such as valine, leucine, and isoleucine generate acetyl-CoA (López-Lara and Geiger 2010). Also at low carbon abundance, the degradation of FA energy stores induces the reversal of FA biosynthesis, the FA β-oxidation pathway also yields acetyl-CoA (Lopez-Lara and Soto 2018). Likewise, NADPH is linked to respiration and is primarily generated in the pentose-phosphate pathway, in the first step glucose-6-phosphate dehydrogenase. Other sources of NADPH include the Ferrodoxin-NADP+ reductase, which is a major source in photosynthetic organisms, and malic enzyme, isocitrate dehydrogenase, and glutamate dehydrogenase (Ohlrogge and Jaworski 1997; Lopez-Lara and Soto 2018). Both these processes therefore put FA biosynthesis in connection with metabolic flux. Malonyl-CoA, on the other hand, is not directly bound to metabolic flux. As shown in Figs. 1.2 and 1.4a, it is produced from acetyl-CoA by ACC, which also provides malonyl-CoA for polyketide synthesis. For this reason, the production of malonyl-CoA is considered the first and only committed step in FA biosynthesis (Wakil et al. 1983; Cronan and Waldrop 2002; Tong 2013).

Considering the intimate linkage of FA biosynthesis, and therefore FAS activity, to the metabolic state of the cell, two immediate consequences arise. First, FA biosynthesis is subject to feedback regulation from metabolic pathways and the ensuing flux. Second, were FAS activity to continue unhindered and unregulated when carbon sources are limiting, FA biosynthesis has the potential to jeopardize the survival of cells. It would deplete ATP, acetyl-CoA, and NADPH stores under conditions where already diminishing energy and carbon stores make these co-substrates sparse and withdraw them from processes vital for the subsistence of cells. This downward cycle would cause cells to starve and ultimately drive them to death. To circumvent this impending doom, FA biosynthesis is subject to feedback metabolic regulation cycles, including transcriptional feedback cycles and multiple

layers of biochemical regulation (Lust and Lynen 1968; Wakil et al. 1983; Schweizer and Hofmann 2004).

Genetic regulatory pathways of FA biosynthesis pathways have been elucidated over the years *via* microbiological, biochemical and genetic methods (Parsons and Rock 2013). In *E. coli*, for instance, the first global regulatory system uncovered was related to the abundance of the global long-chain acyl-ACP and long-chain acyl-CoA pool, which is low under normal homeostatic conditions. However, when, for example, phospholipid biosynthesis is compromised by either poor activity of the glycerolphosphate acyltransferase PlsB system or the stringent response following starvation, this pool is elevated. The effector of the stringent response appears to be the alarmone ppGpp, which inhibits PlsB. There also exists a complex transcriptional regulation of FA biosynthesis through the transcription factors FadR and FabR (Lopez-Lara and Soto 2018; Gago et al. 2019). FadR is tightly bound to DNA under conditions of low long-chain acyl-CoA concentrations and represses transcription from the genes of FA β-oxidation pathway (Cronan John Jr and Subrahmanyam 1998). On the other hand, under low long-chain acyl-CoA concentrations, it enhances transcription from several FA biosynthetic genes, including fabA, fabB, the fabHDG operon, fabF, fabI, acpP, and the accA-D genes. Under high long-chain acyl-CoA concentrations, it dissociates from DNA, eliciting the opposite transcriptional response. FabR acts through a different mechanism, unsaturated long-chain acyl-CoAs or unsaturated long-chain acyl-ACPs promote binding to DNA, shutting of the transcription of fabA/fabB genes (Zhu et al. 2009). Binding of either saturated long-chain acyl-CoAs or saturated long-chain acyl-ACPs to FabR elicit dissociation from DNA and switch on the transcription of fabA and fabB genes. In the yeast *S. cerevisiae*, it has been shown that the expression of FAS and ACC is regulated to FA and inositol supplementation (Schüller et al. 1992; Chirala 1992; Chirala et al. 1994). It also requires the INO2 and INO4 proteins which stimulate transcription, and the effects of FA and inositol are cumulative (Schüller et al. 1992; Chirala et al. 1994). In mammalian cells, sterol regulatory element-binding proteins (SREBPs) play a role in the regulation of FAS, ACC, and stearoyl-CoA desaturase gene expression (Bennett et al. 1995; Lopez et al. 1996; Kim and Spiegelman 1996; Tabor et al. 1999). ACC in mammals is also inhibited by glucagon and epinephrine, while insulin activates the enzyme through transcription regulatory pathways (Witters et al. 1988; Mabrouk et al. 1990; Andreolas et al. 2002).

In addition to genetic regulation, biochemical regulatory processes are in play with regard to FA biosynthesis. Acetyl-CoA synthesis, generated by the oxidative decarboxylation of glycolytic pyruvate, drives fatty acid synthesis (Nelson and Cox 2013). The same acetyl-CoA also promotes the flux through the citric acid cycle. In eukaryotes, the enzymes of the citric acid cycle are located in the mitochondrion and, when citrate levels are high, they inhibit the pyruvate dehydrogenase and the succinate dehydrogenase complexes (Iacobazzi and Infantino 2014). Superfluous citrate is also exported back into the cytosol, where it negatively impacts several glycolytic enzymes, but positively regulates fructose-1,6-bisphosphatase, and ACC in mammals (Nelson and Cox 2013; Iacobazzi and Infantino 2014). The positive effector function of citrate promotes polymerization of mammalian ACC, and

therefore the production of malonyl-CoA (Hunkeler et al. 2018). It, however, appears not to impact yeast ACC (Wei et al. 2016) and in bacterial ACC, where cellular compartmentalization does not exist (Nelson and Cox 2013). However, as mentioned earlier, ATP-citrate lyase is cytoplasmic and can break down citrate to oxaloacetate and acetyl-CoA (Verschueren et al. 2019; Wei et al. 2019; Granchi 2019). Thus, either by the elevated production of malonyl-CoA and/or acetyl-CoA, citrate exerts a positive effect on FA biosynthesis. When carbon sources are low, AMP is generated and activates AMP kinase, which, in turn, universally phosphorylates ACC inhibiting malonyl-CoA production (Wakil et al. 1983; Wei et al. 2016). Another, universal biochemical regulatory process lies in the products of FA biosynthesis, long-chain fatty acids, long-chain fatty-acyl-CoAs, and long-chain acyl-ACPs, exerting feedback inhibition on ACC and FAS (Wakil et al. 1983; Tong 2013; Hunkeler et al. 2018).

Direct Regulation of ACP

The regulatory pathways described in the previous section were indirectly affecting the FA biosynthesis pathway, but no regulatory cues impacting FAS directly were mentioned. Another layer of biochemical regulation relates to the fact that one has to consider the ACP together with its acylated PPant arm as the true substrate for FAS. It is worth mentioning that bacterial and metazoan ACPs are composed of a four-helix bundle (termed the canonical domain), which is extended in fungal FAS by a structural four helix domain (Fig. 1.11). Considering the central pivotal role of the ACP, one could conceive it also to be a central point of regulation. In a sense, it has to fulfill the role of a universal recognition platform for all FAS active site (Chan and Vogel 2010; Beld et al. 2015; Finzel et al. 2015). On the other hand, it also has to protect its growing hydrophobic cargo from the aqueous environment of the cell

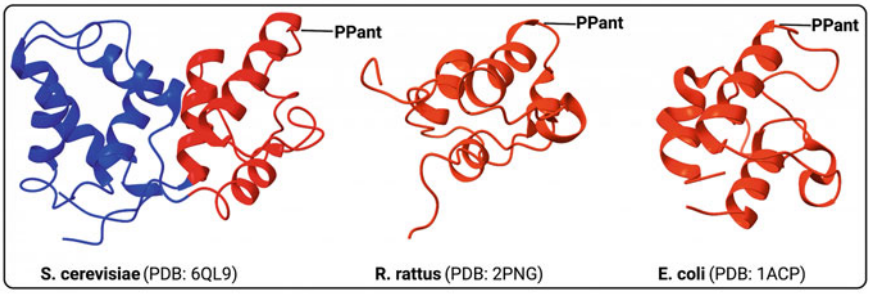

Fig. 1.11 ACP structural conservation. Shown are ACP structures from the Type I yeast FAS (left, PDB-ID: 6QL9), the Type I mammalian FAS (middle, PDB-ID: 2PNG), and the Type II bacterial FAS (right, PDB-ID: 1ACP). Colored in red is the canonical domain of ACPs, which is a four-helix bundle and conserved in all kingdoms of life. The fungal Type I FAS structure contains a four-helix extension called the structural domain (colored in blue)

(Chan and Vogel 2010; Beld et al. 2015; Finzel et al. 2015). To achieve this, a "switchblade" mechanism involving its PPant arm comes into play (Chan and Vogel 2010; Beld et al. 2015; Finzel et al. 2015).

Considering the essential nature of ACP in substrate shuttling and recognition, it is surprising that very little structural knowledge has been gained about its recognition by FAS. Most ACP–FAS interactions seem to be centered around the PPant arm and involve electrostatic interactions (Leibundgut et al. 2007; Lomakin et al. 2007; Gipson et al. 2010; Nguyen et al. 2014; Lou et al. 2019; Lou and Mazhab-Jafari 2020; Mindrebo et al. 2020; Singh et al. 2020). However, if this universally applies to all ACP–FAS enzyme interactions remains to be seen. At least in the yeast Type I FAS, the additional structural domain of the ACP appears to be involved in interactions to ER, KS, DH, and AT domains (Leibundgut et al. 2007; Lomakin et al. 2007; Gipson et al. 2010; Lou et al. 2019; Lou and Mazhab-Jafari 2020; Singh et al. 2020). These interactions involve the structural domain of the ACP and architectural domains of the Type I yeast FAS. To better understand the regulation of FAS activity by ACP regulation, clearly more structures of ACP bound to each FAS enzyme-active site cleft are needed. Ideally, these structures also are of sufficiently high-resolution that the molecular basis of ACP and PPant arm recognition can be deciphered. This raises the issue of biochemically trapping FAS such that homogenous preparations of FA biosynthetic intermediate-ACP adducts can be obtained in preparative amounts. Activity-based probes, based on CoA derivatives as proposed by Burkart and co-workers and employed for Type II FAS systems, appear to be pre-destined for this purpose (Nguyen et al. 2014; Beld et al. 2015; Finzel et al. 2015; Mindrebo et al. 2020).

The importance of ACP regulation is exemplified by the fact that overexpression of apo-ACP is toxic to bacteria (Keating et al. 1995). Thus, PPT activity has to occur faithfully and spatially and makes this group of enzymes crucial for FA biosynthesis and, conversely, valuable drug targets. This is highlighted by recent findings that a mycobacterial PPT, PptT protein, has recently been validated as a drug target and an inhibitory compound has been discovered (Ballinger et al. 2019). Aside from PPT, as shown in Fig. 1.7, holo-ACP hydrolases exist that are able to generate apo-ACP by removing the PPant arm. The *E. coli* enzyme is termed AcpH, and notably the mycobacterial counterpart confers resistance to PptT inhibitors (Ballinger et al. 2019). In mycobacteria, where Type I and Type II FAS systems co-exist, there is a special Type II found called as AcpM that contains an additional C-terminal domain that is unfolded (Wong et al. 2002). What the function of this domain is remains enigmatic until today. Two hypotheses exist: one suggests that this domain helps to carry the longer fatty acid chains that are synthesized by mycobacteria, the second is that this domain mediates the interaction of AcpM with cognate enzymes (Wong et al. 2002). Considering the importance of ACP in FAS, and its modifying enzymes emerging as drug targets, more detailed mechanistic studies into ACP function are warranted.