# 8.07 Fatty Acid Biosynthesis and Oxidation

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## 8.07.1 Introduction

This chapter focuses on the catalytic transformations that result in the cyclic biosynthesis and breakdown of fatty acids. These metabolic pathways will serve as a paradigm for three classes of chemical reactions: carbon–carbon bond formation and cleavage, oxidation and reduction, and hydration–dehydration. The most extensively studied reactions are those involved in microbial fatty acid biosynthesis (Type II fatty acid synthase (FAS-II)) and mammalian fatty acid $\beta$-oxidation. In both pathways, the reactions are catalyzed by separate enzymes that have been cloned and overexpressed, thus providing a ready source of material for structural and mechanistic studies. In contrast, mammalian fatty acid biosynthesis and microbial fatty acid breakdown are catalyzed by multifunctional enzymes (MFEs) that have historically been less amenable to analysis.

The cyclic series of reactions that result in the synthesis and breakdown of fatty acids are shown in **Figure 1**. Fatty acid biosynthesis, exemplified by the pathway from *Escherichia coli*, is initiated by the condensation of malonyl-acyl carrier protein (ACP) with acetyl-CoA by the $\beta$-ketoacyl-ACP synthase (KAS) FabH to generate $\beta$-ketobutyryl-ACP. The $\beta$-keto group is subsequently reduced by the NADPH-dependent $\beta$-ketoacyl-ACP reductase (KAR) FabG followed by the elimination of $H_2O$ catalyzed by FabZ or FabA. The crotonyl-ACP that is formed is reduced by the NADH-dependent FabI and subsequent rounds of elongation are initiated by the action of the KASs FabD and FabF. Enzymes in the fatty acid $\beta$-oxidation pathway catalyze the reverse reactions, a major difference being that the acyl groups are carried by CoA rather than ACP. Fatty acid breakdown is initiated by the action of the flavoenzyme acyl-CoA dehydrogenase (ACD), which oxidizes saturated acyl-CoAs to enoyl-CoAs. This reaction is followed by a hydration, catalyzed by enoyl-CoA hydratase (ECH), followed by a second oxidation, catalyzed by $\beta$-hydroxyacyl-CoA dehydrogenase (HAD), to generate a $\beta$-ketoacyl-CoA. The final step in the pathway is catalyzed by thiolase and involves carbon–carbon bond cleavage leading to the formation of acetyl-CoA and a saturated acyl-CoA that is two carbon atoms shorter.

In discussions on the mechanisms of the enzymes involved in each pathway, there will be a particular focus on three superfamilies: enzymes that share the thiolase fold and catalyze carbon–carbon bond formation and cleavage; reactions catalyzed by NAD(P)-dependent enzymes in the fatty acid biosynthesis pathway involve proteins that are members of the short-chain dehydrogenase reductase (SDR) superfamily; and finally there are mechanistic parallels between the hydration and dehydration reactions in each pathway with a particular focus on the crotonase superfamily.

## 8.07.2   Thiolase Enzyme Homologs

### 8.07.2.1   Fatty Acid Biosynthesis: Carbon–Carbon Bond Formation

Carbon–carbon bond formation is a reaction of fundamental importance to the cellular metabolism of all living systems and includes alkylation reactions involving one and five carbon fragments as well as carboxylation reactions. In addition, a very common method of generating carbon–carbon bonds in biology includes the reactions of enolates and their equivalents (such as enamines) with aldehydes, ketones, keto acids, and esters. Reactions in which the enolate derives from an acyl thioester are Claisen condensations, whereas the remainder are classified as aldol reactions.

### 8.07.2.2   Claisen Condensation Reactions and the Thiolase Superfamily

Claisen condensation reactions are performed by enzymes that are members of the thiolase superfamily based on a three-dimensional fold first characterized in a degradative thiolase from *Saccharomyces cerevisiae*.[1,2] These enzymes primarily form dimers, with each subunit sharing a common superfamily topology,[3] whereas tetramers are observed only in the biosynthetic thiolase subfamily.[4] The monomer is composed of three domains, two core domains, each consisting of a mixed five-stranded $\beta$-sheet covered on each face by $\alpha$-helices, and a loop domain. The two core domains pack together so that the overall fold of the monomer is a five-layered $\alpha\beta\alpha\beta\alpha$ structure. This is shown in **Figure 2(a)** for the KAS II enzyme from *Escherichia coli* (ecFabF). All members of the family have at least one catalytically essential cysteine that becomes covalently modified during the reaction. This cysteine is in the N-terminal domain and lies at the N-terminus of an $\alpha$-helix (**Figures 2(b)–2(d)**), whereas all other catalytic residues are normally contained within the C-terminal domain.

Members of the superfamily may be subdivided based on either function or structural homology and play critical roles in polyketide and isoprenoid biosyntheses, fatty acid biosynthesis, and fatty acid degradation. Wierenga and coworkers[4] have discussed structural features that group thiolase family members into three subfamilies. In the first group are KAS I and KAS II, which initiate each cycle of elongation in fatty acid biosynthesis by condensing a C2 carbon unit derived from malonyl-ACP with the growing fatty acid (**Figure 3**). In addition to the catalytic cysteine, the two other conserved active site residues in the KAS I and KAS II enzymes are the histidine residues (**Figure 2(c)**).

Although most bacteria possess only one elongating KAS, which is generally more similar to KAS II than to KAS I, organisms such as *E. coli* and *Mycobacterium tuberculosis* possess two elongating KAS enzymes. In *E. coli* the
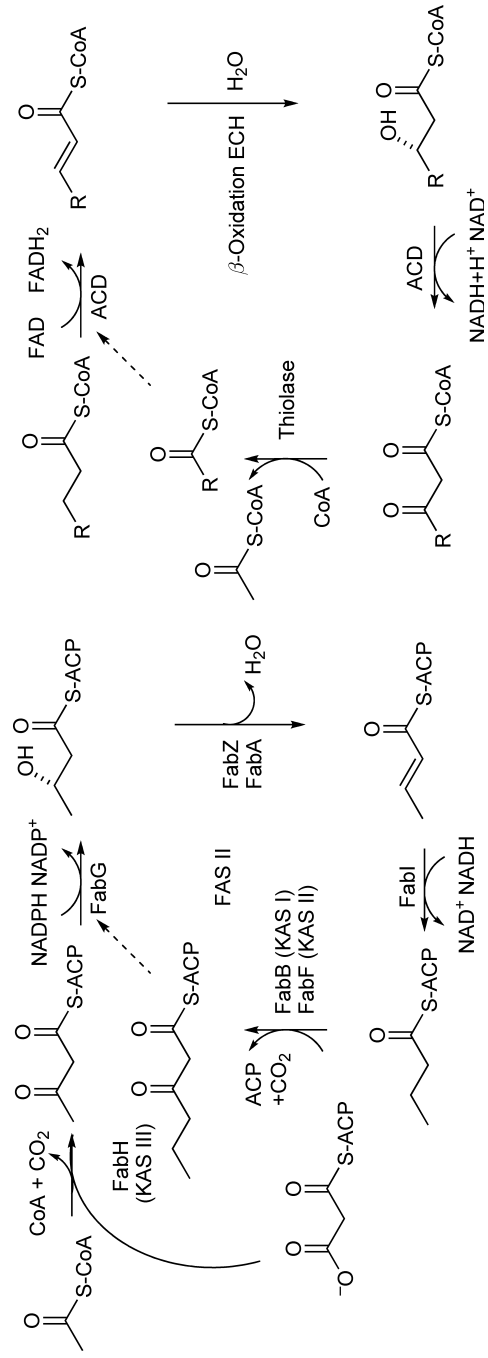
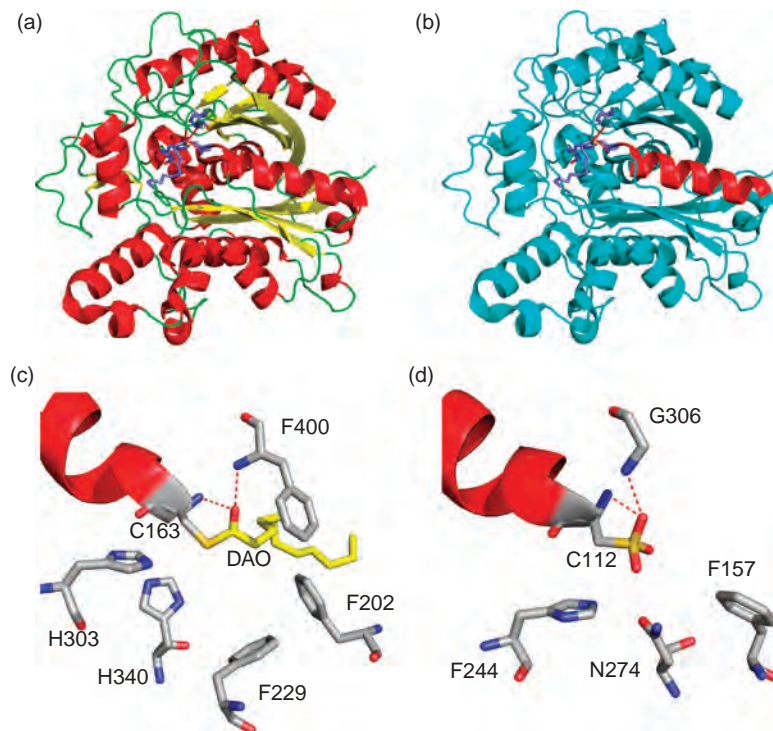**Figure 1** Fatty acid biosynthesis and oxidation.

**Figure 2** Structures of ecFabF (KAS II) and ecFabH (KAS III). (a) Structure of the ecFabF monomer showing the classic thiolase fold (helices, red; sheets, yellow; loops, green). (b) Structure of the ecFabF monomer (cyan) showing the active site $\alpha$-helix (red). (c) Active site of ecFabF showing the catalytic residues and oxyanion hole. The catalytic cysteine (C163) is acylated with a $C_{12}$ fatty acid (DAO, yellow) and lies at the N-terminus of an $\alpha$-helix. (d) Active site of ecFabH showing the catalytic residues and oxyanion hole. Note that C112 in ecFabH has been oxidized to a sulfonic acid. The figures were made using PyMOL[37] and the structures of ecFabF from 2gfy.pdb[26] and ecFabH from 1mzs.pdb.[28]

KAS I and KAS II enzymes are FabB and FabF, whereas in *M. tuberculosis* they are KasA and KasB. FabB is essential for cell viability and required for elongating saturated fatty acids, whereas FabF is nonessential and widely held as the enzyme required for the temperature-dependent regulation of fatty acid biosynthesis.[5–7] In *M. tuberculosis*, KasA and KasB have overlapping substrate specificity with KasB possibly being responsible for the synthesis of very long-chain fatty acids (C54). Both KasA and KasB are important for the growth of *M. tuberculosis in vivo.*[8–10]

The second group contains the Type III KAS, which is the initiating enzyme for fatty acid biosynthesis, as well as the bacterial and plant Type III polyketide synthase (PKS III), such as chalcone synthase and stilbene synthase. 3-Hydroxy-3-methylglutaryl (HMG)-CoA synthase also falls into this category, in which all enzymes have a catalytic triad of Cys-His-Asn (**Figure 2(d)**). The final group of enzymes is the biosynthetic and degradative thiolases that have a Cys-Asn-His catalytic triad together with a second cysteine in the active site.

## 8.07.2.3 Mechanistic Challenges: KAS I–KAS III

The mechanistic challenges faced by the thiolase enzymes include acyl transfer to the active site cysteine, stabilization of oxyanion transition states during each step of the reaction, and generation of the required carbanion nucleophile in the second substrate. In each case the catalytic cysteine lies at the N-terminus of an $\alpha$-helix (**Figure 2(b)**), and, by analogy to proposals advanced in the discussions of enzyme catalysis and protein structure,[11–16] it has been suggested that the helix dipole plays a central role in increasing the nucleophilicity of the cysteine and also providing assistance via transition state stabilization.[17] For enzymes such as KAS I–KAS III that utilize malonyl-ACP as the second substrate, an acetyl-ACP carbanion must be generated by