Molecular Machines

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The Fatty Acid Factory of Yeasts

*Thomas Kolter**

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In the cytoplasm of yeasts, fungi, and animals, fatty acids are synthesized by very large protein complexes. In two recent publications, the closely related X-ray structures of the 2.6-megadalton fatty acids synthases (FAS) of the fungus *Thermomyces lanuginosus*[1] and of yeast[2] were determined with a 3.1-Å resolution. A 4-Å structure of the yeast enzyme gave additional insights.[3] The structures allow a deeper understanding of how these giant molecular factories work.

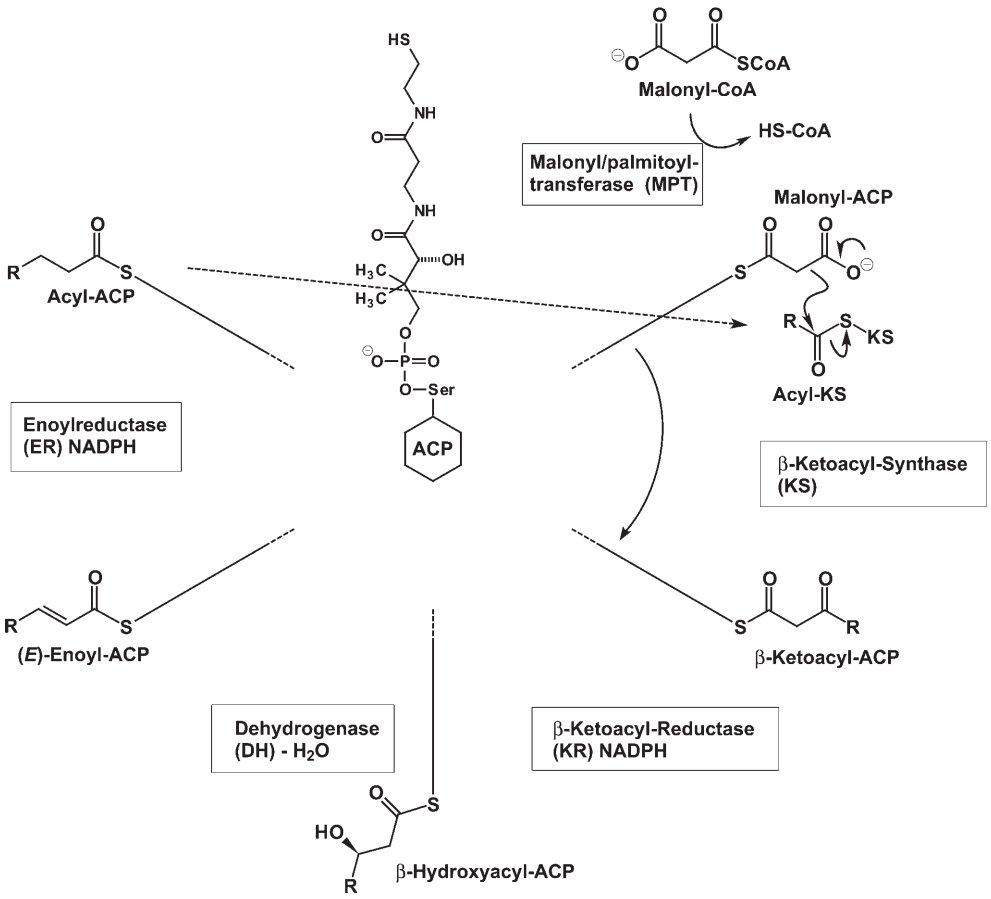
Fatty acids are essential metabolites for all organisms with the exception of Archaea. They serve as structural components of membrane lipids, as the major storage form of metabolic energy, and as components of posttranslationally modified proteins. Although different organisms use the same biosynthetic reaction sequence for the biosynthesis of fatty acids, the structural organization of the enzyme activities is entirely different: The type I fatty acid synthases (FAS I) in the cytoplasm of yeast, fungi, and animals, but also in certain bacteria like corynebacteria and mycobacteria, are megasynthases in which the enzymatic activities are confined to only one or two polypeptide chains. In prokaryotes, plants, but also in mitochondria of animal cells, the different reactions leading to fatty acid formation are catalyzed by independent enzymes that belong to the so-called type II fatty acid synthase system (FAS II).[4] Also the formation of very long chain fatty acids by elongation of medium-chain-length precursors is catalyzed by individual enzymes that are localized in the endoplasmic reticulum, mitochondria, and peroxisomes.

The architecture of FAS I from eukaryotes is entirely different between animals and fungi.[5–8] While the mammalian enzyme is an X-shaped homodimer, encoded by one gene, and with a molecular weight of 540 kDa, the biosynthetic machine of yeast and fungi forms a hexamer of two different polypeptide chains with the stoichiometry $\alpha_6\beta_6$. It is a giant, barrel-shaped factory with a molecular weight of 2.6 megadaltons. Animal and fungal type I synthases have been analyzed for many years,[9,10] but it took until 2006 that the X-ray structures could be refined to 5 Å by the Ban group,[5,6] which allowed a detailed comparison between the animal and the fungal/yeast enzyme.[7,8]

In the cytoplasm of yeast and fungi, fatty acid biosynthesis requires two enzymes: malonyl-CoA synthase and the fatty acid synthase complex, which comprises eight different enzyme activities (Scheme 1). The synthesis of the coenzyme A derivative of palmitic acid, the major product of the synthase, requires 8 mol of acetyl-CoA, from which 7 mol have to be carboxylated to malonyl-CoA before they are utilized by the enzyme. During the synthesis of palmitoyl-CoA, 14 mol of NADPH are consumed for the reduction steps.

FAS I uses a limited number of active sites in an iterative way and works on substrates of increasing chain lengths. Before the fatty acid synthesis can start, a phosphopantetheinyl transferase (PPT) is required for the posttranslational addition of the 18-Å phosphopantetheine arm from coenzyme A to the acyl carrier protein (ACP). In fungi (and yeast), this activity is localized at the C terminus of the α subunit, while in humans it is localized on an individual enzyme.[11] The ACP domain shuttles the thioester-bound reaction intermediates between the different active sites. The reaction cycle starts with the priming reaction, the transfer of an acetyl residue from acetyl-CoA on the thiol group of the ACP arm. In yeast and fungi, this reaction is catalyzed by acetyl transferase (AT). The acetyl residue is then transferred onto a cysteine side chain of the β-ketoacyl synthase (KS) domain. Subsequently, the ACP moves to the malonyl/palmitoyl transferase (MPT) domain, receives a malonyl residue from malonyl-CoA, and shuttles it back to the KS domain. KS, an enzyme domain of the thiolase superfamily,[12] catalyzes the C–C bond-forming step, a decarboxylating Claisen reaction.[13] After decarboxylation, the ACP-bound thioester enolate displaces KS from the acetyl residue. The resulting β-ketoacyl residue is bound to the arm. After a sequence of three steps—the NADPH-dependent reduction of the ketone (β-ketoacyl reductase, KR), dehydratation (dehydrogenase, DH), and NADPH-dependent reduction of the resulting double bond (enoyl reductase, ER)—the ACP-bound acyl residue is ready to be further elongated by repetition of the reaction sequence. It is transferred from ACP to the KS-cysteine and condensed with malonyl-CoA, and the elongation cycle continues until a chain length of 16 carbon atoms is reached. The palmitoyl residue is then transferred onto coenzyme A by the MPT subunit of the fungal enzyme, while the animal enzyme releases the free fatty acid by thioesterase-

[*] Priv.-Doz. Dr. T. Kolter
LiMES—Life and Medical Sciences
Program Unit Membrane Biology and Lipid Biochemistry
Universität Bonn
Gerhard-Domagk-Strasse 1, 53121 Bonn (Germany)
Fax: (+49) 228-73-7778
E-mail: tkolter@uni-bonn.de
Homepage: http://www.uni-bonn.de/~tkolter



Scheme 1. Reactions catalyzed by fatty acid synthase (FAS) from fungi and yeast; activation of ACP by PPT is not shown.

mediated hydrolytic cleavage of palmitoyl-ACP. Other differences between animal and yeast FAS I are that in the priming reaction in animals, acetyl CoA and malonyl-CoA are transferred onto ACP by the same enzyme domain, malonyl-CoA/acetyl-CoA-ACP transacylase, and that the PPT unit is not part of the animal polypeptide chain.

The fungal FAS I factory contains four functional domains per subunit: ACP, KR, KS, and PPT on the α chain, and AT, ER, DH, and MPT on the β chain. Each chain occurs six times in the complex, so that 48 active sites are present. With the exception of ACP and PPT, the domains have been mapped before to the three-dimensional protein structure by cryo-electron microscopy, biochemical data, and lower-resolution X-ray data.

The X-ray structure of FAS I from *Thermomyces lanuginosus* was obtained at 3.1-Å resolution.[1] The knowledge of the structures of the individual bacterial FAS II proteins allowed the interpretation of the electron-density maps. The crystal with space group $P2_1$ had a unit cell of dimensions $216 \times 414 \times 222$ Å^3, a solvent content of 66%, and one FAS molecule per unit cell. 21127 amino acid residues (89%) were resolved, along with six flavin mononucleotides and, in the case of a second, $NADP^+$-soaked crystal, 12 $NADP^+$ molecules in the active sites of KR and ER. The protein has a barrel-like structure of dimensions 270×250 Å. The structure can be regarded as being composed of a central wheel of D_3 symmetry, built up by the six α subunits, and two C_3-symmetric caps, above and below the wheel, that consist of three β subunits each, but also with contributions (3×94 amino acids) from the N terminus of three α chains (Figure 1).[1] The reaction chambers above and below the wheel are accessible by openings from the outside, something that has been seen before in another molecular machine, the pyruvate dehydrogenase complex. The ACP domain, linked to the cap and wheel by flexible linkers, is not visible in the structure. Electron density attributable to time-averaged positions of ACP domains has been obtained by cryoelectron microscopy at 18 Å,[1] but a model of how this domain moves during catalysis had to be derived from the relative positions of the ACP anchor points and those of the active sites. Also the PPT domain is not visible in the structure.

The structure of the yeast synthase revealed information about the ACP, which could not be resolved in the fungal structure.[2] The structure was solved by molecular replacement with the coordinates of the *T. lanuginosus* enzyme. The yeast protein crystallized in space group $P4_12_12$ and had unit-cell dimensions of $231 \times 231 \times 784$ Å^3. Although they crystallized in different space groups, both structures showed only minor differences. A remarkable difference is that the ACP, which was disordered in the fungal structure, was stalled to the KS domain of the yeast protein. In contrast to the lower-resolution structure published later,[3] the phosphopante-

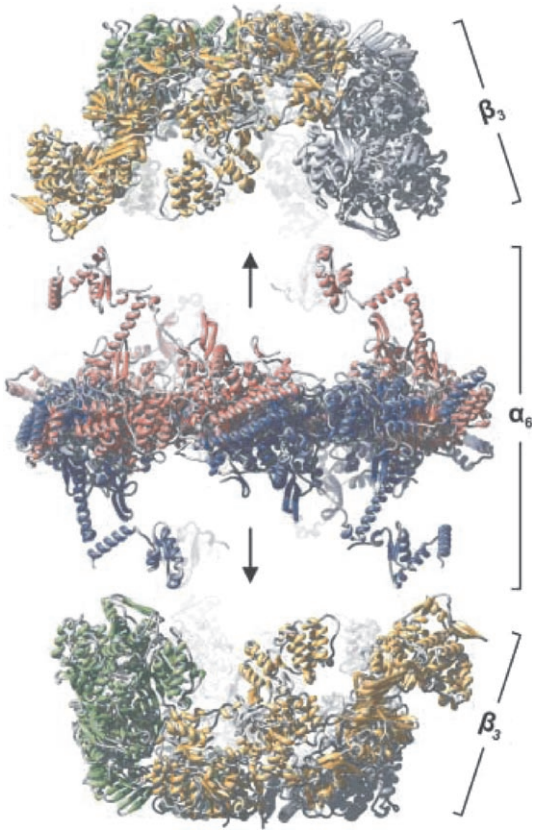


Figure 1. Side view of the quaternary structure and subunit distribution of fungal FAS I.[1] Central wheel and caps have been pulled apart for clarity. The α-subunits are given in blue and pink, the β-subunits in green, brown, and gray.

theine arm was posttranslationally attached to the ACP. The phosphate and pantoic acid moiety of the arm (not the terminal cysteamine and β-alanine part) were resolved. Modeling of the missing part gave a structure in which the arm extends into the catalytic cleft of KS and ends near the cysteine residue of the catalytic CHH triad. This arm, which shuttles the growing acyl chain between the active sites, presumably has a higher affinity for KS, where it was localized, at least under the crystallization conditions. The authors suggest a switch-blade mechanism, by which the arm loaded with the substrate moves from a hydrophobic area of ACP to the active site of KS.

The 4-Å structure of FAS I from yeast[3] was derived from crystals that were obtained by accident when the authors tried to crystallize the yeast 40S ribosomal subunit. In this structure, both the ACP and PPT domains could be assigned to the electron-density maps. Two crystal forms were analyzed, which had space groups $P2_1$ and $P4_32_12$. Ninefold noncrystallographic averaging allowed the development of a model that contained the backbones of 1687 out of 1887 α-subunit amino acids, all of the β subunit, and about 50% of the side chains. According to this model, the priming, elongation, and termination reactions take place in the six reaction chambers, three above and three below the central wheel. Each chamber contains seven catalytic centers, from two α and two β subunits. Again, the ACP was found to be bound to the KS domain. The PPT domain, however, is located outside the particle. Since it is inaccessible for ACP, this domain appears to be in an inactive conformation, and attachment of the phosphopantetheine arm might have to occur prior to assembly of the complex.

What can we learn from these structures? One question is how substrate delivery to the different active sites can be achieved by ACP. According to a fascinating model that was suggested by the authors,[3] after AT-mediated acetyl transfer to ACP at the ceiling of the reaction chamber, ACP lowers its position, thereby shuttling the substrates to the active sites in the direction KS→MPT→KS→KR→DH→ER. Docking to the active sites is mediated by electrostatic interactions between complementary-charged patches on ACP and in the surroundings of the catalytic sites.[1–3] The AT active site is narrower than that of MPT and lacks a positively charged residue; both features explain the preferential use of acetyl-CoA over malonyl-CoA by this domain in the priming reaction. Another question that has been addressed by the authors is how chain elongation is terminated on the stage of chain lengths of 16–18 carbon atoms in the case of ACP-bound palmitoyl or stearoyl residues, which are then transferred onto coenzyme A. The structures show a hydrophobic cavity of around 24 Å in length near the MPT termination site (Figure 2). Stable association of the substrate to this site should require a critical minimal chain length before an acyl transfer on coenzyme A can occur. As proposed more than 30 years ago,[14] the length of the product is determined by comparison of the alkyl chain with a measure distance given by the hydrophic cleft.

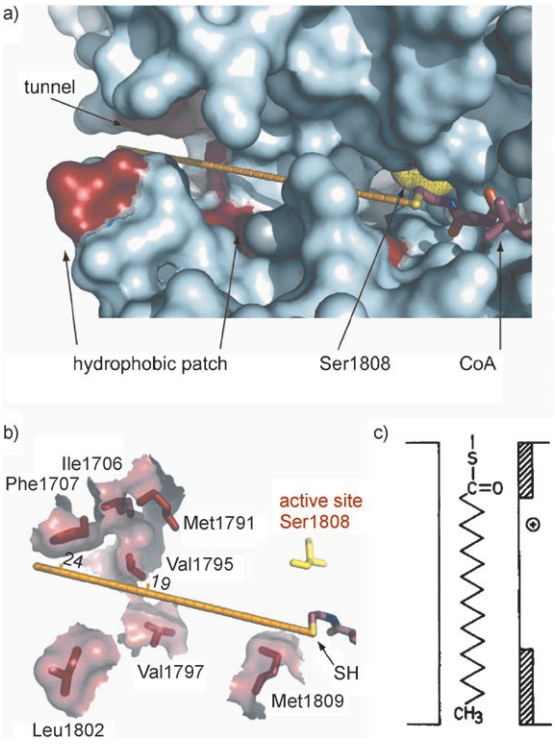


Figure 2. a) MPT palmitoyl termination site and b) hydrophobic patches. The orange rod illustrates the location of the tunnel; distances from the SH group of CoA are given in Å.[3] c) Model for this site including the hydrophobic patches (shaded) proposed by Lynen.[10]

Moreover, the access of malonyl-CoA to the MPT site is hindered in the presence of the long-chain coenzyme A. This hindrance means that the free-swinging arm can only be loaded with acetyl-CoA on the AT site, and not with malonyl-CoA, which synchronizes termination of one reaction cycle with priming of the next one.

The structures are not only amazing examples for a biocatalyst, but structural differences between the animal and fungal FAS I ER domains might be used for the development of antifungal drugs. The contributions[1–3] provide high-resolution pictures of one of the machines that catalyze multistep reactions with the aid of swinging arms or domains,[15] such as pyruvate dehydrogenase, polyketide synthases, and nonribosomal peptide synthetases.

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