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Review

Extending the story of very-long-chain fatty acid elongation

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ABSTRACT

Very-long-chain fatty acids (VLCFAs) are essential molecules produced by all plant cells, and are components or precursors of numerous specialized metabolites synthesized in specific cell types. VLCFAs are elongated by an endoplasmic reticulum-localized fatty acid elongation complex of four core enzymes, which sequentially add two carbon units to a growing acyl chain. Identification and characterization of these enzymes in *Arabidopsis thaliana* has revealed that three of the four enzymes act as generalists, contributing to all metabolic pathways that require VLCFAs. A fourth component, the condensing enzyme, provides substrate specificity and determines the amount of product synthesized by the entire complex. Land plants have two families of condensing enzymes, FATTY ACID ELONGATION 1 (FAE1)-type ketoacyl-CoA synthases (KCSs) and ELONGATION DEFECTIVE-LIKEs (ELO-LIKEs). Our current knowledge of the specific roles of different condensing enzymes is incomplete, as is our understanding of the biological function of a recently characterized family of proteins, CER2-LIKEs, which contribute to condensing enzyme function. More broadly, the stoichiometry and quaternary structure of the fatty acid elongase complex remains poorly understood, and specific phylogenetic and biochemical questions persist for each component of the complex. Investigation of VLCFA elongation in different organisms, structural biochemistry, and cell biology approaches stand to greatly benefit this field of plant biology.

Contents

1. Introduction

Very-long-chain fatty acids (VLCFAs) are imperative to plant survival and have diverse roles throughout the course of plant development. VLCFAs are fatty acids longer than 18 carbons (C18) in length; they are often modified, derivatized, esterified to other molecules, or polymerized to form biologically active products. In most plant cells VLCFAs represent only a fraction of the total fatty

Abbreviations: VLCFA, very-long-chain fatty acid; ACP, acyl carrier protein; CoA, coenzyme A; KCS, KETOACYL-COA SYNTHASE; KCR, KETOACYL-COA REDUCTASE; HCD, HYDROXYACYL-COA DEHYDRATASE; ECR, ENOYL-COA REDUCTASE; ELO, ELONGATION DEFECTIVE; CER2, ECERIFERUM2; LACS, LONG-CHAIN ACYL-COENZYME A SYNTHETASE.

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acid pool, as all cells must produce large quantities of C16 and C18 long-chain fatty acids (LCFAs) for the synthesis of phospholipids and glycolipids that make up the bulk of cellular membranes. However, VLCFAs are required in all plant cells for the production of sphingolipids, and in specific cell types for the synthesis of other VLCFA derivatives such as cuticular waxes, pollen coat, and suberin. In addition, the embryonic cells of the Brassicaceae and jojoba (*Simmondsia chinensis*) accumulate VLCFAs as seed oil storage compounds (triacylglycerols and wax, respectively), which constitute an enormous energy investment by the entire parent plant.

Perhaps because of the involvement of VLCFAs in such diverse metabolic pathways in plant cells, their synthesis has been thoroughly investigated in the past. Great progress has been made in dissecting the genetic components required for VLCFA synthesis, and the structure and function of their protein products. However, it may not be surprising that in discovering the fundamentals of VLCFA formation, studies have also unearthed new questions. Further, the investigation of VLCFA production in other organisms, as well as genomics approaches in biology, have evoked new ideas and questions such that plant VLCFA synthesis remains an active and exciting field of research. In this review we provide an overview of past progress and our present understanding of plant VLCFA synthesis, and focus on the new questions that have arisen and the new directions our field is taking.

2. The fatty acid elongase complex

2.1. Discovery of the fatty acid elongase complex and FAE1

Across different domains of life, *de novo* synthesis and elongation of fatty acids share a basic mechanism. This consists of a sequence of four core reactions that add two carbon units to a substrate molecule. First, either a starter molecule (*e.g.* activated acetate) or a growing acyl chain is condensed with malonate, which may be activated by conjugation to either acyl carrier protein (ACP) or coenzyme A (CoA). This condensation reaction produces an activated β-ketoacyl that is two carbons longer than the initial substrate. Subsequently, the β-keto group is reduced to an alcohol, dehydrated to a β-enoyl, and reduced again to form the $n+2$ acyl product (Fig. 1). Beyond this core sequence of reactions, there is variation in the regulation, substrate activation, localization, mechanism of determining substrate and product specificity, and perhaps most strikingly, in the enzyme(s) involved in both *de novo* synthesis and elongation.

Fatty acid synthases (FAS) are enzymes or enzyme complexes that catalyze the *de novo* synthesis of fatty acids; they can generally be grouped according to whether their catalytic activities are carried out by a single, multifunctional protein, referred to as a type I fatty acid synthase, or whether the activities are encoded on four separate, monofunctional proteins, making up a type II fatty acid synthase. Plants have soluble, plastid-localized type II fatty acid synthases, which elongate fatty acyl groups esterified to acyl carrier protein up to C18. Plant fatty acid synthases are essential for the production of membrane lipids and the long-chain precursors of VLCFAs. For a review of fatty acid synthase structure and function, the reader is directed to [1].

Early work aimed at isolating the enzymes required for the production of VLCFAs in plants revealed that VLCFA elongation differs markedly from the *de novo* synthesis of fatty acids catalyzed by the fatty acid synthase. Extensive biochemical studies using cuticular wax-producing leek (*Allium porrum*) epidermal cells as a model revealed that "elongase" activity is present predominantly in a microsomal cell fraction [2], that the elongase uses malonyl-CoA and acyl-CoA as substrates and NADPH as a reductant [3], and that elongation products are released from the elongase as CoA thioesters [4]. Use of chemical inhibitors in this system demonstrated varied effects of chemicals on elongase activity, suggesting the existence of multiple elongases [5]. Biochemical approaches were also crucial for our early understanding of the nature of the elongase enzyme(s); purification of the leek elongase resulted in enrichment of at least three proteins, suggesting that the elongase is not a single multifunctional protein unit, but rather consists of multiple protein components [6]. For a complete synopsis of early biochemical work on the fatty acid elongase complex, the reader is directed to a review by Cassagne et al. [7]. Additionally, genetic analysis of cuticular wax production in barley had demonstrated that several elongase systems must exist in a given plant, as mutagenesis or treatment of tissues with chemical inhibitors or exposure to different environmental conditions had varied effects on the distribution of cuticular wax monomer chain lengths [8]. Together, both biochemical and genetic evidence suggested a model where multiple heterotetrameric fatty acid elongase complexes carry out VLCFA elongation in the endoplasmic reticulum, with different elongases having substrate specificity tailored to the synthesis of specific downstream products.

The first component of the fatty acid elongase to be genetically identified and characterized in plants was FATTY ACID ELONGATION 1 (FAE1) in a mutant screen for seed VLCFA deficiencies in *Arabidopsis thaliana* [9–11]. A single, semi-dominant nuclear mutation in *FAE1* was sufficient to block the elongation of seed C18:1 to C20:1, C20:1 to C22:1, and C18:0 to C20:0 [11]. As three independent screens for mutants defective in VLCFA elongation in seed oils isolated multiple alleles of only *FAE1*, the generalized elongation model described above developed into two specific schemes. First, FAE1 could be a single, multifunctional elongase analogous to type I fatty acid synthases. FAE1 function would be specific to seed oil production, since none of the *fae1* mutations were lethal, as would be the case if sphingolipid synthesis were compromised. However, this explanation conflicted with evidence from the leek system that suggested plant elongases exist as heteromeric protein complexes. An alternative scheme consistent with all of the data designated FAE1 as a single component of a multimeric elongase. FAE1 would belong to a family of enzymes with distinct substrate specificities and biological roles, while the enzymes that catalyze the remaining three reactions of the elongase would be generalists. Knocking out any generalist component of the elongase would compromise sphingolipid metabolism; therefore, these mutations would be lethal, explaining their absence from the mutant screens.

Positional cloning of the *FAE1* gene revealed that it encodes a protein with sequence similarity to polyketide synthases (PKS) such as chalcone synthase and stilbene synthase, and ketoacyl-ACP synthase IIIs (KASIII) [12]. These enzymes catalyze the condensation of malonyl-CoA, or malonyl-ACP, with a primer molecule, thereby producing a β-ketoacyl-CoA/ACP product. The homology of FAE1 to these monofunctional condensing enzymes provided confirmation of the multimeric fatty acid elongase complex hypothesis suggested above. Characterization of FAE1 has suggested that it uses a similar mechanism to the polyketide pathway-related condensing enzymes and ketoacyl-ACP synthase IIIs [13,14]. FAE1 identification has led to the investigation of its homologs in Arabidopsis; these FAE1-type ketoacyl-CoA synthases, which we will refer to in this review simply as KCSs, have diverse roles in plant metabolism. Our current understanding of KCS function, biological roles, and phylogeny is discussed in detail in Section 3 of this review.

2.2. The four enzymes of the elongase complex

Elongation of VLCFAs in plants shares many parallels with microsomal VLCFA elongation in yeast, and it is undeniable that