



Review article

Review: Metabolic engineering of unusual lipids in the synthetic biology era



Jose A. Aznar-Moreno, Timothy P. Durrett*

Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506, USA

ARTICLE INFO

Keywords:

Unusual fatty acid
Synthetic biology
Transgenic oilseed crop
Hydroxy fatty acid
Acetyl-TAG

ABSTRACT

The plant kingdom produces a variety of fatty acid structures, many of which possess functional groups useful for industrial applications. The species that produce these unusual fatty acids are often not suitable for large scale commercial production. The ability to create genetically modified plants, together with emerging synthetic biology approaches, offers the potential to develop alternative oil seed crops capable of producing high levels of modified lipids. In some cases, by combining genes from different species, non-natural lipids with a targeted structure can be conceived. However, the expression of the biosynthetic enzymes responsible for the synthesis of unusual fatty acids typically results in poor accumulation of the desired product. An improved understanding of fatty acid flux from synthesis to storage revealed that specialized enzymes are needed to traffic unusual fatty acids. Co-expression of some of these additional enzymes has incrementally increased the levels of unusual fatty acids in transgenic seeds. Understanding how the introduced pathways interact with the endogenous pathways will be important for further enhancing the levels of unusual fatty acids in transgenic plants. Eliminating endogenous activities, as well as segregating the different pathways, represent strategies to further increase accumulation of unusual lipids.

1. Introduction

The seeds of plants represent valuable biosynthetic factories, capable of converting photosynthetically derived sugars into large quantities of highly reduced storage lipids. The high energy density of the triacylglycerols (TAG) found in vegetable oil means these molecules are important sources of calories for human and animal nutrition, as well as petroleum alternatives for the chemical industry and for biofuels. The biosynthetic capability of seeds can be further harnessed to synthesize a variety of complex structures with functionalities useful for different applications. Over the past decade, various technological advances have meant that the prospect of using synthetic biology approaches to develop specialized crops producing unusual fatty acids has never been more promising. For example, the development of very cheap DNA sequencing technologies has allowed researchers to query the transcriptome of different seeds and discover key enzymes important for the synthesis of unusual lipids, such as ricinoleic acid and acetyl-TAG [1–4]. Genome editing techniques such as those afforded by the emergence of CRISPR/Cas mediated technology offer the capability to not only oblate gene function in non-model species, but the potential for gene knock-in approaches, as well as the ability to control spatio-temporal gene expression [5]. Decreasing costs of DNA synthesis and improved DNA assembly techniques enable the combinatorial construction of modified or new enzymes and different expression modules

to engineer metabolic networks. These capabilities will allow the dramatic redesign of oil seed biochemical pathways to produce lipids typically produced in other species. Indeed, the combinatorial capabilities of synthetic biology in theory allow the biological production of lipid molecules not found in nature.

However, the successful development of specialized oil seed crops capable of producing high levels of designed lipids will not be possible without understanding how such lipids are synthesized and stored. Here we review the considerable progress that has been made in achieving this goal. In particular, we focus on the realization that a specialized metabolic flux network is required for the accumulation of high levels of unusual lipids. We posit different strategies of how to engineer such segregated biosynthetic pathways with minimal disruption of endogenous metabolism required for seed viability and maximal yield.

2. Valuable oils: structure imparts functionality

The vegetable oil market is dominated by a few established species: soybean, canola, palm and sunflower, which together account for the majority of global vegetable oil production. These oils contain different proportions of just five common fatty acids; palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2) and α -linolenate (18:3). However, these fatty acid structures possess little chemical functionality for many

* Corresponding author.

E-mail address: tdurrett@ksu.edu (T.P. Durrett).

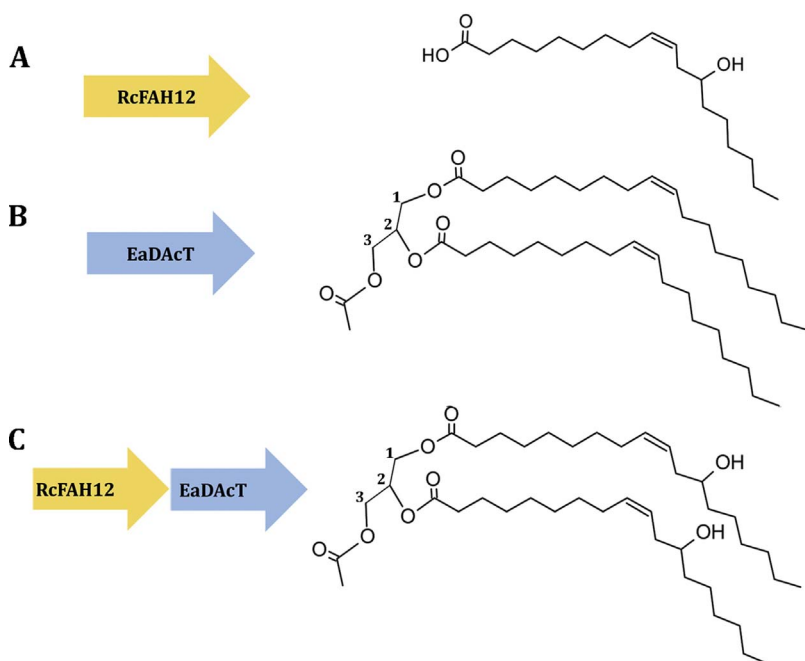


Fig. 1. Synthetic biology will allow the combinatorial production of unusual lipid structures. Shown are structures of ricinoleic acid (A), a representative acetyl-TAG (B) and 3-acetyl-1,2-diricinolein (C), an artificial lipid that could be produced by the coexpression of modules sufficient for the synthesis of ricinoleic acid (FAH12) and acetyl-TAG (EaDAcT).

industrial-chemical applications. In contrast, throughout the plant kingdom, a multitude of fatty acids with different modifications exist (see <https://phylofad.bch.msu.edu/>), many of which are useful for the chemical industry [6,7]. As considerable progress has been made in understanding the synthesis of hydroxy fatty acids, the remainder of this review will focus on these useful molecules, but when relevant will extend discussion to include other compounds.

2.1. Hydroxy fatty acids

A variety of hydroxy fatty acids (HFA) are synthesized in some plants and are useful for the production of lubricants, nylon and plastics [7,8]. For example, 90% of the fatty acids in oil from castor (*Ricinus communis*) are hydroxylated, with ricinoleic acid (18:1-OH; Fig. 1A) the main fatty acid [9]. This high accumulation of HFA means that more than 70% of the TAG molecular species contain HFA at all three positions on the glycerol backbone [10]. Despite the usefulness of its oil, castor is not grown on a large scale due to the fact that the seeds contain the toxin ricin and highly allergenic proteins, increasing the cost of harvest [11]. Other plants not related to castor also accumulate HFA, suggesting this trait might have arisen multiple times during evolution [2,12]. In particular, various *Physaria* species synthesize a variety of HFA including lesquerolic acid, densipolic acid, ricinoleic acid and auricollic acid. *Physaria fendleri* (previously named *Lesquerella fendleri* [13], and historically referred to as *Lesquerella*) for example, contains up to 65 mol% HFA [14,15]. The stereospecific arrangement of these FA is different, with HFA only incorporated to the *sn*-1 and *sn*-3 position of TAG. In contrast, other *Physaria* species such as *P. lindheimeri*, can incorporate HFA into all three positions of TAG, allowing the seeds to accumulate up to 85 mol% HFA [15].

2.2. Acetyl-triacylglycerols

Another group of unusual storage lipids found in some plant species are 3-acetyl-1,2-diacyl-*sn*-glycerols (acetyl-TAG), TAG molecules where the *sn*-3 position is esterified with acetate instead of a long chain fatty acid (Fig. 1B). The presence of the *sn*-3 acetate group means that acetyl-TAG possess different chemical and physical properties compared to regular TAG. For example, acetyl-TAG possess reduced viscosities and lower crystallization temperatures than regular vegetable oils,

suggesting applications as improved biofuel or lubricants [1,16,17]. Acetyl-TAG producing species are particularly abundant in the Celas-traceae (mostly notably in the *Euonymus* genus), but have also been recognised in the Balsaminaceae, Lardizabaleae, Ranunculaceae and Rosaceae [18,19]. EaDAcT, a unique acyltransferase isolated from *Euonymus alatus* (burning bush), uses acetyl-CoA to acetylate diacylglycerol to form acetyl-TAG [1]. Expression of *EaDAcT* in plants and yeast is sufficient for the synthesis of acetyl-TAG [1,16,17,20]; later we discuss strategies that have enabled the accumulation of higher levels of acetyl-TAG.

2.3. The design of artificial lipids

The discovery of different lipid biosynthetic enzymes, combined with modern synthetic biology approaches that allow facile assembly of expression modules containing combinations of these enzymes, allows the contemplation of artificial molecules not encountered in nature. For example, 3-acetyl-1,2-diricinolein could be synthesized by combining modules enabling ricinoleic acid synthesis and incorporation into glycerolipids at *sn*-1 and *sn*-2 with those enabling production of acetyl-TAG (Fig. 1C). Typically, when reacted with diisocyanates, castor oil polymerizes to form thermosetting polyurethanes. In contrast, polymerization of the artificial 3-acetyl-1,2-diricinolein molecules, which possess two rather than three reactive hydroxyls, will give correspondingly more linear rather than cross-linked polyurethanes with dominantly thermoplastic properties. Similarly, the targeted combination of other unusual fatty acids can be used to synthesize lipids with different chemical and physical properties for particular applications. For example, acetyl-TAG containing medium chain fatty acids are predicted to possess further reductions in viscosity due to their lower molecular mass [21]. However, understanding how different unusual fatty acids are synthesized and assembled into TAG will be essential to achieving these ambitious goals.

3. TAG synthesis in developing oilseeds

TAG biosynthesis in developing seeds involves a complex, interconnected pathway (Fig. 2); for a more in-depth description, readers are encouraged to refer to additional review articles [22,23].

In higher plants, the synthesis of fatty acids occurs in the plastid

Download English Version:

<https://daneshyari.com/en/article/5515750>

Download Persian Version:

<https://daneshyari.com/article/5515750>

[Daneshyari.com](https://daneshyari.com)