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Review

Studies on the regulation of lipid biosynthesis in plants: application of control analysis to soybean[☆]

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ABSTRACT

Although there is much knowledge of the enzymology (and genes coding the proteins) of lipid biosynthesis in higher plants, relatively little attention has been paid to regulation. We have demonstrated the important role for cholinephosphate cytidylyltransferase in the biosynthesis of the major extra-plastidic membrane lipid, phosphatidylcholine. We followed this work by applying control analysis to light-induced fatty acid synthesis. This was the first such application to lipid synthesis in any organism. The data showed that acetyl-CoA carboxylase was very important, exerting about half of the total control. We then applied metabolic control analysis to lipid accumulation in important oil crops – oilpalm, olive, and rapeseed. Recent data with soybean show that the block of fatty acid biosynthesis reactions exerts somewhat more control (63%) than lipid assembly although both are clearly very important. These results suggest that gene stacks, targeting both parts of the overall lipid synthesis pathway will be needed to increase significantly oil yields in soybean. This article is part of a Special Issue entitled: Membrane structure and function: Relevance in the cell's physiology, pathology and therapy.

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Abbreviations: CPCT, cholinephosphate cytidylyltransferase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PDAT, phospholipid:diacylglycerol acyltransferase; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol; PUFA, polyunsaturated fatty acids; SQDG, sulphoquinovosyldiacylglycerol; TAG, triacylglycerol

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1. Introduction: a lipid primer for plants

Although plants share many characteristics in common with other eukaryotes, they have certain distinct features with regard to their lipid biochemistry. Plant lipids are located in three main regions – in the extra-cellular domains (wax, cuticle, suberin), in lipid stores (mainly as triacylglycerol) and in cellular membranes [1]. In this article, we will neglect the former but good reviews are available which describe surface coverings and their metabolism [2–6]. The economic importance of plant

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lipids is huge. Each year oil crops produce about 115 million tonnes of oil and this is worth of the order of \$116B [7,8]. A few crops (oilseed rape, palm, soybean and safflower) currently account for about 69% of the total plant production. Interestingly, genetically-modified oil crops already account for a significant output (i.e. 170 million ha in 2012 including nearly 90% of the soybean crop).

There is one commercial crop that produces a different type of stored lipid, jojoba (*Simmondsia chinensis*). In the fruits (nuts) from this species, the oil is a wax ester of 40–42 carbon atoms, which has its main uses in the cosmetic industry [7].

Plants contain phosphoglycerides of the same types as other eukaryotes. In the extra-plastidic membranes, phosphatidylcholine and phosphatidylethanolamine are major components with smaller amounts of phosphatidylinositol and phosphatidylserine [1] (Table 1). Phosphorylated derivatives of phosphatidylinositol which, as in animals, have signalling functions, are found in small amounts, mainly in the plasma membrane [9,10]. Diphosphatidylglycerol (cardiolipin) is confined to the inner mitochondrial membrane [11] where it is a major component (Table 1). Phosphatidylglycerol, in contrast to animal tissues, is a major constituent because it is the only significant phospholipid in chloroplast (plastid) thylakoids. Because of the dominance of chloroplasts in green tissues, phosphatidylglycerol is often present in comparable amounts to phosphatidylcholine (Table 1).

One aspect of membrane composition that sets plant and algae apart from other eukaryotes are their chloroplast thylakoids. The major lipids of these are three glycosylglycerides – monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulphoquinovosyldiacylglycerol (SQDG, the plant sulpholipid). The structures of MGDG, DGDG and SQDG are shown in Fig. 1. Interestingly, all oxygen-evolving photosynthetic organisms, including cyanobacteria, contain rather similar thylakoid membrane compositions with about 45% MGDG, 29% DGDG, 7% SQDG and 9% phosphatidylglycerol in different leaves (see Table 1). The reason for this is unclear although there have been some hypotheses put forward [12,13]. Because of its prevalence in thylakoids, MGDG is the most abundant membrane lipid on earth, although many textbooks fail to even acknowledge its existence!

Sphingolipids are found in plants but, again, with their own distinctiveness compared to animals and yeast. Of particular interest is their enrichment in membrane rafts [14]. Plant sphingolipids are summarised well in [15] and their metabolism is up-dated in [16]. There are also a number of novel lipids in plants which may be present in appreciable quantities in certain tissues. These would include molecules like *N*-acyl-phosphatidylethanolamine and sterol glycosides [17]. Plant sterols themselves are dominated by β -sitosterol and stigmasterol (rather than cholesterol in animals). However, a whole host of minor compounds, including cholesterol, are found widely [17,18].

Table 1
Acyl lipid composition of selected plant tissues.

% total lipids	PC	PE	PI	PG	MGDG	DGDG	SQDG
Leaf lipids	10	5	3	8	40	28	6
Mito. lipids – outer	68	24	5	2	n.d.	n.d.	n.d.
Mito. lipids – inner ^a	29	50	2	1	n.d.	n.d.	n.d.
PM lipids	32	46	19	tr.	n.d.	n.d.	n.d.
Thylakoid lipids	2	tr.	n.d.	10	48	31	8
Root microsomes	35	28	14	n.d.	n.d.	n.d.	n.d.
Cyanobacteria	n.d.	n.d.	n.d.	19	56	14	11

Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PM, plasma membrane; SQDG, sulphoquinovosyldiacylglycerol; n.d., none detected; tr., trace (<0.5); Mito., mitochondrial.

^a Diphosphatidylglycerol (cardiolipin) is located to the inner mitochondrial membrane (14–20% total lipids). Microsomes included several other lipids such as cardiolipin. See [1] for further details.

Most membrane (and storage) lipids are acylated compounds. Saturated fatty acids themselves are made in plants, as in other organisms, by the combined action of (a multi-protein) acetyl-CoA carboxylase and (a Type II) fatty acid synthase. In most tissues, palmitate and stearate are produced in an approximately 1:5 ratio. Exceptions could be in cases where short chain fatty acids are produced as a result of the activity of a special thioesterase (e.g. in coconut) or where a higher percentage of palmitate is produced (e.g. in palm oil). More details of *de novo* fatty acid synthesis, which is localised in the plastid stroma, will be found in [19].

The long-chain (16 or 18C) saturated fatty acids are converted to unsaturated acids by the action of desaturases. While the desaturation of stearate to oleate is catalysed by a soluble stearoyl-ACP $\Delta 9$ -desaturase (in the stroma) [20], other plant desaturases use complex lipid substrates. For such reactions both the desaturase proteins and the substrates are found in membranes. Given the prevalence of stearate as a product of plant fatty acid synthase, it is not surprising that the main unsaturated fatty acids in plants are the 18C molecules – oleate, linoleate, and α -linolenate (Table 2). Therefore, major reactions in fatty acid processing by higher plants are the three successive desaturations of stearate and its products at the $\Delta 9$, $\Delta 12$ and $\Delta 15$ positions. The latter two reactions are particularly important because they give rise to the “essential fatty acids”, linoleate and α -linolenate, which are the simplest members of the *n*–6 and *n*–3 series of polyunsaturated fatty acids (PUFA), respectively. The *n*–6 and *n*–3 PUFA cannot be made by mammals and almost no animals (a few protozoa and insects are exceptions [7]) but are needed to produce very long chain (20C, 22C) PUFA that give rise to signalling molecules like eicosanoids, resolvins and protectins [21,22].

As mentioned above, linoleate and α -linolenate are produced by $\Delta 12$ - and $\Delta 15$ -desaturases, respectively, using complex lipid substrates. The idea that complex lipids could act as substrates for fatty acid desaturation came from work in Tony James' lab. at Unilever [23,24] and from Morris Kates' group in Ottawa [25]. We provided the first direct demonstration in higher plants [26] when we showed that linoleoyl-MGDG could be used by isolated chloroplasts to synthesise α -linolenate. At that time there was some controversy because Roughan and co-workers believed that phosphatidylcholine was the substrate for PUFA production [e.g. 27]. However, Roughan and co-workers were using developing oil crops and we were using leaves and this was the main difference for the data and their interpretation. In fact, identification of desaturase genes has allowed us to define the pathways for desaturation. These are shown in Fig. 2 where it will be seen that fatty acid desaturase genes for $\Delta 12$ and $\Delta 15$ desaturations are located in plastids (*fad 6*, *fad 7/8*) where they use mainly MGDG as a substrate, while the endoplasmic reticulum contains *fad 2* and *fad 3*-encoded enzymes which perform the same reactions using mainly phosphatidylcholine (Fig. 2) [28]. In the diagram, which shows *Arabidopsis* pathways, it will be noted that there are also desaturations at the 16C level because this plant belongs to the group of “16:3-plants” that produce all *cis*- $\Delta 7$, 10, 13-hexadecatrienoic acids (*n*–3–16:3) (see Table 2 also).

So, it turned out that, despite controversy, our finding that MGDG was the substrate for α -linolenate production, first suggested by radiolabelling studies [29], turned out to be fully validated. Moreover, we and others suggested a cooperation of the membranes of the endoplasmic reticulum and plastids in producing the world's most prevalent fatty acid, α -linolenate [see 30].

1.1. Assembling acyl lipids

As noted above, the most abundant complex lipids in plants are acyl lipids such as the storage lipid triacylglycerol and the membrane lipids (glycosylglycerides, phosphoglycerides). The major route for their synthesis is based on the glycerol 3-phosphate pathway first elucidated by Eugene Kennedy in the late 50s [31]. The first two reactions involve

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