Acyl-CoA measurements in plants suggest a role in regulating various cellular processes
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Abstract
Acyl-CoA esters have been shown to be involved in regulating metabolism and cell signalling in bacteria, yeast and mammalian cells, but little is known about their role in plants. Using a new method for the sensitive detection and quantification of acyl-CoA esters, we have recently shown that acyl-CoA pools can be dramatically altered in transgenic oilseed rape embryos, engineered to produce medium-chain fatty acids, and in mutant Arabidopsis seedlings that are unable to mobilize storage lipid. The consequences of these alterations are discussed in the context of oil yield and organelle biogenesis and the possible role of acyl-CoAs in regulating these processes.

Roles for acyl-CoAs in various organisms
In addition to serving as important intermediates in the synthesis and breakdown of lipids and fatty acids, acyl-CoA esters play a role in an array of other processes in living cells, as summarized in Figure 1 (reviewed in [1,2]). Proposed roles for acyl-CoAs include allosteric regulation of a number of enzymes, including acetyl-CoA carboxylase [3] and hexokinase [4]. In plants, long-chain acyl-CoAs have been shown to inhibit fatty acid synthesis in plastids through the inhibition of the plastidial glucose 6-phosphate transporter and the adenylate transporter [5,6]. In mammalian cells, long chain acyl-CoAs have also been shown to regulate the opening of ATP-sensitive K+ channels [7] and activation of Ca2+-ATPases, thereby regulating insulin secretion. Other proposed roles for acyl-CoAs include signal transduction through protein kinase C [8], inhibition of retinoic acid-induced apoptosis [9], involvement in budding

Key words: Arabidopsis, oilseed, β-oxidation, peroxisome.
Abbreviations used: ACBP, acyl-CoA binding protein; AtACh, Arabidopsis thaliana acyl-CoA thioesterase; cts/l, comatose I mutant; pedl, peroxisome-deficient I mutant; X-ALD, X-linked adrenoleucodystrophy.
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Acyl-CoA levels in plants

To aid our work on the metabolic engineering of lipid metabolism in *Brassica napus* (oilseed rape), and on determination of gene function in *Arabidopsis*, we developed a new method for the sensitive detection and quantification of acyl-CoA esters [18]. This method relies on the conversion of acyl-CoAs into fluorescently active acyl-etheno-CoA derivatives and, when coupled with HPLC, allows the entire cellular pool of acyl-CoAs to be profiled and quantified. Using this method, the acyl-CoA content of developing *B. napus* embryos during the lipid synthesis phase, and *Arabidopsis* seedlings in the lipid mobilization phase, were found to be in the range of 3 to 6 μM [18]. In mammalian cells, the total cellular long-chain acyl-CoA concentration has been reported to be in the range of 5 to 160 μM, with concentrations varying significantly depending on the metabolic state [1]. We have recently shown that developing embryos of *B. napus* plants transformed with thioesterase genes that result in the synthesis of either 10:0 or 12:0 fatty acids, accumulate a disproportionately high concentration of 10:0 CoA or 12:0 CoA during seed development compared to long-chain acyl-CoAs isolated from the same tissues [19]. We conclude from this study that *B. napus* plants that have been modified to synthesize medium-chain fatty acids may lack the necessary mechanisms, such as specific acyltransferases, to incorporate these fatty acids efficiently into seed lipids. Remarkably, the levels of 10:0 CoA in the engineered embryos can represent as much as 60 mol% of the total, with the acyl-CoA pool increasing by approx. one third above that of the parental lines. On an overall basis, lipid accumulation does appear to be compromised in these lines, and studies aimed at establishing additional changes in the metabolome and transcriptome of these embryos should help establish if the altered acyl-CoA levels are affecting processes in plant cells in a manner similar to that shown to occur in other organisms.

We have also found that acyl-CoA levels are significantly altered in *Arabidopsis* mutants that are disrupted in the breakdown of fatty acids derived from storage lipid in young seedlings. For example, metabolite profiling of seedling extracts from the *kat2* mutant that is disrupted in a gene encoding the 3-ketoacyl-CoA thiolase enzyme (EC: 2.3.1.16) of β-oxidation revealed that long-chain (C₁₆−C₂₀) acyl-CoAs are not catabolized and are present at elevated levels in extracts [20]. The *kat2* mutant is unable to mobilize storage triacylglycerol and it requires exogenous sugars for growth [20]. After 5 days' growth on sucrose media, *kat2* seedlings become photosynthetic but lipid bodies persist in the green cotyledons. Interestingly, the peroxisomes of *kat2* seedlings are enlarged and some of these contain unusual membrane inclusions, and chloroplasts and mitochondria are also modified in some cells. The *kat2* mutant is allelic to the peroxisome-deficient 1 (*ped1*) mutant and electron microscopy of *ped1* has shown that it also contains enlarged abnormal peroxisomes with tubular structures that contain many electron-dense vesicles [21,22]. A schematic representation of the effects of the *kat2* mutant are shown in Figure 2. Disruption of the multifunctional protein of peroxisomal β-oxidation in *Yarrowia lipolytica* also results in enlarged peroxisomes, thus implicating fatty acid β-oxidation in the control of peroxisome size and number in yeast [23]. Although we have not directly established the subcellular location of the increased amounts of acyl-CoAs in the *kat2* mutant, our assumption is that the significant increases observed will occur in peroxisomes since these organelles are the site of fatty acid β-oxidation in plants. The *Arabidopsis* catcomate (*cts1*) mutant is disrupted in a peroxisomal protein of the ABC transporter class with significant identity with the human X-linked adrenoleucodystrophy (X-ALD) protein [24]. As with X-ALD patients, *cts1* mutant embryos and seedlings exhibit pleiotropic phenotypes associated with perturbation of fatty acid metabolism. In common with the *kat2* mutant, *cts1* requires sucrose for seedling growth, lipid bodies persist in green cotyledons of 5-day-old seedlings that have been rescued on sucrose and long-chain acyl-CoAs accumulate [24] (Figure 2). However, unlike in *kat2*, peroxisomes in *cts1* are normal even though acyl-CoA levels are elevated and lipid
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bodies persist (Figure 2). The fact that acyl-CoAs do accumulate in cts1 indicates that the CTS1 ABC protein transports acyl-CoAs rather than fatty acids. In cts1, the assumption is that the acyl-CoAs accumulate in the cytosol. We hypothesize that it is this different subcellular localization of the elevated acyl-CoA pools between the kat2 and cts1 mutants that results in the kat2 mutant having enlarged peroxisomes, possibly due to the increased peroxisomal acyl-CoA concentration in kat2. This results in much greater enlargement of the peroxisome than is common in cotyledon cells during post-germinative growth of oilseeds [25,26].

Since acyl-CoAs appear to play an important role in regulating a diverse array of cellular processes, it is not surprising that mechanisms exist to regulate their levels. Acyl-CoA binding protein (ACBP) binds long-chain acyl-CoAs with high affinity and is believed to play an important role in intracellular acyl-CoA transport and pool formation, and therefore also for the function of acyl-CoAs as metabolites and regulators of cellular functions [27]. ACBP appears to act as an intracellular acyl-CoA transporter and pool former for medium- and long-chain acyl-CoAs only, and also appears to protect acyl-CoAs from hydrolytic degradation. Turnover through the action of acyl-CoA hydrolases or thioesterases represents an additional important point of regulation and these enzymes have been shown to occur in the cytosol, mitochondria and peroxisomes of mammalian cells [2]. Two families of acyl-CoA thioesterases are present in Arabidopsis [28]; the first, consisting of AtACH (Arabidopsis thaliana acyl-CoA thioesterase) 1 and AtACH2, appears to be peroxisomal, as they have type-I peroxisomal targeting sequences, and the second, consisting of AtACH4 and AtACH5, resides in the endoplasmic reticulum, as shown by green fluorescent protein studies [28]. A cDNA encoding a 92-amino-acid ACBP has been identified from B. napus that has high homology with ACBP sequences from yeast, cow, man and fruit fly [29]. Northern blot analysis showed that this class of ACBP genes is expressed strongly in developing embryo, flowers and cotyledons of seedlings and, to a lesser extent, in leaves and roots [29]. The high degree of sequence conservation across species of this class of small cytosolic ACBPs suggests that their physiological roles have been preserved during evolution. A second class of membrane associated ACBPs, designated ACBP1, have subsequently been identified in Arabidopsis [30]. ACBP1 was described as a 216-amino-acid glycosylated, membrane-associated protein with an acyl-CoA binding domain at the C-terminus and a transmembrane domain at the N-terminus [30]. ACBP1 appears to participate in intermembrane lipid transport from the endoplasmic reticulum via vesicles to the plasma

Figure 2
Summary of the biochemical phenotypes of the kat2 and cts1 phenotypes compared with a normal wild-type cotyledon cell from 5-day-old seedlings grown on exogenous sucrose

FFA, non-esterified ('free') fatty acids; TAG, triacylglycerol.
membrane, where it could maintain a membrane-associated acyl pool [31]. A BLAST search of the complete Arabidopsis protein database using the B. napus cytosolic ACBP sequence [29] revealed genes for six proteins that contain a 60-amino-acid, highly conserved domain of the cytosolic ACBP (Figure 3). At1g21812 encodes the Arabidopsis homologue of the B. napus cytosolic ACBP. At5g53470 corresponds to the ACBP1 previously described [30]. Based on full-length cDNA annotation, the ACBP1 protein encoded by At5g53470 is made up of 338 amino acids with the acyl-CoA binding domain in the middle of the protein. A second gene, At4g27780, that is highly homologous to At5g53470, is present in Arabidopsis. Both of these genes encode proteins that contain ankyrin repeats at the C-terminal region (Figure 3). Ankyrin repeats are tandemly repeated modules of about 33 amino acids that occur in a large group of functionally diverse proteins and are thought to function as protein–protein interaction domains. Two further genes, At3g05420 and At5g27630, encode proteins that are significantly larger than the previously described ACBP1; they both contain the ACBP domain at the N-terminus and a series of kelch repeats (Figure 3). Kelch is a 50-residue motif named after the Drosophila mutant in which it was first identified. As with ankyrin repeats, kelch repeats occur in functionally diverse proteins and appear to perform a role in cytoskeletal association and also appear to be involved in other interactions. The sixth gene, At4g24230, encodes a protein that contains an ACBP domain at the C-terminus and lacks any other obvious structural motifs (Figure 3).

Given that the ankyrin and kelch repeats present on four out of the six Arabidopsis ACBPs are likely to be involved in promoting protein–protein interactions, it is interesting to speculate that such interactions could provide a mechanism for acyl-CoAs to transduce signals to other proteins. Since it is now possible to functionally characterize the role of Arabidopsis genes through reverse genetics approaches, the study of these different classes of ACBPs should provide new insight into the regulation of acyl-CoA pools, and the role of acyl-CoAs in regulating metabolism and other cellular processes in plant tissue.

References
Manipulating desaturase activities in transgenic crop plants
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Abstract
The properties of edible vegetable oils are determined to a large extent by the relative content of the triacylglycerol fatty acids. The degree of saturation of these fatty acids can determine the functional, sensory and nutritional value of the oil.

One method of altering the unsaturated fatty acid content of oilseeds is by manipulating the expression of desaturase genes of these plants. Manipulating the expression of desaturase genes in transgenic crops such as soybean, maize and canola (oilseed rape) has led to oils with improved functionality and nutrition. We have also been successful in manipulating the fatty acid content of domesticated oilseed plants by expressing heterologous desaturase and desaturase-related genes from exotic plants that produce unusual fatty acids. We have discovered that metabolic regulation, the number of genetic alleles that encode fatty acid biosynthetic enzymes, and the movement of fatty acids between complex lipids in the cell, all have a role in determining the effect of a transgene on the phenotype of the crop plant and the fatty acid composition of its seed oil.

Introduction
The functional properties of both animal fats and vegetable oils are largely determined by the properties of the fatty acids that are esterified to triacylglycerol [1]. For example, vegetable oils are usually rich in unsaturated fatty acids, which have low melting points and are usually liquid at room temperature. In contrast, most animal fats are rich in saturated fatty acids and are solid at room temperature. The number of double bonds in their constituent fatty acids also determines other important functional properties of vegetable oils, such as oxidative stability, and hence shelf life and cooking properties [2]. Fatty acids also determine many of the nutritional and health properties of edible fats and oils. For example, vegetable oils that are rich in unsaturated fatty acids are generally considered better for cardiovascular health than fats that are rich in saturated fatty acids. In addition, functional groups related to fatty acid double bonds, such as hydroxy and epoxy groups, as well as non-methylene-interrupted double bonds, confer important functional properties to oils used in non-edible applications, such as castor and Tung oils [3].

Key words: flux, gene family.
Abbreviations used: ACP, acyl-carrier protein; DAGAT, diacylglycerol acyltransferase; EST, expressed sequence tag.

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Received 18 July 2002.

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