Commentary

As simple as ACB – new insights into the role of acyl-CoA-binding proteins in Arabidopsis

Lipid metabolism is often considered to be a hard topic, involving a great deal of complicated biochemistry represented by zigzag lines. The situation in plants is made even worse with the presence of plastids, which compartmentalize and bisect the biosynthetic process into different organelles. Many ground-breaking biochemical studies were carried out in the 1960s and 1970s to try and better understand the fundamental processes, such as fatty acid biosynthesis, lipid desaturation and the synthesis of triacylglycerol (TAG), significantly driven forward by the vision of Paul Stumpf who is rightly considered the founding father of plant lipid biochemistry. These studies led to the realization that plant lipid metabolism operated a twin-track system, with plastidial fatty acid synthesis utilizing acyl-carrier protein (ACP)-linked substrates (analogous to prokaryotic metabolism), whereas microsomal (extraplastidial) reactions required acyl-CoA substrates (Stumpf, 1994). Importantly, the primary pathway for the synthesis of phospholipids and neutral lipids, such as TAG, was shown to require acyl-CoA forms of substrate (Stumpf, 1994). Subsequent pioneering molecular genetic studies by Somerville & Browse (Ohlrogge *et al.*, 1991) established Arabidopsis as the workhorse model for dissecting plant lipid metabolism, and their brute-force screens for mutant plants altered in fatty acid metabolism continues to provide new insights right up to this day (Lu *et al.*, 2009). In particular, the *fad* mutants (Arabidopsis lines in which the desaturation of different lipids and fatty acids was altered) have proved crucial in providing a molecular basis to understand the two-compartment basis of fatty acid modification, allowing the identification of closely related enzymes that carry out similar modifications to discrete lipid types (Somerville & Browse, 1996).

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One long-standing conundrum associated with compartmentalized fatty acids is how these hydrophobic carbon chains can be moved around the cell (Ohlrogge *et al.*, 1991). Like lunches, fatty acids are rarely free, usually existing as either the ACP- or CoA-esters, as already mentioned (Ohlrogge *et al.*, 1991; Stumpf, 1994), or as the acyl-chains of different lipids (Somerville & Browse, 1996; Lu *et al.*, 2009). Co-ordinating the delivery of fatty acids for the synthesis of extraplastidial glycerolipids requires the action of 'lipid chaperones', such as the acyl-CoA-binding proteins first described in yeast (Gaigg *et al.*, 2001) and now known to be ubiquitous in eukaryotes (Faergeman *et al.*, 2007). In the model yeast *Saccharomyces cerevisiae*, the genome encodes a single acyl-CoA-binding protein, ACB1, which, upon deletion, leads to profound defects in vesicular transport, membrane organization and sphingolipid metabolism (Gaigg *et al.*, 2001). The precise biochemical substrates and ligands of ACB1 in yeast are still unclear, although the amounts of very long chain fatty acids (VLCFAs) were found to be significantly reduced, commensurate with the microsomal elongase (the complex that generates VLCFAs) utilizing acyl-CoA substrates (Gaigg *et al.*, 2001). In Arabidopsis, six orthologues (designated *ACBP1–6*) of the yeast *ACB1* gene have been identified (Xiao & Chye, 2009), and previous studies have indicated that members of this gene family have discrete localizations (cytosol, microsomal membranes), and that over-expression of some individual ACBPs results in increased tolerance of abiotic stresses, such as freezing and heavy metals (Chen *et al.*, 2008; Xiao *et al.*, 2008; Xiao & Chye, 2009). The underlying mechanisms delivering these enhancements are still unclear, but clues based on the observation that ACBPs not only bind acyl-CoAs, but also phospholipids, link the response to membrane biosynthesis and/or homeostasis (Yurchenko *et al.*, 2009).

In the latest paper from the group of Mee-Len Chye, in this issue of *New Phytologist* (Chen *et al.*, 2010, pp. 843–855), the importance of acyl-CoA-binding proteins is emphatically demonstrated through Arabidopsis reverse-genetics. Chen *et al.* (2010) isolated homozygous insertion mutations for the Arabidopsis *ACBP1* and *ACBP2* genes. These two genes encode acyl-CoA-binding proteins, which additionally contain ankyrin repeat domains (involved in protein–protein interactions) and both proteins have previously been shown to be located in the endomembrane system (Xiao & Chye, 2009). Importantly, *ACBP1* was shown to be strongly expressed during embryo development and also seed maturation, two distinct stages likely to be

dependent on acyl-CoA processes such as membrane lipid biogenesis. In fact, previous genetic screens in Arabidopsis for genes that were essential for embryo development identified several mutations in genes encoding acyl-CoA-dependent enzyme activities, most notably *PAS2* (the 3-hydroxy-acyl-CoA-dehydratase of the microsomal elongase; Bach *et al.*, 2008) and *PAS3/ACC1* (cytosolic acetyl-CoA carboxylase; Baud *et al.*, 2004), confirming the central nature of these metabolic pathways. Chen *et al.* (2010) examined the phenotype of *acbp1* and *acbp2* insertion mutants after demonstrating that the homozygous mutants were lacking in transcripts from these genes; however, no morphological phenotype was observed in these single mutant plants, and no alteration to embryo development was seen. This was not unexpected, because transcript profiling of *ACBP1* and *ACBP2* indicated a strong overlap in their expression patterns. Moreover, Chen *et al.* (2010) showed that the disruption of one gene led to a compensatory increase in the expression of the other (i.e. *ACBP2* expression increased in *acbp1* mutants). Thus, *ACBP1* and *ACBP2* were likely to be under some common (homeostatic) regulation. Perhaps the most significant observation of this study arises from the authors' inability to recover an *acbp1 acbp2* double mutant – the dual loss of both of these acyl-CoA-binding proteins resulted in embryo lethality and it was never possible to recover mutants disrupted in both genes (although it was possible to obtain *acbp1 ACBP2*$^{+/-}$ hemizygotes). Attempts to culture embryos from double mutants *in vitro* also failed to yield any viable calluses. Thus, the *acbp1 acbp2* double mutant is embryo-lethal, probably failing at very early stages of development.

Analysis of the lipid profiles of the *acbp1* and *acbp2* single mutants provides some tantalizing insights into the likely role of these binding proteins, at least in terms of the biochemical lesions their absence generates. These current and previous studies have shown that plant acyl-CoA-binding proteins not only bind these ester-linked fatty acids, but also bind specific phospholipids (Yurchenko *et al.*, 2009; Chen *et al.*, 2010). In view of this, a detailed phospholipidomic analysis of the two mutants was carried out, revealing perturbations to both the amounts and composition of different phospholipids (Chen *et al.*, 2010). Importantly, this was observed in siliques, but not in rosette leaves, consistent with the expression profile of the two genes. Perhaps most intriguingly, the *acbp1* mutant had strongly decreased amounts of VLCFAs in phosphatidylserine. Phosphatidylserine is the one phospholipid that in plants contains a significant amount of VLCFAs, and in that respect, the phospholipidomic chemotype of the Arabidopsis *acbp1* mutant is analogous to the yeast *acb1*Δ mutant. Acyl-CoA profiling (a sensitive analytical technique that can give quantitative measurements of this femtomolar metabolic 'hub') of the *acbp1* mutant revealed an increase in 18:0-CoA in this mutant background, whereas the *acbp2* mutant did not differ from the wild type in the acyl-CoA species examined. Collectively, these data identify ACBP1 and ACBP2 as key players in the channelling of substrate acyl-CoAs to the glycerolipid biosynthetic pathways active in the endomembrane system during the early stages of embryo development.

So what is the wider significance of this study? First, it serves to demonstrate that acyl-CoA-binding proteins play an essential role in plant lipid metabolism and that they are not dispensable. Perhaps fatuously, it is sometimes thought that ACBPs are the *in vivo* equivalent of the BSA we add to our *in vitro* enzyme assays, in the belief that BSA will help to maintain the solubility of fatty acid substrates. Whilst there is good biochemical evidence to support such a role for ACBPs, it is also clear from this present reverse-genetic study that ACBP1 and ACBP2 have very precise functions, given the specific alterations seen in lipid composition. The precise role of these two proteins still awaits further elucidation, but it is interesting to speculate on their role in VLCFA biosynthesis. As already noted, two *PAS* (*PASTICCINO*) genes are known to be involved in the synthesis of VLCFAs (Baud *et al.*, 2004; Bach *et al.*, 2008). Recently, a third *PAS* mutant, *pas1*, was characterized as an immunophilin, which is required for the optimal synthesis of VLCFAs (Roudier *et al.*, 2010). PAS1 physically interacts with components of the microsomal elongase, such as PAS2, probably representing a cohort or scaffold protein around which this complex is assembled (Roudier *et al.*, 2010). One possible role for ACBP1/2 is the delivery of substrates (such as 18:0-CoA) to the elongase, perhaps by interacting with PAS1. Alternatively, ACBP1/2 may be involved in mediating the (dynamic) exchange between the acyl-CoA pool and phospholipids. The channelling of acyl-CoAs into glycerolipids via the Kennedy pathway is still only partially understood and a role of ACBPs is possible. Equally, a different role for these proteins could be in helping to mediate the well-documented reverse reaction of *lyso*-phosphatidylcholine acyltransferases (LPCAT), by which fatty acids are returned to the acyl-CoA pool from the *sn-2* position of phosphatidylcholine (Stymne & Stobart, 1984). Given the binding of phosphatidylcholine by ACBP1, it is possible to hypothesize that this protein helps to drive LPCAT in this reverse reaction, perhaps through the generation of local concentration gradients. Whatever the situation, it would be interesting to determine if ACBP1/2 physically interacts with proteins such as PAS1 or LPCAT, and it would be worthwhile to examine the role of ACBP1 in LPCAT activity. In addition, it would be useful to determine the composition of other lipid species that contain VLCFAs such as sphingolipids in the Arabidopsis *acbp1* and *acbp2* mutants, since these lipids are well known to play a key role in many cellular processes.

In conclusion, the acyl-CoA-binding proteins of Arabidopsis are clearly important in embryo development,

almost certainly co-ordinating some aspect of acyl-CoA-dependent lipid metabolism. Whilst their precise functions still remain to be determined, it is now obvious that they play a crucial and specific role in contributing to the phospholipid synthesis and homeostasis required for embryo development.

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Carbonyl sulfide: a new tool for understanding the response of the land biosphere to climate change

The global climate is changing mostly because of the anthropogenic emission of greenhouse gases, especially CO_2, into the atmosphere. The rate of atmospheric CO_2 growth in the future will depend on the balance between emissions and sink strengths. The importance of land photosynthesis in dampening the CO_2 increase in the atmosphere is now well recognized and both climate and global carbon (C) models incorporate photosynthesis–climate feedbacks. However, we still lack robust tools for partitioning different component fluxes (e.g. photosynthesis, respiration, decomposition) and for assessing the effects of climate change and atmospheric CO_2 increase on the C-sequestration potential of the biosphere. The paper by Stimler and coworkers in this issue of *New Phytologist* (pp. 869–878) shows that carbonyl sulfide (COS) represents a useful tracer of gross photosynthesis. This offers the perspective of an additional independent tool to study the terrestrial C cycle and to investigate ecosystem responses to global change.

About three-quarters of the anthropogenic emission of CO_2 is caused by the burning of fossil fuel, with a small contribution from cement production, while the remaining emissions are attributable to deforestation and land-use change. At present, the rate of increase of $[CO_2]$ in the atmosphere is about half that of CO_2 emissions, because some of the CO_2 emitted is dissolved in the oceans, some is taken up by land vegetation and a certain amount of 'missing C' may be ascribed to an underestimation of belowground allocation (Burgermeister, 2007). The sink