Chapter 15
Acyl-CoA-Binding Proteins (ACBPs) in Plant Development

Shiu-Cheung Lung and Mee-Len Chye

Abstract Acyl-CoA-binding proteins (ACBPs) play a pivotal role in fatty acid metabolism because they can transport medium- and long-chain acyl-CoA esters. In eukaryotic cells, ACBPs are involved in intracellular trafficking of acyl-CoA esters and formation of a cytosolic acyl-CoA pool. In addition to these ubiquitous functions, more specific non-redundant roles of plant ACBP subclasses are implicated by the existence of multigene families with variable molecular masses, ligand specificities, functional domains (e.g. protein-protein interaction domains), subcellular locations and gene expression patterns. In this chapter, recent progress in the characterization of ACBPs from the model dicot plant, *Arabidopsis thaliana*, and the model monocot, *Oryza sativa*, and their emerging roles in plant growth and development are discussed. The functional significance of respective members of the plant ACBP families in various developmental and physiological processes such as seed development and germination, stem cuticle formation, pollen development, leaf senescence, peroxisomal fatty acid β-oxidation and phloem-mediated lipid transport is highlighted.

Keywords Acyl-CoA esters • Fatty acids • Lipid trafficking • Phospholipid metabolism • Transporters

Introduction

In eukaryotic cells, the metabolism of fatty acids (FAs) takes place at various subcellular locations and requires extensive metabolite exchange *via* intracellular lipid trafficking. Plant FAs are synthesized *de novo* predominantly in the stroma of plastids, which houses acetyl-CoA carboxylase for acetyl-CoA carboxylation as the first committed step and FA synthetase for subsequent acyl-chain extension (Rawsthorne 2002). These nascent FAs are either utilized directly for plastidial lipid biosynthesis *via* the prokaryotic pathway (Roughan et al. 1980; Sparace and Mudd 1982; Heinz

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and Roughan 1983) or exported into the cytosol after activation into CoA-esters by acyl-CoA synthetase at the outer membrane of the plastidial envelope (Andrews and Keegstra 1983; Block et al. 1983). The plant mitochondria are also capable of limited FA synthesis, mainly for the production of lipoic acid (Wada et al. 1997; Gueguen et al. 2000). At the endoplasmic reticulum (ER) membrane, the cytosolic acyl-CoA pool fuels the assembly of triacylglycerols (TAGs) and extraplastidial membrane lipids such as phosphatidylcholine (PC) *via* the eukaryotic pathway (Slack and Roughan 1975; Simpson and Williams 1979; Dubacq et al. 1983). During this process, an acyl group is released from PC by the reverse reaction of acyl-CoA:lyso-PC acyltransferase (LPCAT) or phospholipase A, and the resulting lyso-PC can be reacylated by the forward reaction of LPCAT (Stymne and Stobart 1984; Wang 2001). The net outcome of this deacylation-reacylation cycle, often known as "acyl editing", is an exchange of acyl groups between the cytosolic acyl-CoA pool and PC at the ER (Bates et al. 2007; Bates and Browse 2012). PC, after the removal of its phosphocholine headgroup, can return to the diacylglycerol (DAG) pool at the ER membrane for TAG assembly, or it can enter the DAG pool at the inner membrane of plastid envelope for the assembly of thylakoid glycerolipids (Browse et al. 1986; Benning 2009). In the ER of epidermal cells, an acyl-CoA elongase catalyzes the formation of very-long-chain (VLC; C22 or longer) acyl-CoA esters (Lessire et al. 1985; Bessoule et al. 1989), which can be further metabolized to wax constituents *via* the primary alcohol and alkane pathways (Rowland et al. 2006; Greer et al. 2007). The wax molecules are delivered from the plasma membrane into the apoplast for cuticle formation (Samuels et al. 2008). During seed germination, FAs are liberated from TAGs by lipase activities on oil bodies, translocated across the single-membraned glyoxysomes (i.e. specialized peroxisomes) in the form of free FAs or activated CoA-esters, and eventually β-oxidized to acetyl-CoA molecules (Beisson et al. 2001; Fulda et al. 2002, 2004). In view of the subcellular nature of plant FA metabolism in the compartmentation of discrete pathways, highly dynamic lipid transfer mechanisms are essential to route the substrates, intermediates and products to destinations including the endomembrane system, the cytosol, plastids, mitochondria, peroxisomes and the extracellular space.

Due to their hydrophobic nature and lack of sorting signals in contrast to nascent polypeptides, lipids and their derivatives are delivered intracellularly in a facilitated manner. In vesicular trafficking, lipophilic metabolites are sorted between organelles *via* vesicle budding and fusion. Movement of a shorter distance (i.e. a few nm) across organellar membranes can be mediated by transbilayer flip-flop mechanisms or direct contact between the membranes (Raggers et al. 2000; Sprong et al. 2001; Holthuis and Levine 2005; van Meer et al. 2008; Toulmay and Prinz 2011). In a membrane-independent mode of transfer, lipids and their derivatives rely on protein facilitators or transporters such as ATP-binding cassette (ABC) transporters (Zolman et al. 2001; Kim et al. 2013), lipid transfer proteins (Kader 1997; Yeats and Rose 2008) and acyl-CoA-binding proteins (ACBPs; Xiao and Chye 2011a). For an in-depth discussion of the various mechanisms of lipid transfer within the plant cell and how they affect organelle biogenesis, readers are referred to recent review

articles (Benning 2009; Hurlock et al. 2014). This chapter focuses on new progress in the biochemical and functional characterization of plant ACBPs and their emerging roles in various facets of plant growth and development.

Conservation of ACBP Families in Eukaryotes

ACBPs, virtually found in all eukaryotic species and a few pathogenic prokaryotes, represent a multigene family conserved phylogenetically and functionally (Burton et al. 2005; Faergeman et al. 2007). ACBPs bind acyl-CoA esters (C12–C26) with high specificities and affinities in a non-covalent, reversible manner (Rasmussen et al. 1993; Faergeman and Knudsen 1997; Chye 1998; Chye et al. 2000; Knudsen et al. 2000; Burton et al. 2005; Leung et al. 2004, 2006; Hsiao et al. 2014a; Xue et al. 2014). The prototype form in ACBP families is represented by highly-conserved, low molecular mass (ca. 10-kDa) cytosolic proteins, which may be accompanied by larger variants identified previously from the majority of eukaryotic phyla except the fungi (Kragelund et al. 1999; Faergeman et al. 2007). The yeast (*Saccharomyces cerevisiae*) genome encodes a single-copy ACBP homolog, and its depletion adversely affected FA elongation as well as membrane assembly and organization (Gaigg et al. 2001), whilst its overexpression significantly altered acyl-CoA pool size and composition (Mandrup et al. 1993; Knudsen et al. 1994). The first characterized ACBP was purified from rat brain as a neurotransmitter termed diazepam-binding inhibitor (Guidotti et al. 1983), which shares sequence homology with the ACBPs subsequently purified from rat and bovine livers (Mogensen et al. 1987; Knudsen et al. 1989).

Following the discoveries of mammalian ACBPs, the prototype 10-kDa ACBP homologs were identified from a number of plant species, including *Brassica napus* (oilseed rape; Hills et al. 1994; Brown et al. 1998), *Ricinus communis* (castor bean; van de Loo et al. 1995), *Arabidopsis thaliana* (thale cress; Engeseth et al. 1996), *Gossypium hirsutum* (cotton; Reddy et al. 1996), *Digitalis lanata* (foxglove; Metzner et al. 2000), *Oryza sativa* (rice; Suzui et al. 2006) and *Vernicia fordii* (tung tree; Pastor et al. 2013). In addition to the 10-kDa ACBP isoforms, the acyl-CoA-binding (ACB) domain is conserved in larger multi-domain proteins from *A. thaliana* (Chye 1998; Chye et al. 2000; Leung et al. 2004, 2006; Xiao and Chye 2009), *Agave americana* (century plant; Guerrero et al. 2006), *O. sativa* (Meng et al. 2011, 2014; Meng and Chye 2014), *V. fordii* (Pastor et al. 2013) and *Vitis vinifera* (grape; Takato et al. 2013). A recent BLASTP search using sequences of Arabidopsis paralogs retrieved 84 ACB domain-containing sequences comprising 66–682 amino acid residues from 14 plant species and 2 green algae (Meng et al. 2011). The majority of these proteins possess a conserved *N*-terminal ACB domain accompanied by highly dissimilar *C*-terminal regions (Meng et al. 2011). Based on molecular mass and domain architecture, Meng et al. (2011) classified plant ACBPs into four subgroups (Fig. 15.1; Table 15.1), namely small ACBPs (Class I), ankyrin-repeat ACBPs (Class II), large

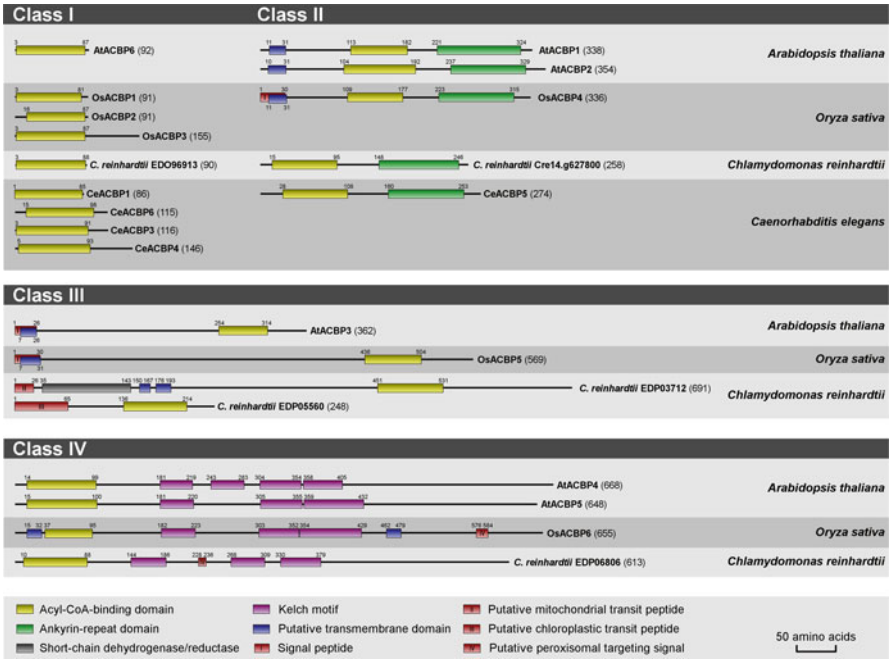

Fig. 15.1 Domain architecture of Classes I–IV ACBP members from plant and non-plant species. Representative members from Arabidopsis (*Arabidopsis thaliana*; At), rice (*Oryza sativa*; Os), green alga (*Chlamydomonas reinhardtii*; Cr) and the nematode worm (*Caenorhabditis elegans*; Ce). Classes III and IV ACBPs were not identified from *C. elegans*. Protein queries were submitted to NCBI's protein BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to scan for the presence of conserved domains and their intervals were annotated by alignment with the Conserved Domain Database (CDD) collection including acyl-CoA-binding domain (cd00435), ankyrin-repeat domain (cd00204), short-chain dehydrogenase/reductase (pfam01113) and kelch motif (pfam01344, pfam07646, pfam13415, pfam13418 and pfam13854). Amino acid residues are indicated in parentheses. AtACBPs, OsACBPs and CeACBPs are numbered according to Xiao and Chye (2009), Meng et al. (2011), and Elle et al. (2011), respectively. Their sequence data can be found under the following GenBank accession number: AtACBP1 (AED96361), AtACBP2 (AEE85391), AtACBP3 (AEE84874), AtACBP4 (AEE74237), AtACBP5 (AED93708), AtACBP6 (AEE31396), OsACBP1 (BAG86980), OsACBP2 (BAG86809), OsACBP3 (ABF97253), OsACBP4 (BAF16206), OsACBP5 (BAG93201), OsACBP6 (ABF99748), CeACBP1 (CCD67306), CeACBP3 (CAA91987), CeACBP4 (CCD61830), CeACBP5 (CAB03343) and CeACBP6 (CAA19448)

ACBPs (Class III) and kelch-ACBPs (Class IV), which are each represented in 12 of the 13 higher plant species analyzed (except *Picea sitchensis*) using genome sequence information available to date. The indispensability of the diverse subclasses of plant ACBPs implicates their functional non-redundancy with respect to their specific roles for normal plant growth and development.

Over the years, a combination of techniques including molecular genetics, microscopy, cell biology, biochemistry and mass spectrometry have been utilized in this laboratory to unravel the discrete functions of the six ACBP members in

Table 15.1 Properties of four classes of ACBPs from various plant sources

Class	Member	Locus ID	Protein size		pI	Tissue specificity[a]	Subcellular localization	Binding preferences			References
								Acyl-CoA			
			Residues	M_r (kDa)				By Lipidex assays	By quantitative assays[b] (K_d)	Phospholipid	
I	AtACBP6	At1g31812	92	10.4	4.91	High in leaves, stems, developing seeds, siliques and flowers (esp. microspores, tapetum, pollen)	Cytosol	18:2 > 20:4 > 18:1 ≈ 16:0 > 18:3	16:0 (35.9 nM) 18:2 (36.4 nM) 14:0 (38.7 nM) 18:1 (45.3 nM) 18:0 (60.6 nM) 18:3 (84.1 nM)	di 16:0-PC di 18:0-PC di 18:1-PC di 18:2-PC	Engeseth et al. (1996), Chen et al. (2008) and Hsiao et al. (2014a, 2015)
	OsACBP1	Os08g06550	91	10.2	4.86	High in leaves and roots		18:1 > 18:2 > 18:3 > 16:0	N.D.	di 18:0-PA/PC di 18:1-PA/PC di 18:2-PC	Meng et al. (2011, 2014)
	OsACBP2	Os06g02490		10.3	4.69	High in leaves, roots and soft dough seeds		18:3			
	OsACBP3	Os03g37960	155	17.7	9.05	High in leaves, roots and seeds at anthesis	Cytosol and others[c]				
	BnACBP	Bna.2377	92	10.2	5.50	High in developing embryos, flowers and cotyledons	Cytosol	18:2 > 18:1 > 18:3		N.D.	Hills et al. (1994), Engeseth et al. (1996) and Yurchenko et al. (2009)

(continued)

Table 15.1 (continued)

Class	Member	Locus ID	Protein size		pI	Tissue specificity[a]	Subcellular localization	Binding preferences			References
								Acyl-CoA			
			Residues	M_r (kDa)				By Lipidex assays	By quantitative assays[b] (K_d)	Phospholipid	
II	AtACBP1	At5g53470	338	37.5	4.32	High in developing seeds, siliques, vasculature, root primordia, flowers (not in petals/ stamens), stem epidermis and trichomes	ER and PM	20:4 > 18:2 > 18:3 > 18:1 > 16:0	18:3 (0.44 μM) 18:1 (0.76 μM) 18:2 (0.83 μM) 25:0 (1.69 μM) 26:0 (1.94 μM) 24:0 (2.14 μM)	di 16:0-PA di 18:0-PA di 18:1-PA/PC di 18:2-PC	Chye (1998), Chye et al. (1999), Leung et al. (2006), Xiao et al. (2008b), Gao et al. (2009), Chen et al. (2010), Du et al. (2010a, 2013a) and Xue et al. (2014)
	AtACBP2	At4g27780	354	38.5	4.22	High in roots (esp. vasculature), pollen, developing embryos, siliques and guard cells		18:2 > 18:3 > 20:4 ≈ 16:0 > 18:1	N.D.	16:0-lysoPC di 18:1-PC di 18:2-PC	Chye et al. (2000), Li and Chye (2003), Kojima et al. (2007), Gao et al. (2009, 2010a), Chen et al. (2010) and Du et al. (2013b)
	OsACBP4	Os04g58550	336	36.0		High in leaves, roots and seeds at anthesis	ER	18:2 > 16:0 > 18:3		di 16:0-PA di 18:0-PA/PC di 18:1-PA/PC di 18:2-PC	Meng et al. (2011, 2014) and Meng and Chye (2014)

III	AtACBP3	At4g24230	362	39.3	3.96	High in shoots, roots (not in root hair/tip), vasculature and flowers (esp. stigma)	ER and apoplast	20:4 > 18:3 > 18:2 > 18:1 ≈ 16:0	22:0 (29.9 nM) 20:0 (66.0 nM) 24:0 (80.6 nM) 18:2 (788 nM)	di 16:0-PC/PE di 18:0-PC/PE di 18:1-PC/PE di 18:2-PC/PE	Leung et al. (2004, 2006), Xiao et al. (2010), Zheng et al. (2012) and Xie et al. (2015)
	OsACBP5	Os03g14000	569	61.2	3.98	High in leaves and seeds at all stages	ER	18:3 > 16:0	N.D.	di 18:0-PA/PC di 18:1-PA/PC di 18:2-PC	Meng et al. (2011, 2014) and Meng and Chye (2014)
IV	AtACBP4	At3g05420	668	73.2	4.95	High in leaves, roots, siliques and flowers (esp. pollen grains; not in microspores/ tapetum)	Cytosol	18:1 > 16:0 > 18:2 > 18:3	18:0 (2.7 μM) 16:0 (23.5 μM) 14:0 (65.3 μM) 18:2 (73.8 μM) 18:1 (95.2 μM) 18:3 (189 μM)	di 18:1-PC di 18:2-PC	Leung et al. (2004), Xiao et al. (2008a, 2009a) and Hsiao et al. (2014a, 2015)
	AtACBP5	At5g27630	648	71.0	5.86	High in leaves, roots and flowers (esp. microspores, tapetum; not in pollen grains)			18:0 (35.4 μM) 16:0 (35.7 μM) 14:0 (41.9 μM) 18:2 (64.1 μM) 18:1 (74.4 μM) 18:3 (92.4 μM)		
	OsACBP6	Os03g61930	655	71.4	5.05	High in leaves, roots, developing and germinating seeds	Peroxisomes	18:3 > 18:2	18:3 (32.3 μM)	di 18:0-PA/PC di 18:1-PA/PC di 18:2-PC	Meng et al. (2011, 2014)

ER endoplasmic reticulum, M_r relative molecular mass, *N.D.* not determined, *PA* phosphatidic acid, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PM* plasma membrane

[a]All ACBPs were detectable in all tissues tested albeit at varying expression levels

[b]The dissociation constants (K_d) were determined by microscale thermophoresis (for rAtACBP3) or isothermal titration calorimetry (otherwise)

[c]Irregular membranous and punctate structures

Arabidopsis, constituting the first comprehensive study on an ACBP family in the Plant Kingdom (Xiao and Chye 2009). Our recent work on the rice ACBP family has also provided insights into similarities and differences between the representative dicot (Arabidopsis) and monocot (rice) ACBP families (Meng et al. 2011, 2014). The important features and specific roles of the Arabidopsis and rice ACBP subclasses in plant growth and development are described herein.

Arabidopsis ACBPs

The Arabidopsis genome contains six *ACBP* paralogs (i.e. *AtACBP1–AtACBP6*), all of which encode functional proteins as demonstrated *in vitro* through the binding of *Escherichia coli*-expressed recombinant (r) ACBPs to acyl-CoA esters (Engeseth et al. 1996; Chye 1998; Chye et al. 2000; Leung et al. 2004, 2006; Gao et al. 2009; Xiao et al. 2009a, 2010; Xue et al. 2014; Hsiao et al. 2014a; Xie et al. 2015). In addition to the prototype 10-kDa AtACBP6 (Class I), five other AtACBPs ranging from 37.5 to 73.2 kDa exist (Xiao and Chye 2009). Class II members, AtACBP1 and AtACBP2, are highly homologous sharing 71 % amino acid sequence identity, and are transmembrane proteins that are targeted to the ER and plasma membrane (Chye 1998; Chye et al. 1999, 2000; Li and Chye 2003). Both proteins harbor a *C*-terminal domain of ankyrin repeats (Li and Chye 2004; Gao et al. 2009, 2010a; Du et al. 2013a), a potential domain for protein-protein interactions (Michaely and Bennett 1992; Bork 1993), subsequently substantiated for AtACBP2 (Gao et al. 2009, 2010a). AtACBP3 (Class III) contains an *N*-terminal cleavable signal peptide which directs the protein to the endomembrane system and the apoplastic space, as verified using confocal laser scanning microscopy of green fluorescent protein (GFP)-tagged proteins and subcellular fractionation experiments followed by Western blot analysis using AtACBP3-specific antibodies (Leung et al. 2006; Xiao et al. 2010). The location of an ACB domain within the *C*-terminal region of AtACBP3 contrasts with the more common appearance of this domain at the *N*-termini in the other ACBPs (Meng et al. 2011; Xiao and Chye 2011a). Besides the prototype AtACBP6, Class IV AtACBP4 and AtACBP5 are the two other cytosolic isoforms, and they represent the largest (i.e. 73.2 and 71.0 kDa, respectively) members in the AtACBP family (Leung et al. 2004; Xiao et al. 2008a). The core regions of AtACBP4 and AtACBP5 invariably feature kelch motifs (Leung et al. 2004; Li et al. 2008), that constitute potential sites for protein-protein interactions (Adams et al. 2000).

Sequence alignment of the ACB domains from the six AtACBPs and other plant ACBPs shows high conservation of the ‘‘YKQA’’ and ‘‘KWDAW’’ motifs (Xiao and Chye 2011a), which correlates well with the X-ray and nuclear magnetic resonance structures of ligand-bound ACBP complexes from cow, man, *Plasmodium falciparum* and yeast (Kragelund et al. 1993; van Aalten et al. 2001; Faergeman et al. 2007; Taskinen et al. 2007). Site-directed mutagenesis of either one of these two motifs affected the binding of rAtACBP2 to palmitoyl-CoA ester (Chye et al.

2000; Leung et al. 2004) and rAtACBP4 and rAtACBP5 to oleoyl-CoA ester (Leung et al. 2004), but not rAtACBP1 or rAtACBP3 to arachidonyl-CoA ester, which was instead disrupted by amino acid substitution of an arginine residue upstream of the "YKQA" motif (Leung et al. 2006). Thus, the amino acid determinants of ACB functions in AtACBPs appear to be isoform- and ligand-dependant. In fact, the six AtACBPs, which show more or less sequence variations within their ACB domains, exhibit varying ligand specificities. Both rAtACBP1 and rAtACBP2 displayed preferences for linoleoyl- and linolenoyl-CoA esters (Gao et al. 2009), and recent data from isothermal titration calorimetry (ITC) also revealed high affinities of rAtACBP1 to VLC acyl-CoA esters (Xue et al. 2014). Also, rAtACBP3 bound VLC acyl-CoA esters (Xie et al. 2015), and exhibited higher affinity for arachidonyl-CoA ester than linoleoyl- and linolenoyl-CoA esters (Leung et al. 2006; Xiao et al. 2010). Recently, the thermodynamics of interactions between the three cytosolic AtACBPs and long-chain (C16–C18) acyl-CoA esters were quantitatively compared using ITC (Hsiao et al. 2014a), and the binding affinities of rAtACBP4 and rAtACBP5 (dissociation constants K_d in the micromolar range) were reported to be much weaker than that of rACBP6 (K_d in the nanomolar range). Other than acyl-CoA esters, AtACBPs exhibited affinities for phospholipids, as typified by the binding of all six rAtACBPs to PC *in vitro* (Chen et al. 2008, 2010; Xiao et al. 2009a, 2010). In addition, rAtACBP1 and rAtACBP2 bind phosphatidic acid (PA; Du et al. 2010a) and lyso-PC (Gao et al. 2010a), respectively, whilst rAtACBP3 binds phosphatidylethanolamine (PE; Xiao et al. 2010).

While the differential subcellular localization and ligand specificities of AtACBPs for different acyl-CoA esters and (lyso-)phospholipids *in vitro* are in favor of the hypothesis that individual ACBPs play non-redundant roles, studies of protein interactome have provided insights on their functions (Du and Chye 2013). Bimolecular fluorescence complementation (BiFC) and yeast 2-hybrid (Y2H) assays indicated that one of the protein partners of the ankyrin repeat-containing isoform, AtACBP1, is phospholipase Dα1 (PLDα1; Du et al. 2013a), which hydrolyzes structural phospholipids to produce PA as a second messenger in abscisic acid (ABA) signaling (Jacob et al. 1999; Zhang et al. 2004; Li et al. 2009). The studies of Arabidopsis AtACBP1-overexpressors (AtACBP1-OEs) and the *acbp1* knockout mutant revealed the potential of AtACBP1 in promoting PLDα1 activity related to ABA signaling during seed germination and seedling development (Du et al. 2013a; see Sect. AtACBPs in the Regulation of Seed Germination and Seedling Development for details). Using AtACBP2, another ankyrin repeat-containing isoform, as bait in Y2H screening of an Arabidopsis cDNA library identified a member of the ethylene response factor (ERF) family, termed ethylene-responsive element-binding protein (AtEBP) or RELATED TO APETALA2.3 (RAP2.3), as a protein interactor (Li and Chye 2004). The AtACBP2-AtEBP interaction was confirmed by an *in vitro* pull-down assay, and their fluorescent fusion proteins were found to co-localize at the plasma membrane (Li and Chye 2004). The same interactor AtEBP/RAP2.3 was also found in another Y2H screen using AtACBP4 as bait, and this interaction was confirmed by co-immunoprecipitation and fluorescence resonance energy transfer, the latter of which revealed that they interact predominantly in the cytosol (Li et al.

2008). In Northern blot analysis, the concomitant induction of *AtACBP4* and *AtEBP* expression by the treatment with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), methyl jasmonate (MeJA) or the pathogen *Botrytis cinerea* suggested that AtACBP4 may play a role in AtEBP-mediated defense response *via* ethylene and/or jasmonate (JA) signaling (Li et al. 2008). AtACBP1 and AtACBP2 were also demonstrated to interact with another related ERF member, RAP2.12, using BiFC and Y2H assays (Licausi et al. 2011). Under aerobic conditions, AtACBP1 and AtACBP2 sequester the soluble RAP2.12 at the plasma membrane and protect it from degradation *via* the ubiquitin-dependent *N*-end rule pathway, whereas low oxygen concentration triggers the release of RAP2.12 from the plasma membrane into the nucleus for activation of hypoxia-responsive gene expression (Licausi et al. 2011). More recently, a role for AtACBP3 was identified in VLCFA-mediated response to hypoxic stress in conjugation with salicylic acid (SA) and ethylene signaling (Xie et al. 2015).

AtACBP2 also interacts at the plasma membrane with other stress-responsive proteins including a farnesylated protein AtFP6 (Gao et al. 2009) and a lysophospholipase AtLYSOPL2 (Gao et al. 2010a), both of which have roles in conferring plant tolerance to heavy metal stress. AtFP6 is a member of the isoprenylated protein family (i.e. farnesylated proteins) featuring at least one characteristic metal-binding motif (core sequence: M/LXCXXC; Dykema et al. 1999). In roots, *AtFP6* mRNA expression was induced by cadmium and zinc (Gao et al. 2009), whereas *AtACBP2* expression was lead-inducible (Xiao et al. 2008b). The *in vitro* binding of AtFP6 and AtACBP2 to lead, cadmium and copper, in conjunction with the enhanced cadmium-tolerance of Arabidopsis overexpressing either protein, led to the assumption that the AtACBP2-AtFP6 interaction at the plasma membrane of root cells facilitates the transport of these heavy metal ions (Xiao et al. 2008b; Gao et al. 2009). Another AtACBP2-interactor, AtLYSOPL2, is one of the few known plant LYSOPLs to degrade lyso-phospholipids into FAs and glycerolphosphate derivatives (Wang and Dennis 1999), representing an important step in the detoxification of lyso-phospholipids, the intermediates of phospholipid metabolism post-stress (Flieger et al. 2002; Ryu 2004). In Arabidopsis, the overexpression of *AtLYSOPL2* or *AtACBP2* improved plant tolerance to cadmium and hydrogen peroxide (H_2O_2), whilst *lysopl2* knockout mutants were more sensitive to zinc and H_2O_2 (Gao et al. 2009, 2010a), suggesting a protective role of AtLYSOPL2 and AtACBP2 against metal-induced oxidative stress (Gao et al. 2010a). Considering the binding activity of rAtACBP2 to linoleoyl-CoA ester, linolenoyl-CoA ester, PC and lyso-PC (Gao et al. 2010a), and the tight association of AtACBP2 with two metal-inducible proteins AtFP6 and AtLYSOPL2, it is possible that AtACBP2 has a role in phospholipid repair following lipid peroxidation upon heavy metal-induced stress (Gao et al. 2009, 2010a, b). In another study, quantitative real-time PCR (qRT-PCR) further identified the up-regulation of *AtACBP1* and *AtACBP4*, besides *AtACBP2*, mRNAs in Arabidopsis shoots and roots upon lead (II) treatment (Du et al. 2015). The overexpression of *AtACBP1* or *AtACBP4* in transgenic *Brassica juncea* culminated in an enhanced accumulation of lead (II) in the cytosol of root cells without increasing lead (II) tolerance (Du et al. 2015).

Other than hypoxia, heavy metal and oxidative stresses, some members of the AtACBP family function in responses to other abiotic stresses including low temperature and drought. Chen et al. (2008) indicated relatively higher mRNA and protein levels of AtACBP6 in rosettes of wild-type Arabidopsis upon cold treatment. The freezing-tolerant phenotype of AtACBP6-OEs was attributed to the up-regulated expression of *PLDδ* in rosettes, leading to the elevation of PA and the decline in PC (Chen et al. 2008), but the freezing-tolerant flowers of the same AtACBP6-OEs were recently shown to be directly associated with elevations of PC and monogalactosyldiacylglycerol and decline in PA (Liao et al. 2014). On the other hand, the overexpression of AtACBP1 rendered transgenic Arabidopsis more sensitive to freezing stress accompanied by a decrease in PC and an increase in PA (Du et al. 2010a). Reciprocally, the Arabidopsis *acbp1* mutant plants exhibited freezing tolerance accompanied by PC accumulation and PA reduction in rosette leaves, possibly due to suppression in hydrolysis of PC to PA as evident from the down-regulated expression of *PLDα1* (Du et al. 2010a). In spite of the high homology to AtACBP1, AtACBP2 was not associated with the freezing response (Du et al. 2010b). Instead, it has been ascribed a role in drought response (Du et al. 2013b). Du et al. (2013b) showed that the overexpression of *AtACBP2* in Arabidopsis altered the expression profiles of ABA signaling genes, including the up-regulation of Respiratory Burst Oxidase Homolog D (*AtRBOHD*) and *AtRBOHF* and the down-regulation of *HYPERSENSITIVE TO ABA1*. The over-production of ABA-mediated reactive oxygen species in the guard cells induced stomatal closure and reduced transpiration loss leading to an enhanced drought tolerance in *AtACBP2-OEs*, in comparison with *acbp2* mutant plants which were more sensitive to drought than the wild type (Du et al. 2013b).

In addition to abiotic stresses, AtACBPs have been linked to defense responses against pathogens. Expression of both *AtACBP3* and *AtACBP4* was shown to be inducible by treatment with the defense-related phytohormones including MeJA and ACC and necrotrophic fungus *B. cinerea* (Li et al. 2008; Xiao and Chye 2011b). *AtACBP3* expression was also up-regulated by treatment with SA, arachidonic acid (a fungal elicitor) and bacterial pathogen *Pseudomonas syringae* pv *tomato* DC3000 (Xiao and Chye 2011b). These phytohormones and pathogens induced β-glucuronidase (*GUS*) expression in transgenic Arabidopsis expressing *GUS* from *AtACBP3* (Zheng et al. 2012). Electrophoretic mobility shift assays (EMSA) further revealed that an S-box of AT-rich sequence (−516/−512) binds nuclear extracts from pathogen-infected leaves (Zheng et al. 2012). In Arabidopsis AtACBP3-OEs, the constitutive activation of pathogenesis-related (*PR*) gene expression and the induction of cell death and hydrogen peroxide production likely account for the enhanced resistance to *P. syringae* DC3000 (Xiao and Chye 2011b). Conversely, Arabidopsis *acbp3* knock-out mutant and *AtACBP3* RNA interference (RNAi) transgenic plants were more susceptible to *P. syringae* attack and exhibited lower mRNAs of the defense-related *PR1*, *PR2* and *PR5* (Xiao and Chye 2011b). Consistently, transgenic Arabidopsis expressing an *AtACBP3* homolog from grape (i.e. *VvACBP*)

showed resistance to *P. syringae* DC3000 and a hemibiotrophic fungus, *Colletotrichum higginsianum* (Takato et al. 2013).

Besides the stress-responsiveness of *AtACBPs*, the expression of some is subject to diurnal oscillation during light/dark cycles (Xiao et al. 2009a, b, 2010; Zheng et al. 2012; Hsiao et al. 2014b). In Arabidopsis rosettes, the expression patterns of *AtACBP4* and *AtACBP5* in light/dark cycling (Xiao et al. 2009a, b) correlated well with that of *FAD7* which encodes an Arabidopsis plastidial ω-3 FA desaturase (Nishiuchi et al. 1995). All three genes are expressed at higher levels during the day than night, and their expression was rapidly silenced in continuous darkness (Xiao et al. 2009a). On the other hand, an opposing pattern was observed for *AtACBP3* and *AtACBP6*, both of which were up-regulated in the dark (Xiao et al. 2009a). Similar RNA gel blot analysis indicated that *AtACBP3* was highly expressed in continuous darkness (Xiao et al. 2010). By using transgenic Arabidopsis expressing GUS driven by various deletions of the *AtACBP3* promoter, the *cis*-elements responsible for the dark-induced expression of *AtACBP3* were mapped to a 160-bp 5′-flanking region (–434/–274), within which a DNA-BINDING WITH ONE FINGER (DOF) box and a GT-1 motif were shown to interact specifically with nuclear proteins from dark-treated Arabidopsis leaves in EMSA and DNase I footprinting assays (Zheng et al. 2012). In Arabidopsis seedlings, the biological clock regulates *AtACBPs* transcriptionally, amongst which *AtACBP3* most diurnally oscillates (Hsiao et al. 2014b). The diurnal patterns of all six *AtACBPs* appeared slightly altered in an arrhythmic line (i.e. *cca1lhy* double mutant) with defects in internal clock functions (Hsiao et al. 2014b).

Rice ACBPs

As our studies on AtACBPs and their stress-responsive protein interactors have opened new possibilities in protecting plant against abiotic stresses and phytopathogens (Xiao and Chye 2011a; Chen et al. 2014), we sought to investigate if genetic engineering strategies could be extended to agronomically important species including those taxonomically distant from the eudicot Arabidopsis. In this regard, rice represents an excellent candidate as a monocot model plant that is arguably the most important staple crop in Asia. The completion of the rice genome sequence (Goff et al. 2002; Yu et al. 2002) facilitated the retrieval of homologous sequences from available rice genome databases. BLASTP searches using the amino acid sequences of AtACBP1, AtACBP3, AtACBP4 and AtACBP6 as query identified six rice ACBP homologs designated as OsACBP1 to OsACBP6, respectively (Meng et al. 2011). Meng et al. (2011) studied the phylogeny, gene expression and biochemical analyses of the rice ACBP family. The characterization of the six rice ACBPs in comparison to AtACBPs has revealed similarities and differences (Meng et al. 2011, 2014; Meng and Chye 2014), as summarized below.

Resembling the prototype AtACBP6 (Class I), OsACBP1, OsACBP2 and OsACBP3 are of relatively low molecular masses (i.e. 10.2–17.7 kDa) (Meng et al.

2011). In contrast to Class I in which three members exist in rice, the remaining three classes (Classes II, III and IV) of OsACBPs are each represented by a single member (Meng et al. 2011). The 36.0-kDa OsACBP4 resembles the ankyrin-repeat-containing Class II AtACBP1 and AtACBP2. The 61.2-kDa OsACBP5 belongs to Class III (large ACBPs), whilst the largest (71.4-kDa) kelch-motif-containing OsACBP6 falls under Class IV.

Analysis by qRT-PCR on organ-specificity and stress-responsiveness of the rice *ACBP* family provided the initial clues on their possible functions (Meng et al. 2011). In terms of spatial expression, transcripts of the six rice *ACBPs* were detectable in all organs tested, in descending order of relative expression levels from leaves, roots to stems in 7-week-old plants (Meng et al. 2011). The expression patterns of the six rice *ACBP*s varied during seed development (Meng et al. 2011). Both *OsACBP1* and *OsACBP5* were stably expressed throughout the entire reproductive phase, but *OsACBP3* and *OsACBP4* expression were elevated at anthesis, and *OsACBP2* expression peaked at the more mature soft dough-staged seeds (Meng et al. 2011). On the other hand, *OsACBP6* expression was higher in anthesis- and soft dough- than milk-staged seeds (Meng et al. 2011). The highest expression of Class III *OsACBP5* occurred during seed development, resembling Class II *AtACBP1* and *AtACBP2* (Chen et al. 2010), and deviated from *AtACBP3* (Class III) which was expressed at a low level in siliques (Zheng et al. 2012). When the regulation of rice *ACBP* expression in response to abiotic and biotic stresses was tested by qRT-PCR (Meng et al. 2011), they appeared to be consistent with microarray data (http://www.ricearray.org/). *OsACBP4* and *OsACBP5* were induced by high-salinity treatment and *OsACBP4* was also induced by drought (Meng et al. 2011). Upon cold stress, *OsACBP6* expression slightly declined after 24 h, whilst down-regulation of the other five *OsACBPs* occurred within 12 h. After wounding, the transcripts of the three Class I *OsACBPs* and Class II *OsACBP4* declined but *OsACBP5* and *OsACBP6* were induced at 30 min post-treatment followed by a significant decrease below the basal level. Inoculation with the rice blast fungus *Magnaporthe grisea* up-regulated *OsACBP5* but reduced the expression of the other five *OsACBPs* (Meng et al. 2011). These varying expression patterns of respective rice *ACBPs* in response to environmental and biotic stresses appeared to differ from their Arabidopsis counterparts within the same subclasses (see Sect. Arabidopsis ACBPs). Differential spatial and stress-responsive expression patterns between rice and Arabidopsis *ACBPs* indicate functional divergence of ACBPs following gene duplication during the evolution of monocot and eudicot genomes (Meng et al. 2011).

In vitro binding assays confirmed that the six *OsACBP*s encode functional proteins (Meng et al. 2011, 2014). In rice, as in Arabidopsis, individual ACBP homologs exhibited differential binding preferences for acyl-CoA species (Meng et al. 2011), implicating that rice ACBPs play non-redundant physiological roles. Meng et al. (2011) tested the binding of rOsACBPs to four selected acyl-CoA species representing saturated (i.e. palmitoyl-CoA ester), monounsaturated (i.e. oleoyl-CoA ester) and polyunsaturated (i.e. linoleoyl- and linolenoyl-CoA esters) fatty acyl-CoA esters. While only rOsACBP1 binds oleoyl-CoA ester, all six rOsACBPs bind linolenoyl-CoA ester (Meng et al. 2011). Also, rOsACBP1, rOsACBP4 and

rOsACBP5 showed binding activities to palmitoyl-CoA ester (Meng et al. 2011). Linoleoyl-CoA ester was bound by rOsACBP1, rOsACBP4 and rOsACBP6 (Meng et al. 2011). In addition to acyl-CoA esters, the interactions of rOsACBPs with phospholipids were tested (Meng et al. 2014). While all rOsACBPs bind 18:0-PC, 18:1-PC and 18:2-PC, none bind 14:0-PC, 16:0-PC or lyso-PC (Meng et al. 2014). All rOsACBPs also bind 18:0-PA and 18:1-PA but only rOsACBP4 binds 16:0-PA (Meng et al. 2014). Although rAtACBP3 binds PE (Xiao et al. 2010) and rAtACBP2 binds lyso-PC (Gao et al. 2010a), no rOsACBP emerged to bind PE, lyso-PC or phosphatidylserine (PS) (Meng et al. 2014).

In terms of subcellular localization, differences were also observed between Arabidopsis and rice ACBPs within the same subclasses (Meng et al. 2014; Meng and Chye 2014). The Class I ACBPs from rice (i.e. OsACBP1, OsACBP2 and OsACBP3) were localized to the cytosol of agroinfiltrated tobacco leaf epidermal cells as well as the cotyledonary and root cells of transgenic Arabidopsis using fluorescent protein fusions (Meng et al. 2014). In addition, GFP-tagged OsACBP3 which has a 64-amino acid *C*-terminal extension was localized to some irregular membranous structures and randomly-scattered punctate structures in transgenic Arabidopsis (Meng et al. 2014). The ER localization of Class II OsACBP4 resembled AtACBP1 and AtACBP2 belonging to the same class, but OsACBP4, unlike its Arabidopsis counterparts, was not sorted to the plasma membrane. However, Class III OsACBP5 was confined to the ER and was not targeted extracellularly (Meng et al. 2014), unlike Class III AtACBP3 which was localized to the endomembranes and the apoplastic space (Leung et al. 2006; Xiao et al. 2010). In transgenic Arabidopsis seedlings, both OsACBP4 and OsACBP5 were visualized at the peripheral tubular ER, whereas OsACBP4 was also observed at the central and peripheral cisternal ER structures (Meng et al. 2014; Meng and Chye 2014), which are specialized for ribosome binding and cotranslational translocation of proteins (Friedman and Voeltz 2011). In addition, both OsACBP4 and OsACBP5 were localized to the membranes of ER bodies (Meng and Chye 2014), which have been identified as ribosome-surrounded, spindle-shaped ER structures in healthy seedlings or wounded/JA-treated rosette leaves of Arabidopsis (Hayashi et al. 2001; Matsushima et al. 2003). Interestingly, OsACBP6 represented the first plant ACBP demonstrated to be targeted to the peroxisomes (Meng et al. 2014). OsACBP6::GFP fusion proteins were confirmed to colocalize with the peroxisomal marker DsRed::SKL in primary roots and leaves of transgenic Arabidopsis as well as particle-bombarded rice sheath cells (Meng et al. 2014). Proteomic analysis of rat liver and mouse kidney peroxisomes had previously identified 56.6-kDa ACBP homologs (Kikuchi et al. 2004; Wiese et al. 2007).

Roles of ACBPs in Plant Development

Lipids are vital macromolecules acting as a major constituent of biological membranes and other structural components, fuel for many metabolic processes and key mediator of signal transduction (Wang 2004). Apart from the involvement of plant

ACBPs in different stress responses to environmental and biotic cues (Xiao and Chye 2011a), accumulating evidence suggests that ACBPs are crucial for normal plant growth and development (Table 15.2). The current knowledge of the physiological roles of individual plant ACBP homologs in plant development is discussed.

ACBPs in Seed Oil Biosynthesis

In oleaginous plants, TAG is the major form of seed storage lipids. Its synthesis involves the sequential incorporation of three FA moieties onto a glycerol backbone *via* a series of acyl-CoA-dependent acylation steps in the Kennedy pathway (Kennedy 1961). This pathway starts from the acylation of glycerol-3-phosphate to form lyso-PA by the action of glycerol-3-phosphate acyltransferase (GPAT) (Kennedy 1961). Lyso-PA acyltransferase (LPAAT) catalyzes the acylation of lyso-PA to PA, which is dephosphorylated into DAG by the action of phosphatidate phosphatase (Kennedy 1961). The final acylation of DAG to form TAG is catalyzed by DAG acyltransferase (DGAT), although acyl-CoA-independent reactions also exist (Lung and Weselake 2006). During the acyl transfer reactions, ACBP facilitates TAG assembly by maintaining a cytosolic acyl-CoA pool as a source of FAs, while the free acyl-CoA concentration in the cytosol is typically maintained below 10 nM (Knudsen et al. 1999). The ACBP:acyl-CoA ratio regulates the feedback inhibitory effect of substrates on enzyme activities (Rasmussen et al. 1993). ACBP also protects long-chain acyl-CoA esters from degradation by microsomal acyl-hydrolases (Rasmussen et al. 1993; Engeseth et al. 1996; Jolly et al. 2000). The tendency of ACBP to associate with acyl-CoA esters on the membrane periphery suggests its potential role in intermembrane acyl-CoA transport (Simonsen et al. 2003), and in the protection of membranes and membrane-associated enzymes from the deleterious detergent effect of amphiphilic acyl-CoA esters (Rasmussen et al. 1990). More specifically, mammalian ACBPs are known to modulate the activities of GPAT (Jolly et al. 2000; Kannan et al. 2003; Huang et al. 2005), acyl-CoA:lysophospholipid acyltransferase (Fyrst et al. 1995) and acyl-CoA:cholesterol acyltransferase (Kerkhoff et al. 1997; Chao et al. 2003).

In *B. napus*, the activities of TAG biosynthetic enzymes are also regulated by a 10-kDa cytosolic ACBP (Brown et al. 1998; Hobbs and Hills 2000; Yurchenko and Weselake 2011). GPAT activity was stimulated *in vitro* in the presence of rBnACBP if its concentration exceeded that of the [^{14}C]-oleoyl-CoA substrate; otherwise GPAT activity was inhibited (Brown et al. 1998). In fact, the level of BnACBP was elevated 12-fold during seed maturation coinciding with peak TAG accumulation, implicating an important role of ACBP in seed oil biosynthesis (Engeseth et al. 1996). In contrast, BnACBP was near undetectable in desiccating seeds at maturity (Brown et al. 1998). In addition, rBnACBP stimulated the activity of BnDGAT, when expressed in *B. napus* microspore-derived cell suspension cultures or in a

Table 15.2 Summary of ACBP functions in plant development

Function	Species	ACBP(s) involved	Binding partner(s) involved	Effect of knockout mutation, silencing (RNAi) and overexpression in Arabidopsis			References
				Line	Phenotypic effects	Regulation of associated genes	
Seed oil biosynthesis	*Brassica napus*	BnACBP	LC acyl-CoAs	*phaP*::*BnACBP*	Higher 18:2 and 18:3 at the expense of MUFAs and SFAs in seed oils at maturity	N.D.	Yurchenko et al. (2009, 2014)
				phaP::*OleoH3P-BnACBP*	Higher 18:2 at the expense of MUFAs and SFAs in seed oils at maturity		
	Arabidopsis thaliana	AtACBP4; AtACBP5; AtACBP6	18:1-CoA	*acbp6*	Higher 18:1-CoA in cotyledonary-staged embryos		Hsiao et al. (2014a)
				acbp4acbp5acbp6	Lower seed weight compared to double mutants and wild type		
Peroxisomal fatty acid β-oxidation	*Oryza sativa*	OsACBP6	18:3-CoA	*35S*::*OsACBP6*/*pxa1*	Restored IBA sensitivity, normal root length, hypocotyl elongation and wound-induced JA production compared to *pxa1*	Up-regulation of JA-responsive gene *VSP1* upon wounding	Meng et al. (2014)

Seed germination and seedling development	*Arabidopsis thaliana*	AtACBP1	PA; PLDα1 protein	*acbp1*	Lower ABA sensitivity during seed germination and seedling development	Down-regulation of *ABA2* and of *PLDα1* and *AREB1* under ABA treatment	Du et al. (2013a)
				35S::*AtACBP1*	Higher ABA sensitivity during seed germination and seedling development; higher PA at the expense of PC in rosettes	Up-regulation of *PLDα1* and down-regulation of *ABI1* under ABA treatment; up-regulation of ABA signaling genes (i.e. *AREB1*, *RD29A*, *MYC2*, *PLDα1*, *NCED3*, *ABA2*, *AtrbohD*, *AtrbohF*) in seedlings	
		AtACBP4; AtACBP5; AtACBP6	LC acyl-CoAs	*acbp6*	Higher 18:1- and 18:2-CoA in 5-days-old seedlings	N.D.	Hsiao et al. (2014a)
				acbp4acbp5	Hypersensitivity to ABA during seed germination		
				acbp5acbp6			
				acbp4acbp6			
				acbp4acbp5acbp6			

(continued)

Table 15.2 (continued)

Function	Species	ACBP(s) involved	Binding partner(s) involved	Effect of knockout mutation, silencing (RNAi) and overexpression in Arabidopsis			References
				Line	Phenotypic effects	Regulation of associated genes	
Embryo development	*Arabidopsis thaliana*	AtACBP1; AtACBP2	PC	*acbp1*	Higher MGDG at the expense of PC, PI & PS and higher levels of most polyunsaturated species of phospholipids in siliques	Up-regulation of *AtACBP2*	Chen et al. (2010)
				acbp2	No phenotype	Up-regulation of *AtACBP1*	
				acbp1acbp2	Embryo-lethal	N.D.	
Pollen development	*Arabidopsis thaliana*	AtACBP4; AtACBP5; AtACBP6	N.D.	*acbp4acbp6*	Aborted and vacuolated pollen; reduced seed number	N.D.	Hsiao et al. (2015)
				acbp5acbp6	*Ditto*		
				acbp4acbp5acbp6	*Ditto*; aberrant exine formation; fewer oil bodies in pollen; reduced pollen activity		

Cuticle formation	*Arabidopsis thaliana*	AtACBP1; AtACBP3; AtACBP4; AtACBP6	LC & VLC acyl-CoAs	*acbp1*	Fewer epicuticular wax crystals, ruptured cuticle membrane and altered cuticular wax composition in stems; more susceptible to *Botrytis cinerea* infection	Down-regulation of wax (i.e. *CER8*, *KCR1*, *ECR*, *CUT1*/*KCS6*) & cutin (i.e. *LACS2*, *CYP86A2*/*4*, *GPAT8*) biosynthetic genes	Xue et al. (2014)
				acbp6	Leaky leaf cuticle; faster water loss under drought; aberrant cuticle morphology; altered cuticular wax composition in leaves; more susceptible to *Pseudomonas syringae* infection; failed to generate SAR signals	N.D.	Xia et al. (2012)
				acbp3	*Ditto*; reduced levels of cutin monomers in leaves		
				acbp4	*Ditto*		

(continued)

Table 15.2 (continued)

Function	Species	ACBP(s) involved	Binding partner(s) involved	Effect of knockout mutation, silencing (RNAi) and overexpression in Arabidopsis			References
				Line	Phenotypic effects	Regulation of associated genes	
Leaf senescence	*Arabidopsis thaliana*	AtACBP3	PE	*acbp3*	Delayed dark-induced leaf senescence; lower PE in rosettes	N.D.	Xiao et al. (2010) and Xiao and Chye (2010)
				AtACBP3-RNAi	*Ditto*		
				35S::AtACBP3	Early age-dependent and dark/N_2-starvation-induced leaf senescence; higher PE and PI at the expense of PC and PI in rosettes; higher oxylipin-containing polar lipids; inhibited autophagosome formation	Up-regulation of senescence-associated genes (i.e. *SAG12*, *SEN1*, *SAG101*, *PLDδ*, *PLDγ*, *PLDζ2*)	

ABA abscisic acid, *IBA* indole-3-butyric acid, *LC* long-chain, *MUFAs* monounsaturated fatty acids, *N.D.* not determined, *PA* phosphatidic acid, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *PS* phosphatidylserine, *SAR* systemic acquired resistance, *SFAs* saturated fatty acids, *VLC* very-long-chain

yeast knockout mutant, at an ACBP:oleoyl-CoA ratio of 1:3, but DGAT activity declined if the ratio exceeded 1:1 (Yurchenko and Weselake 2011). Also, rBnACBP showed a subtle stimulatory effect on AtDGAT activity when expressed in insect cells at all (5, 15 or 30 μM) oleoyl-CoA ester concentrations tested (Hobbs and Hills 2000).

Apart from being incorporated into the glycerol backbone during glycerolipid production, palmitoyl, stearoyl and oleoyl-CoA esters exported from the plastids are subject to further modifications at the ER membrane *via* two major pathways for VLCFA and polyunsaturated FA (PUFA) syntheses (Harwood 1988). While FA elongases use acyl-CoA substrates (Pollard et al. 1979; Pollard and Stumpf 1980a, b), the newly synthesized oleic acid for desaturation into linoleic acid and α-linolenic acid must first be incorporated into PC (Slack et al. 1979; Citharel et al. 1983; Stymne et al. 1983; Murphy et al. 1985), predominantly at the *sn*-2 position (Bates et al. 2007, 2009; Tjellström et al. 2012). The resulting PUFAs may be released from PC by the reverse reaction of LPCAT, and its forward reaction can then incorporate another FA during the next round of acyl editing, leading to the enrichment of polyunsaturated acyl-CoA esters in the cytosolic pool for TAG assembly (Stymne and Stobart 1984; Wang 2001). In transgenic Arabidopsis, this acyl exchange between acyl-CoA esters and PC was stimulated by the expression of the cytosolic 10-kDa BnACBP under the control of seed-specific phaseolin promoter, resulting in a higher PUFA (linoleic and linolenic acids) seed content at the expense of eicosenoic acid and saturated FAs (Yurchenko et al. 2009). Similar acyl compositional change was reported in transgenic lines expressing an ER-targeted BnACBP, despite lower linolenic acid contents in both the acyl-CoA pool and seed oil compared with the cytosolic BnACBP lines (Yurchenko et al. 2014). As rBnACBP stimulated AtLPCAT activity *in vitro,* it has been proposed that a higher number of available ACB sites in the cytosol or on the ER may favor the partitioning of oleoyl-CoA ester into PC for subsequent desaturation rather than elongation (Yurchenko et al. 2009, 2014). To support this hypothesis, protein-protein interactions of BnACBP with acyl-CoA metabolic enzymes have been proposed for future investigations (Yurchenko and Weselake 2011).

In Arabidopsis, the three cytosolic isoforms (i.e. AtACBP4, AtACBP5 and AtACBP6) are potential candidates as cytosolic acyl-CoA pool formers that can facilitate acyl transfer from plastids to the ER for glycerolipid synthesis (Xiao and Chye 2009). ITC revealed that all three proteins bind palmitoyl-, oleoyl-, linoleoyl- and linolenoyl-CoA esters *in vitro,* although rAtACBP6 showed much stronger affinities than rAtACBP4 and rAtACBP5 (Hsiao et al. 2014a). The cotyledonary-staged embryos of the *acbp6* mutant accumulated a higher level of oleoyl-CoA ester (Hsiao et al. 2014a), in agreement with its reduction in developing seeds (20 days after flowering) in *BnACBP*-overexpressing Arabidopsis in comparison with the wild type (Yurchenko et al. 2014). Given that GUS was also strongly expressed in the cotyledonary-staged embryos of transgenic *AtACBP6pro*::*GUS* lines, AtACBP6 appears to have a potential role in seed oil biosynthesis (Hsiao et al. 2014a). On the other hand, the possibility that AtACBP4 and AtACBP5 play overlapping roles with AtACBP6 cannot be ruled out. *AtACBP4* and *AtACBP5* expression, coincided with

that of *FAD7* (Nishiuchi et al. 1995), and were up-regulated during the day (Xiao et al. 2009a, b), consistent with the light/dark cycles of acetyl-CoA production from carbon fixation that fuels plastidial FA biosynthesis (Sasaki et al. 1997; Harmer et al. 2000). In fact, the depletion of all three cytosolic AtACBPs in a triple mutant resulted in the most notable reduction in seed weight when compared with the double mutants, implicating the involvement of AtACBP4, AtACBP5 and AtACBP6 in seed development (Hsiao et al. 2014a).

OsACBP6 in Peroxisomal FA β-Oxidation

A novel ACBP function in plant lipid catabolism has been proposed recently after observations that OsACBP6 is targeted to the peroxisomes (Meng et al. 2014). In higher plants, the peroxisomes are microbodies specialized for a variety of metabolic functions, including β-oxidative breakdown of FAs into acetyl-CoA units which can be further metabolized into succinate through the glyoxylate cycle, hence converting FAs into carbohydrates (Tolbert and Essner 1981; Olsen 1998; Graham and Eastmond 2002). Despite the presence of peroxisomal FA β-oxidation in most plant tissues (Gerhardt 1983), it is most active during seed germination and early seedling growth when FAs are liberated from TAGs in oil bodies and activated into CoA-esters as substrates for β-oxidation (Fulda et al. 2004). The import of FAs into the peroxisomes, despite the uncertainty whether it is in the form of free acids or CoA esters (Fulda et al. 2004; van Roermund et al. 2012; De Marcos Lousa et al. 2013), is mediated by a peroxisomal membrane-localized ABC transporter encoded by a single gene, *COMATOSE* (*CTS*; Footitt et al. 2002), which is also named *PEROXISOMAL ABC TRANSPORTER1* (*PXA1*; Zolman et al. 2001) and *PEROXISOME DEFICIENT3* (*PED3*; Hayashi et al. 2002).

In the Arabidopsis *pxa1* mutant, carbon reserves from seed oil cannot be mobilized into sugars owing to the defects in peroxisomal FA β-oxidation, resulting in the arrest of seedlings during post-germinative growth if no sucrose were to be supplemented (Zolman et al. 2001). In fact, the *ped3* mutants cannot degrade seed reserve lipids for gluconeogenesis as verified by its unaltered lipid content 5 days post-germination (Hayashi et al. 2002). In addition, root elongation of the *pxa1* mutant was not inhibited by the auxin precursor indole-3-butyric acid (IBA) because it cannot be converted into active indole-3-acetic acid when peroxisomal β-oxidation activities are impaired (Zolman et al. 2000, 2001). Similarly, the *ped3* mutants were resistant to the herbicide precursor 2,4-dichlorophenoxybutyric acid as it could not be β-oxidized into toxic 2,4-dichlorophenoxyacetic acid (Hayashi et al. 1998, 2002). On the other hand, the overexpression of *OsACBP6* complemented the *pxa1* mutant phenotypes (Meng et al. 2014). In the *OsACBP6-OE*/*pxa1* lines, hypocotyl elongation of seedlings was restored in a sucrose-free medium, and the number and diameter of rosettes from 3-week-old plants appeared similar to the wild type (Meng et al. 2014). Root elongation of the *OsACBP6-OE*/*pxa1* lines was inhibited signifi-

cantly by 10 μM IBA, in contrast to the subtly-affected root length of the *pxa1* mutant (Meng et al. 2014).

In addition to acyl-CoA esters, 12-oxo-phytodienoic acid (OPDA) is another potential import substrate of CTS (Theodoulou et al. 2005). In chloroplasts, the JA biosynthetic pathway starts with the phospholipase-catalyzed hydrolysis of membrane lipids to release linolenic acid, which is oxidized and further modified to form OPDA (Vick and Zimmerman 1983). In the peroxisomes, OPDA is subject to several rounds of β-oxidation in JA formation (Vick and Zimmerman 1983). Despite its membrane-permeable nature, OPDA enters the peroxisomes in a transporter-regulated manner (Stenzel et al. 2003). CTS is believed to facilitate the transfer of OPDA as the basal and wound-induced JA levels in the *cts* mutant were detected to be lower than the wild type (Theodoulou et al. 2005). The expression of a JA-responsive gene, *VEGETATIVE STORAGE PROTEIN1* (*VSP1*), was also down-regulated by nearly 50 % in flowers of the *cts* mutant in comparison with the wild type (Theodoulou et al. 2005). On the other hand, the overexpression of *OsACBP6* in the *pxa1* mutant led to the recovery of induced JA production 1.5 h after wound-treatment (Meng et al. 2014). Concomitantly, *VSP1* expression in the *OsACBP6-OE/pxa1* lines was rapidly up-regulated 0.5 h post-wounding (Meng et al. 2014).

ITC was further used to test if rOsACBP6 binds potential CTS substrates including linolenoyl-CoA ester, OPDA and IBA (Meng et al. 2014). Although rOsACBP6 binds linolenoyl-CoA ester in ITC consistent with previous data from Lipidex 1000 binding assays (Meng et al. 2011, 2014), it did not bind IBA or OPDA (Meng et al. 2014). Hence, the possibility of IBA and OPDA being transported into the peroxisomes in the form of CoA-thioesters cannot be ruled out (Hu et al. 2012). Especially, given the potential existence of an alternative CTS-independent route which accounts for the low but detectable JA levels in the *cts* mutant (Theodoulou et al. 2005; Dave et al. 2011), OsACBP6 may emerge to be such a candidate that regulates peroxisomal FA β-oxidation (Meng et al. 2014).

AtACBPs in the Regulation of Seed Germination and Seedling Development

While a new role has been ascribed to OsACBP6 (Class IV) in lipid catabolism during seed germination and seedling growth, no such peroxisome-localized homolog was identified in Arabidopsis. However, another ACBP isoform, AtACBP1 (Class II), is linked to ABA signaling during seed germination and seedling development in Arabidopsis (Du et al. 2013a). ABA is an osmotic stress-related phytohormone that plays important roles in adaptive responses to abiotic and biotic stresses (Hirayama and Shinozaki 2007; Ton et al. 2009; Cutler et al. 2010), as well as in plant growth and development such as seed maturation and dormancy (Cheng et al. 2002; Fujii and Zhu 2009; Nakashima et al. 2009). The ABA receptors, REGULATORY COMPONENTS OF ABA RECEPTOR/PYRABACTIN

RESISTANCE1/PYRABACTIN RESISTANCE1-LIKE, have been recently identified as members of the START domain superfamily (Ma et al. 2009; Park et al. 2009; Klingler et al. 2010; Nishimura et al. 2010). Upon ABC transporter-mediated uptake into the cells (Kang et al. 2010; Kuromori et al. 2010; Boursiac et al. 2013), ABA binds to the cytosolic receptors, which in turn suppress phosphatase activity of type 2C protein phosphatase (PP2C), a negative regulator of ABA responses (Ma et al. 2009; Park et al. 2009; Santiago et al. 2009; Szostkiewicz et al. 2010). PP2C inhibition in the presence of ABA triggers the release of SNF1-related protein kinases 2, which phosphorylate and regulate the activity of downstream transcription factors, ABA-RESPONSIVE ELEMENT-BINDING PROTEIN/FACTOR (Nakashima and Yamaguchi-Shinozaki 2013). During seed germination, PA is produced as an important second messenger in ABA responses (Katagiri et al. 2005). The signaling PA molecules are generated by the PLD-catalyzed hydrolysis of structural phospholipids or by the sequential action of phospholipase C and DAG kinase (Testerink and Munnik 2005). It has been previously shown that PLD and its product, PA, inhibit the function of the negative regulator ABA INSENSITIVE1 (ABI1; Zhang et al. 2004), a member of the PP2C family (Gosti et al. 1999).

In Arabidopsis seedlings, *ACBP1* is highly expressed and its expression is inducible by ABA or drought treatment (Du et al. 2013a). In addition, the overexpression of *AtACBP1* up-regulated *PLDα1* and three ABA/stress-responsive genes including *AREB1*, *RESPONSE TO DESICCATION 29A* (*RD29A*) and *bHLH-TRANSCRIPTION FACTOR MYC2* (*MYC2*) with or without ABA treatment, whilst *PLDα1* and *AREB1* were suppressed in the *acbp1* mutant upon ABA treatment (Du et al. 2013a). These observations are consistent with the higher accumulation of PA in the ABA-treated germinating seeds of AtACBP1-OEs and its lower level in the *acbp1* mutant (Du et al. 2013a). Functionally, the overexpression of *AtACBP1* rendered the freshly-harvested seeds more dormant than the wild type, whereas the *acbp1* mutant seeds became less dormant (Du et al. 2013a). The dry after-ripened seeds from AtACBP1-OEs were more sensitive to inhibition by exogenous ABA during germination in comparison with the wild type, while the *acbp1* mutant seeds became more resistant (Du et al. 2013a). Thus, AtACBP1 likely plays a role in ABA signaling during seed dormancy, germination and seedling development. As rAtACBP1 is the sole member of the AtACBP family that binds both PC and PA *in vitro* (Du et al. 2010a, 2013a), and that AtACBP1 and PLDα1 proteins were identified to interact at the plasma membrane in BiFC assays (Du et al. 2013a), AtACBP1 probably promotes PLDα1 activities *via* protein-protein interactions during ABA-mediated regulation of seed germination and seedling development in Arabidopsis.

Recently, Hsiao et al. (2014a) reported the accumulation of oleoyl- and linoleoyl-CoA esters in Arabidopsis *acbp6* seedlings in comparison to the wild type. During seed germination in the presence of exogenous ABA, double mutants of *acbp4acbp5*, *acbp4acbp6* and *acbp5acbp6* and the *acbp4acbp5acbp6* triple mutant exhibited a lower germination rate than the wild type, implicating an overlapping role of the three cytosolic AtACBPs in seed germination (Hsiao et al. 2014a).

AtACBP1 and AtACBP2 in Embryo Development

In the attempt to unravel the physiological functions of AtACBPs using a reverse genetics approach, embryo lethality of the *acbp1acbp2* double mutant represents the only severe phenotype observed thus far (Chen et al. 2010). On the other hand, neither *acbp1* nor *acbp2* single mutant plants exhibited morphological anomaly during seed development or seedling growth (Chen et al. 2010). It is plausible that AtACBP1 and AtACBP2 share an overlapping function in lipid metabolism during embryogenesis as both proteins are endomembrane-localized and highly conserved with 71 % sequence identity (Chye 1998; Chye et al. 1999, 2000; Li and Chye 2003). Microarray data also suggested that the temporal expression pattern of *AtACBP1* during seed development coincides with *AtACBP2* (Chen et al. 2010), and immunohistochemical staining confirmed that both proteins accumulated in cotyledonary-staged embryos (Chye et al. 1999; Chen et al. 2010). The ovules of *acbp1acbp2* double mutants aborted during early embryo development at either zygotic, two-cell or eight-cell stages, preceding the active phase of FA and TAG accumulation (Chen et al. 2010). The aborted embryos could not be induced to form calli, confirming that the embryos were arrested at the very early stages of development (Chen et al. 2010). Lipid profiling further revealed that siliques of the *acbp1* single mutant accumulated more galactolipid monogalactosyldiacylglycerol and stearoyl-CoA ester than the wild type, but most of the polyunsaturated species of phospholipids, including PC, PE, PS and phosphatidylinositol (PI), were notably reduced (Chen et al. 2010). As rAtACBP1 binds both acyl-CoA esters and phospholipids (PC and PA) *in vitro* (Chye 1998; Gao et al. 2009; Du et al. 2010a, 2013a; Xue et al. 2014), AtACBP1 possibly contributes to acyl-CoA metabolism and membrane phospholipid biogenesis during embryo development. AtACBP1- and AtACBP2-mediated acyl exchange between the acyl-CoA and phospholipid pools is one such possibility (Napier and Haslam 2010).

The importance of normal lipid metabolism during seed development can be reflected by the embryo-lethality phenotypes observed in several loss-of-function mutants that have impaired acyl-CoA-dependent enzymes in Arabidopsis. For instance, embryo development was arrested at the heart-torpedo stage by a T-DNA insertion within the *LPAAT1* gene that encodes a plastid LPAAT for the acylation of lyso-PA into PA (Kim and Huang 2004). In addition, depletion of the 3-hydroxyacyl-CoA dehydratase PASTICCINO2 (PAS2), a component of the microsomal fatty acyl elongase complex, affected cotyledon development as visualized at the torpedo stage (Bach et al. 2008; Bach and Faure 2010). Multiple allelic mutants in the *PAS3*/*ACC1* gene that encodes an acetyl-CoA carboxylase also resulted in defective embryo morphogenesis (Baud et al. 2003, 2004). In the *pas2* and *acc1* mutants, the VLCFA content significantly declined or was barely detectable in seed storage TAGs, respectively (Baud et al. 2003; Bach et al. 2008). Similarly, insertional mutants in *AtKCR1* which encodes β-ketoacyl-CoA reductase in the fatty acyl elongase complex also resulted in embryo lethality (Beaudoin et al. 2009). Collectively, reverse genetics studies indicate a link between embryogenesis and the

synthesis of VLCFAs, which constitute a significant proportion (i.e. 13–21 %) of total FAs in Arabidopsis seeds (O'Neill et al. 2003). Although the modified content of VLCFAs in the TAG pool may not severely influence plant development, their altered compositions in phospholipids and complex sphinogolipids may lead to profound consequences in plant development affecting embryogenesis (Bach and Faure 2010). With respect to the phospholipid profile of siliques from the *acbp1* mutant (Chen et al. 2010), the levels of VLCFAs esterified to PS (i.e. C40:3- and C40:4-PS) were particularly reduced (Napier and Haslam 2010). Given our recent evidence that rAtACBP1 binds VLC acyl-CoA esters *in vitro* (Xue et al. 2014), an alternative role of AtACBP1 in VLCFA formation in seed development may now be proposed. However, it is noteworthy that the differences in lipid profile and acyl-CoA content between the *acbp2* mutant and the wild type were statistically insignificant (Chen et al. 2010). Whether AtACBP2 acts concertedly with AtACBP1 in binding VLC acyl-CoA esters that may account for the embryo lethality of the *acbp1acbp2* double mutant remains to be investigated.

AtACBPs in Cuticle Formation

In terrestrial plants, the cuticle is a highly hydrophobic layer that covers the surface of all aerial organs as a protective barrier against non-stomatal water loss (Riederer and Schreiber 2001) and pathogens (Barthlott and Neinhuis 1997), and as an interface for interaction with the environment in stress responses and adaptations (Bernard and Joubès 2013). It also has a role in contact-mediated cell-cell interactions such as pollen-stigma interaction and in the prevention of fusions between different plant organs (Pruitt et al. 2000; Sieber et al. 2000). The cuticle is composed of cutin and wax, both of which are synthesized exclusively in the epidermal cells (Bernard and Joubès 2013; Lee and Suh 2013). Cutin forms the structural skeleton of the cuticle and is a polyester built from monomers of hydroxylated and epoxy-hydroxylated C16 and C18 FAs, glycerol and minute amounts of phenolic compounds (Heredia 2003; Nawrath 2006; Samuels et al. 2008). Intracuticular waxes are embedded within the cutin in the cuticle membrane which is covered by an outermost layer of epicuticular waxes (Jenks et al. 2002; Kunst and Samuels 2003). On the ER membrane, cuticular waxes are produced by the extension of leucoplast-synthesized C16 and C18 FAs as CoA-esters to form VLCFAs, which are reduced into primary alcohols and wax esters *via* acyl reduction or modified into aldehydes, alkanes, secondary alcohols and ketones *via* the decarbonylation pathway (Jenks et al. 2002; Nawrath 2006; Samuels et al. 2008; Lee and Suh 2013).

The binding specificities of rAtACBP1 to C18 and VLC acyl-CoA esters *in vitro* coinciding with strong GUS expression in stem epidermis from the *AtACBP1pro*::*GUS* transformants led to investigations to address whether ER-localized AtACBP1 facilitates cutin and wax biosyntheses from such acyl-CoA precursors (Xue et al. 2014). This novel role of AtACBP1 was supported by phenotypic abnormalities of its loss-of-function mutant (Xue et al. 2014). Scanning and transmission electron microscopy showed fewer epicuticular wax crystals and a ruptured cuticle mem-

brane in Arabidopsis *acbp1* mutant stems (Xue et al. 2014). Gas chromatography-mass spectrometry (GC-MS) further revealed an aberrant composition of cuticular wax in *acbp1* mutant stems with significantly reduced levels of C29 alkane, C28 and C30 primary alcohols, and C29 secondary alcohol and ketone (Xue et al. 2014). GC-MS also indicated lower levels of C18:1 and C18:2 ω-hydroxyl and dicarboxylic FAs, which are the constituents of cutin monomers (Xue et al. 2014). Concomitantly, the expression of several wax and cutin biosynthetic genes was down-regulated in stems of the *acbp1* mutant in comparison with the wild type and *acbp1*-complemented lines (Xue et al. 2014). Although AtACBP1 may not play a direct role in leaf wax synthesis as evident from the lack of *AtACBP1pro*::*GUS* signal in leaf epidermis, the wax content was reduced in *acbp1* mutant leaves, which became more susceptible to *B. cinerea* infection (Xue et al. 2014). As cuticular wax biosynthesis is tightly controlled by a number of transcription factors in response to diverse external cues (Aharoni et al. 2004; Zhang et al. 2007; Cominelli et al. 2008; Lü et al. 2009; Seo et al. 2011), defective stem cuticle formation in the *acbp1* mutant may possibly alter the status of the plant as a whole and exert an indirect effect on leaf wax synthesis (Xue et al. 2014).

In another study, AtACBP3, AtACBP4 and AtACBP6 were shown to be crucial for normal leaf cuticle development as knockout mutant plants exhibited higher water loss under drought stress, increased folding on the adaxial leaf surface, irregularity in cuticular appearance and permeability of leaves to toluidine blue staining (Xia et al. 2012). The aberrant cuticle structures of knockout mutant plants were linked to higher levels of several components of cuticular wax including FA (C16:0, C18:0), alkanes (C29, C31 and C33) and primary alcohols (C28 and C32), and reduced levels of cutin monomers including C16:0, C18:1 and C18:2 dicarboxylic FAs (Xia et al. 2012). The defective cuticles of the *acbp3*, *acbp4* and *acbp6* mutant plants also account for their higher susceptibility to *P. syringae* (both virulent DC3000 and avirulent *avrRpt2* strains) and, occasionally, to *B. cinerea* and *C. higginsianum* (Xia et al. 2012). When petiole exudates from *avrRpt2*-challenged leaves of the wild type were infiltrated into *acbp3*, *acbp4* and *acbp6*, the mutant plants were protected against virulent bacteria inoculated at distal leaves away from the exudate-infiltrated leaves (Xia et al. 2012). On the other hand, the petiole exudates from *acbp3*, *acbp4* and *acbp6* did not confer comparable protection of the wild type against virulent bacteria in similar experimental set-ups (Xia et al. 2012). Accordingly, it was concluded that AtACBP3, AtACBP4 and AtACBP6 are essential for the formation of an intact cuticle and the generation of mobile systemic acquired resistance (SAR) signals in response to microbial pathogens but not for the perception of SAR signals (Xia et al. 2012).

Cytosolic AtACBPs in Pollen Development

Lipids form a structurally and metabolically essential constituent in pollen, which contain extracellular lipidic deposits (i.e. tryphine) covering the exine wall surface (primarily composed of the chemically-stable polymer sporopollenin) and

intracellular lipid pools as storage oil bodies and extensive membranous structures (Evans et al. 1991, 1992; Piffanelli et al. 1997; Murphy 2006). The importance of pollen lipids is evident from aberrant pollen development resulting by the manipulation of gene expression affecting acyl lipid metabolism, including intracellular storage lipid accumulation (Zhang et al. 2009), extracellular pollen lipid secretion (Zheng et al. 2003), wax deposition (Aarts et al. 1995, 1997; Millar et al. 1999; Ariizumi et al. 2003; Chen et al. 2003; Kurata et al. 2003) and membrane lipid catabolism (Kim et al. 2011). As potential intracellular acyl transporters involved in lipid metabolic processes, the three cytosolic AtACBPs (i.e. AtACBP4, AtACBP5 and AtACBP6) were recently investigated with respect to their roles in pollen lipid formation (Hsiao et al. 2015).

Normal pollen development in single mutants of the three cytosolic AtACBPs has been attributed to the overlapping roles of AtACBP4 and AtACBP5 with AtACBP6, as *AtACBP6pro*::*GUS* signals coincided with *AtACBP5pro*::*GUS* in microspores and tapetal cells, and with *AtACBP4pro*::*GUS* in pollen grains at later stages (Hsiao et al. 2015). On the other hand, the *acbp4acbp6* and *acbp5acbp6* double mutants, but not *acbp4acbp5*, exhibited morphological aberrance in floral development, such as reduction in the number of normal pollen, appearance of aborted pollen, reduction in seed number per silique and more prominent cytoplasmic vacuolation in mature pollen grains (Hsiao et al. 2015). The morphology of flowers and siliques appeared to be the most affected in the *acbp4acbp5acbp6* triple mutant (Hsiao et al. 2015). Scanning and transmission electron microscopy further confirmed defects in the exine of the triple mutant in a smoother pollen surface and an irregular arrangement between the bacula and tryphine in comparison with the wild type, whilst lower abundance of oil bodies in its pollen was in agreement with the decreased pollen activity (Hsiao et al. 2015).

Although the composition of the sporopollenin polymer and its biosynthetic pathways in the tapetum remain elusive, the inter-organellar transport (e.g. from plastids to ER) and some modifications (e.g. acyl reduction) of the acyl-lipid intermediates require their esterification into CoA-esters, as mutation of Arabidopsis *ACYL-COA SYNTHETASE5* led to defective pollen exine formation (de Azevedo Souza et al. 2009; Grienenberger et al. 2010; Ariizumi and Toriyama 2011). The subsequent secretion of lipidic sporopollenin precursors across the plasma membrane of tapetal cells onto the surface of developing microspore walls is mediated by ABC TRANSPORTER G26 (ABCG26), and *abcg26* mutants showed reduced male fertility and exine-less microspores (Quilichini et al. 2010; Choi et al. 2011). The comparable but less severe phenotypic changes in pollen of the *acbp4acbp5acbp6* triple mutant implicate overlapping roles for the three cytosolic AtACBPs in sporopollenin biosynthesis, potentially by intracellular transport of the acyl-lipid precursors (Hsiao et al. 2015).

AtACBP3 in Leaf Senescence

During senescence and nutrient starvation, dysfunctional or unwanted organelles and cellular constituents are rapidly recycled to remobilize the supply of carbon and nitrogen for sustaining growth and survival in a turnover process known as autophagy (Li and Vierstra 2012). The sophisticated machinery for autophagy is now known to be conserved from yeast to higher eukaryotes including plants (He and Klionsky 2009; Liu and Bassham 2012). Autophagy is mediated by the sequestration of intracellular components in double-membrane autophagosomes, which subsequently fuse with lysosomes or plant vacuoles for breakdown and recycling of degradation products (He and Klionsky 2009). The biogenesis of autophagosomes requires two ubiquitination-like conjugation systems. The ubiquitin-like proteins ATG8 and ATG12 conjugate to PE and ATG5, respectively (Geng and Klionsky 2008; Liu and Bassham 2012). ATG8-PE constitutes a scaffold for membrane expansion of autophagosomes, the size of which correlates with the amount of ATG8 (Geng and Klionsky 2008; Xie et al. 2008). The lipidation of ATG8 is facilitated by the ATG12-ATG5 conjugate, and is reversible by the action of PLD (Fujioka et al. 2008; Chung et al. 2009, 2010).

In Arabidopsis, the ATG8-PE association during starvation-induced and age-dependent leaf senescence may also be modulated by AtACBP3, which has been demonstrated to bind PE *in vitro* and its expression was up-regulated in continuous darkness and senescing rosettes (Xiao et al. 2010). The overexpression of *AtACBP3* led to higher accumulation of PE in rosettes, whereas its level decreased in the *acbp3* mutant and *AtACBP3-RNAi* transgenic lines (Xiao et al. 2010). AtACBP3-OEs exhibited faster leaf senescence upon dark treatment or nitrogen starvation, and they also showed early senescence of 5-week-old rosettes in a SA signaling-dependent manner under normal light/dark condition (Xiao et al. 2010). These stress-inducible and age-dependent senescence phenotypes of AtACBP3-OEs resemble those of Arabidopsis *atg2*, *atg5*, *atg7*, *atg10* and *atg18a* mutants defective in the autophagic pathway (Doelling et al. 2002; Thompson et al. 2005; Xiong et al. 2005; Phillips et al. 2008; Yoshimoto et al. 2009). Consistently, the *acbp3* mutant and *AtACBP3-RNAi* transgenic lines showed delayed dark-induced leaf senescence (Xiao et al. 2010). The root cells of AtACBP3-OE seedlings had fewer autophagosome structures as visualized by the expression of the autophagy marker GFP-ATG8e or in monodansylcadaverine staining in comparison with the wild type (Xiao et al. 2010). As protein degradation of GFP-ATG8e was also stimulated in an AtACBP3-OE background, AtACBP3 may regulate autophagosome formation by controlling ATG8-PE lipidation, possibly *via* competition for a common ligand, PE (Xiao et al. 2010; Xiao and Chye 2010). Apart from the modulation of ATG8 stability, AtACBP3 may affect membrane lipid metabolism during leaf senescence as membrane phospholipids (e.g. PC and PI) were lost and their degradative and oxidative products (e.g. PA, lyso-phospholipids, arabidopsides) increased in dark-treated and premature senescing AtACBP3-OE plants (Xiao et al. 2010; Xiao and Chye 2010).

ACBPs in Systemic Transport via the Phloem

In vascular plants, the phloem conducts long-distance translocation of phytohormones, inorganic ions and assimilates such as sugars, amino acids and organic acids. A number of soluble mobile proteins have also been identified from the phloem sap in earlier studies (Fisher et al. 1992; Ishiwatari et al. 1995; Hayashi et al. 2000), prompting subsequent comprehensive analyses of the protein complements in phloem exudates by peptide sequencing and tandem mass spectrometry-based proteomic studies (Walz et al. 2004; Suzui et al. 2006; Guelette et al. 2012). Partial peptide sequences from the phloem exudates of cucumber (*Cucumis sativus*) and pumpkin (*Cucurbita maxima*) were highly homologous to ACBP and some other defense-related proteins (Walz et al. 2004). In another study, ACBP was identified as a major phloem sap protein in rice, and its existence was also confirmed in the phloem exudates of winter squash (*C. maxima*), *B. napus* and coconut palm (*Cocos nucifera*) by Western blot analysis using an anti-BnACBP antibody (Suzui et al. 2006). More recently, proteomic analysis of the Arabidopsis phloem exudate revealed the identities of 65 proteins, 11 of which were lipid-binding proteins (Guelette et al. 2012). The same study also identified several FAs and lipids, some of which function as signaling compounds (e.g. PA and phosphatidyl bisphosphate), leading to a hypothesis that some lipid-binding proteins in the phloem sap may play a role in long-distance transport of lipid signals (Guelette et al. 2012). As to whether the ACBPs present in phloem exudates play a role in systemic lipid trafficking remains to be determined. In Arabidopsis, *AtACBP3*, which was expressed in the phloem as indicated by promoter-*GUS* fusions in transgenic Arabidopsis (Zheng et al. 2012), presents a promising candidate for future investigations.

Conclusion

Lipids have widespread biological roles in energy storage and provision, cellular structure formation and signal transduction. ACBPs, as “lipid chaperones”, are emerging to be versatile proteins that contribute to a number of different processes in plant growth and development, besides their elucidated functions in stress responses. In addition to the binding of acyl-CoA esters as prototypical ligands highlighting the pertinence of ACBPs to acyl-CoA-dependent biosyntheses of lipids (e.g. seed oils and cuticle), the phospholipid-binding affinities of ACBPs further diversify their functions in the regulation of specific plant developmental events such as seed germination, seedling development and leaf senescence. Whilst the vast majority of novel ACBP functions were identified in the eudicot model plant Arabidopsis, recent studies in the monocot rice have unravelled substantial differences between the two ACBP families in terms of ligand specificities, domain architecture, subcellular localization patterns and stress-responsiveness in gene expression. The possible involvement of the peroxisome-localized OsACBP6 in

FA catabolism represents the first example of a unique rice ACBP function not previously reported in Arabidopsis, marking a contrast between rice and Arabidopsis. Further dissection of the functions of rice ACBP homologs and phloem-mobile ACBPs from other species will provide a more thorough understanding of the developmental roles of plant ACBPs in the future.

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