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Expression of ACBP4 and ACBP5 proteins is modulated by light in Arabidopsis

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In our recent paper in *Plant Physiology and Biochemistry*, we reported that the mRNAs encoding *Arabidopsis thaliana* cytosolic acyl-CoA-binding proteins, ACBP4 and ACBP5, but not ACBP6, are modulated by light/dark cycling. The pattern of circadian-regulated expression in *ACBP4* and *ACBP5* mRNAs resembles that of *FAD7* which encodes omega-3-fatty acid desaturase, an enzyme involved in plastidial fatty acid biosynthesis. Recombinant ACBP4 and ACBP5 proteins were observed to bind oleoyl-CoA ester comparably better than recombinant ACBP6, suggesting that ACBP4 and ACBP5 are promising candidates in the trafficking of oleoyl-CoA from the plastids to the endoplasmic reticulum (ER) for the biosynthesis of non-plastidial membrane lipids. By western blot analyses using the ACBP4 and ACBP5-specific antibodies, we show herein that the levels of ACBP4 and ACBP5 proteins peak at the end of the light period, further demonstrating that they, like their corresponding mRNAs, are tightly controlled by light to satisfy demands of lipids in plant cells.

Key words: acyl-CoA-binding protein, ACBP4, ACBP5, lipid trafficking, phosphatidylcholine-binding

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Introduction

Acyl-CoA-binding proteins (ACBPs) are conserved at the acyl-CoA-binding domain and possess the ability to bind long-chain acyl-CoA (LCA-CoA) esters.[1] In *Arabidopsis thaliana*, six genes designated *ACBP1* to *ACBP6* have been identified to encode ACBPs.[1] Among them, ACBP1 and ACBP2 are membrane-associated proteins,[2-5] ACBP3 is extracellularly-targeted,[6] and the remaining three (ACBP4, ACBP5 and ACBP6) are cytosolic proteins.[7,8] The *C*-terminal ankyrin repeats in ACBP1 and ACBP2 and the kelch motifs in ACBP4 and ACBP5 have been reported to mediate protein-protein interaction.[9-11] Given that both ACBP1- and ACBP2-overexpressing transgenic Arabidopsis showed improved tolerance to heavy metals (Pb or Cd) stress, we have suggested that these two plasma membrane-localized ACBPs may be involved in the repair of the phospholipid bilayer membrane following heavy metal stress.[9,12]

Recombinant ACBP4 and ACBP5 have been previously reported to preferentially bind oleoyl-CoA rather than palmitoyl-CoA or arachidonyl-CoA esters.[13] Since these two larger cytosolic ACBPs (ACBP4 and ACBP5) resembles ACBP6 in binding palmitoyl-CoA and oleoyl-CoA esters in vitro,[14] they are potential candidates for the shuttling of acyl-CoAs from the plastid to the ER for the biosynthesis of non-plastidial membrane lipids. We have also observed that recombinant ACBP6 binds phosphatidylcholine (PC) in vitro and that transgenic Arabidopsis overexpressing ACBP6 are altered in levels of PC and phosphatic acid and display an enhanced freezing tolerance phenotype.[7] To further establish the functions of these three ACBPs in plant lipid metabolism, we have presented recent evidence that the levels of *ACBP4* and *ACBP5* (but not *ACBP6*) mRNAs increase in the light and are dampened-off upon darkness.[15] This mirrors the expression pattern of the mRNA encoding FAD7, an omega-3-fatty acid desaturase involved in plastidial lipid metabolism.[16] Taken together, we suggest

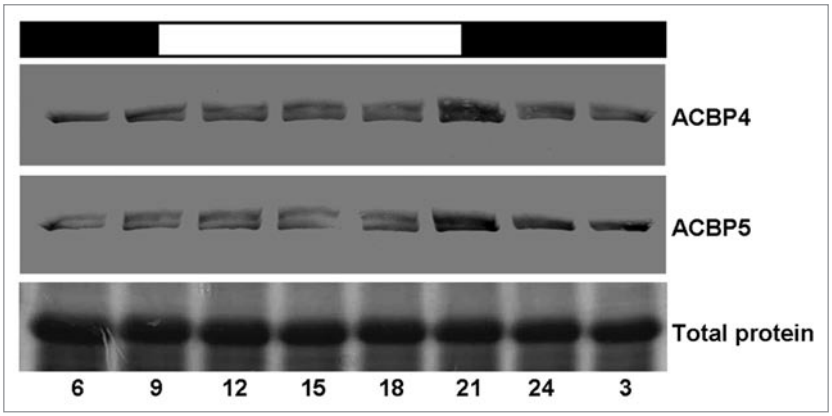

Figure 1. Western blot analysis shows circadian-regulated expression of ACBP4 and ACBP5 in Arabidopsis grown under 12 h light/12 h dark cycles. Arabidopsis seeds were germinated and grown in a growth chamber under 12 h light [09:00 to 21:00]/12 h dark [21:00 to 09:00] cycles. Total protein was extracted from four-week-old rosettes harvested at 3-h intervals. The ACBP4- and ACBP5-specific antibodies were used in western blot analysis have been previously described.[8] The cross-reacting 73.1-kDa ACBP4 and 71-kDa ACBP5 bands were detected using the Amplified Alkaline Phosphatase Assay Kit. Bottom, gel identically loaded and stained with Coomassie blue. Bottom numbering indicates time of day (24-h clock) and top white and black bars indicate light and dark periods, respectively.

that ACBP4 and ACBP5 likely function in oleoyl-CoA transfer between the chloroplasts and the ER.

Light-Regulated Expression of ACBP4 and ACBP5 Proteins

To further investigate the circadian-regulated expression of *ACBP4* and *ACBP5* mRNAs reported in Xiao et al. (2009),[15] the expression levels of ACBP4 and ACBP5 proteins were measured by western blot analysis of total proteins from four-week-old rosettes of wild-type Arabidopsis (Col-0) collected at 3-h intervals. Protein extracts were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blot analysis using ACBP4-specific and ACBP5-specific polyclonal antibodies.[8] Results from western blot analysis showed that both ACBP4 (molecular mass of 73.1 kDa; **Fig. 1**, upper) and ACBP5 (molecular mass of 71-kDa; **Fig. 1**, middle) increased during the light period (from 09:00 to 21:00), peaking at the end of the light period at 21:00. Subsequently, their expression declined in the dark (from 21:00 to 09:00) (**Fig. 1**). Also western blot analysis revealed the possibility of post-translational modification in ACBP4 and ACBP5 proteins during the light period, as suggested by the doubling of bands between 09:00–21:00. This preliminary observation requires further in-depth investigations to better understand the regulation of ACBP4 and ACBP5.

Other Implications of ACBP4 and ACBP5 in Circadian Control

Our observations have revealed that cytosolic ACBP4 and ACBP5 are transcriptionally[15] and translationally (reported herein) modulated by light in Arabidopsis rosettes. In plants, plastidial fatty acid biosynthesis is largely dependent on carbon fixation in the chloroplasts for the generation of acetyl-CoA[17,18] and is controlled by light under a circadian rhythm.[19] The production of acetyl-CoA oscillates daily within light/dark cycles, i.e., increases in the light and decreases in darkness.[17,18] Consistently, some of the genes encoding proteins associated with plastidial fatty acid biosynthesis such as acetyl-CoA carboxylase (ACCase) in pea, and the chloroplast omega-3-fatty acid desaturase (FAD7) in Arabidopsis are transcriptionally controlled by light.[16,20] It is still unclear how these genes are regulated to date. A recent study has revealed that the genes encoding the β-subunit of ACCase (*accD*) and a long-chain acyl-CoA synthetase are upregulated in transgenic Arabidopsis overexpressing soybean Dof proteins GmDof4 and GmDof11, respectively.[21] Hence, the Dof family proteins, which are a large family of plant transcription factors involved in diverse processes such as light signals, defense responses as well as plant development,[22] likely function in the transcriptional regulation of genes in plant lipid metabolism.

By analysis of the putative promoter sequences (c.a. 1.5-kb) obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/), we have located some putative elements that may be relevant in the regulation of ACBP4 and ACBP5. In particular, four duplicated copies of the Dof element (WAAAG) occur in the *ACBP4* and *ACBP5* 5'-flanking regions, suggesting that their circadian-regulated expression may also be subject to control by the conserved Dof family proteins in lipid metabolism. Furthermore, many other putative regulatory elements, particularly light-responsive elements and circadian rhythm-related elements have been identified too. For example, two circadian rhythm related elements (CAANNNNATC) are observed in the *ACBP4* 5'-flanking region, but are absent from the *ACBP5* 5'-flanking region. In contrast, other putative light-responsive elements that are present in both *ACBP4* and *ACBP5* 5'-flanking regions include the AE-box (AGAAACAA), box I (TTTCAAA), box 4 (ATTAAT) and the ACE element (CTAACGTATT). The presence of these putative sequences in the 5'-flanking regions of *ACBP4* and *ACBP5* are consistent with our observations of circadian- and light-regulated expression of these two genes. Further analysis of these sequences need to be carried out to confirm their functionality in the regulation of *ACBP4* and *ACBP5* expression.

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