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Arabidopsis acyl-CoA-binding proteins ACBP1 and ACBP2 show different roles in freezing stress

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In our recent paper in Plant Physiology, we reported that recombinant *Arabidopsis thaliana* acyl-CoA-binding protein ACBP1 binds phosphatidic acid (PA) in vitro and *acbp1* mutant plants are conferred freezing tolerance. ACBP1-overexpressors were freezing sensitive and accumulated more PA, in contrast to *acbp1* mutants which had reduced PA and elevated PC levels. Such changes in PC and PA were consistent with the expression of the mRNA encoding phospholipase Dα1 (PLDα1), a major enzyme that promotes the hydrolysis of PC to PA. In contrast, the expression of *phospholipase Dδ* (*PLDδ*), which plays a positive role in freezing tolerance, was upregulated in *acbp1* mutants and downregulated in ACBP1-overexpressors. Reduced *PLDα1* expression and decreased hydrolysis of PC to PA may have enhanced membrane stability in the *acbp1* mutants. Given the PA- and acyl-CoA-binding abilities of ACBP1, the expression of *PLDα1* and *PLDδ* could be subject to regulation by PA or acyl-CoA esters maintained by ACBP1, if ACBP1 were to resemble the yeast 10-kD ACBP in modulating gene expression during stress responses. Interestingly, another membrane-associated ACBP, ACBP2, which shows high (76.9%) conservation in amino acid homology to ACBP1, did not appear to be involved in the freezing response.

Key words: acyl-CoA-binding protein, ACBP1, ACBP2, freezing tolerance, phosphatidic acid-binding, lipid profiling

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Introduction

In Arabidopsis, a gene family encodes acyl-CoA-binding proteins (ACBPs) that show conservation at the acyl-CoA-binding domain.[1] These ACBPs, designated ACBP1 to ACBP6, range in size from 10.4 to 73.1 kD.[1] ACBP1 and ACBP2 are membrane-associated proteins with *N*-terminal membrane targeting domains and *C*-terminal ankyrin repeats.[2-5] ACBP3 is localized extracellularly[6] while the others (ACBP4, ACBP5 and ACBP6) are cytosolic proteins.[7,8] Recombinant ACBP1 to ACBP6 have been demonstrated to bind long-chain acyl-CoA esters with varying affinities.[1] The ankyrin repeats in ACBP1 and ACBP2 and the kelch motifs in ACBP4 and ACBP5 can mediate protein-protein interactions.[9-11] It has been previously demonstrated that the overexpression of ACBP6 increases freezing tolerance in transgenic Arabidopsis, accompanied by an elevation in *PLDδ* mRNA.[7] Our recent findings indicate that another ACBP (ACBP1) is also associated with freezing stress.

ACBP1, PA and Freezing Sensitivity

The *acbp1* mutant plants were tolerant to freezing (-8°C) and had accumulated PC (with reduced PA) while ACBP1-overexpressing plants displayed enhanced freezing sensitivity accompanied by decrease in PC and increase in PA. An increase in the ratio of PC to PA has been reported to protect the plasma membrane during freezing stress, enhancing freezing tolerance.[12,13] Differences in PC and PA contents in *acbp1* mutants and ACBP1-overexpressors seem to be related to changes in the mRNA encoding PLDα1, which promotes the hydrolysis of PC to PA.[12,13] Expression of another Arabidopsis *phospholipase*, *PLDδ*, which plays a positive role in

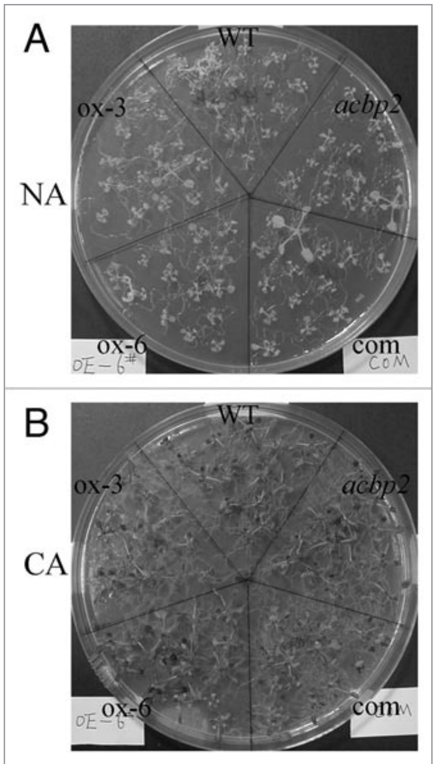

Figure 1. The *acbp2* mutant, ACBP2-overexpressing and ACBP2-complemented seedlings resemble wild type in response to freezing stress. NA (A) and CA (B) 11-d-old wild-type (WT), *acbp2* mutant, ACBP2-overexpressing (ox-3 and ox-6) and ACBP2-complemented (com) seedlings were treated at -12°C for 1 h. After thawing overnight at 4°C, the seedlings were left to recover in a growth chamber (16-h-light [23°C]/8-h-dark [21°C]) for 7 d before photography.

freezing tolerance,[13,14] declined in ACBP1-overexpressors but was enhanced in *acbp1* mutants during stages of cold acclimation, freezing and recovery. Since protein-lipid binding assays indicate that ACBP1 binds PA in vitro and it has been previously shown to bind acyl-CoA esters,[2,4,6,9] ACBP1 could maintain a membrane-associated PA or acyl-CoA pool, mimicking the yeast ACBP-acyl-CoA ester complex which modulates the expression of genes associated with stress responses and the biosyntheses of fatty acid and phospholipids.[15] Furthermore, ACBP1, PLDα1 and PLDδ have all been shown expressed at the plasma membrane.[2,3,5,16,17]

ACBP2 does not Resemble ACBP1 during Freezing Stress

ACBP2 is a homologue of ACBP1 sharing 76.9% identity.[1] Like ACBP1, ACBP2 is localized to the plasma membrane and the ER.[2,3,5] ACBP1 and ACBP2 are highly conserved in their domains, each containing an *N*-terminal membrane-associated domain, an acyl-CoA-binding domain and a *C*-terminal ankyrin repeat.[1] To investigate the role of ACBP2 in the freezing response, a T-DNA knockout mutant of *ACBP2* (*acbp2*)[18] and two independent transgenic lines of *ACBP2*-overexpressors (ox-3 and ox-6)[9] were subject to freezing treatment. Also, the *acbp2* mutant was complemented using Agrobacterium-mediated transformation by a construct expressing *ACBP2* from the cauliflower mosaic virus (CaMV) *35S* promoter and resulting T_4 complemented lines (com) were subsequently subject to freezing treatment following previous procedures.[7] Briefly, nonacclimated (NA) and cold-acclimated (CA) 11-d-old seedlings were subjected to a temperature drop from 4°C to -2°C at 2°C h^{-1}. After incubation at -2°C for 2 h, ice crystals were placed on the plates. The temperature was subsequently lowered to -12°C at 2°C h^{-1} and held at -12°C for 1 h. The seedlings were thawed at 4°C overnight and were photographed after 7-d recovery. As shown in **Figure 1**, both NA (**Fig. 1A**) and CA (**Fig. 1B**) seedlings from the *acbp2* mutant, *acbp2*-complemented line and ACBP2-overexpressors appeared similar to wild type under freezing stress, indicating that ACBP2 does not seem to be involved in the freezing response, unlike ACBP1.

Conclusions and Perspectives

ACBP1 and ACBP2 are highly conserved homologues in Arabidopsis.[1] ACBP1- and ACBP2-overexpressing plants show enhanced tolerance to heavy metals (Pb and Cd, respectively).[9,19] Based on the ability of their recombinant proteins in binding [^{14}C]linoleoyl-CoA and [^{14}C]linolenoyl-CoA in vitro, these two plasma membrane-associated ACBPs could participate in the repair of the peroxidised membrane lipids following heavy metal stress.[9,19] Our recent observations have revealed that of these two similar proteins, only ACBP1 plays a role in freezing stress. In vitro filter-binding assays have shown that of the Arabidopsis ACBPs tested,[7,20] only ACBP1 is able to bind PA. PA is believed to be an important stress-signaling lipid participating in multiple stresses including freezing stress.[21,22] Other studies have demonstrated that PA is a considerable negative curvature and fusigenic lipid which may be harmful to cell membranes by promoting the formation of nonlamellar phase.[13,23] Interaction of ACBP1 with membrane-associated PA may possibly reduce membrane stability and increase freezing sensitivity, while alterations in ACBP2 expression do not appear to affect freezing tolerance, perhaps as a consequence of its inability to bind PA. Since ACBP1 binds PA, which serves as a vital messenger in multiple stresses, ACBP1 could play a role in other plant stresses besides those that have been described.

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