ARTICLE ADDENDUM

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Protein interactors of acyl-CoA-binding protein ACBP2 mediate cadmium tolerance in Arabidopsis

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In our recent paper in the *Plant Journal*, we reported that *Arabidopsis thaliana* lysophospholipase 2 (lysoPL2) binds acyl-CoA-binding protein 2 (ACBP2) to mediate cadmium [Cd(II)] tolerance in transgenic Arabidopsis. ACBP2 contains ankyrin repeats that have been previously shown to mediate protein-protein interactions with an ethylene-responsive element binding protein (AtEBP) and a farnesylated protein 6 (AtFP6). Transgenic Arabidopsis ACBP2-overexpressors, lysoPL2-overexpressors and AtFP6-overexpressors all display enhanced Cd(II) tolerance, in comparison to wild type, suggesting that ACBP2 and its protein partners work together to mediate Cd(II) tolerance. Given that recombinant ACBP2 and AtFP6 can independently bind Cd(II) in vitro, they may be able to participate in Cd(II) translocation. The binding of recombinant ACBP2 to [^{14}C]linoleoyl-CoA and [^{14}C]linolenoyl-CoA implies its role in phospholipid repair. In conclusion, ACBP2 can mediate tolerance to Cd(II)-induced oxidative stress by interacting with two protein partners, AtFP6 and lysoPL2. Observations that ACBP2 also binds lysophosphatidylcholine (lysoPC) in vitro and that recombinant lysoPL2 degrades lysoPC, further confirm an interactive role for ACBP2 and lysoPL2 in overcoming Cd(II)-induced stress.

Acyl-CoA-binding proteins (ACBP1 to ACBP6) are encoded by a multigene family in *Arabidopsis thaliana*.[1] These ACBP proteins are well studied in Arabidopsis in comparison to other organisms,[1-4] and are located in various subcellular compartments.[1] Plasma membrane-localized ACBP1 and ACBP2 contain ankyrin repeats that have been shown to function in protein-protein interactions.[5,6] ACBP1 and ACBP2 which share 76.9% amino acid identity also confer tolerance in transgenic Arabidopsis to lead [Pb(II)] and Cd(II), respectively.[1,5,7] Since recombinant ACBP1 and ACBP2 bind linolenoyl-CoA and linoleoyl-CoA in vitro, they may possibly be involved in phospholipid repair in response to heavy metal stress at the plasma membrane.[5,7] In contrast, ACBP3 is an extracellularly-localized protein[8] while ACBP4, ACBP5 and ACBP6 are localized to cytosol.[9,10] ACBP1 and ACBP6 have recently been shown to be involved in freezing stress.[9,11] ACBP4 and ACBP5 bind oleoyl-CoA ester and their mRNA expressions are light-regulated.[12,13] Besides acyl-CoA esters, some ACBPs also bind phospholipids.[9,11,13] To investigate the biological function of ACBP2, we have proceeded to establish its interactors at the ankyrin repeats, including AtFP6,[5] AtEBP[6] and now lysoPL2 in the Plant Journal paper. While the significance in the interaction of ACBP2 with AtEBP awaits further investigations, some parallels can be drawn between those of ACBP2 with AtFP6 and with lysoPL2.

AtFP6 and lysoPL2 are Protein Partners of ACBP2 in Mediating Cd(II) Tolerance

Similar to lysoPL2, the heavy-metal-binding protein AtFP6 was first identified to interact with ACBP2 in yeast two-hybrid analysis.[5] Subsequently, Arabidopsis AtFP6-overexpressors demonstrated improved Cd(II) tolerance in comparison to wild type.[5] AtFP6 belongs to the AtFP family of proteins that posses an M/LXCXXC domain participating in heavy metal binding.[5] The *AtFP6* mRNA is Cd(II)-inducible in Arabidopsis roots, in vitro translated AtFP6 was observed to bind Pb(II), Cd(II) and copper [Cu(II)], and $(His)_6$-AtFP6 binds Pb(II).[5] Hence, AtFP6 may possibly mediate Pb(II), Cd(II) and Cu(II) transport in Arabidopsis roots.[5] Given that $(His)_6$-ACBP2 binds [^{14}C]linoleoyl-CoA and [^{14}C]linolenoyl-CoA, the precursors for phospholipid repair following lipid peroxidation from heavy metal stress at the plasma membrane, and that *ACBP2*-overexpressors are more tolerant to H_2O_2 than wild type, a role for ACBP2 in post-stress membrane repair has been previously proposed.[5]

We identified another interactor of ACBP2 that resembles AtFP6 in its ability to confer tolerance to Cd(II) when overexpressed in transgenic Arabidopsis. This other ACBP2 protein interactor, Arabidopsis lysophospholipase lysoPL2, showed 33% to 37% amino acid identity to Arabidopsis lysoPL1 (At2g39400) and five lysoPL1-like proteins by BLASTP analysis. Lysophospholipases are enzymes that catalyze the degradation of lysophospholipids to produce fatty acids and glycerolphosphate derivatives.[14] They are well-characterized in mammals and bacteria[15,16] but few plant lysophospholipases have been reported.[17,18] Although the expression of Arabidopsis *lysophospholipase 1 (lysoPL1)* has been demonstrated to be induced by pathogen treatment,[18] its in vivo biological functions as well as those of other plant lysophospholipases remain to be elucidated. In our recent study, we have demonstrated that lysoPL2 and ACBP2 function together to promote degradation of lysoPC in response to Cd(II)-induced oxidative stress.

ACBP2 Interactors (AtEBP, AtFP6 and lysoPL2) are Stress-Inducible

All three protein interactors of ACBP2, so far identified, are encoded by stress-inducible genes. *AtEBP* mRNA is induced by ethylene and pathogen[19] while the mRNA expression of *AtFP6* is Cd(II)- and zinc [Zn(II)]-inducible.[5] The mRNA of *lysoPL2* has been shown to be Zn(II)- and H_2O_2-inducible and its protein, Cd(II)- and Zn(II)-inducible. Both *AtFP6*,[5] and *lysoPL2* displayed spatial expression patterns that overlap that of ACBP2, with higher expression in root, stem and flower and lower expression in silique and leaf. However *AtEBP* showed a slightly different expression pattern with higher mRNA accumulation in leaf and stem.[19] By using an ACBP2 derivative lacking the ankyrin repeat, we have shown that all three protein partners interact at the ankyrin repeats of ACBP2.[5,6] Although the significance in the interaction of AtEBP with ACBP2 is currently less clear, both AtFP6 and lysoPL2 have emerged to work with ACBP2 in mediating Cd(II) tolerance.

Conclusions and Perspectives

Many proteins including P_{1B}-type heavy metal ATPases, ABC transporter, phytochelatins, methallothioneins and oxidative stress-related proteins have been associated with heavy metal stress.[20-23] AtHMA4, a P_{1B}-type heavy metal ATPase, translocates Zn(II) and Cd(II) from root to shoot.[20] AtOXS3, an oxidative stress-related protein, confers tolerance to Cd(II) and oxidizing chemicals.[22] Phytochelatins and methallothioneins bind Cd(II) to form complexes.[23] Our recent findings present a mechanism involving lysoPL2 recruitment by ACBP2 to remove toxic lysoPC. This promotes repair of peroxidized phospholipids arising from Cd(II)-induced oxidative stress at the plasma membrane.

Heavy metals including Cu(II), iron [Fe(III)], nickel [Ni(II)], Cd(II) and Zn(II) are known to induce oxidative stress at high concentrations.[24-27] Cu(II) and Fe(III) belong to transition metals that induce lipid peroxidation and oxidative stress via Fenton-type reactions.[24] Cd(II) and Zn(II) are not transition metals, however they can also activate reactive oxygen species to induce lipid peroxidation.[25-27] Since lysoPL2 has been shown to confer tolerance to Cd(II)-induced oxidative stress, it will be worthwhile to investigate if it could also be used in protection against a wider range of metals in future studies.

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