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Distinct expression profiles of acyl-CoA-binding proteins AtACBP4 and AtACBP5 during pollen development

Flower development is a process during which plants must progress from sexual immaturity to maturity. Throughout these phases, the differential expression of many genes and proteins is evident. In angiosperms, the pollen generates male sperms to facilitate pollination and fertilization but the molecular mechanisms leading to pollen development that are related to lipid changes have remained largely elusive. Recently, we investigated the underlying molecular regulatory mechanisms of two kelch-motif-containing acyl-CoA-binding proteins, AtACBP4 and AtACBP5, during flower development. These two proteins, which shared 81.4% amino acid identity and contain an acyl-CoA-binding domain that binds lipids, were distinctly expressed, and exhibited diversified and complementary roles in lipid metabolism during pollen development.

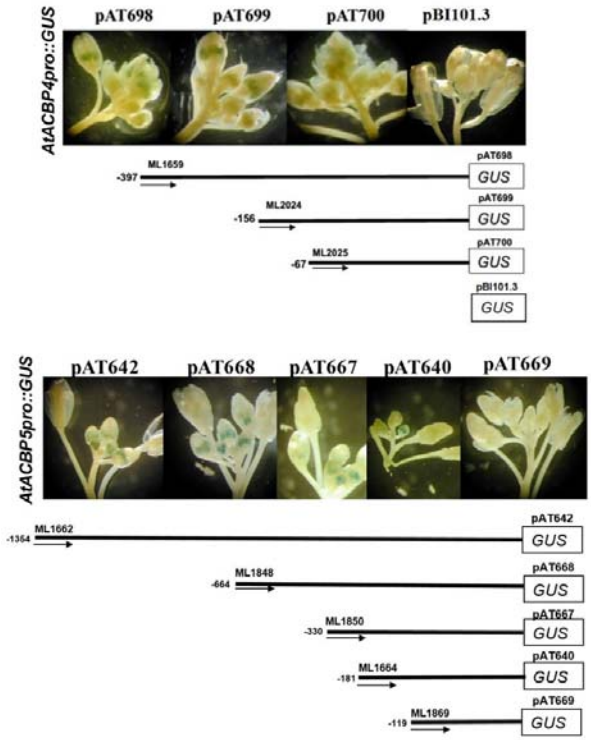

Fig. 1. Analysis of GUS expression in transgenic Arabidopsis flowers transformed with AtACBP4pro::GUS and AtACBP5pro::GUS and their deletion derivatives.
Histochemical GUS stains of transgenic Arabidopsis 8-week-old flowers transformed with AtACBP4pro::GUS constructs (pAT698, pAT699 and pAT700) and AtACBP5pro::GUS constructs (pAT642, pAT668, pAT667, pAT640 and pAT669). Inflorescences from 8-week-old Arabidopsis transformants were stained with substrate X-gluc.

The earlier expression of *AtACBP5* in anther development was detected in the microspores as well as the tapetum, endothecium and epidermis. *AtACBP5* was most highly expressed before stage 9 when

the petal primordia elongate. During stages 9-10 when the microspores vacuolate to expand their size, *AtACBP5* was more highly expressed in the tapetal cells than the microspores. When the tapetal cells started to degrade at stage 10, *AtACBP5* was no longer detected on immunoelectron microscopy using anti-AtACBP5 antibodies. On the other hand, *AtACBP4* was expressed during the later stages (11-14) of anther development, in the pollen grains and the endothecium.

When the lipid-associated roles of AtACBP4 and AtACBP5 in pollen development were investigated, wax analysis of *acbp4* and *acbp4acbp5* mutant flower buds showed a significant increase in C29-alkanes in comparison to Col-0. Fatty acid profiling demonstrated a decrease in stearic acid and an increase in linolenic acid in the *acbp4* and *acbp4acbp5* buds, respectively, over Col-0. Analysis of inflorescences from *acbp4* and *acbp5* revealed that there was an increase of *AtACBP5* expression in *acbp4*, and an increase of *AtACBP4* expression in *acbp5*. Interestingly, α-amylose content decreased in *acbp5* but not *acbp4*. Given that our previous study had revealed that recombinant AtACBP4 and AtACBP5 could bind oleoyl-CoA *in vitro* and that oleoyl-CoA is known to passively regulate starch synthesis, it would be interesting to follow up on how AtACBP4 could affect starch synthesis.

To investigate the regulation of stage-specific expression for *AtACBP4* and *AtACBP5* in anthers, deletion analysis of their 5'-flanking regions was carried out (Fig. 1). Our results defined the minimal promoter regions for *AtACBP4* (-145/+103) and *AtACBP5* (-181/+81). Electrophoretic mobility shift assays (EMSAs) further identified a pollen-specific *cis*-acting element POLLEN1 (AGAAA) at *AtACBP4* (-157/-153) which interacted with nuclear proteins from flower, and this was substantiated by DNase I footprinting assays. The POLLEN1 box, mapped in the *AtACBP4* 5'-flanking region, represents the first functional POLLEN1 to be reported in Arabidopsis. Thus far, specific elements that control the early-staged expression of *AtACBP5* remain unindentified and warrants future investigations.

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Publication

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