Characterization of Sphingolipid Metabolism and Function in Arabidopsis Using Mutants Defective in Sphingoid Long-Chain Base Synthesis

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Sphingolipids are major structural components of plasma membrane, tonoplast, and other endomembranes of plant cells. Sphingolipid metabolites such as ceramides and long-chain base phosphates have also been shown to have bioactive properties in the regulation of a number of physiological processes in plants, including programmed cell death and guard cell closure. A goal of our research is to understand the function and metabolism of sphingolipids in plants by the characterization of Arabidopsis T-DNA with alterations in the synthesis of sphingolipid long-chain bases. One target of our research is serine palmitoyltransferase (SPT), which catalyzes the first step in sphingolipid synthesis. Using yeast complementation, we demonstrated that Arabidopsis SPT is a heteromeric enzyme composed of LCB1 and LCB2 subunits. Homozygous T-DNA mutants lacking either subunit were not recoverable, indicating that sphingolipids are essential in Arabidopsis. In addition, partial RNAi suppression of the *LCB1* gene was accompanied by reductions in growth of plants due to decreased cell expansion. However, the total content of long-chain bases on a per plant weight basis was unchanged, suggesting that plants adjust growth to compensate for the availability of long-chain bases. To examine the effect of alterations in sphingolipid long-chain base composition, T-DNA mutants for the C-4 long-chain base hydroxylase were generated that completely lack tri-hydroxy long-chain bases. By comparison, approximately 90% of the sphingolipids in wild-type plants contain tri-hydroxy long-chain bases. The C-4 hydroxylase null mutants were severely dwarfed and did not progress beyond the vegetative stage of growth. Analysis of sphingolipid molecular species indicated that all sphingolipid classes in the mutants had greatly reduced content of very long-chain fatty acids. These results demonstrate that relatively small changes in long-chain base structure can have profound and unexpected effects on plant growth and development and on global sphingolipid composition.