Regulation of Neutral Lipid Accumulation in Vegetative Tissues of Plants

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Project Goals: Our research project aims to examine the cellular machinery that influences the accumulation of lipid droplets in non-seed tissues of plants. Our initial efforts are focused on a homolog of a mammalian hydrolase, *CGI-58*, that in humans is the causative gene for a neutral lipid storage disease. A loss-of-function mutation in this gene in Arabidopsis results in accumulation of triacylglycerols (TAGs) in leaf tissues that normally do not store neutral lipids. We proposed to 1) examine the role of AtCGI58 gene products in lipid droplet formation in Arabidopsis vegetative tissues 2) characterize the biochemical and cellular properties of CGI58 in the context of lipid metabolism. An improved understanding of the factors that regulate the accumulation of neutral lipids in vegetative tissues of plants may help to identify new strategies to enhance the energy content of crop plants.

Confocal laser scanning fluorescence imaging experiments indicated that Arabidopsis *cgi-58* loss-of-function mutants had significantly more lipid droplets in leaf mesophyll cells compared with wild-type cells. These lipid droplets were determined to be cytosolic and isolated lipid droplets were shown to contain triacylglycerols (TAG) with leaf-type fatty acids. While the numbers of lipid droplets were somewhat variable in leaf tissues of mature plants, there was a ten-fold increase in TAG levels in *cgi-58* mutants compared with wild-type plants. This lipid droplet/TAG phenotype was similar to that reported for peroxisomal mutants deficient in the uptake or catabolism of fatty acids (e.g., acylCoA oxidase, *acx1*, *acx2*; FA transporter, *cts1*, *pxa1*). There were no statistical differences in TAG levels of *cgi-58* seeds suggesting that the CGI-58 protein may act to influence lipid droplet abundance in non-seed tissues only, unlike the known peroxisomal mutants that confer a sugar-dependent germination phenotype. TAGs of *cgi-58* mutants were specifically enriched in molecular species that contained 18:3 (linolenic acid) fatty acid moieties. The enrichment in 18:3-containing lipids also was observed in major membrane galactolipids and phospholipids, suggesting that CGI-58 may participate specifically in the turnover of 18:3-containing glycerolipid species.

In animal systems, the CGI-58 protein is known to activate triacylglycerol lipases, and to transiently interact with the lipid droplet surface to mediate its functions. While the details of lipase activation in animal cells are not clear, we asked if the AtCGI-58 protein interacts with partner proteins to accomplish its function in lipid droplet biogenesis in plants. We implemented an unbiased approach to screen for interacting partners of CGI-58 using a yeast two-hybrid system to screen an Arabidopsis cDNA library. Several candidate interacting proteins were identified and we are currently following up one in detail—At4g39850 (*PXA1*, encoding peroxisomal fatty acid transporter 1, previously named *comatose*, *cts*, above). The interaction of CGI-58 with PXA1 might not be entirely unexpected, since the phenotype of the loss of *pxa1* (*cts*) function mutant shows an increase in cytosolic lipid droplets in leaves, similar to the *cgi-58* knockouts. Our interaction assays revealed that only the C-terminus of PXA1 interacts with CGI-58, specifically the so-called Walker B motif of nucleotide binding domain 2 (NBD2). This domain has been hypothesized by others to be involved in the regulation of the PXA1 transporter activity. Interaction between CGI-58 and PXA1 *in planta* was demonstrated through nuclear re-localization assays. In these experiments, the so-called Walker B motif of NBD2 (but not a mutated form of the Walker B motif, the Walker A motif

nor the NBD1 domain of PXA1) containing a nuclear localization sequence (NLS) was able to redirect AtCGI-58-GFP protein to the nucleus when these two proteins were co-expressed in tobacco BY-2 cells, indicating that the Walker B motif of the PXA1 NBD2 domain is sufficient for CGI-58 interaction and that this interaction indeed occurs in the plant cytosolic environment. Additional transient expression assays in leaves with prolonged incubation have shown that in addition to a cytosolic location, CGI-58-GFP co-localizes to peroxisomes, consistent with a PXA1 interaction *in vivo*.

To test the functional association of CGI-58 and peroxisomal PXA1 beyond TAG accumulation, we turned to another "reporter" of PXA1 function, which is the uptake of 12-*oxo*-phytodienoic acid (oPDA) for its conversion to jasmonic acid. Indeed, whole seedlings or mature leaves of *cgi-58* knockout plants, when wounded, produced significantly less jasmonic acid compared with wild type plants. The reduction in wound-inducible jasmonate was to about 60% of wild-type levels, and there were no significant differences in jasmonate levels in unwounded tissues, suggesting that plants may have mechanisms in place to generate jasmonate independent of CGI-58. However, CGI-58 clearly influences the efficiency of jasmonate formation, likely through facilitating the uptake of oPDA by PXA1. These results corroborate our protein-protein interaction findings, and broaden the importance of CGI-58 function in plants to include modulation of signaling lipid metabolism in addition to regulating neutral lipid accumulation.

References (supported by this project)

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Supported by the Office of Biological and Environmental Research in the DOE Office of Science (DE-SC0000797).