145. Regulation of Neutral Lipid Compartmentalization in Vegetative Plant Tissues

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Project Goal: The overall goal of our research program is to identify, characterize and manipulate the cellular machinery that influences the accumulation and compartmentalization of neutral lipids in vegetative tissues of plants.

Our research has focused on identifying the subcellular machinery that plants use to compartmentalize neutral lipids in cells of vegetative tissues. Virtually all cells in plants (and other organisms) synthesize triacylglycerols (TAGs) and deposit them in cytosolic lipid droplets (LDs), yet little is known about the molecular mechanisms responsible for the biogenesis and function of LDs, especially in cells of non-seed tissues. While seed tissues accumulate large amounts of TAGs in specialized, oleosin protein-coated LDs, most non-seed tissues do not. The energy density of TAGs is approximately twice that of carbohydrate on a mass basis, and as such, the seed oils of plants are a major source of calories for animal and human nutrition. By contrast, TAGs normally accumulate to a very small percentage in vegetative tissues like leaves and stems. In recent years, the use of increased amounts of seed TAGs as a bioenergy feedstock has placed energy and food demands at increasing conflict. Hence, the mechanisms in vegetative tissues that limit the accumulation TAGs represent key targets to expand the total TAG/energy content of plants, thereby reducing the competition between oilseeds for food and biomass for energy uses.

Our previously-funded research uncovered a gene in the plant *Arabidopsis thaliana* that, when disrupted, resulted in the hyperaccumulation of TAG-containing LDs in leaves. This gene is a homologue of human comparative gene identification-58 (CGI-58), which when mutated causes Chanarin-Dorfman syndrome, a neutral lipid storage disorder that also results in an increase in cellular accumulation of LDs in human tissues, particularly in cell types that typically lack high amounts of TAG such as skin and blood cells. Follow-up work this year with *Arabidopsis* has pointed to a central role for CGI-58 in regulating the turnover of cellular fatty acids, thus influencing the availability of lipids for membrane production, energy storage, and signaling pathways. We are now focused on other genes known to be involved in LD biogenesis in mammalian cells and testing their ability to modulate LD formation in *Arabidopsis*, including several endogenous *Arabidopsis* homologues of mammalian proteins (e.g., seipins), or other genes encoding foreign proteins that are not found in plants (e.g., the mammalian fat-inducing transmembrane protein 2, FIT2). Furthermore, our recent proteomics analysis of non-seed LDs, derived from avocado tissues, revealed a new class of LD-associated proteins that are highly conserved in plants, and we are currently investigating the ability of these proteins to promote the formation and/or regulation of LDs in leaves of *Arabidopsis*. Collectively, this research has important bioenergy applications, but also will help to unravel the complex machinery in eukaryotes that is deployed for the metabolism and maintenance of neutral lipids in LDs. Our results are also expected to stimulate new ideas about the dynamic interplay between lipid storage, mobilization and signaling in eukaryotic systems -- an understanding that will be important to achieving the broader BER goal of "sustainable and affordable production of renewable biofuels in an environmentally conscientious manner".

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3) **Horn PJ, Silva JE, Anderson D, Fuchs J, Borisjuk L, Nazarenus TJ, Shulaev V, Cahoon EB, Chapman KD** (2013) Imaging heterogeneity of membrane and storage lipids in transgenic *Camelina sativa* seeds with altered fatty acid profiles. *Plant Journal*, Oct;76(1):138-50. doi: 10.1111/tpj.12278. Epub 2013 Aug 5.
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