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Center for Advanced Biofuel Systems (CABS)
Final Technical Report

Benning Lab

To explore and identify factors involved in TAG regulation and turnover in the microalgae *Nannochloropsis oceanica*, random insertional mutagenesis was performed by plasmid disruption. Over 4,000 independent mutants were produced and further incubated under nitrogen (N) sufficient or N-limited conditions, followed by an initial lipid mutant screen using gas chromatography. About 60 out of 3,000 mutant strains with significant changes in lipid composition (especially in TAG) were further examined by thin-layer chromatography coupled with gas chromatography. Of these, 31 mutants showed a reproducible lipid phenotype. These mutants were tested by southern blot for single-transgene insertions, and 15 resultant mutants with single copy insertions were analyzed by plasmid rescue and site-finding PCR to identify the disrupted genes. Currently, a gene encoding a putative acyltransferase has been identified from a mutant that produces 50% less TAG compared to the wild type under N deprivation. Further investigations will be carried out to characterize the function of this gene, as well as the other disrupted genes from the selected mutants.

To determine the physiological role of TAG accumulation following nutrient deprivation in *Chlamydomonas reinhardtii*, a complete characterization of the *pgd1* mutant was carried out by multiple approaches such as microscopy, low-temperature fluorescence emission spectra and P700 absorption spectra. The results revealed the significance of membrane lipid remodeling and TAG accumulation for the survival of Chlamydomonas cells under various environmental stresses and the importance of lipid composition of the thylakoid membrane for photosynthesis. A paper summarizing this work is being written.

Gang Lab

In order to understand the large-scale structural organization of regulatory networks by which the green microalga *Chlamydomonas reinhardtii* accumulates triacyglycerols (TAG) and other lipids in response to nitrogen starvation, a time course –based comparative multi-omics effort (transcriptomic, proteomic, metabolite and flux changes) was established to characterize in details the response of Chlamydomonas to nitrogen deprivation. Results of the combined datasets, systems level investigation indicated that C. reinhardtii cells sense and respond on a large scale within 30 min to a switch to N-deprived conditions with a drastic metabolic repatterning followed a biphasic modality, centered on the initiation of TAG synthesis between 4 and 6 h after N depletion (1). The combined omic results increase our understanding of the chronological transcriptional, proteomic and metabolite profiling changes that occur before and after TAG accumulation initiates and allows us to propose that future metabolic engineering projects may benefit from attempts to characterize the regulatory networks of this biphasic response during N deprivation in Chlamydomonas. Therefore, lipidomic and primary metabolite profiling generated compound accumulation levels that were integrated with the transcript dataset and transcription factor profiling to produce a time-transcriptional regulatory networks. Evaluation of these proposed regulatory networks led to the identification of several time-regulatory hubs that are candidates for control of many aspects of cellular metabolism, from N assimilation and metabolism, to central metabolism, photosynthesis and lipid metabolism, during the two major phases of the response to N deprivation (2). However, the massive TAG accumulation that can occur in microalgae, during N deprivation, is correlated with a reduced in abundance of many transcripts and proteins involved in photosynthesis, carbon fixation and

chlorophyll synthesis (3). This leads ultimately to cessation of cell division and growth. The light reactions of photosynthesis may be a limiting factor with regards to TAG synthesis. To better understand the mechanisms that regulate photosynthesis under N deprivation conditions, we identified and characterized a photosynthesis regulatory hub (TAB2) that reroutes the external carbon source to the TAG accumulation by maintaining an adequate reductant pool during N deprivation (4). Future work that identifies and characterizes the role of these specific regulatory hubs, should provide a clear understanding of how the switch to N-depleted medium is indeed sensed and then transduced to large metabolic changes that enable this aquatic single-celled organism to survive large environmental stresses. Such knowledge now enables synthetic biology approaches to alter the response to the N-depletion stress and lead to rewiring of the regulatory networks so that lipid accumulation could be turned on in the absence of N-deprivation, allowing for the development of algal production strains with highly enhanced lipid accumulation profiles. Beyond providing insights into the regulatory systems-level organization of Chlamydomonas metabolism during nitrogen deprivation, we believe that our dataset and approach sets the stage for an emerging series of studies that will decipher the dynamic regulatory network in other microalgae.

(1) *Jeong-Jin Park, Hongxia Wang, Mahmoud Gargouri, Rahul Deshpande, Jeremy N. Skepper, F. Omar Holguin, Matthew Juergens, Yair Shachar-Hill, Leslie M. Hicks, and David R. Gang. The response of Chlamydomonas reinhardtii to nitrogen deprivation: A systems biology analysis (Revision in Plant Journal)*

(2) *Mahmoud Gargouri, Jeong-Jin Park, F. Omar Holguin, Min-Jeong Kim, Hongxia Wang, Rahul Deshpande, Yair Shachar-Hill, Leslie M. Hicks and David R. Gang. Identification of regulatory network hubs that control lipid metabolism in Chlamydomonas reinhardtii. (Revision in Journal of Experimental Botany)*

(3) *Matthew Juergens, Rahul R. Deshpande, Ben F. Lucker, Jeong-Jin Park, Hongxia Wang, Mahmoud Gargouri, Omar Holguin, Bradley Disbrow, Tanner Schaub, Jeremy N. Skepper, David M. Kramer, David R. Gang, Leslie M. Hicks, and Yair Shachar-Hill1. The Response of Photosynthetic Structure and Function to N Deprivation in Chlamydomonas reinhardtii (Submitted)*

(4) *Mahmoud Gargouri, Philip D. Bates, Jeong-Jin Park, Helmut Kirchhoff, David R. Gang. TAB2 a knot regulator that maintains a proper energy balance promoting triacylglycerol accumulation during nitrogen depletion in Chlamydomonas reinhardtii (Submitted)*

Cahoon Lab

The goals of the research are (1) to understand how certain non-agronomic species (e.g. Cuphea sp.) have evolved specialized fatty acid biosynthetic and metabolic systems to accumulate high-levels of short/medium chain-length fatty acids (C8-C16) in their seed oils and (2) to use this knowledge to develop seed oils with Jet A fuel functionality in engineered camelina. To accomplish this, 454 transcriptomes were generated from developing seeds of *Cuphea viscosissima* and *Cuphea pulcherrima*. The seed oil of these species contain>90% C8 and C10 fatty acids. From this deep collection of next-generation sequence information, candidate genes were mined for enzymes suspected to have evolved for short-/medium-chain fatty acid

metabolism, including novel FatB thioesterase, glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAT), and diacylglycerol acyltransferase (DGAT) genes. Acyl-CoA independent phospholipid: diacylglycerol acyltransferase (PDAT) genes have also been isolated and identified in *Cuphea viscosissima* and *Cuphea pulcherrima*. To date, we have assembled >145 seed-specific gene expression constructs containing Cuphea candidate genes and introduced these into camelina. Camelina lines with seed oils as high as 50 mol% C14-C16 FAs, 40 mol% C12-C16 FAs, and 32% C8-C16 FAs have been developed, without significant impact on total seed oil content. These oils are anticipated to mimic Jet A fuel functionality and also generate intellectual property for the utility of the novel acyltransferases. We are now exploring enzyme specialization in fatty acid synthase of Cuphea to achieve higher levels of short/medium chain-length fatty acid production in engineered oilseeds. To further accumulate MCFA in TAG, down-regulation of competitive genes in the fatty acid biosynthesis pathway such as Camelina KASI, KASII, and plastid acyl-ACP synthetase (AAE) genes have also been applied to obtain further increases in MCFAs in Camelina seed oil.

Major Accomplishments

- **Camelina developing seed transcriptome database released**
  In depth transcript analysis of Camelina developing seeds was built from 2047 Sanger ESTs and more than 2 million 454-derived sequence reads. The transcriptome of approximately 60K transcripts from 22,597 putative genes includes camelina homologues of nearly all known seed expressed genes. Sequence information and searchable sequence databases were publicly disseminated at www.camelinagenome.org.
- ***Cuphea pulcherrima* and *Cuphea viscosissima* developing seed transcriptomes were generated**
  Transcriptome sequencing and assembly of over 2 million 454 pyrosequencing reads were obtained from developing of *Cuphea pulcherrima* and *Cuphea viscosissima* seeds, which accumulate up to 95% of the C8 and C10 short-/medium-chain length fatty acids. These sequences were mined to identify candidate genes for enzymes evolved for short-/medium-chain fatty acid synthesis and metabolism for use in camelina metabolic engineering efforts. Candidate genes identified included genes encoding acyltransferases glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAT), and diacylglycerol acyltransferase (DGAT) genes as well as fatty acid synthase genes such as 3-ketoacyl-ACP synthase I and II and the wrinkled1 transcription factor.
- **Functional analysis of Cuphea acyltransferases reveals novel enzymes incorporation of C10 fatty acids at the *sn*-2 and *sn*-3 positions of the triacylglycerol backbone**
  Three seed-specific lysophosphatidic acid acyltransferase (LPAT) genes were identified among seven total LPAT genes in the *Cuphea pulcherrima* and *Cuphea viscosissima* seed transcriptomes. One of these encodes CvLPAT2, an LPAT2-type enzyme, which displayed specificity for CoA esters of saturated fatty acids, particularly C10:0 upon expression in camelina seeds. In contrast, all previously-identified LPAT2 enzymes almost exclusively use CoA esters of C18 unsaturated substrates. One seed-specific diacylglycerol acyltransferase (DGAT) gene was identified in the *Cuphea pulcherrima* seed transcriptome. The corresponding enzyme CpuDGAT1 was active with 10:0-CoA and 10:0-containing diacylglycerol substrates and enhanced accumulation of C10, C12, C14 and C16 fatty acids in triacylglycerol (TAG) upon expression in camelina seeds. Co-expression of genes for CvLPAT2 and CpuDGAT1 resulted in synergistic increases in short-/medium-chain fatty

acid accumulation in the total TAG and in the TAG *sn*-2 position of engineered camelina seeds.

- **Camelina lines with JetA fuel-type oil compositions were identified from 44 transgenic lines expression combinations of specialized Cuphea genes.**
  Over 145 seed-specific gene expression constructs containing *Cuphea* candidate genes involved in short and medium chain fatty acid biosynthesis and accumulation were prepared and used to generate 44 homozygous camelina transgenic lines. These lines accumulated seed oils with an array of short-/medium-chain fatty acid compositions, depending on the FatB thioesterase and acyltransferase gene combinations. The best three lines accumulated up to 50 mol% C14-C16 fatty acids, 40 mol% C12-C16 FAs, and 45 mol % C10-C16, without significant impact on total seed oil content. These oils are anticipated to mimic Jet A fuel functionality. Qualitative and quantitative assessment of seed oil from the best homozygous lines showed that stacking Cuphea genes is an effective approach for achieving enhanced accumulation of short and medium-chain fatty in engineered camelina seeds.
- **Transformation of best short- and medium-chain fatty acid producing homozygous camelina transgenic line with best aromatic hydrocarbon producing constructs in 2.5 mg/g seed limonene (monoterpene) and 1 mg/g cadenine (sequeterpene) accumulation.**
- **Down-regulation of genes in competing pathways for short-/medium-chain fatty acid synthesis enhances JetA fuel-type fatty acid compositions**
  Seed-specific suppression of camelina 3-ketoacyl-ACP synthase (KAS) II genes resulted in increases of C16 fatty acids to ~20% of the total fatty acids. Seed-specific suppression of camelina acyl-ACP synthetase, which regulates recycling of short-chain fatty acids to the plastid fatty acid synthase, resulted in a 5-6% increase of C10 fatty acid accumulation.
- **Retargeting of mitochondrial acyl carrier protein (ACP) to chloroplasts inhibits fatty acid synthesis**
  Retargeting mitochondrial ACP to chloroplasts in camelina seeds resulted in the inhibition of fatty acid synthesis and decreases in accumulation of short- and medium-chain fatty acids.

Hicks Lab

1) **Quantitative Proteomics Profiling**: Detection, identification, and characterization of biomolecules remains an essential component in the elucidation of complex cellular processes, and is still one of the most challenging areas in contemporary scientific arena. Progress in the field of analytical instrumentation continually advances current capabilities and the ease by which these efforts can be carried out. The Hicks lab has established and continues to implement *robust, quantitative proteomics platforms*, including but not limited to both labeling-based and label-free quantification techniques that utilizes gel- and/or liquid-based separation approaches (e.g. 2-DE, DIGE, iTRAQ, label-free). We continue to optimize sample preparation, enrichment strategies and data acquisition methods for increased proteome coverage, with the long-term goal of being able to robustly detect and characterize very low abundance proteins and their PTMs currently not detected due to dynamic range limitations. Relevant recent publications include:

CABS -omics collaboration to investigate the mechanism by which Chlamydomonas transitions to oil production during N starvation at very early time points: the Hicks lab has employed the established iTRAQ global, quantitative profiling to analyze proteome changes at 0, 1, 2, 4, 6, 12

and 24 hour time points following N starvation of the wild type *cw15* (a cell wall-deficient *C. reinhardtii* strain).

Park, J. J., Wang, H., Gargouri, M., Deshpande, R. R., Skepper, J. N., Holguin, F. O., Juergens, M. T., Shachar-Hill, Y., Hicks, L. M.*, and Gang, D. R.* (2015) The response of Chlamydomonas reinhardtii to nitrogen deprivation: a systems biology analysis. *Plant J* 81, 611-624
*Joint corresponding author

Juergens, M. T., Deshpande, R. R., Lucker, B. F., Park, J. J., Wang, H., Gargouri, M., Holguin, F. O., Disbrow, B., Schaub, T., Skepper, J. N., Kramer, D. M., Gang, D. R., Hicks, L. M., and Shachar-Hill, Y. (2015) The Regulation of Photosynthetic Structure and Function during Nitrogen Deprivation in Chlamydomonas reinhardtii. *Plant Physiol* 167, 558-573

Gargouri M, Park JJ, Holguin FO, Kim M-J, Wang H, Deshpande R, Shachar-Hill Y, Hicks LM, Gang DR (2015) Identification of regulatory network hubs that control lipid metabolism in Chlamydomonas reinhardtii. *J Exp Botany*, 66, 4551-4566

A similar iTRAQ approach was applied to gain an understanding of the basis of increased yield and stress tolerance in transgenic lines of *Camelina sativa* seeds that overexpress Arabidopsis G-protein γ subunit 3 *(AGG3)*:

Alvarez S, Roy Choudhury S, Sivagnanam K, Hicks LM, Pandey S (2015) Quantitative proteomics analysis of *Camelina sativa* seeds overexpressing *AGG3* gene to identify the proteomic basis of increased yield and stress tolerance. *J. Proteome Res*, 14, 2606-2616

Post-translational Modifications: Essential processes for growth and response are increasingly revealed to be regulated by post-translational modifications of proteins that impact protein complex formation, enzyme catalysis, and structure/function relationships.

Thiol-Based Regulatory Switches. In many organisms the general role of redox environment as a regulatory factor in cellular processes is only beginning to be examined in detail. In response to changes in cellular redox environment, the formation of disulfide bonds provides a mechanism for rapidly modulating protein function. Although the generation of reactive oxygen species and alteration of cellular redox state can lead to changes in disulfide bond formation in redox-sensitive proteins, the actual number and types of these proteins in plants remain largely unknown. In plants, oxidative regulation and redox signaling occur in photosynthesis, translation, transcription, apoptosis, and seed development, but on-going efforts to identify proteins using thiol-based redox switches promise to expand the redox proteome. The Hicks lab is interested in the development and application of both gel- and liquid-based targeted proteomics methods to this end to both identify redox regulated proteins and characterize the site-specific modifications. We have recently published an optimized liquid-based redox quantification approach, the development and application of which will be used to elucidate biochemical responses to changes in cellular and environmental conditions and to discover new redox regulatory mechanisms in plants.

Slade, W. O., Werth, E. G., McConnell, E. W., Alvarez, S., and Hicks, L. M. (2015) Quantifying reversible oxidation of protein thiols in photosynthetic organisms. *Journal of the American Society for Mass Spectrometry* 26, 631-640

Abstract: Efforts to quantify thiol modifications on a global scale have relied upon peptide derivatization, typically using isobaric tags such as TMT, ICAT, or iTRAQ that are more expensive, less accurate, and provide less proteome coverage than label-free approaches – suggesting the need for improved experimental designs for studies requiring maximal coverage and precision. Herein, we present the coverage and precision of resin-assisted thiol enrichment coupled to label-free quantitation for the characterization of reversible oxidative modifications on protein thiols. Using *C. reinhardtii* and Arabidopsis as model systems for algae and plants, we quantified 3662 and 1641 unique cysteinyl peptides, respectively, with median CVs of 13% and 16%. Further, our method is extendable for the detection of protein abundance changes and stoichiometries of cysteine oxidation. Finally, we demonstrate proof-of-principle for our method, and reveal that exogenous hydrogen peroxide treatment regulates the *C. reinhardtii* redox proteome by increasing or decreasing the level of oxidation of 501 or 67 peptides, respectively. As protein activity and function is controlled by oxidative modifications on protein thiols, resin-assisted thiol enrichment coupled to label-free quantitation can reveal how intracellular and environmental stimuli affect plant survival and fitness through oxidative stress.

Phosphorylation. Protein phosphorylation, is ubiquitously found in eukaryotes and revealed to be critical for growth, adaptation and survival in photosynthetic organisms. Uncovering when and how phosphorylation occurs requires specialized strategies, of which those based on liquid-chromatography mass-spectrometry (LC-MS) have increasingly become the tool of choice due to the breadth, depth, and specificity of coverage afforded. Phosphoproteomics in photosynthetic organisms has the potential to not only inform on how life is sustained on earth but also how an organism responds to their environment, with insights ranging from agriculture, biofuels, and medicine.

Recently, the Hicks lab published the richest exploration of the *C. reinhardtii* phosphoproteome to date and a review article on phosphoproteomics in photosynthetic organisms. This resource is being used as we extend our work using a quantitative phosphoproteomics approach to delineate specific kinase pathways in Chlamydomonas relevant to nutrient sensing and lipid accumulation.

Wang, H. X., Gau, B., Slade, W. O., Juergens, M., Li, P., and Hicks, L. M. (2014) The Global Phosphoproteome of Chlamydomonas reinhardtii Reveals Complex Organellar Phosphorylation in the Flagella and Thylakoid Membrane. *Mol Cell Proteomics* 13, 2337-2353
***Highlighted as 'Data Set of the Week' (2014/6/23) by the Global Proteome Machine**

Slade, W. O., Werth, E. G., Chao, A., and Hicks, L. M. (2014) Phosphoproteomics in photosynthetic organisms. *Electrophoresis* 35, 3441-3451 (Review)

Kutchan Lab

Many plant-derived compounds of high value for industrial or pharmaceutical applications originate from plant species that are not amenable to cultivation. Biotechnological production in low-input organisms is an attractive alternative. Several microbes are well established as biotechnological production platforms, however, their growth requires fermentation units, energy input and nutrients. Plant-based production systems potentially allow the generation of high value compounds on arable land with minimal input. We have explored whether *Camelina sativa* (camelina), an emerging low-input non-foodstuff Brassicaceae oilseed crop grown on marginal lands or as a rotation crop on fallow land, can successfully be refactored to produce and store novel compounds in seed. As proof-of-concept, we used the cyclic monoterpene hydrocarbon (4*S*)-limonene and the bicyclic sesquiterpene hydrocarbon (+)-δ-cadinene, which have potential biofuel and industrial solvent applications. Posttranslational translocation of the recombinant enzymes to the plastid with concurrent overexpression of 1-deoxy-D-xylulose-5-phosphate

synthase (*DXS*) resulted in the accumulation of (4*S*)-limonene and (+)-δ-cadinene up to 7 mg g^{-1} seed and 5 mg g^{-1} seed, respectively. This study presents the framework for rapid engineering of camelina oilseed production platforms for terpene-based high value compounds.

Augustin, J.M., Higashi, Y. Feng, X. and **Kutchan, T.M.** Production of mono- and sesquiterpenes in *Camelina sativa* oilseed. *Planta* **242,** 693-708 (2015).

Sayre Lab

Major accomplishments:

1. Demonstration that overexpression of a cyanobacterial FBPase with reduced Km for FBP relative to the Chlamydomonas FBPase enhances algal growth (dry weight) in photobioreactors by 40% over wild-type algae.
2. Discovery that a bacterial carbonic anhydrase, when overexpressed in the stroma of Arabidopsis chloroplasts, enhanced aerial photosynthetic rates by 30% and biomass accumulation by two-fold.
3. Developed stable nuclear gene expression systems with enhanced gene expression in Chlamydomonas (Kumar et al., 2013).
4. Analyses of thermodynamics and kinetics of biomass production in algae demonstrated that carbohydrate production is 10% more efficient and faster than oil (TAG) production. When these results are considered in light of the fuel conversion efficiency outcomes for hydrothermal liquefaction of algae they suggest that making more carbohydrate and less oil may yield a greater energy return on investment for biofuels (See Sayre, 2010; Subramanian et al., 2013; Blankenship et al., 2011; Perrine et al., 2012).
5. Elucidated the impact of HLA3 (plasmamembrane localized, ATP-dependent bicarbonate transporter) expression on a futile ATP-dependent inorganic carbon pumping cycle. This lead to the development of alternative strategies to facilitate algal CCM operation in Camelina.

Shachar-Hill Lab

Major Accomplishments:

1) Establishment of embryo culture system and metabolic flux analysis of Camelina during oil formation. Discovery of low carbon conversion efficiency under in planta conditions and demonstration that this is due to hyperactive Oxidative Pentose Phosphate Pathway (Carey et al.).
2) In collaboration with Cahoon lab, metabolic flux analysis of Camelina embryos engineered to accumulate short chain fatty acids (four constructs with different fatty acid profiles). Demonstration that altering fatty acid profiles significantly influences fluxes through upstream central metabolism.
3) In collaboration with Gang and Hicks lab completed multiomic and functional analyses of nitrogen metabolism, photosynthesis, lipid metabolism, and the regulation of gene expression during nutrient limitation and the indiction of oil accumulation in Chlamydomonas (Gargouri et al., “Identification of regulatory”; Higashi et al., “Production of terpene-“; Park et al., 2014).
4) Establishment of kinetic metabolic flux analysis methods of lipid metabolism in camelina embryos during oil accumulation. Discovery and quantification of turnover of triacylglycerol due to fatty acid replacements during oil accumulation.
5) Characterization of the dose response curves for light and of carbon availability on the rates of photosynthetic fluxes and oil and starch accumulation during nutrient deprivation.

Discovery that the predictions of the current view of oil accumulation by algae during nutrient stress is not quantitatively supported.

6) In collaboration with non-CABS researchers - development of tools for high throughput metabolic flux analysis in plants (Poskar et al., 2014; Poskar et al., 2012; O'Grady et al., 2012; Chen, Shachar-Hill, 2012)

Umen Lab

Major accomplishments:

-In collaboration with Jaworski laboratory completed quantitative analysis of starch and lipid accumulation of *vip1*-1 under autotrophic, mixotrophic and nitrogen starvation conditions. Found increased neutral lipids (up to six-fold) and decreased starch (~20-30%) for *vip1-1* under all conditions compared with wild-type parental strain. A provisional patent has been filed based on this discovery.

-In conjunction with Brad Evans and the Mass Spectrometry and Proteomics Facility at Danforth Center developed mass spectrometry-based detection method for inositol polyphosphates (IPs) from *Chlamydomonas*. Also successfully adapted gel-electrophoresis based detection method for IPs from *Chlamydomonas*.

-Tested induction of autophagy in *vip1-1* and *itpk1-1* but found no relationship or enhancement in the mutants, thereby ruling out this pathway as a source of increased lipids in *vip1-1*.

-Successfully complemented *vip1-1* and *itpk1-1* with epitope-tagged *VIP1* and *ITPK1* constructs, thus enabling detection and localization of the proteins.

-Tested additional TOR kinase inhibitors on Chlamydomonas and identified two molecules that inhibit the pathway more strongly than rapamycin. Found that *vip1-1* and *itpk1-1* are both hypersensitive to these new classes of inhibitors.

Wang Lab

Major Accomplishments:

Identified transcription factors (TFs) that interact with lipid metabolites and act as potential lipid sensors. We identified nine transcription factors that interact with lipid metabolites, and showed that a specific MYB-domain TF requires PA to enter the nucleus (Yao et al., 2013).

Discovered direct interactions between lipid metabolites and enzymes of central carbon metabolism. We found that phospholipase Ddelta and phosphatidic acid (PA) binding to the glycolytic glycraldehyde-3-phosphate dehydrogenases (Guo et al., "Cytosolic glyceraldehyde-3-"; Kim, Guo, Wang, 2013). This interaction provides a potential link for the coordinated control of carbohydrate and lipid metabolism. Manipulation of the cytosolic glycraldehyde-3-phosphate dehydrogenases perturbs cellular metabolism and seed oil accumulation (Guo et al., 2014).

Showed that phosphatidylcholine turnover promotes TAG production and suppresses cellulose deposition. We identified that a patatin-related phospholipase A family, *pPLAIII*, promote TAG formation and the increase in lipid production is at the expenses of cellulose

deposition (Li et al., 2013). The finding provides direct evidence for a role of lipid turnover in promoting seed oil accumulation and also a foundation to explore the metabolic control of carbon partitioning between lipids and carbohydrates.

Improve TAG production in camelina. We have manipulated a number of genes that impact seed oil accumulation in camelina (Li et al., "Overexpression of patatin").

Developed capacity to profile quantitatively TAG molecular species. Vegetable oils are comprised of many TAG molecular species, which can be quantitatively analyzed using electrospray ionization mass spectrometry (Lie et al., 2014).

Woodford Thomas Lab

Major Accomplishments:

- Development of new classroom activities for inquiry-based learning including new tools for teaching classical and molecular genetics (e.g. Ds-Red expressing *Camelina*),
- Shared the Backyard Biofuels Project (bioprospecting for oil-producing algae) with schools and other academic institutions through standards-based curricular modules and a "how to" manual,
- Professional development workshops for K-12 teachers on the topics of photosynthesis, energy capture and transfer, carbon assimilation and bioenergy,
- Production of a new education video on oilseed plants for Gr. 6-12 classrooms,
- Dissemination of bioenergy information and learning activities at public STEM events for a wide range of audiences, including a "teach-in" during the 16th International Congress on Photosynthesis Research, the USA Science and Engineering Festival and other ShareFairs.
- Conducting classroom tours of CABS laboratories and making scientific presentations to students and teachers on bioenergy research,
- Mentoring of high school students doing independent research projects related to CABS science.
- In June 2013, CABS Science and Education Outreach personnel, along with their collaborative partners at the Saint Louis Science Center, were Invited to participate in the White House Office of Science and Technology Policy and "Champions of Change" event on Citizen Science for recognition of their successful and impactful citizen science program "Backyard Biofuels". Valuable contributions were made to a new OSTP report to the President on how to implement successful citizen science programs that recruit a wide range of participants at all stages in inquiry, particularly underserved youth, in order to promote better public understanding of science and scientific research.

PUBLICATIONS

1.) Solely Supported by CABS

Wang, Y., Chen, H., and **Yu, O**. (2010). Metabolic engineering of resveratrol and other longevity boosting compounds. Biofactors 36, 394-400 [10.1002/biof.126].

Wang, H., Alvarez, S., and **Hicks, L.M.** (2012). Comprehensive comparison of iTRAQ and label-free LC-based quantitative proteomics approaches using two *Chlamydomonas reinhardtii* strains of interest for biofuels engineering. J Proteome Res 11, 487-501[10.1021/pr2008225]

Slade, W. O., Werth, E. G., Chao, A., and **Hicks, L. M.** (2014) Phosphoproteomics in photosynthetic organisms. *Electrophoresis* 35, 3441-3451 (Review article)

Wang, H. X., Gau, B., Slade, W. O., Juergens, M., Li, P., and **Hicks, L. M.** (2014) The Global Phosphoproteome of Chlamydomonas reinhardtii Reveals Complex Organellar Phosphorylation in the Flagella and Thylakoid Membrane. *Mol Cell Proteomics* 13, 2337-2353

Augustin, J.M., Higashi, Y. Feng, X. and **Kutchan, T.M.** Production of mono- and sesquiterpenes in *Camelina sativa* oilseed. *Planta* **242,** 693-708 (2015).

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Juergens, M. T., Deshpande, R. R., Lucker, B. F., Park, J. J., Wang, H., Gargouri, M., Holguin, F. O., Disbrow, B., Schaub, T., Skepper, J. N., Kramer, D. M., Gang, D. R., **Hicks, L. M.**, and Shachar-Hill, Y. (2015) The Regulation of Photosynthetic Structure and Function during Nitrogen Deprivation in Chlamydomonas reinhardtii. *Plant Physiol* 167, 558-573

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Slade, W. O., Werth, E. G., McConnell, E. W., Alvarez, S., and **Hicks, L. M.** (2015) Quantifying reversible oxidation of protein thiols in photosynthetic organisms. *Journal of the American Society for Mass Spectrometry* 26, 631-640

2.) Multiple Sources of Support

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