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Session 1: Fatty acids and glycerolipids: biosynthesis, modification, biosynthetic evolution

*Michael Burkart

University of California, San Diego

Mike Burkart is professor and chair of chemistry and biochemistry at University of California San Diego. Originally from Texas, he received his B.A. in Chemistry from Rice University and Ph.D. from the Scripps Research Institute. Following postdoctoral studies at Harvard, he initiated his own research group at UC San Diego in 2002. His research includes natural product biosynthesis and development, spanning fundamental enzymology / structural biology to drug development and renewable chemicals. He is a co-founder of startup companies Algenesis Materials and Aspera Biomedicines.

Synopsis:
The modular metabolic pathways encoding fatty acid, non-ribosomal peptide, and polyketide synthases offer a glimpse of how evolution of an iterative multienzyme pathway can diverge into the rich pharmacopeia of secondary natural products. While their basic mechanisms have been understood for decades, the molecular details of structure and catalysis have remained largely uncharted. We have developed a set of chemical biology tools that has allowed elucidation of the complex interactions within carrier protein-dependent synthases, beginning with the E. coli fatty acid synthase and expanding beyond into other bacteria and into secondary metabolism.

Additional Speakers and Presentations

John Shanklin: "The mechanism of SnRK1 sensor kinase in the regulation of lipid synthesis"

Somnath Koley: "Does fatty acid turnover occur concurrently with plant lipid synthesis?"

Peter Eastmond: "Applying rare-variant association analysis to a wheat mutant population identifies a hypermorphic allele of ACETYL-CoA CARBOXYLASE 1 that increases leaf lipid content in grasses"

Basil Nikolau: "Acetyl-CoA: A crucial central metabolite that juxtaposes between anabolism and catabolism in multiple cellular and subcellular compartments"

Yuki Nakamura: "A pair of PAP and LPAT playing a distinct role in glycerolipid metabolism in siliques"

Mónica Venegas-Calerón: "Sunflower ACYL-COA:LYSO-PHOSPHATIDIC ACID ACYLTRANSFERASE and its contribution to fatty acid distribution in triacylglycerols"

Ida Lager: "The interplay of diacylglycerol acyltransferases and phosphatidylcholine:diacylglycerol cholinephosphotransferases facilitates high hydroxy fatty acyl content in triacylglycerols"

Hyojin Kim: "Polyketide synthase-like functionality acquired by plant fatty acid elongase"

Angel Baca-Porcel: "Algal homologs of the plant CER1 and CER3 proteins are functional hydrocarbon-forming enzymes"

The Mechanism of SnRK1 Sensor Kinase in the Regulation of Lipid Synthesis

Jantana Blanford [1†], Zhiyang Zhai [1†], Marcel D. Baer [2†], Gongrui Guo [1], Hui Liu [1], Qun Liu[1], Simone Raugei [2], John Shanklin [1*]

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Many enzymes and factors that affect lipid accumulation, including WRI1, DGAT, and LEC2 contain SUCROSE-NON-FERMENTING1-RELATED PROTEIN KINASE1 (SnRK1) target sequences. When intracellular sugar concentrations are low, SnRK1, a central plant metabolic sensor kinase, phosphorylates its target proteins, initiating their proteasomal degradation, thereby reducing their fatty acid and lipid production. The SnRK1 kinase subunit KIN10 is itself activated by phosphorylation of T175 in its T-loop by GEMINIVIRUS REPINTERACTING KINASE1 (GRIK1). Under conditions of high sugar, activation of KIN10 by GRIK1 is inhibited, preventing the phosphorylation and degradation of its target proteins. Trehalose 6-phosphate (T6P) is a proxy for cellular sugar status and a potent inhibitor of SnRK1. We previously reported that T6P binds to KIN10 weakening its affinity for GRIK1. We now present the molecular details of T6P inhibition of KIN10. A combination of molecular dynamics simulations and other computational analyses with microscale thermophoresis analysis and in vitro phosphorylation assays identified the T6P binding site on KIN10 and the molecular motions of the T-loop necessary for KIN10 activation. Under high-sugar conditions, T6P binds to KIN10, blocking the reorientation of its activation loop and preventing its phosphorylation and activation by GRIK1. Under these conditions, SnRK1 maintains only basal activity, minimizing phosphorylation and degradation of its target proteins including WRI1, DGAT and LEC2, which consequently accumulate, stimulating lipid production.

Supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, specifically through the Physical Biosciences program of the Chemical Sciences, Geosciences, and Biosciences Division. J.S. was supported under contract no. DE-SC0012704 and SR under DE-AC05-76RL01830. Computer time was provided by the US DOE National Energy Research Scientific Computing Center (NERSC at LBL) and (MSCF at PNNL).

Does fatty acid turnover occur concurrently with plant lipid synthesis?

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Plant lipid turnover is an essential process for germinating seedlings, and occurs in senescing tissues, but has not been characterized in developing seeds. We observed simultaneous lipid biosynthesis and turnover at multiple stages of seed-filling in Camelina. Our results indicate an active carnitine shuttle and non-intuitive oxidation in mitochondria at later seed-filling stages when peroxisomal oxidation is inactive. Importantly, the different subcellular locations may signify the relevance of co-products including metabolic water for seed germination or reductant and energy for seed-filling. Tests in canola and Arabidopsis seeds and engineered high-oil tobacco leaves and camelina seeds indicate the observation is not the exception but the rule and may partially explain underperforming engineered lines of the past.

Funding source: U.S. Department of Agriculture-Agricultural Research Service; National Science Foundation award (IOS-1829365); U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research (BER), Grant no. (DE-SC0023142; DE-SC0022207); U.S. Department of Agriculture-National Institute of Food and Agriculture grant award (2017-67013-26156; 2021-67013-33778).

Applying rare-variant association analysis to a wheat mutant population identifies a hypermorphic allele of *ACETYL-CoA CARBOXYLASE 1* that increases leaf lipid content in grasses

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Acetyl-coenzyme A carboxylase (ACCase) catalyses a key step in fatty acid synthesis (FAS). Two types of ACCase are found in nature; a heteromeric ('prokaryotic') form composed of four dissociable subunits and a homomeric ('eukaryotic') form consisting of a single multidomain polypeptide. As a result of endosymbiosis, plants generally harbour both forms; the heteromeric form functions in the plastid, which is the sole site of long-chain FAS in plants, while the homomeric form is cytosolic. However, in several plant lineages the homomeric ACCase has undergone gene duplication and a copy has become targeted to the plastid. In grasses (Poaceae) the heteromeric form of ACCase has subsequently been lost and the plastid-targeted homomeric form has taken over its function in supporting FAS. In plants that retain the heteromeric ACCase, this form of the enzyme plays a major role in governing the rate of FAS and its activity is tightly regulated by mechanisms that have been well studied. By contrast, comparatively little is known about how plastid targeted homomeric ACCase (and thus FAS) is regulated in grasses. We have screened an exome sequenced wheat ethyl methanesulfonate mutant population and used rare-variant association analysis methods we recently developed (doi: 10.1111/pbi.13890) to identify several genes that control lipid content. Among these genes we found that the *ACETYL-CoA CARBOXYLASE 1* (*ACC1*) homoeologous triad, encoding plastid-targeted homomeric ACCase, are strongly associated with lipid content. The wheat population contains >600 non-synonymous mutations in the *ACC1* triad and we identified several hypermorphic (gain-of-function) alleles. Expression of one of these *ACC1* mutant alleles in transgenic wheat and rice can double total leaf lipid content, compared to expression of wild type. Our data suggest that *ACC1* is a major determinant of lipid content in grasses and that hypermorphic mutations in *ACC1* can enhance lipid accumulation, by mechanisms that require further study, and which could help reveal how FAS is regulated in grasses.

Acetyl-CoA: A crucial central metabolite that juxtaposes between anabolism and catabolism in multiple cellular and subcellular compartments.

Basil J. Nikolau

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As a nexus in metabolism, acetyl-CoA links many processes, including respiration, fatty acid metabolism, polyketide biosynthesis, amino acid metabolism and the biosynthesis of mevalonate-derived terpenes. Additional complexity is associated with the fact that acetyl-CoA metabolism is differentially distributed among different subcellular compartments, including mitochondria, plastids, peroxisomes, nuclei and cytosol. Furthermore, tissue differentiation and environmental stimuli provide additional basis for differential regulation of acetyl-CoA metabolism; e.g., the epidermis of the aerial organs produces the acetyl-CoA-derived cuticle and anthocyanins, which are processes stimulated by stress conditions. Since the early 1960's, with the discovery that radioactively labeled acetate can efficiently label fatty acids, it was assumed that acetyl-CoA synthetase is the primary *in planta* source of acetyl-CoA for *de novo* biosynthesis of fatty acids. However, over the past 25-years, several research groups, including my own, have deciphered different mechanisms for generating acetyl-CoA in different subcellular compartments. Thus, in mitochondria, in addition to the classical pyruvate dehydrogenase complex that generates acetyl-CoA for the TCA cycle, leucine catabolism also generates acetyl-CoA. The process of β-oxidation of fatty acids generates acetyl-CoA in peroxisomes. In plastids, the site of *de novo* fatty acid biosynthesis, there are two enzymatic mechanisms that generate acetyl-CoA: a) the plastidic isoform of the pyruvate dehydrogenase complex, and b) acetyl-CoA synthetase. Reverse genetic strategies and coupled with isotope labelling experiments have demonstrated that the pyruvate dehydrogenase complex provides the acetyl-CoA needed for *de novo* fatty acid biosynthesis, whereas the metabolic function of acetyl-CoA synthetase is to provide a mechanism for detoxifying acetate. This latter function is particularly significant in the recovery of carbon following periods of anaerobiosis when anaerobic fermentation generates ethanol. This carbon can be recovered into central metabolism via a three-reaction process (i.e., ethanol → acetaldehyde → acetate → acetyl-CoA), which requires acetyl-CoA synthetase. Finally, in the cytosol acetyl-CoA is generated by ATP-citrate lyase. This enzyme is well characterized as part of lipogenesis system in animals, but was discovered in plants in only 2002. The plant enzyme consists of two different subunits, in a A_4B_4 tertiary organization, and in Arabidopsis three genes encode the A-subunit, and 2 genes encode the B-subunit. Metabolomics and transcriptomics analyses of null alleles of these ATP-citrate lyase subunit genes have identified gene dosage effects in determining different mutant phenotypes associated with reduced ATP-citrate lyase expression. These findings are consistent with the expectation that the ATP-citrate lyase-generated acetyl-CoA pool acts as the precursor for a plethora of metabolic end-points (e.g., cuticular waxes, polyketides, terpenes), including the acetylation of histones in nuclei.

Sunflower ACYL-COA:LYSO-PHOSPHATIDIC ACID ACYLTRANSFERASE and its contribution to fatty acid distribution in triacylglycerols

Mónica Venegas-Calerón*, Mónica Villoslada-Valbuena, Rosario Sánchez, Ana Mapelli-Brahm, Juan Diego Fernández-García, José Antonio Aznar-Moreno, Rafael Garces, Enrique Martínez-Force, Joaquín J. Salas.

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Oilcrops such as sunflower accumulate triacylglycerols (TAGs) in large amounts in seeds to feed the embryo. Their dynamic synthesis occurs in the endoplasmic reticulum through the successive acylation of glycerol-3-phosphate (G3P) dependent of acyl-CoA, and also other acyl-CoA-independent pathways interconnected. However, the contributions of these pathways to the final TAG composition differ depending not only on the species or tissue, but also due to changes in environmental conditions and/or developmental stages. The final composition of TAGs and the fatty acid distribution will determine their physical properties and industrial applications.

The fatty acid composition and distribution of TAGs is not random. The sn-2 position binds mostly unsaturated fatty acids, whereas the positions sn-1/3 can be esterified to saturated fatty acids at different extension producing TAGs with fatty acid distributions specific of each species. The distribution of fatty acids is controlled by several factors that have not been investigated in deep, such as the substrate specificities of enzymes involved (acyltransferases and phospholipases) and the composition of the donor acyl-CoA pool.

As part of our studies, we are interested in the enzymes involved in TAG synthesis to find the factors enhancing or hampering the accumulation of certain fatty acids. In this regard, the enzyme 2-lysophosphatidic acid acyltransferase (LPAAT) seems to play a crucial role in the biosynthesis of TAG. The substrate specificity of this enzyme varies depending on the species and clearly influences both the stereospecific distribution of fatty acids in TAGs. In the present work, we study the putative LPAAT family in sunflowers. The implication of these enzymes on the final composition of sunflower oil was discussed attending the results.

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A pair of PAP and LPAT playing a distinct role in glycerolipid metabolism in siliques

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Glycerolipid biosynthesis pathways are present in plastids and the endoplasmic reticulum (ER). Major phospholipids and triacylglycerol (TAG) are synthesized in the ER; however, how plastid-localized glycerolipid biosynthetic enzymes contribute to the ER-localized pathway in non-photosynthetic organs remain elusive. In siliques of *Arabidopsis thaliana*, we recently found that a pair of phosphatidic acid phosphatases, LPPα2 and LPPε1 localized at the ER and plastid outer envelope, redundantly function in TAG biosynthesis. Overexpression of plastid LPPε1 had the similar effect with that of ER-localized LPPα2 on TAG proeuction, suggesting a role of plastidic enzyme in ER-localized TAG biosynthesis. We further investigated the role of LPAT1 and LPAT2, the major LPAT isoforms in plastid and ER pathways. Partial suppression of *LPAT2* gave distinct effect in different organs, and seed-specific suppression of plastidic *LPAT1* primarily affected ER phospholipid biosynthesis. These observations imply a possible new route of TAG biosynthesis via plastid-localized enzymes in siliques.

This work was supported by GteX Program Japan Grant Number JPMJGX23B0.

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Algal homologs of the plant CER1 and CER3 proteins are functional hydrocarbon-forming enzymes.

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Various organisms, including plants and algae, have the ability to synthesize hydrocarbons (linear alkanes and alkenes) from fatty acids. In plants, very-long-chain (VLC) alkanes C25-C35 are secreted onto the surface of the epidermis of aerial organs and often form the major component of the waterproof layer of cuticular waxes. The key components of plant VLC alkane biosynthesis are the two homologous membrane-bound proteins ECERIFERUM 1 and 3 (CER1 and CER3). It is known that these two proteins interact, but it has not yet been demonstrated that they both have enzymatic activity, or what this (these) activity(ies) is (are). Alkanes play a vital role in preventing desiccation in the terrestrial environment and the ability to synthesize and secrete them is thought to have represented a key event in the conquest of land by the algal ancestors of plants.

In algae, it has been shown that C15-C17 linear alkanes and alkenes found in the thylakoid fraction are produced from C16-C18 free fatty acids by the photoenzyme fatty acid photodecarboxylase (FAP). FAP appears to be an algal-specific protein and has thus been lost in plants. Interestingly, a few algal species that accumulate alkanes do not possess FAP homologs, but instead possess a single protein homolog of CER1 and CER3.

In this work, we report the functional characterization of CER1/3 proteins from various groups of algae. Results show that some CER1/3 proteins efficiently mediate HC biosynthesis and that contrary to land plant CER1 and CER3, are likely to be bifunctional enzymes. These findings are thus consistent with the idea that the land plant alkane-forming CER1/CER3 complex has evolved from an algal enzyme by gene duplication.

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Session 2: Extra-plastidic membrane lipids: phospholipids, sterols, sphingolipids

*Nan Yao
Sun Yat-sen University

Nan Yao received her B.A. from Beijing Agriculture University (China) in 1988, and studied at Kobe University, Japan (M.S. and Ph.D.) in plant pathology under the mentorship of Dr. Shigeyuki Mayama. While a graduate student, she characterized morphological features of apoptotic cell death in an oat-victorin system. She began her research on mechanisms of plant programmed cell death while a post-doctoral fellow with Dr. Jean Greenberg at University of Chicago in 2002. There, she contributed to the understanding of cell death related proteins using Arabidopsis as a model system. Dr. Yao joined the faculty of School of Life Science, Sun Yat-sen University in Guangzhou, China, at the end of year of 2005. Yao's laboratory focuses on metabolism and roles of plant sphingolipids important for programmed cell death and abiotic and biotic stresses.

Synopsis:
Yao will present recent progress regarding sphingolipid metabolism impacts the endogenous circadian rhythm and discovery a systemic signal that enhances de novo sphingolipid biosynthesis and leads to sphingolipid-dependent cell death in Arabidopsis distal leaves.

Additional Speakers and Presentations

Tristen Mier: "Functional divergence of ceramide synthases contributed to diversification of sphingolipids in green plants"

Tegan Haslam: "Investigating contributions of GIPCs to fundamental cell functions using mutants of the moss *Physcomitrium patens*"

Koichiro Awai: "A betaine lipid in Euglena can substitute phospholipids under phosphate deficiency"

Hubert Schaller: "Phytosterol metabolism and homeostasis"

A Systemic Signal Enhances *de novo* Sphingolipid Biosynthesis and Triggers Cell Death

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In the field, plants may experience multiple stresses simultaneously; understanding the crosstalk between biotic and abiotic stress responses helps us understand how plants confront environmental changes. Here, we combined an abiotic stress, NaCl irrigation, with the fungal toxin Fumonisin B1 (FB1, an inhibitor of ceramide synthase), which induces cell death in plants. We found that in Arabidopsis, treatment with FB1 and NaCl induced a signal that is transported over long distances and causes cell death in systemic leaves. The systemic cell death was dependent on *de novo* sphingolipid biosynthesis, not salicylic acid or jasmonate. SERINE-PAMITOYL-COA TRANSFERASE (SPT) activity is vital for this cell death, which also involves Ca^{2+} signaling. Thus, our findings reveal the existence of a systemic signal that increases sphingolipid biosynthesis, providing new insight into the regulation of stress responses and sphingolipid metabolism in plants. We will discuss the possible function of the systemic signal which transported from local to distal in stress resilience.

Functional Divergence of Ceramide Synthases Contributed to Diversification of Sphingolipids in Green Plants

Tristen J. Mier[a,b], Dongdong Zhang[a,b], Miguel Avila Garcia[a,b], Rebecca Cahoon[a,b], Jennifer Markham[a,b], and Edgar B. Cahoon*[,a,b]

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Sphingolipids are a class of lipid that are universal across eukaryotes with an astonishing structural diversity that contributes to a diverse set of functions. Much of our knowledge about sphingolipid metabolism in plants originates from studies on *A. thaliana,* resulting in many questions on its applicability to other important plant species. Here we present sphingolipidomic data from 36 plants and demonstrate overarching motifs present in plant sphingolipid metabolism as well as the diversity between species. One of these overarching motifs is that the identify of long chain base and length of the acyl chain largely determines if the ceramide accumulates as GlcCers or GIPCs. We hypothesize that an early gene duplication and functional divergence of an ancestral ceramide synthase contributed to the refinement of this pathing and present supportive phylogenetic evidence. We further demonstrate that the last loop of plant ceramide synthases controls the substrate specificity of the enzyme and that swapping this domain between enzymes results in an enzyme whose specificity is dictated by the characteristics of the donor enzyme. The result of this work illustrates overarching motifs central to plant sphingolipid metabolism and suggests that the functional divergence of ceramide synthases by mutations in the last loop contributed to the establishment of these motifs.

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Investigating contributions of GIPCs to fundamental cell functions using mutants of the moss *Physcomitrium patens*

Tegan M. Haslam[1,*], Linus Wegner[2], Katrin Ehlers[2], Cornelia Herrfurth[1,3], Ivo Feussner[1,4]

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Essential membrane lipids, such as complex phosphosphingolipids (in plants, glycosyl inositol phosphorylceramides, GIPCs), are difficult to functionally characterize due to non-viable phenotypes of mutants affected in their synthesis. We previously developed a CRISPR/Cas9-based method for generating knock-down mutants of essential genes, which retain sufficient activity to be viable, using the moss *Physcomitrium patens*. Following isolation and biochemical characterization of our mutants, we now report some of the developmental phenotypes observed in the most interesting alleles. Helpfully, some of these mutants displayed altered GIPC levels without corresponding increases in their free ceramide precursors, allowing for uncoupling of phenotypes associated with product deficit versus substrate accumulation, which in this case is a potent cell death signal. Additionally, the relatively uncomplicated development and organ structure of the moss simplified observation of morphological and anatomical defects. In our mutants, we observed alteration in cell division, expansion, and differentiation, as well as changes to intercellular cytosolic connectivity. Specific aspects of these phenotypes and their implications for our understanding of GIPC functions and localization will be discussed.

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Phytosterol metabolism and homeostasis

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Sterols are mandatory cellular components, as building blocks of membranes and as precursors of phytohormones, both functions having specific molecular structural requirements. Sterol homeostasis is an essential process in eukaryotic development. Strikingly, genetic mechanisms implied in sterol homeostasis in mammals, fungi and plants are different. However, a common aspect between plants and other organisms is a key role of upstream enzymes implied in the mevalonate/isoprenoid pathway. To identify major components of the regulatory circuits at play in isoprenoid and phytosterol homeostasis, we have characterized high sterol esters producers in *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Nicotiana tabacum.* In the latter species, a high phytosterol producer previously isolated in the laboratory carries a semi-dominant mutation responsible for sterol esters accumulation in lipid droplets. Genetic reprogramming in this tobacco mutant and the phenotypes of its hypersterolemic leaves and lipid droplets are investigated to determine processes and proteins associated with sterol ester formation and mobilization at the cellular level.

Funded by Horizon2020-MSCA-IF-EF-RI # 897283 *High Phytosterol variants towards improved feedstocks and biofortification of crops*

Session 3: Plastidic lipids: glycolipids, phospholipids, isoprenoids

*Christoph Benning
Michigan State University

Christoph Benning has been working for over 30 years on different aspects of lipid metabolism in photosynthetic organisms. His Department of Energy Plant Research Laboratory discovered, and studies, proteins involved in the biosynthesis of polar lipids of the photosynthetic membrane, the exchange of membrane lipid precursors between the ER and the chloroplast envelope membranes and the respective contact sites, and proteins involved in lipid remodeling as adaption to abiotic stresses. His lab discovered a transcription factor governing the biosynthesis of storage lipids in plant embryos and used it for biotechnological applications. The Benning lab has also applied genomic and genetic approaches to identify key regulatory factors and enzymes required for triacylglycerol biosynthesis, lipid droplet formation, and lipid turnover in microalgae.

“Chloroplast lipids as stress mitigators and sensors”
Synopsis:
Whenever photosynthetic organisms are confronted with adverse conditions, the photosynthetic electron transport reactions can become imbalanced and consequently, toxic side products are formed mainly in the form of different reactive oxygen species (ROS). Aside from the proteins and pigments of the electron transport chain, the thylakoid membrane lipids are first to experience damaging effects of ROS. Thus, it seems reasonable to hypothesize that products of the reactions between ROS and membrane lipids and their turnover are sensed to adjust the rate of photosynthetic electron transport to current conditions. He will discuss work in progress to test this hypothesis.

*Koichi Kobayashi

Osaka Metropolitan University

Koichi Kobayashi obtained his Ph.D. in 2007 from the Tokyo Institute of Technology under the mentorship of Prof. Hiroyuki Ohta, working on the roles of three isoforms of monogalactosyldiacylglycerol synthase in Arabidopsis thaliana. After two postdoc periods for studying the regulation of chlorophyll biosynthesis, he joined Prof. Hajime Wada's research group at the University of Tokyo as an assistant professor and studied mainly the role of phosphatidylglycerol in plants and cyanobacteria. He is currently an associate professor at Osaka Metropolitan University and leads the Plant Physiology Laboratory in the Department of Biology, Graduate School of Science. His research group focuses on understanding the molecular mechanisms that regulate chloroplast development and photosynthesis in plants and is particularly interested in the involvement of thylakoid membrane lipids in the regulation.

"Thylakoid biogenesis and photosynthetic activation driven by plastid lipid biosynthesis"
Synopsis:
The lipid bilayer of thylakoid membranes of cyanobacteria and plant chloroplasts mainly consists of four lipid classes—monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and phosphatidylglycerol. Besides providing the hydrophobic membrane matrix, these lipids function directly in photosynthesis as structural components of the several photosynthetic complexes including photosystem II and photosystem I. In plants, moreover, each lipid has specific roles in membrane-associated processes during the development of chloroplasts. Kobayashi will discuss how these lipids function in chlorophyll biosynthesis, thylakoid formation, and photosynthetic activation during chloroplast development, particularly focusing on the indispensable roles of phosphatidylglycerol in these processes.

Additional Speakers and Presentations

Yang Xu: "Arabidopsis rhomboid-like protein 10 and acyl carrier protein 4 act independently in chloroplast phosphatidic acid assembly"

Arabidopsis rhomboid-like protein 10 and acyl carrier protein 4 act independently in chloroplast phosphatidic acid assembly

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Plant lipid biosynthesis involves precise subcellular compartmentation of different biosynthetic pathways and their complex interplay. While many proteins related to plant lipid metabolism are known, our understanding of the dynamic protein interactomes in lipid biosynthesis remains limited, though rapidly evolving. Previously, the Arabidopsis rhomboid-like protein10 (RBL10) was discovered to influence the acyl chain composition of monogalactosyldiacylglycerol (MGDG) and impact thylakoid lipid assembly through the plastid pathway. However, the exact mechanism underlying this effect is not fully understood. Our recent research revealed that RBL10 forms a large protein complex and directly interacts with acyl carrier protein4 (ACP4), which serves as a carrier for *de novo* chloroplast fatty acid synthesis. To understand the influence of the RBL10 and ACP4 interaction on chloroplast lipid metabolism, we examined *acp4 rbl10* double mutants and *ACP4* overexpression lines in the *rbl10* mutant background and found that RBL10 and ACP4 act in MGDG and phosphatidylglycerol (PG) biosynthesis independently. Notably, defects in phosphatidic acid (PA) turnover observed in the *rbl10* mutant were partially rescued in the *acp4 rbl10* double mutant. These changes appear to be associated with alterations in the size and profiles of PA, diacylglycerol (DAG), and acyl-ACP precursor pools, which likely involves a direct protein-protein interaction network. Ongoing research delving into the nature of the interactomes using proximity labeling and the regions of interaction will be discussed in further detail.

Session 4: Trafficking of lipids within and among cells and tissues

*Mike Henne

University of Texas Southwestern Medical Center

Dr. Henne is an associate professor and endowed scholar at UT Southwestern Medical Center. His lab studies lipid droplet functional diversity and the spatial organization of metabolism.

Synopsis:
The fruit fly Drosophila melanogaster stores lipids as fats in lipid droplets within the fat body, the adipose-hepatic hybrid tissue of insects. Although fat stores compose much of the biomass of Drosophila larvae, genetic depletion of these fat stores still permits larvae to grow, pupate, and become flies with almost no detectable fat deposits. Using genetic tools, proteomics profiling, and metabolomics, we dissect how these Drosophila rewire their metabolism to promote development in the absence of de novo lipogenesis and fat storage and identify new signaling molecules coordinating this metabolism rewiring.

*Gerhard Liebisch

University Hospital of Regensburg

Gerhard Liebisch obtained his Ph.D. at the University of Regensburg. He is research associate at the Institute of Clinical Chemistry and Laboratory Medicine at the University Hospital of Regensburg and responsible for the instrumental analytics lab (https://lipidomics-regensburg.de/).

His research interests focus on the development of mass spectrometric methods for quantification of lipid species. For more than 20 years, these methods have been applied in large-scale clinical studies and basic research including the use of

stable isotope labelled lipid(s)/-precursor to trace the transport and metabolism of lipids. He is a co-author of more than 250 papers in peer reviewed international journals, editorial board member of The Journal of Lipid Research.

He is a co-founder of the Lipidomics Standards Initiative and a board member in the International Lipidomics Society.

“The lipidomics checklist: Current status”
Synopsis:
This presentation will discuss the structure and rationale of the Lipidomics Minimal Reporting Checklist (https://lipidomicstandards.org/reporting_checklist/). The checklist is a web-based questionnaire based on consensus-driven guidelines for lipidomics. Its main purpose is to describe essential steps of lipidomics experiments in a standardized way to increase transparency in lipidomics research. Furthermore, it can be considered as a guideline for “good lipidomics practice” for both new and experienced lipidomics researchers.

Additional Speakers and Presentations

Pan Liao: "Cuticle thickness and non-specific lipid transfer protein-mediated release of volatile organic compounds"

Katrin Philippar: "Structure and function of FAX proteins – subcellular fatty acid distribution in the light of land plant evolution"

Noemí Ruiz-López: "A novel lipid transport protein at ER-chloroplast contact sites, NTMC2T5, is required for chloroplast development during germination"

Cuticle thickness and non-specific lipid transfer protein-mediated release of volatile organic compounds

Pan Liao[1,2,3,4], Itay Maoz[5], Meng-Ling Shih[6], Ji Hee Lee[2], Xing-Qi Huang[2], Shaunak Ray[6], Benoît Boachon[7], John A Morgan[6], Natalia Dudareva[2]

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Plant-emitted volatile organic compounds (VOCs) are crucial for pollination, seed dispersal, and environmental interactions, offering protection against parasites, pathogens, and herbivores. They also add to the fragrance of fruits, vegetables, flowers, and leaves. VOCs primarily consist of phenylpropanoids/benzenoids, terpenoids, fatty acid derivatives, and amino acid derivatives. Despite advances in VOC biosynthesis knowledge, the mechanisms of their cell-to-environment release remain less understood. Historically, this release was thought to be passive diffusion, but recent evidence demonstrates an ABC transporter in petunia flowers actively mediates this process. In our studies using *Petunia hybrida*, which emits high levels of phenylalanine-derived volatiles, we employed reverse-genetic and chemical methods to discover that a cell-wall localized non-specific lipid transfer protein (nsLTP), PhnsLTP3, aids VOC release across the cell wall to the cuticle. Downregulation of *PhnsLTP3* reduced VOC emission and resulted in VOC redistribution without impacting their total pools, with less VOCs approaching the cuticle. Furthermore, our work shows that the cuticle, despite its resistance, acts as a VOC sink/concentrator. Intracellular VOC buildup curtails their biosynthesis through feedback inhibition, limiting phenylalanine precursor supply to prevent toxicity from these hydrophobic compounds. These findings offer insights for metabolic engineering to modulate VOC release and mitigate unwanted plant odors.

Structure and function of FAX proteins – subcellular fatty acid distribution in the light of land plant evolution

Alexander Banguela-Castillo, Jens Neunzig, *Katrin Philippar

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In plant cells, fatty acid (FA) synthesis occurs in the plastid stroma and thus requires plastid FA-export for lipid assembly in the endoplasmic reticulum (ER). Further on, precursors for extracellular lipid compounds are shuttled from the ER towards the plasma membrane. In this context, we described the membrane-intrinsic protein FAX1 to mediate FA-export across the plastid inner envelope (IE). In Arabidopsis, FAX1 function is crucial for pollen cell wall formation, male fertility and cellular lipid homeostasis (Li et al. 2015, doi: 10.1371/journal.pbio.1002053). In addition, our most recent data show that At-FAX1 is a key player in cold acclimation (John et al. 2024, doi.org/10.1101/2023.10.26.564161). Based on evolutionary conserved structural features and sequence motifs, we now define the plant FAX-protein family. Besides their Tmemb14 membrane-spanning domain, the plastid IE-intrinsic FAX1, FAX2, FAX3 contain distinct N-terminal stretches. Among them, the apolipoprotein-like α-helical bundle of FAX1/FAX2, which evolved during the plants' conquest of land, presumably for metabolic adaptation to stress, is the most prominent. Besides plastid envelope FAX proteins, we find FAX5/6 and FAX7 to be targeted to ER/secretory pathway membranes. Overall, two basic sets of FAX proteins in plastids and ER/secretory pathway membranes with corresponding structure/function appear to be conserved in Viridiplantae evolution: namely FAX1 + FAX5/6 and FAX3 + FAX7. In Chlamydomonas, we can show that the function of FAX1 and FAX5/6 is crucial for triacylglycerol oil production (Peter et al. 2022, doi: 10.3389/fmolb.2022.939834). In Arabidopsis, FAX1-FAX3 are forming hetero oligomeric complexes and thereby function together in vegetative leaf growth (Bugaeva et al. 2023, doi.org/10.1101/2023.02.09.527856). Our newest results on FAX5/6 in Arabidopsis point to possible roles in pollen tube germination, seed coat and cutin assembly as well as regulation of cell death.

Session 5: Emerging methods for lipid research and crop design

*Sandra Correa
Universität Potsdam

Sandra Correa received her M.Sc. degree in Biology and the Ph.D. in Biotechnology from the University of Antioquia. She joined the Bioinformatics group of the University of Potsdam in 2022 as Research Assistant. She has recently assumed the position of junior group leader as part of the Collaborative Research Center 1644 funded by the German Research Foundation (DFG).

Her research focus has been in the field of Systems Biology, developing computational pipelines for the integration of heterogeneous data sets into metabolic models and the implementation of computational modeling approaches to study different aspects of plant metabolism. Her most recent work has focused on the development and validation of computational tools to model lipid metabolism in plants, which are beginning to be used to elucidate the molecular mechanisms of the plasticity of plant lipid metabolism to environmental cues.

Synopsis:
Despite decades of research into lipid metabolism, there remain significant knowledge gaps that prevent its rational engineering. She will present how building constraint-based models, which gather all existing knowledge about lipid metabolism in plants, can help address the pressing challenge of identifying genes involved in lipid metabolism. In addition, Correa will show how the integration of lipidomics data into these models can help the characterization of the role of genes in lipid metabolism and its regulation. She will conclude the talk by showing how constraint-based approaches can be used for studying lipid metabolism responses to environmental temperature.

*Kim Ekroos
International Lipidomics Society

Kim Ekroos is one of the pioneers in the field of lipidomics, with more than 20 years of experience in the academic, industry and regulatory disciplines of lipidomics and lipid biology. His work has among others resulted in the first-ever establishment of lipidomics based lipid biomarkers in clinical diagnostics. He received his Ph.D. in biology from the Technical University in Dresden, Germany, in 2003. In 2004 he started his postdoctoral work at AstraZeneca in Mölndal, Sweden. He joined Zora Biosciences Oy in 2008 and was promoted to Chief Technology Officer in 2013. In 2016 he founded Lipidomics Consulting Ltd., a consulting business providing unique services for customers globally by designing conclusive mass spectrometry and experimental approaches to make inroads into cell, disease, biomarker and drug research and development. Today, he is the president of the International Lipidomics Society (ILS; www.lipidomicssociety.org) and spearheading the Lipidomics Standardization Initiative.

“Orthogonal high resolution mass spectrometry for characterizing content and localization of complex ganglioside phenotypes in Parkinson’s disease”
Synopsis:
Mutations in GBA1, encoding lysosomal glucocerebrosidase (GCase), are a risk factor for Parkinson’s disease. Reduced GCase function results in accumulation of glycosphingolipid substrates and imbalances in downstream ganglioside metabolites. Here we performed in-depth characterization and region-specific localization of brain gangliosides in a GBA mutant mouse model using next-generation ion mobility instrumentation and mass spectrometry imaging. We identify dark corners of the ganglioside lipidome and identify their region-specific localization in brain. Ongoing work evaluates how well the lipid findings translate to PD patient brain specimens. Our work opens new avenues to tackle the dysfunctional glycolipid metabolism accompanying neurodegeneration.

Additional Speakers and Presentations

Rajib Saha: "Advancing plant systems biology: Dynamic metabolic modeling for understanding genetic and biochemical redundancy, pathway regulation, and cuticle protection"

Susanne Hoffmann-Benning: "A Tale of Three Worlds: Integrating molecular biology, optogenetics and molecular dynamics to understand protein-lipid interactions in signaling"

Michael Smanski: "Towards engineering two-directional barriers to gene flow in plants"

Advancing Plant Systems Biology: Dynamic Metabolic Modeling for Understanding Genetic and Biochemical Redundancy, Pathway Regulation, and Cuticle Protection

Abraham B. Osinuga[1], Lohani Beer[1] and Rajib Saha[1*]

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The integrity and functionality of the maize plant cuticle, a critical barrier composed of cutin polymers and cuticular waxes, are essential for plant survival under adverse environmental conditions. Yet, the detailed mechanisms governing its biosynthesis and assembly remain poorly understood. Here, we introduce an interdisciplinary approach that combines computational simulations with experimental methodologies to elucidate the cuticle biosynthesis processes in maize. Our strategy employs dynamic metabolic modeling and kinetic analysis to dissect the complex transcriptional, metabolic, and transporter pathways that orchestrate cuticle biosynthesis. By leveraging dual synthetic biology platforms, we reconstitute these pathways in non-native systems, including *A. thaliana* roots and *S. cerevisiae* strains, to provide a comparative insight into cuticle assembly mechanisms. A comprehensive genome-scale metabolic model (GMM) of the *Arabidopsis* root system and a re-engineered yeast model showcasing the refactored cuticular wax pathway stand at the core of our study. Currently, these models facilitate an in-depth exploration of the very-long-chain fatty acids (VLCFAs) biosynthesis, a precursor to cuticular wax components, emphasizing the fatty acid elongation module (FAE pathway). Utilizing 'omics' data and kinetic modeling, our research focuses on delineating the dynamic metabolic fluxes and regulatory networks involved. We examine, *in silico*, the maximum catalytic capacity of each enzyme combination in the bioengineered yeast strains, including using a deep-learning framework to estimate the catalytic efficiencies (k_{cat}, K_M values) of enzyme-substrate interactions within our synthetic systems. This integrative approach sheds light on the previously obscure aspects of cuticle formation and demonstrates the potential of synthetic biology and metabolic modeling in resolving complex biological puzzles. Our findings offer promising avenues for agricultural innovations, particularly in engineering crops towards improved resilience against environmental stresses. This work lays a foundational stone for future explorations into plant systems biology, paving the way for advanced metabolic engineering and synthetic biology applications in plant science.

A Tale of Three Worlds: Integrating molecular biology, optogenetics and molecular dynamics to understand protein-lipid interactions in signaling.

Susanne Hoffmann-Benning[1], Amanda M. Koenig[1,4], Allison Barbaglia[1,4], Evan Kurtz[1,2], Curtis Chen[1], Kun Tang[3], Duncan Boran[1,4], Dayna Olson[1,4], Martin Kulke[4], Josh Vermaas[4], Matias Zurbriggen[3]

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One goal of the United Nations is to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture". However, this is a daunting issue in the face of population increases, climate change, and the resulting scarcity of arable land. Given the current climate change and the urgency to balance our need for food and fuel, it is essential to understand how plants signal environmental conditions not only locally but also to distal parts of the plant and adjust development accordingly. My lab has identified a number of putative lipid-binding proteins in the plant phloem. We characterized their lipid-binding properties, localization and stress response. One of these proteins, PLAFP appears to confer drought-tolerance in plants. But does it move? And what is the mechanism of protein-lipid interaction? In this talk I will present how a combination of three vastly different approaches (biochemical, synthetic biology, computational) guided our path of discovery of the protein and its function. Our optogenetics using a light-regulated promoter shows protein movement throughout the plant. Molecular dynamics simulations suggest how protein and lipid interact in the membrane and later in the phloem.

This research was supported by NSF-IOS grant #1841251 *"EAGER: Lipids on the move"* and by USDA NIMSS MICL 04237 to SHB

Towards engineering a two-directional barrier to gene flow in plants

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Abstract: In this talk, I will introduce the concept and mechanism of Engineered Genetic Incompatibility (EGI). EGI is an approach for engineering species-like barriers to gene flow in sexually reproducing organisms that has been demonstrated to work in microbial species and insects. In crop species, EGI would provide a layer of transgene containment by preventing hybridization of an engineered line with wildtype or other crop lines. I will describe the progress and challenges of translating this technology into plants. I will also describe useful tools for plant engineering that have emerged as tangential side-products from this research.

Session 6: Surface lipids: biosynthesis and regulation of plant protection

*Ivo Feussner

Georg-August-Universität Göttingen

Ivo Feussner studied chemistry and graduated with Helmut Kindl working on lipid droplets in plants. After joining the groups of Claus Wasternack and Uwe Sonnewald, he is now a full professor for plant biochemistry in Göttingen, Germany. His expertise ranges from lipid-derived signaling connected to stress, lipidomics, lipid peroxidation processes, metabolic pathways as well as working on structure-function relationships of lipid metabolizing enzymes. He was named a fellow of the German National Academy of Sciences in 2022.

Synopsis:
The plant cuticle is a hydrophobic barrier, which seals the surface of above-ground organs. Its biosynthesis has been intensively studied in angiosperms, but knowledge in non-vascular plants is scarce. While in *Arabidopsis thaliana* alkanes are major constituents of the cuticle, in bryophytes primarily wax esters were found. Although bryophyte cuticles harbor similar compound classes as angiosperms, their biosynthesis seems not to be conserved. Our results suggest that the biosynthetic enzymes involved in primary alcohol and wax ester formation in land plants have either evolved multiple times independently or undergone pronounced radiation followed by the formation of lineage-specific toolkits.

Additional Speakers and Presentations

Joaqun Salas: "Synthesis of wax esters in sunflower (Hellianthus annuus) seeds"

Shrikaar Kambhampati: "Suberin - a recalcitrant biopolymer for increased CO2 drawdown and storage: Lessons learned from Typha spp. adapted to wetland ecosystems"

Athanas Guzha: "A lipid transfer protein isoform modulates both neutral lipid abundance in embryos and deposition of cuticular waxes on aerial organs"

Divergent evolution of wax biosynthesis among bryophytes

Alisa Keyl[1], Cornelia Herrfurth[1], Garima Pandey[2], Ryeo Jin Kim[2], Lina Helwig[1], Tegan M. Haslam[1], Sophie de Vries[3], Jan de Vries[3], Nora Gutsche[4], Sabine Zachgo[4], Mi Chung Suh[2], Ljerka Kunst[5] and Ivo Feussner[1]

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The plant cuticle is a hydrophobic barrier, which seals the epidermal surface of most above-ground organs. The cuticle biosynthesis of angiosperms has been intensively studied, but knowledge about its existence and composition in non-vascular plants is scarce. While in *Arabidopsis thaliana* alkanes are major constituents of the cuticle, in bryophytes primarily wax esters were found. Therefore, we isolated and characterized homologs of *Arabidopsis thaliana* fatty acyl-CoA reductase (FAR) ECERIFERUM 4 (*At*CER4) and bifunctional wax ester synthase/acyl-CoA:diacylglycerol acyltransferase 1 (*At*WSD1) in the liverwort *Marchantia polymorpha* (MpFAR2 and MpWSD1) and the moss *Physcomitrium patens* (PpFAR2A, PpFAR2B, and PpWSD1). Although bryophyte harbor similar compound classes as described for angiosperm cuticles, their biosynthesis may not be fully conserved between the bryophytes *M. polymorpha* and *P. patens* or between these bryophytes and angiosperms. While PpFAR2A and PpFAR2B contribute to the production of primary alcohols in *P. patens*, loss of Mp*FAR2* function does not affect the wax profile of *M. polymorpha*. In contrast, MpWSD1 acts as the major wax ester-producing enzyme in *M. polymorpha*, whereas mutations of Pp*WSD1* do not affect the wax ester levels of *P. patens*. Our results suggest that the biosynthetic enzymes involved in primary alcohol and wax ester formation in land plants have either evolved multiple times independently or undergone pronounced radiation followed by the formation of lineage-specific toolkits.

Synthesis of Wax Esters in Sunflower (*Hellianthus annuus*) Seeds

Joaquín J. Salas[1*], Cristina de Andrés Gil[1], Mónica Villoslada-Valbuena[1], Antonio J. Moreno-Pérez[2], Fred Beaudoin[3], Rafael Garcés[1], Enrique Martínez-Force[1], Mónica Venegas Calerón[1]

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Sunflower is one of the main oil crops, standing out for its adaptability, productivity and the high quality of the oil extracted from its seeds. Sunflower seeds are achenes consisting of a fibrous cover or hull and an oil-rich kernel. The hulls contain a significant amount of wax esters (WEs) that protect the embryo from moisture and pathogen attack. These WEs also have technological importance as they cloud the oil extracted from whole seeds. Sunflower seed WE are saturated with chain lengths from C40 to C62, including small amounts of odd chain species. WE are synthesized from acyl-CoAs by the enzymes fatty acyl reductase (FAR) and wax synthase (WS). The coding genes for FAR and WS were located in the sunflower genome and the forms specifically expressed in developing seed coatings were determined (4 FAR forms and 3 WS forms). These genes were cloned and functional studies were performed. While *Ha*WSs were clearly localized in the endoplasmic reticulum (ER), studies in *Ha*FARs showed ambiguous results consistent with a cytosolic localization while maintaining some interaction with the ER. The substrate specificity of those genes was determined by expression in yeast. The *Ha*FARs were highly specific and each form synthesized only a few species of very long chain saturated alcohols. *Ha*WSs on the other hand showed very broad specificity profiles, being able to produce waxes with short chain alcohols. When both enzymes were simultaneously expressed in yeast it was possible to reconstruct the synthesis of WEs present in sunflower hulls. Possible applications and improvements that could be applied in sunflower cultivation were discussed based on the results.

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Suberin - a recalcitrant biopolymer for increased CO_2 drawdown and storage: Lessons learned from *Typha spp.* adapted to wetland ecosystems.

Shrikaar Kambhampati[1], Anna De Angelis[1], Leo Andrade[1], Justin Pacheco[1], Anna Gauthier[1], Bradley Abramson[1], Todd P. Michael[1], Suzanne Thomas[1], James J. La Clair[1,2], Joseph P. Noel[1*]

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Plants exhibit a plethora of adaptive root traits in response to changes in their environment often impacted by climate, including drought, waterlogging, salinity, metal toxicity and/or oxygen deprivation. One such trait is the deposition of suberin, a protective barrier surrounding the cell walls of specialized root tissues, such as endodermis, exodermis and periderm. Suberin is a complex biopolymer that is predominantly composed of esterified, glycerol, cinnamates and very long chain fatty acid-derived monomers. While the structure and composition of suberin is not completely understood, much of what we know today comes from studies with *Arabidopsis*, potato wound healing, and cork oak bark. Our research investigates the roots of several plant species that accumulate higher amounts of suberin due to their adaptation to various dryland and wetland ecosystems focusing on structural and compositional differences between species. Using *Typha spp.*(cattails), adapted to wetland environments as model species, we explore the thermal stability, decomposition resistance, and metabolic regulation linked to suberin's potential for long term carbon storage in soils and sediments. To accomplish these goals, we use $^{13}CO_2$ labeling to trace carbon movement through central carbon, specialized and lipid metabolic networks from shoot to root. This quantitative approach is used to assess the potential for CO_2 drawdown and carbon sequestration by *Typha spp.* in wetlands, the latter of which are arguably the most efficient and largest carbon sinks on earth. Using new computational and experimental tools for labeling analyses, we identify gaps in our knowledge of suberin biosynthetic networks, and construct *de novo* metabolic maps, with the goal to quantify bottlenecks in carbon flow towards suberin to inform the selection and engineering of climate adaptable wetland and crop species.

A lipid transfer protein isoform modulates both neutral lipid abundance in embryos and deposition of cuticular waxes on aerial organs

Athanas Guzha[1], Julius Ver Sagun[1], Tatiana Garcia[2], Allison Barbaglia-Hurlock[2], Payton Whitehead[1], Erich Grotewold[2], Ana Paula Alonso[1] and Kent D. Chapman[1]*

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Pennycress (*Thlaspi arvense* L.) has been identified as a promising alternative crop for aviation fuel production. While pennycress benefits from the fully sequenced genome and research tools of the closely related model plant *Arabidopsis thaliana*, there are still significant challenges associated with establishing gene function that would make pennycress a more viable bioenergy oilseed crop. An unbiased transcriptome wide-association study revealed that expression of a Lipid Transfer Protein isoform (LTP6) was positively associated with lipid content in developing seeds of 22 accessions of pennycress. Ectopic transient expression of *LTP6* in *Nicotiana benthamiana* leaves induced a proliferation of cytoplasmic neutral lipids droplets (LDs). Similarly, the constitutive expression of *LTP6* in Arabidopsis results in increased LD abundance in the leaves. Analysis of infiltrated *N. benthamiana* leaves using GC-MS indicated that the overexpression of this protein increased the total neutral lipid composition of the infiltrated leaves. GFP fused LTP6 protein products localized either to LDs or LD-ER junctions. Interestingly, Arabidopsis *ltp6* mutant lines revealed a disrupted deposition of wax crystals on some aerial organs and the production of large aberrant LDs in mature seeds, suggesting that LTP6 may be important for both storage lipid compartmentation in embryos and the distribution of surface lipids on aerial organs.

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Session 7: Lipid signaling: molecules, metabolism, mechanisms

*Amélie Bernard

Centre National de la Recherche Scientifique

Amélie Bernard obtained her Ph.D. in 2011 during which she worked on the synthesis of cuticular lipids (Laboratory of Membrane Biogenesis, Bordeaux, France). During her postdoc she started studying autophagy by examining the regulation of autophagy-related genes in yeast (2012-2016; Laboratory of Daniel Klionsky, LSI, University of Michigan, Ann Arbor, USA). In 2016, A. Bernard joined the CNRS as an independent researcher in the Laboratory of Membrane Biogenesis. Her team studies the interrelation between lipids and autophagy to resolve the molecular bases of this critical process for plant acclimation to environmental stresses. In 2020 A. Bernard obtained an ERC starting grant for her project 'LIP-ATG'. In 2023 she was awarded the CNRS Bronze Medal.

"Lipid/protein interplay in membrane formation and remodeling during plant autophagy"

Synopsis:

Autophagy is an intracellular degradation process essential for plants tolerance to environmental changes. During autophagy, portions of the cells are sequestered by a growing membrane which ultimately forms a specialized vesicle prior to cargo delivery to the lytic vacuole. To resolve this orchestrated series of membrane remodeling events, we recently established the molecular footprint of autophagy compartments, unravelling its singularity and providing targets for structural and functional studies. Notably, the characterization of atypical phospholipases now shades light on the mechanism by which autophagy vesicles are disrupted to ensure cargo liberation at the antepenultimate step of the autophagy pathway.

Additional Speakers and Presentations

Teun Munnik: "Polyamines trigger new form of endocytosis, involving distinct lipid signaling pathways"

Sebastien Mongrand: "New insights on Arabidopsis thaliana plant plasma membrane asymmetry"

Kenji Matsui: "Mechanism of plant perception of lipid-derived green leaf volatiles"

Polyamines trigger new form of endocytosis, involving distinct lipid signaling pathways

Xavier Zarsa[1], Nataliia Gnyliukh[2], Ringo van Wijk[1], Lana Shabala[3], Sergey Shabala[3], Jiří Friml[2], Teun Munnik[1*]

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Polyamines are small, polycationic molecules, essential for growth and survival in all eukaryotes. Most common are putrescine^{2+} (Put), spermidine^{3+} (Spd), and spermine^{4+} (Spm). In plants, polyamines promote growth responses and stress tolerance and since they achieve this at higher concentrations (µM-mM) than phytohormones (nM), they are considered as *Plant Growth Regulators*. Nonetheless, still very little is known about their molecular mode of action (*see* Zarsa *et al*., 2019, 2020).

Treatment of *Arabidopsis thaliana* seedlings with polyamines triggered the formation of two lipid second messengers: phosphatidic acid (PA; Zarsa *et al*., 2019) and phosphatidylinositol 4,5-bisphosphate (PIP_2; Zarsa *et al*., 2020). Using KO mutants, evidence is obtained that PA is predominately generated by activation of phospholipase Dδ (PLDδ), while PIP_2 through activation of PIP5-kinase 7 (PIP5K7), PIP5K8 and PIP5K9 (Zarsa *et al*., 2019, 2020, unpublished).

Since both lipids had been implicated in endocytosis (De Jong & Munnik, 2021; Doumane *et al*., 2021), specific experiments were designed to investigate this. Following the internalization of the lipophilic, fluorescent dye, FM4-64, polyamines were discovered to trigger endocytosis in Arabidopsis roots, in particular at the root transition- and elongation zones. When co-incubated with Brefeldin A (BFA), both number and size of BFA bodies increased, again proving of polyamine-induced endocytosis (PIE).

PIE was most effectively triggered by Spm, occurring at low µM concentrations; significantly lower than endogenous, basal levels in plants, and much lower than what is typically used in literature (high µM/low mM). The potency of polyamine species to trigger PIE followed the number of amino groups and positive charges: Spm^{4+} = $tSpm^{4+}$ > Spd^{3+} >> Put^{2+}, and was identical to the lipid responses. In addition, evidence was obtained that PIE was not clathrin- or sterol-mediated, and appeared to be fluid-phased.

While both *pldδ*- and triple *pip5k7/8/9*- KO mutants lost their respective lipid responses, PIE was not affected. Instead, looking closer at phospholipid changes triggered by Spm, a third lipid response was noticed: a small but significant increase in the level of PI4P. Moreover, double *pi4kβ1 pi4kβ2*-KO mutants revealed a strong reduction in PIE as well as in their PI4P- and PIP_2 responses, while PA levels were unaffected. Individual *pi4kβ1*- and *pi4kβ2* mutants exhibited reduced responses too, though less than double mutant. Together, results imply that PI4Kβ1 & -β2 function upstream of PIE, and that the conversion of PI4P into $PI(4,5)P_2$ by PIP5k7/8/9 is downstream, *e.g.* reflecting exocytosis, to counterbalance the increased endocytosis. Since PA levels were not affected in either *pi4k*- or *pip5k* mutants, activation of PLDδ must reflect an independent, coinciding polyamine-triggered event.

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News insights on Arabidopsis thaliana plant plasma membrane asymmetry

Christophe Der[2], Robert Franck [2], Patricia Gerbeau-Pissot [2], Ghislaine Recorbet [2], Adiilah Mamode Cassim [2], Laetitia Fouillen [1], Jérôme Fromentin [2], Françoise Simon-Plas [2]*, Sébastien Mongrand [1]*

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Plasma membrane (PM) is an essential barrier between the cell and the external environment. PM is a stable bilayer, highly adaptable: a feature crucial for signal perception and transmission of information. Together with glycerolipids, sphingolipids and sterols are key elements of the PM organization, but they are not organized symmetrically organized between in the two leaflets, leading to two fundamentally biophysical properties of the outer and inner monolayer. This has been thoroughly described in animal blood cells [1,2], but evidences in plant PM remains very scares with only 2 publications [3,4].

In this talk, I will highlight experimental news insights on plant plasma membrane asymmetry of Arabidopsis thaliana suspension cell culture. First, using environmental-sensitive fluorescent probes with spectral confocal and FLIM, we determined the asymmetry of PM lipid packing on Arabidopsis suspension cells. We clearly evidenced that lipid packing of the apoplastic leaflet is much higher that the one of the cytoplasmic leaflet. Thus, as observed in animal cells, there is a gradient[1,2] of packing from the ordered apoplastic leaflet to the endosome membranes, the cytoplasmic leaflet being in a intermediate state. Second, we showed that this difference in lipid packing of the two leaflets in not dependent on cytoskeleton. Third, we provide state-of-the-art approaches the first combined reference of the plant PM lipidome and proteome[5]. We identified and quantified a reproducible core set of 2165 proteins, which is by far the largest set of available data concerning this plant PM proteome. We showed that the trans membrane spanning proteins display a structural asymmetry of the PM reflected in the asymmetric structures of protein transmembrane domains. Fourth, we determined with an unprecedented repertoire of 405 molecular species of lipids showing that sterols, phospholipids, and sphingolipids are present in similar proportions in the plant PM. Within each lipid class, the precise amount of each lipid family and the relative proportion of each molecular species were further determined, allowing to establish the complete lipidome of Arabidopsis PM, and highlighting specific characteristics of the different molecular species of lipids. Fifth, using Phospholipase A treatment on Right Side Out purified PM, we showed that phosphoglycerolipids are asymmetrically distributed between the 2 leaflets of PM.

These structural asymmetries of the PMs are conserved throughout eukaryota, suggesting fundamental membrane principles.

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Mechanism of plant perception of lipid-derived green leaf volatiles

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Most green plants on land produce galactolipid hydroperoxides by adding oxygen to chloroplast galactolipids as soon as plant tissues are injured by, for example, feeding damage. Through the cleavage reaction of the resulting hydroperoxides, 6-carbon volatile compounds called green leaf volatiles (GLVs) are produced. Laboratory and field observations have shown that plants surrounding an injured plant perceive GLVs emitted from the injured individual, which enhances the plant's ability to defend itself against upcoming attacks by herbivores, based on its knowledge of the damage to the neighboring plants. However, the mechanism of the pathway from such volatile-perception to induced defense response has not been fully clarified. We have confirmed that the expression of a cystatin gene, encoding a protease inhibitor, is induced when maize seedlings accept relatively low concentrations of GLVs. We performed structure-activity relationship analysis using (*Z*)-3-hexenyl acetate as a representative of GLVs and its structural analogs, using the cystatin gene expression level in maize seedlings as an indicator. As a result, it was confirmed that maize strictly recognizes the 3-hexenol structure of GLVs. We also found that MAPK is transiently phosphorylated in maize seedlings in response to GLVs. It is suggested that maize has a structure-specific factor sensing GLVs, and that this factor activates the MAPK-mediated signal transduction pathway to induce cystatin gene expression when maize seedlings perceive GLVs.

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Lichtenthaler Lecture

*James Letts

University of California, Davis

James Letts earned his Ph.D. in Biochemistry and Biophysics from Rockefeller University where he worked in the laboratory of Dr. Roderick Mackinnon on the human voltage-gated proton channel. He began his work on respiratory complexes a postdoctoral fellow in Dr. Leonid Sazanov's group first at the Mitochondrial Biology Unit of the Medical Research Council in Cabridge UK and then at the Institute of Science and Technology Austria. As an associate professor at UC Davis in the Department of Molecular and Cellular Biology, his group combines single particle cryogenic electron microscopy and functional analysis to study mitochondrial electron transport chain complexes and supercomplexes from diverse organisms. The lab is pioneering a bottom-up structural proteomics approach to explore the biodiversity of the respiration across eukaryotes.

Synopsis:
The plant mitochondrial electron transport chain (mETC) is a core bioenergetic pathway important for plant health and biomass accumulation. The mETC is made up of four large membrane protein complexes that couple electron transfer reactions to proton pumping across the inner mitochondrial membrane. Due to the relatively low abundance of mitochondria in plants, biochemical and structural work on the plant mETC has lagged behind that of other organisms. Dr. Letts will present work examples from his lab that have overcome these challenges to produce some of the first structures plant mETC complexes and their higher order associations into supercomplexes.

Awards Talk

*John Harwood

Cardiff University

John Harwood graduated from Birmingham University (England) with a B.Sc. (Medical Biochemistry) in 1966 and a Ph.D. (inositol lipids) in 1969. He was awarded a D.Sc. in 1979 for research achievements. After completing postdoctorates in Davis, California and Leeds University, he was appointed a lecturer in Cardiff in 1973, where he has served as head of research, deputy director and director of the School of Biosciences. He has published over 650 scientific publications including 5 authored and 15 edited books. He has served on committees for several learned societies, research councils and editorial boards. He has received a number of awards (including Phytochemical Society of Europe, Terry Galliard, Supelco/Pelick, Chevreul Medal, Morton Lecture, Stephen Chang) and has been elected a Fellow of several learned societies.

Synopsis:
“The ISPL and its Awards”
The idea for the ISPL began in 1973 when Paul Stumpf from the University of California, Davis visited the U.K. An informal meeting was arranged with researchers from Tony James’ group at Unilever, Colworth House. Conversation turned to holding an international symposium and Terry Galliard, who had worked with Stumpf, organized the first ISPL in Norwich in 1974. Following Terry’s death, his friend Jean-Claude Kader proposed a Terry Galliard medal and, later, the Paul Stumpf award was instigated for promising early career researchers.

The ISPL and its Awards

John L. Harwood

Cardiff University, Wales, UK

The beginning of the ISPL meetings (and, hence, the associated awards) really started in 1973 when Paul Stumpf visited the UK. At that time, the two main labs. focusing on plant lipids were Stumpf's in Davis, California and that of Tony James (co-inventor with A.J.P.Martin of the gas-liquid chromatogram) in Colworth House, Unilever. An informal meeting was arranged by Terry Galliard (Food Research Institute) and Mike Gurr of Unilever. Although small, the meeting was very enjoyable and conversation turned to organizing an international conference in the next year.

Terry (together with Mike Gurr, Mike Rhodes and Ian Mercer) organized the first ISPL in Norwich in April 1974. With plentiful sponsorship, the meeting was very inexpensive and over half the attendees were students of very young post-doctorals. The ISPLs continued every two years, at first in Europe but, later, worldwide. Unfortunately, Terry died in 1993 and his good friend Jean-Claude Kader proposed a Terry Galliard Award during the subsequent ISPL in Paris. This award is given 'to recognize the career achievements of a distinguished plant lipid scientist' with the first recipient being Norio Murata in 1994.

In 2007, Paul Stumpf passed away (like Terry also of cancer) and in order to honour his landmark contributions, the Paul Stumpf award was instigated. This is given to promising early career plant lipid researchers and is fitting because of Paul's encouragement of many such individuals over the years.

In my talk, I will give some more details (and stories behind) of the history of the ISPL and the two awards.

Stumpf Award Lecture

*Lucas Busta

University of Minnesota Duluth

Lucas Busta is an assistant professor at the University of Minnesota Duluth (UMD) in the Swenson College of Science and Engineering, Department of Chemistry and Biochemistry. He completed his undergraduate studies at UMD, earned a Ph.D. in chemistry from the University of British Columbia while working with Reinhard Jetter, then was a postdoctoral fellow at University of Nebraska mentored by Dr. Edgar B. Cahoon, sponsored by the National Science Foundation's Plant Genome Research Program. He is fascinated by the unique chemistries that biological systems use to survive harsh environments. His research uses informatics to unite classical analytical chemistry with emerging high-throughput DNA sequencing technologies to understand the molecular structures and biosynthesis of plant chemicals, polymers, and composites. His goal is to use this approach to develop and apply new knowledge about chemical biology to sustaining and improving human life while protecting the planet.

Synopsis:
Specific plant lineages create thick layers of visible epicuticular surface crystals, referred to as wax blooms, which defend against environmental stress. Here, with help from citizen scientists and middle school students, we surveyed the wax bloom chemistry of 78 species spanning dicot, monocot, and gymnosperm lineages. Many genera produced fatty acid-derived wax blooms, but some, including Kalanchoe, generated triterpenoid-rich wax blooms. We built a phylogeny for 19 Kalanchoe species, assessed their wax chemistry, and sequenced multiple epidermis-specific transcriptomes from Kalanchoe. This presentation will integrate these data to shed light on triterpenoid wax bloom genesis with a focus on triterpenoid transport.

Galliard Medal Lecture

*Xuemin (Sam) Wang
University of Missouri-St. Louis

Xuemin (Sam) Wang obtained his B.Sc. from Huazhong Agricultural University, M.Sc. from The Ohio State University, and Ph.D. from the University of Kentucky, followed by postdoctoral training at Louisiana State University. He joined Kansas State University as assistant professor in the Department of Biochemistry, rose to the rank of professor, and served as the founding director of the Kansas Lipidomics Research Center. Currently, Dr. Wang is Desmond Lee Endowed Professor of Plant Sciences in the University of Missouri-St. Louis and Member Principal Investigator at Donald Danforth Plant Science Center.

“Phospholipases and Lipid-Mediated Signaling: Connecting Stress Cues to Physiological Responses for Crop Improvement”
Synopsis:
We investigate how various phospholipases and derived lipid mediators function as molecular switches to modulate plant growth, development, and stress responses, and how lipid metabolic processes are regulated. Ongoing projects include determining the effect of lipid signaling on crop P use efficiency, unraveling molecular conduits interconnecting lipid metabolism and the circadian clock, and elucidating the role of phospholipases and lipid modulation in reproductive architectures and haploid seed production in cereal crops. Advances in understanding lipid-mediated signaling and phospholipases have potential for new strategies to improve crop nutrient use efficiency, lipid production, and resilience to stress and changing environments.

Session 8: Triacylglycerol: metabolism, biosynthetic regulation, and storage

*Till Ischenbeck
University of Münster

Till Ischebeck has been working in the plant lipid field since joining Peter Dorman's lab as an undergraduate student in 2002. Since then, he has worked for John Shanklin, Ingo Heilmann and Ivo Feussner with station in the Max-Plank-Institute in Golm, the Brookhaven National Laboratory, the Universities of Göttingen and Vienna before being appointed as associate professor at the University of Münster. During this time, he has worked on phytol and fatty acid metabolism and lipid signaling before focusing his work on lipid droplets. He applies a mixture of proteomics, lipidomics, cell biology and biochemistry to elucidate the role of lipid droplets across tissues and species.

Synopsis:
Plant lipid droplets are organelles surrounded by a phospholipid monolayer that stores hydrophobic compounds such as triacylglycerols but also secondary metabolites. In addition, proteins are associated with lipid droplets. One protein family is a family of triacylglycerol lipases. We study by molecular dynamics simulations how this lipase family might bind to the monolayer. Furthermore, we could show that these lipases are not involved in triacyl breakdown in seedlings but are important for the synthesis of oxylipin-derived volatiles in Arabidopsis. Furthermore, this protein family might be involved in the degradation of triacylglycerol in Physcomitrium patens spores.

Additional Speakers and Presentations

Yonghua Li-Beisson: "The α/β hydrolase domain-containing protein 1 (ABHD1) acts as a lysolipid lipase and is involved in lipid droplet formation"

Yingqi Cai: "Dynamin-Related Protein 1A Interacts with SEIPIN1 and LDIP to Modulate Storage Lipid Compartmentation in Plant Cells"

Abraham J. Koo: "Increasing oil production in leaves by engineering plastidial phospholipase A1"

Thiya Mukherjee: "Customizing carbon partitioning: A pathway to enhance soybean seed value and yield"

Philip Bates: "Triacylglycerol remodeling: Discovery and bioengineering to increase hydroxy- and polyunsaturated fatty acids in Arabidopsis and Camelina seed oils"

Wei Ma: "ZINC FINGER PROTEIN condensates mediate seed loading by affecting funiculus"

Yang-Tsung Lin: "Engineering Oleaginous Green Algae for a Healthy and Sustainable Human Milk Fat Substitute in Infant Formulas"

Amira Rasoul: "Fueling the future: Evaluating carbon conversion efficiency and biosynthetic pathways in fae1-3 pennycress (Thlaspi arvense) embryos"

Que Kong: "Phase separation of a MYB transcription factor mediates seed oil biosynthesis in Arabidopsis"

The α/β hydrolase domain-containing protein 1 (ABHD1) acts as a lysolipid lipase and is involved in lipid droplet formation

Ismael Torres-Romero[1]†, Bertrand Légeret[1]†, Marie Bertrand[1], Damien Sorigue[1], Alicia Damm[2], Stéphan Cuiné[1], Florian Veillet[1], Carla Blot[1], Sabine Brugière[3], Yohann Couté[3], Matthew G. Garneau[4], Hari K. Kotapati[4], Yi Xin[5]‡, Jian Xu[5], Philip D. Bates[4], Abdou R. Thiam[2], Fred Beisson[1], **Yonghua Li-Beisson[1]***

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Abstract

Lipid droplets (LDs) are the major sites of lipid and energy homeostasis. However, few LD biogenesis proteins have been identified. Using model microalga *Chlamydomonas*, we show that ABHD1, an α/β-hydrolase domain-containing protein, is localized to the LD surface and stimulates LD formation through two actions: one enzymatic and one structural. The knockout mutants contained similar amounts of triacylglycerols (TAG) but their LDs showed a higher content of lyso-derivatives of betaine lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS). Over-expression of *ABHD1* increased LD abundance and boosted TAG content. Purified recombinant ABHD1 hydrolyzed lyso-DGTS, producing a free fatty acid and a glyceryltrimethylhomoserine. In vitro droplet-embedded vesicles showed ABHD1 promoted LD emergence. Taken together, these results identify ABHD1 as a new player in LD formation by its lipase activity on lyso-DGTS and by its distinct biophysical property. This study further suggests that lipases targeted to LDs and able to act on their polar lipid coat may be interesting tools to promote LD assembly in eukaryotic cells.

Increasing oil production in leaves by engineering plastidial phospholipase A1

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Bioengineering efforts aimed at enhancing the production of oil from non-storage vegetative tissues, which constitute the majority of plant biomass, hold great potential for providing an alternative source of renewable biofuel and industrial feedstocks. While plants typically do not accumulate significant amounts of triacylglycerol (TAG) in their vegetative tissues, the expression of a plastid-localized phospholipase A1 (PLA1) protein, DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1), led to a substantial increase in leaf TAG in Arabidopsis. The inducible system controlling the expression of DAD1 circumvented toxicity issues associated with overexpressing lipases and facilitated a rapid burst of TAG within several hours. The increase of TAG was accompanied by the formation of oil bodies in the leaves, petioles, and stems, but not in the roots. Lipid analysis indicated that the increases in TAG were mostly at the expense of plastidial galactolipids, and that the fatty acid (FA) composition of TAG resembled that of leaf lipids rather than seed lipids, predominantly consisting of 18:3 FAs. Expression of DAD1 in the fad3fad7fad8 mutant, devoid of 18:3 FAs, still resulted in comparable TAG accumulation with 18:2 as the major FA constituent, reflecting the flexible in vivo substrate use of DAD1. The transient expression of either DAD1 or NbDAD1 in *Nicotiana benthamiana* leaves stimulated the accumulation of TAG, demonstrating cross-species translational potential of this technology. In summary, these findings illustrate a novel approach to metabolic engineering for enhanced oil production in vegetative tissues and offer valuable tools for investigating lipid remodeling initiated by plastidial lipases.

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ZINC FINGER PROTEIN condensates mediate seed loading by affecting funiculus

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The plant funiculus is the anchor connecting the developing seed to the placenta within the inner dorsal pod strands of the silique wall and acts as the direct pathway for transporting nutrients to the seed. The secondary cell wall (SCW) is a crucial component of funiculus cells. However, the molecular mechanism underlying SCW biosynthesis in funiculus remains elusive. Here, we identified a previously uncharacterized C2H2-type zinc finger protein, ZINC FINGER PROTEIN (ZFP), which plays an essential role in mediating funiculus function. We found that *ZFP* was highly expressed in the Arabidopsis non-seed tissues of siliques, including funiculus. The *ZFP* loss-of-function mutants exhibited various morphological changes in siliques, such as crinkled and shorter siliques, as well as reduced seed size and seed oil content. Comparative transcriptomic analysis indicated that various genes involved in SCW biosynthesis, including the transcription factor *NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1* (*NST1*), were upregulated in the *zfp* mutant. Dual-Luciferase assays in *Nicotiana benthamiana* leaves revealed that ZFP significantly repressed the activity of the *NST1* promoter (*proNST1*). Electrophoretic mobility shift (EMSA) assay showed ZFP directly bound to *proNST1*. The *in situ* analysis of SCW distribution revealed that *zfp* exhibited ectopic lignified SCW in funiculus. In developing seeds, seed oil accumulation in *zfp* was significantly reduced. We found the glucosinolate contents were substantially lower in *zfp* compared to WT in course of seed developmental stages, suggesting ZFP affected seed loading, possibly through repressing *NST1*. Intriguingly, ZFP displayed hallmarks of intrinsic disorder and

formed phase-separated condensates with liquid-like properties *in planta*. Protein phase separation forms membraneless compartments that are vital for cellular functions and transcriptional regulation. We also demonstrated reconstituted ZFP was phase-separated *in vitro*. Collectively, our findings suggest that repression of *NST1* by nuclear ZFP condensates fine-tunes *NST1* expression, thus controlling the development of funiculus and seed loading.

Engineering Oleaginous Green Algae for a Healthy and Sustainable Human Milk Fat Substitute in Infant Formulas

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In 2020 only 25% of U.S. infants under 6 months were exclusively breastfed, and this percentage was even lower among low-income families. During early lactation infants are dependent on human milk fat (HMF) for more than 50% of their calories, and the lipid component of infant formula is similarly essential for proper nutrition. The distinctive features of HMF compared to common vegetable oils include 1) enrichment for rapidly metabolizable medium-chain fatty acids (MCFA, C8:0-C14:0), and 2) preference for esterification of palmitic acid (C16:0) at the triacylglyceride (TAG) *sn*-2 position, which is crucial for the absorption of both palmitic acid and calcium. These characteristics can only by mimicked in formula by blending and enzymatic conversion of multiple different vegetable oils, increasing production costs and demands on land usage. In this study we address these challenges through development of a HMF substitute by metabolic engineering of *Auxenochlorella protothecoides* (*A. pro.*), a model green alga for synthetic biology, with rapid biomass accumulation, high lipid content, and a simple genome structure compared to most oil crops. We modified *A. pro.* fatty acid biosynthesis by introducing heterologous chain-length-determining fatty acyl-ACP thioesterases to increase the levels of MCFA and palmitic acid. The relative abundance of stearic acid (C18:0) and oleic acid (C18:1) was altered through targeted deletion of one allele of the *FAB2A* gene, encoding stearoyl-ACP desaturase. In addition, C16:0-specific lysophosphatidic acid acyltransferases were introduced to increase the regioisomeric positioning of palmitic acid at *sn*-2 in TAG. These results demonstrate the feasibility of sustainably producing a nutritious HMF substitute in *A. pro*.

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Session 9: Plant lipids in abiotic and biotic stress resilience

*Rebecca Roston

University of Nebraska-Lincoln

Rebecca Roston is a plant biochemist and associate professor at the University of Nebraska-Lincoln where she also helps direct the Center for Plant Science Innovation. She earned her B.S. in 2003 and her Ph.D. in 2009, both from the University of California, Davis, where she focused on chloroplast protein targeting under the guidance of Kentaro Inoue. From 2009 to 2014, she was a postdoctoral researcher at Michigan State University with Christoph Benning, specializing in plant lipid metabolism and transport. At UNL, her team researches plant membranes and their ability to tolerate stress, grow, develop, and enhance photosynthetic yield. They couple membrane lipid analysis with plant physiology measurements and add standard biological fractionation and molecular biology techniques to determine lipid changes needed for development and stress tolerance and the molecular mechanisms through which they occur. New directions include contributing to our understanding of how redox homeostasis affects stress tolerance and stress tolerance engineering.

Synopsis:
Low temperatures challenge plants, restricting their distribution and causing agricultural losses. Maintaining functional membranes is crucial for cold tolerance, and lipid distribution in membranes is highly temperature-sensitive. The mechanisms by which plants translate low temperatures into membrane remodeling are not fully understood. This talk describes two investigations: the differential responses of TAG acyltransferase family members to specific temperature regimens and the identification of kinases that fine-tune the activity level of SENSITIVE TO FREEZING 2 (SFR2), a key enzyme for chloroplast membrane remodeling, in response to cold.

Additional Speakers and Presentations

Haruhiko Jimbo: "Elucidation of phosphatidylglycerol turnover during PSII repair by using chemically synthesized ether-linked lipids"

Liang Guo: "Phospholipid metabolism-mediated phosphate recycling in plants"

Yasuyo Yamaoka: "Exploring stress responses and lipid homeostasis in microalgae"

Ruth Welti: "Natural variation in Arabidopsis thaliana FATTY ACID DESATURASE 2 is associated with latitude and freezing tolerance"

Mie Shimojima: "Modulation of membrane glycerolipids in response to low temperature and acidic stress in Marchantia polymorpha"

Eric Marechal: "Role of membrane and storage lipids in the adaptation of the ubiquitous snow alga Sanguina nivaloides (Chlorophyta) to life in melting snowfields"

Sruthi Narayanan: "Lipid modulation contributes to heat stress adaptation in peanut"

Richard Haslam: "Feeling the heat: Investigating the dual assault of Zymoseptoria tritici and heat stress on wheat (*Triticum aestivum*)"

Abstract

Elucidation of phosphatidylglycerol turnover during PSII repair by using chemically synthesized ether-linked lipids

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Phosphatidylglycerol (PG) is essential for the function of photosystem II (PSII) in photosynthetic organisms. Crystallography of the PSII dimer from *Thermosynechococcus vulcanus* shows that PSII contains 5 PGs per reaction center (Umena et al. 2011); however, the function of PGs in PSII is still unclear. Many lipids including PGs associate with D1 protein, a reaction center of PSII. D1 is rapidly turned over under strong light, thus lipids in D1 seem to be replaced as well during the repair of damaged PSII. In the present study, we investigated the effects of PG turnover on the photoinhibition of PSII. We used a *pgsA* mutant of *Synechocystis* sp. PCC 6803 lacking PG synthesis and applied chemically synthesized ether-linked PGs, which cannot be metabolized in *Synechocystis* cells. *PgsA* cells utilized chemically synthesized PGs to grow photoautotrophically. However, one synthesized PG (ET2), which includes an ether-bond between the fatty acid chain at the *sn*-2 position in glycerol backbone, inhibited the repair of photodamaged PSII. By contrast, another synthesized PG (ET1) in which the ether bond is at the *sn*-1 position, did not affect photoinhibition. Pulse-chase experiment with radiolabeled ^{35}S-Metionine/Cysteine showed that ET2 inhibited degradation of D1 under strong light. Therefore, PG is degraded at the *sn*-2 position to support the degradation of D1 protein during the PSII repair.

Investigating lipid metabolism regulation and stress response for environmental adaptation in *Chlamydomonas reinhardtii*

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Microalgae represent promising prospects for sustainable lipid accumulation due to their capacity to accumulate substantial oil levels under stress. Yet, the underlying regulatory pathways governing lipid metabolism in stress responses remain partially elucidated. We are currently investigating such lipid accumulation mechanism using a model microalgae *Chlamydomonas reinhardtii.*

The MYB1 transcription factor demonstrated substantial induction under diverse stressors in Chlamydomonas. In comparison to the parental strain, *myb1* mutants exhibited a reduced production of both total fatty acids and storage lipids during nitrogen depletion, indicating the crucial role of MYB1 as a positive regulator of lipid accumulation in Chlamydomonas under nitrogen-depleted conditions. Further recent investigations expanded our understanding of MYB1, recognizing it as a positive facilitator of lipid accumulation under the multiple stress conditions.

We also explored the ER stress response, triggered by misfolded protein accumulation in Chlamydomonas. The CrIRE1/CrbZIP1 pathway emerged prominently, stimulating the UPR and activating ER-resident lipid components in ER stress. Recent studies underscored its regulatory impact on sterols and sphingolipids, highlighting its pivotal role as an indispensable component of the microalgae ER stress response.

Our study provides valuable insights into unravel stress response mechanisms and regulators of lipid metabolism in Chlamydomonas.

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Phospholipid metabolism-mediated phosphate recycling in plants

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Phosphate is a macronutrient necessary for plant growth and development and its availability and efficient use greatly affect crop growth and yield. Membrane phospholipids constitute approximately one third of all organic phosphate in plants, serving as an important cellular Pi reservoir. Lipidomic analysis reveals that the level of phospholipid and phosphosphingolipid dramatically decreases upon phosphate starvation or during leaf senescence. Our results indicate that up to 90% of lipid phosphorus is recycled from senescent leaves before falling off the plants. *Nonspecific phospholipase C4* (*NPC4*) and *phospholipase Dζ2* (*PLDζ2*) are highly induced by phosphate starvation or leaf senescence. We firstly show that NPC4 is the key enzyme hydrolyzing glycosylinositolphosphorylceramide (GIPC) supported by genetic, biochemical and lipidomic analysis. Knockouts of *PLDζ2* or/and *NPC4* decrease the loss of membrane phospholipids and impact plant growth and yield. Furthermore, we identify that a transcription factor PHR1 directly regulates the expression of *NPC4*, which impacts the sphingophospholipid hydrolysis in response to phosphate limitation. These results indicate that PLDζ2- and NPC4-mediated membrane phospholipid hydrolysis promotes phosphorus recycling and the phospholipid hydrolysis-mediated phosphorus recycling improves phosphorus use efficiency in plants.

Natural variation in *Arabidopsis thaliana FATTY ACID DESATURASE 2* is associated with latitude and freezing tolerance

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FATTY ACID DESATURASE 2 (FAD2) converts 18:1 on phosphatidylcholine (PC) to 18:2. Early work demonstrated that FAD2 activity is necessary for *Arabidopsis thaliana* growth at 6°C and that prolonged exposure of plants lacking FAD2 activity to 6°C results in plant death (Miquel et al., 1993, PNAS 90, 6208). A genome wide association study carried out under cold-temperature conditions, using levels of intact lipid molecular species as the measured phenotypes, found that natural variation in the 5'UTR of the gene *FAD2* (*At3g12120*, SNP at Chr3_3862612) is associated with levels of several phospholipids, including the FAD2 substrate, PC 18:1_18:3. The group of accessions with the minor allele G at position 3862612 on chromosome 3 had, on the average, higher levels of 18:1-containing FAD2 substrate lipids, lower levels of 18:2-containing FAD2 product lipids, and lower FAD2 expression compared to the group of accessions with the major allele G at the same position. The accessions in the minor allele group originated at lower latitudes (average 41.7°) and showed less tolerance to freezing than those in the major allele group (average 46.7° latitude). Taken together, the results confirm the importance of FAD2 activity in cold/freezing tolerance and imply that FAD2 natural variation is important in adaptation to environmental temperature.

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Modulation of membrane glycerolipids in response to low temperature and acidic stress in *Marchantia polymorpha*

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Modulation of the membrane lipid composition is one of the systems for plants to overcome abiotic stress. Here we analyzed glycerolipid biosynthesis in the bryophyte model plant *Marchantia polymorpha* under low temperature and acidic conditions with acetic acid. In Marchantia, one of the three non-specific phospholipase Cs (NPCs) was elucidated to involve in low-temperature stress from the gene expression. Under low temperature, phospholipids containing C20:5 (EPA) increased with decreasing that of plastid glycolipids. NPC affected the EPA ratio in phospholipids without changes in membrane glycerolipid composition. Under acidic stress with acetic acid, Marchantia plants accumulated oligogalactolipids. In Arabidopsis, oligogalactolipids are synthesized under freezing stress by galactolipid:galactolipid galactosyltransferase (GGGT), namely SFR2. We identified a gene for Marchantia GGGT, produced the knock-out mutants, and found that the Mp*gggt* mutant was more sensitive to the acidic conditions than wild-type plants. The results indicated that MpGGGT plays a crucial role for Marchantia growth under acidic stress conditions which might be encountered during land colonization.

Funded by a Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Role of membrane and storage lipids in the adaptation of the ubiquitous snow alga *Sanguina nivaloides* (Chlorophyta) to life in melting snowfields

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Snowfields with a vivid red color occur at high elevations in mountain ranges worldwide, as well as in sub-Arctic and sub-Antarctic regions, due to the proliferation of pigmented single-cell algae. The taxonomic assessment of the main species forming red snow blooms has remained elusive only until the recent discovery of a novel genus of Chlamydomonadales, named *Sanguina*, producing cysts enriched in carotenoids, with high level of astaxanthin [1]. The mechanisms enabling *Sanguina nivaloides* to live in the snowpack for months, and to form red blooms, are unknown. Addressing these questions is challenging, as *Sanguina nivaloides* is still non-cultivable. Based on high-resolution 3D imaging of *S. nivaloides* cysts collected in the snow [2], combined with physiological, biochemical and lipidomic analyses, we have shown that *Sanguina* cysts populate liquid water at the periphery of ice grains, are photosynthetically active, can survive for months, and are sensitive to freezing. These properties are consistent with the dynamics of blooms detected in the Alps by satellite monitoring of astaxhantin [3]. Cysts developing in the snow harbor a wrinkled plasma membrane expanding the interface with environment. Ionomic analysis supports a cell efflux of K^+, and assimilation of phosphorus. Glycerolipidomic analysis confirms a phosphate limitation. The chloroplast contains thylakoids oriented in all directions optimizing the capture of light scattered in the snow; it fixes carbon in a central pyrenoid and produces starch in peripheral protuberances. Analysis of cells kept in the dark shows that starch is a short-term carbon storage. The biogenesis of cytosolic droplets in *S. nivaloides* cysts shows that they are loaded sequentially with triacylglycerol and carotenoids for long-term carbon storage and protection against oxidative stress [2]. These analyses combined with preliminary genomic data highlight some of the adaptive mechanisms allowing *Sanguina* to thrive in snowfields worldwide, pave the way to future studies on the acquisition of these remarkable traits in the evolution of Chlorophyta, and give insights on their capacity to survive in the current context of climate and environmental changes.

1. Prochazkova L, Leya T, Krizkova H, Nedbalova L (2019) Sanguina nivaloides and Sanguina aurantia gen. et spp. nov. (Chlorophyta): the taxonomy, phylogeny, biogeography and ecology of two newly recognised algae causing red and orange snow. ***FEMS Microbiol Ecol*** 95 (6). doi:10.1093/femsec/fiz064

2. Ezzedine JA, Uwizeye C, Si Larbi G, Villain G, Louwagie M, Schilling M, Hagenmuller P, Gallet B, Stewart A, Petroutsos D, Devime F, Salze P, Liger L, Jouhet J, Dumont M, Ravanel S, Amato A, Valay JG, Jouneau PH, Falconet D, Marechal E (2023) Adaptive traits of cysts of the snow alga Sanguina nivaloides unveiled by 3D subcellular imaging. ***Nat Commun*** 14 (1):7500. doi:10.1038/s41467-023-43030-7

3. Roussel L, Dumont M, Gascoin S, Monteiro D, Bavay M, Nabat P, Ezzedine JA, Fructus M, Lafayssa M, Morin S, Maréchal E. Melt duration controls red algal blooms in the snow of the European Alps. ***Proc Natl Acad Sci U S A under rev.***

Lipid Modulation Contributes to Heat Stress Adaptation in Peanut

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Abstract

Heat stress, which most commonly accompanies drought stress, is one of the major factors that limit peanut (*Arachis hypogaea*) yield and profitability. At the cellular level, membrane damage is a fundamental cause of yield loss at high temperatures. Our investigations on a subset of a peanut recombinant inbred line population demonstrated that the membrane lipid remodeling occurring at high temperatures is consistent with homeoviscous adaptation to maintain membrane fluidity. A major alteration in the leaf lipidome at high temperatures was the reduction in the unsaturation levels, primarily through reductions of 18:3 fatty acid chains, of the plastidic and extra-plastidic diacyl membrane lipids. In contrast, levels of 18:3-containing triacylglycerols (TGs) increased at high temperatures, consistent with a role for TGs in sequestering fatty acids when membrane lipids undergo remodeling during plant stress. Polyunsaturated acyl chains from membrane diacyl lipids were also sequestered as sterol esters (SEs). The removal of 18:3 chains from the membrane lipids decreased the availability of susceptible molecules for oxidation, thereby minimizing oxidative damage in membranes. Our results suggest that transferring 18:3 chains from membrane diacyl lipids to TGs and SEs is a key feature of lipid remodeling for high-temperature adaptation in peanut. Further, in our investigations with six selected genotypes with varying heat response, we found that the expression of *Fatty Acid Desaturase 3-2* (*FAD3-2*; converts 18:2 fatty acids to 18:3) decreased under high temperatures in the heat-tolerant genotype but not in the susceptible genotype. This result suggests that reducing *FAD3* expression for decreasing the levels of 18:3 fatty acids is likely another heat-acclimation mechanism in peanut. Finally, QTL-seq allowed the identification of a genomic region associated with heat-adaptive lipid remodeling, which would be useful for identifying molecular markers for heat tolerance. The information generated from these studies on lipid remodeling for high-temperature adaptation will aid in breeding heat-tolerant peanut varieties.

Session 10: Lipid biotechnology: oilseeds, algae, vegetative organs, emerging platforms

*Laura Wayne
Corteva Agriscience

Laura Wayne is the seed composition discovery leader at Corteva Agriscience, in Johnston, Iowa. She leads a team of scientists within R&D Trait Discovery researching ways to increase oil and enhance seed composition. Her overall research interest is in understanding the underlying mechanisms of plant metabolism to develop useful products from plants, providing food, fuel, and sustainable feedstocks for our growing population. Laura received her Ph.D. in molecular plant science from Washington State University in Pullman, Washington, and a B.S. with honors in biotechnology from the State University of New York College of Environmental Science and Forestry (SUNY-ESF) in Syracuse, New York.

“Increasing Oil in Soybean”
Synopsis:
To increase total oil in soybean seeds, modifications in the diacylglycerol acyltransferase (DGAT) enzyme were examined in model systems and then transgenically overexpressed in soybean. Over-expression of DGAT1b with three amino acid substitutions results in increases in total oil and protein from multi-location field testing. In addition to amino acid substitutions in DGAT1b, a strong promoter is needed to drive high oil accumulation. Two DGAT1b variants were selected for insertion into soybean via CRISPR-Cas9 editing. These edits contain the beta-conglycinin promoter driving DGAT1b variant and have increases in seed oil and protein content.

Additional Speakers and Presentations

Prasad Parchuri: "Deciphering diacylglycerol enantiomer specificities of DGAT isoforms: Insights into TAG remodeling in lipid metabolism of different species"

Timothy Durrett: "Complete remodeling of TAG composition to generate novel oils in transgenic oilseeds"

Payton Whitehead: "Lipid droplet packaging proteins from jojoba (Simmondsia chinensis) improve the compartmentalization of wax esters"

Peter Doermann: "Engineering Synechocystis sp. PCC 6803: A genetic approach to modulate the triacylglycerol content for enhanced bio-production"

Jorg Schwender: "Elucidation of triacylglycerol overproduction in the C4 bioenergy crop Sorghum bicolor by constraint-based analysis"

John Sedbrook, Illinois State University, Pennycress (Thlaspi arvense L.) seed size mutants affect seed oil and protein accumulation differently

LIPID DROPLET PACKAGING PROTEINS FROM JOJOBA (*SIMMONDSIA CHINENSIS*) IMPROVE THE COMPARTMENTALIZATION OF WAX ESTERS

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Simmondsia chinensis, better known as jojoba, is a desert shrub native to the southwestern United States and northern Mexico. Jojoba is unusual in that instead of primarily storing triacylglycerols (TAGs) in its seeds, it instead stores large quantities of liquid wax esters (WEs) as its major seed storage lipid. The principal subcellular location for storage of these neutral lipids (TAGs or WEs) is in organelles known as lipid droplets (LDs). These LDs are spherical structures composed of a phospholipid monolayer derived from the ER, surrounding a core of hydrophobic compounds. In addition to the phospholipid monolayer and the hydrophobic core, these LDs have a host of proteins that associate with the outer surface of the LD promoting the formation, stabilization, and ultimately degradation of the LD. Here we have identified several Jojoba homologs of Arabidopsis LD proteins, with some having significantly differential expression in tissues of Jojoba seeds that are specific for WE accumulation. Confocal microscopic and mass spectrometric analysis of *Nicotiana benthamiana* leaves transiently expressing jojoba LD

proteins in conjunction with WE-synthesizing enzymes, have revealed that an isoform of the LD protein, Lipid Droplet Associated Protein 1 (LDAP1), was capable of more efficiently releasing WEs from the ER into LDs compared to other jojoba LD proteins or LDAP1 isoforms from other plant species. A single amino acid residue within one amphipathic α-helix of jojoba LDAP1 was both necessary and sufficient to promote proper wax packaging. In addition, jojoba LDAP1 was shown to correct subcellular defects in stable Arabidopsis lines accumulating WEs supporting a critical role for LDAP1 in WE packaging. Ultimately, these results may provide new insights into the mechanistic roles that LD proteins play in the biogenesis of LDs with varied neutral lipid compositions, which may support biotechnology strategies to over produce WEs in heterologous systems.

Funded by the U.S. Department of Energy, Office of Science, BES-Physical Biosciences Program (DE-SC0016536).

Engineering *Synechocystis* sp. PCC 6803: A Genetic Approach to Modulate the Triacylglycerol Content for Enhanced Bio-production

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Synechocystis sp. PCC 6803 is a model cyanobacterium which can be genetically manipulated, owing to its well characterized genome, short generation time and transformation efficiency. Under stress like nitrogen limitation, *Synechocystis* displays reduced chlorophyll synthesis, impaired photosynthetic activity and metabolic reprogramming, including the accumulation of carbon reserves in the form of glycogen, polyhydroxyalkanoates (PHA), and small amounts of triacylglycerol (TAG). We recently showed that the gene *slr2103* is involved in the synthesis of TAG and phytyl esters in *Synechocystis*. In a biotechnological approach, we found that TAG accumulates even further in *Synechocystis* mutants affected in glycogen and PHA biosynthesis, indicating that carbon can be redirected between different carbon reserves. Furthermore, we screened different slr2103-like containing single cell and filamentous cyanobacteria for their capacity to produce TAG, phytyl esters, and other acylated products, to study the substrate specificity of the slr2103-like acyltransferases.

Elucidation of triacylglycerol overproduction in the C_4 bioenergy crop *Sorghum bicolor* by constraint-based analysis

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Upregulation of triacylglycerols (TAG) in vegetative plant tissues such as leaves has the potential to drastically increase the energy density and biomass yield of bioenergy crops. In this context, constraint-based analysis has the promise to improve metabolic engineering strategies. Here we present a core metabolism model for the C4 biomass crop *Sorghum bicolor* (*iTJC1414*) Extending *iTJC1414* to a four-cell diel model we simulate C4 photosynthesis in mature leaves with the principal photo-assimilatory product being replaced by TAG produced at different levels. Independent of specific pathways and per unit carbon assimilated, energy content and biosynthetic demands in reducing equivalents are about 1.3 to 1.4 times higher for TAG than for sucrose. For plant generic pathways, ATP- and NADPH-demands per CO_2 assimilated are higher by 1.3- and 1.5-fold, respectively. If the photosynthetic supply in ATP and NADPH in *iTJC1414* is adjusted to be balanced for sucrose as the sole photo-assimilatory product, overproduction of TAG is predicted to cause a substantial surplus in photosynthetic ATP. This means that if TAG synthesis was the sole photo-assimilatory process, there could be an energy imbalance that might impede the process. Adjusting *iTJC1414* to a photo-assimilatory rate that approximates field conditions, we predict possible daily rates of TAG accumulation, dependent on varying ratios of carbon partitioning between exported assimilates and accumulated oil and in dependence of activation of futile cycles of TAG synthesis and degradation. We find that, based on the capacity of leaves for photosynthetic synthesis of exported assimilates, mature leaves should be able to reach a 20% level of TAG per dry weight within one month if only 5% of the photosynthetic net assimilation can be allocated into oil. From this we conclude that high TAG levels should be achievable if TAG synthesis is induced only during a final phase of the plant life cycle.

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Poster Abstracts

Viewing Guide

Topic Area	Board Assignments
Emerging methods for lipid research and crop design	A-E
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Fatty acid and glycerolipid: biosynthesis, modification, biosynthetic evolution	L-X
Lipid biotechnology: oilseeds, algae, vegetative organs, emerging platforms	Y-JJ-1
Lipid signaling: molecules, metabolism, mechanisms	JJ-2 - MM-1
Plant lipids in abiotic and biotic stress resilience	MM-2 - UU-2
Plastidic lipids: glycolipids, phospholipids, isoprenoids	VV-ZZ
Surface lipids: biosynthesis and regulation of plant protection	AAA-CCC
Trafficking of lipids among cells and tissues	DDD
Triacylglycerol: metabolism, biosynthetic regulation and storage	EEE-LLL
Other Category	E-2

Presenters

1. Emerging methods for lipid research and crop design

Fnu Alisha
Sabin Dahal
Paliav Singh
Manish Sridhar Immadi
Wenhao Shen
Chunhui Xu
Jiahong Zhou
Li-Hua Zhu/Oliver Moss
Doug Allen

Characterization of Pennycress seeds engineered to synthesize Medium Chain Fatty Acids

FNU Alisha, Timothy P. Durrett

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Pennycress (Thlaspi arvense) is an emerging oilseed crop weed that belongs to the mustard family of Brassicaceae with a rapid lifecycle. It has a diploid genome and is closely related to the model plant Arabidopsis thaliana. Pennycress is easily transformable, making it one of the unique platforms for the synthesis of biotechnology-derived compounds such as Medium Chain Fatty Acids, which are abundant in tropical nuts such as coconut, and other plant species such as Cuphea where they accumulate up to ~94mol%. Pennycress seeds accumulate high levels of triacylglycerols (TAGs, oil), ~33% by dry weight and primarily composed high erucic acid (22:1, ~33%), linolenic acid (18:3, ~15%), linoleic acid (18:2, ~20%) and oleic acid (18:1, ~13%) but do not naturally produce MCFAs. Fatty acyl thioesterases (FatB) enable the synthesis of these MCFAs. Two acyltransferases, CvLPAT (Lysophosphatidyl acyltransferase) and CpuDGAT (Diacylglycerol acyltransferase) that specifically transfer these MCFAs into TAG molecules, increase the incorporation of MCFA. We looked at the germination, Ds-Red and MCFAs levels for selection of the best lines with expression of FLD (CvFatB+CvLPAT2+CpuDGAT1) gene combination, which yields 10:0 levels of 6.3 mol% and UC (UcFatB+CnLPAT) gene combination, which yields 12:0 levels of 25.81 mol%. These levels are quite lower than in naturally occurring MCFAs plant species. A systems biology approach on these developing transgenic seeds will learn more about the enzymatic bottlenecks responsible for such lower levels.

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2. Extra-plastidic membrane lipids: phospholipids, sterols, sphingolipids

Ed	Cahoon
Marina	Gavilanes-Ruiz
Ariadna	Gonzalez Solis
Hiro	Imai
Toshiki	Ishikawa
Abraham	Osinuga
Qiong	Xiao

Modeling Pulse-Chase Radiolabeling Data with a User-Friendly Computational Platform to Assess Lipid Metabolism

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Abstract:
Lipid flux has historically been considered through radioisotopes (^{14}C, ^{3}H). Transient labeling reflects the precursor-product relationships of metabolites in biochemical networks; however distinguishing models based on the inspection of labeling curves is difficult and not always intuitive given the complexity of lipid metabolism. A user-friendly computational modeling platform was designed to aid the interpretation of complex labeling descriptions that depict metabolic flux through multiple subcellular locations. Modeling the transient labeling of metabolite pools is a powerful, unbiased approach that provides a quantitative assessment of multiple subcellular or even more localized pools that can be resolved and would otherwise be difficult if not impossible to measure experimentally. Ordinary differential equations were used to describe pulse and pulse-chase labeling experiments, based on mass action kinetics. The modeling platform is being used to inform lipid engineering strategies through a design-build-test learn strategy. As other modeling platforms for pulse-chase labeling data do not currently exist, we expect this user-friendly GUI-platform will be easily adapted by those interested in labeling kinetics and flux well beyond the field of lipid metabolism.

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Redesigned KBCommons – v2.0 framework for enhanced multi-omics data integration and visualization for diverse organisms

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KBCommons v2.0 represents an upgraded iteration of its precursor, providing a robust platform for the storage, sharing, analysis, and visualization of genomics and integrative multi-omics data across diverse organisms. Its four modules encompass data storage, processing, access, and a user-friendly web interface. Prominent functionalities comprise the ability to generate new knowledge bases (KBs) for any organism, contribute multi-omics data, and update genome versions.

KBCommons v2.0 comes with the modern new design and interactive user interference with several new tools integrated into it to support multi-omics data integration. The 3D Omics Studio tool includes a Differential Expression Tool that augments data analysis capabilities, enabling users to discern subtle gene/protein/metabolite expression patterns across various experimental conditions with enhanced accuracy and efficiency. The Comparative and Cross-species Multi-Omics Translation (CCMT) tool allows for comparative analysis of similar or different multi-omics data between organisms and within the same organism. It offers insights into transcriptomics, proteomics, metabolomics, and gene regulatory network comparisons.

Furthermore, the Allele Catalog tool available for certain plant species facilitates efficient exploration and analysis of large-scale resequencing data for discovering new alleles and phenotypic changes based on accession categories. Lastly, the GenVarX tool focuses on transcription factor binding sequences, copy number variations, SNPs, Indels, and their impact on phenotypes. These aid in understanding phenotypic variations, particularly for precision agriculture.

With the increasing rate of generated genomics and multi-omics data, KBCommons is an essential framework for all organisms. It is publicly available at https://kbcommons.org/

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scINTGrate: Interactive Multiomics Data Analytics, Inference and Visualization Portal

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We introduce scINTGrate, a comprehensive web portal for integrating, analyzing, visualizing, and deriving inferences from bulk and single-cell multiomics data from biomedical and plant research, seamlessly accessible to a wide range of users through integration with KBCommons. The portal addresses the challenges associated with the growing volume and complexity of omics datasets by providing a user-friendly interface. scINTGrate enables the integration of single-cell RNA sequencing (scRNA-seq) and bulk RNA-seq data, facilitating the exploration of cellular heterogeneity and the comparison of gene expression patterns using CIBERSORTx across different experimental conditions. The portal offers a range of clustering and differential expression analysis tools, along with interactive visualization modules for intuitive data exploration. scINTGrate is built using the Django web framework and utilizes a MySQL database for efficient data management. The portal's modular architecture ensures scalability and extensibility, accommodating the growing needs of multiomics data integration, analysis, and inference generation in various research fields, including biomedical and plant sciences. Through a case study about studying multi-stress combinations in Arabidopsis, soybean and tomato, we demonstrate scINTGrate's potential to uncover novel insights, drive hypothesis generation, and facilitate easy analysis of comparisons across the different species, making it a valuable resource for the scientific community working with biomedical and/or plant datasets.

SoyHub - A hub for soybean-applied genomics prediction tools based on diverse soybean re-sequenced accessions

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SoyHub serves as a pivotal resource for advancements in the field of soybean genomics, providing a suite of tools designed to enhance research through data-driven insights. These tools, including the Allele Catalogue, GenVarX, AccuTool, MADis, and SNPViz, are instrumental in deciphering the complex genetic and phenotypic landscapes of soybeans by utilizing whole genome resequencing data from ~2939+ soybean accessions.

The Allele Catalogue leverages extensive genotype and phenotype datasets to visualize alleles across various accessions, facilitating an understanding of allele groupings and their implications for soybean improvement.

GenVarX focuses on uncovering genomic variations such as copy number variations and allele differences in promoter regions, which are crucial for regulatory mechanisms and phenotypic alterations.

AccuTool, powered by the comprehensive Soy775 data panel, allows for precise accuracy computations between wild-type and mutant alleles, enabling the identification of variant positions with significant associations.

MADis aids in the discovery of multiple alleles affecting the same phenotype through sophisticated combinatorial computations, offering insights into the relative importance of each allele combination.

Finally, SNPViz v2.0 presents an interface for exploring large-scale haplotype blocks, integrating SNP and Indel data with gene models, and correlating them with phenotype-genotype accuracy, Gene Ontology annotations, protein families, and their functional implications.

Collectively, these tools represent a significant advanced and a forward stride in soybean genomic research, offering researchers the means to navigate the genetic complexities of soybeans with unprecedented clarity and specificity.

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SIMPEL 2.0: Automating Untargeted Isotope Labeling Analysis and Pathway Elucidation

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Lipids fill crucial biochemical roles as signaling molecules and establishing membranes that define organelles. Additionally, lipids are the most energy dense storage reserve with twice the energy content of carbohydrates and valued for many food and biotechnological applications. Understanding the dynamics and fluxes of lipid metabolism in plants is crucial to support engineering efforts that enable production of biofuels, renewable feed stocks to supplant petroleum, and vegetable oils for food applications. Isotopes are commonly used to deduce fluxes through metabolism; however, despite significant advances in stable isotope labeling techniques, data analysis is laborious, and if performed manually, prone to error, due to the number of peaks created when isotopes are incorporated into molecules. Traditional metabolomics data analysis methods with untargeted workflows cannot handle isotopes and do not provide any additional information on metabolic pathways. SIMPEL, a computational tool designed to streamline the analysis of stable isotope labeling data in an untargeted manner, addresses these shortcomings. SIMPEL automates isotopologue binning, ensuring accurate identification and characterization of labeled compounds within high-resolution mass spectrometry (HRMS) datasets. By integrating preprocessed HRMS data and extracting labeling information from all identified compounds, the tool considers the unique properties and characteristics of isotopologue distributions and automatically generates a suite of plots to help with interpretation of complex data. SIMPEL can offer valuable insights into context-specific lipid metabolism in plants but is also being employed to investigate other challenging areas in metabolism, including plant -microbe interactions, nutrient exchange with the environment and photosynthesis. The tools offer an unbiased and rigorous approach which will advance isotope labeling studies and our understanding of metabolism.

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Improving the seedcake quality of rapeseed by using RNP-mediated CRISPR gene editing

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Rapeseed is the second most important oil crop in the world, and the only major oil crop to grow in Northern Europe. Apart from its important oil, the seeds of rapeseed contain also a high quality of proteins similar to the soybean protein. Once oil is pressed from the seeds, the high protein seedcake is left behind. The protein content of the seedcake is up to 40% of the total dry matters in the seeds, however it is currently used only as feed in a limited scale with a lower price than the soybean meal, and it can't be used as food due to the presence of high levels of anti-nutritional compounds. Sinapine is one of such compounds, which makes the seedcake taste bitter and gives the eggs of chickens that consume it a fishy taste. One of our research focuses in rapeseed is to reduce the sinapine level in the seeds by knocking out functions of the specific genes involved in the biosynthesis of sinapine by using the RNP (ribonucleoprotein) - based CRISPR/Cas9 approach. This method enables generation of transgene free mutants, which are considered as non-GM in more and more countries worldwide and would play an important role in future agricultural production. We have worked with a couple of target genes with known functions for reducing the sinapine content in rapeseed and have produced a large number of edited lines with functional mutations on the target genes. Preliminary results on chemical analysis have shown a significant reduction of sinapine in the seeds by up to 70% in some lines compared with the wild type. The better performing mutation lines are grown in biotron for obtaining homozygous lines and further phenotypic analysis.

Funded by 1) SLU-Grogrund – Centre for Breeding of Food Crops; 2) Swedish Government supported program: Trees and Crops for the Future.

Sphingolipid Homeostasis—How Do Cells Know When Enough is Enough?

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Sphingolipid homeostatic regulation is important for balancing plant life and death. Plant cells finely tune sphingolipid biosynthesis to ensure sufficient levels to support growth through their basal functions as major components of endomembranes and plasma membrane. Conversely, accumulation of sphingolipid biosynthetic intermediates, long-chain bases (LCBs) and ceramides, is associated with programmed cell death (PCD). Limiting these apoptotic intermediates is important for cell viability; while overriding homeostatic regulation permits cells to generate elevated LCBs and ceramides to respond to pathogens to elicit the hypersensitive response in plant immunity. Key to sphingolipid homeostasis is serine palmitoyltransferase (SPT), an ER-associated, multi-subunit enzyme catalyzing the first step in the biosynthesis of LCBs, the defining feature of sphingolipids. Across eukaryotes, SPT interaction with its negative regulator ORM is critical for sphingolipid biosynthesis. The recent cryo-electron microscopy structure of the Arabidopsis SPT complex indicates that ceramides bind ORMs to competitively inhibit SPT activity. This system provides a sensor for intracellular ceramide concentrations for sphingolipid homeostatic regulation. Combining the newly elucidated Arabidopsis SPT structure and mutant characterization, we present a model for the role of the two functionally divergent Arabidopsis ceramide synthase classes to produce ceramides that form repressive (trihydroxy LCB-ceramides) or non-repressive (dihydroxy LCB-ceramides) ORM interactions to influence SPT activity. We describe how sphingolipid biosynthesis is regulated by the interplay of ceramide synthases with ORM-SPT when “enough is enough” and override homeostatic suppression when “enough is not enough” to respond to environmental stimuli such as microbial pathogen attack.

Sphingolipid Imbalance Produces an Increase in the Activity of the Plasma Membrane H^+-ATPase from Arabidopsis Seedlings

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Sphingolipids constitute one third of the plant plasma membrane lipids (Bahammou et al. 2024. Plant J. May 18). Presumably, their abundance, together with structural features, impact properties and proteins of the membrane. In this work, we have studied the effect of sphingolipids on the activity of the H^{+-}ATPase, an essential primary pump of the plasma membrane from plants. Our approach was to take advantage of an Arabidopsis mutant (*lcb2b hp/lcb2a*), which shows decreased levels of total endogenous sphingolipids upon silencing of the gene encoding one of the subunits (LCB2b) from the serine palmitoyltransferase, which catalyzes the first reaction of sphingolipid synthesis. This subunit is a necessary component of one dimeric form that yield an active enzyme. In this line, the other subunit (LCB2a) is not expressed. As a consequence, the rosette total sphingolipids decreased down to 64% as compared to the wt plants at certain time (Dietrich et al. 2008. Plant J, 54: 284-298). However, total sphingolipids from the plasma membranes isolated from adult plants of the *lcb2b hp/lcb2a* line increased 2-fold. When the vanadate sensitive H^+-ATPase activity (correspondent to the plasma membrane ATPase) was studied in microsomal fractions from the leaf seedlings, the activity increased 2-fold as compared to the controls (wt and non-silenced lines). This effect was dependent on the time elapsed from the addition of the silencing inducer. The activity measured in the roots from the seedlings was unaltered. When the levels of the H^+-ATPase were estimated by Western blot, same amounts were found in the microsomal fractions from all Arabidopsis genotypes. Reconstitution of the microsomal fractions with an extract of endogenous sphingolipids revealed that some species may be involved in the increase of activity of the H^+-ATPase. These results suggest that membrane bulk- or adjacent-sphingolipids have an important role in the activity of this H^+ pump.

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Studying the role of sphingolipids in membrane trafficking and plasma membrane dynamics in plants

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The plant cell membrane functions as a platform for signal perception from the environment and other cells. This membrane constantly changes its molecular composition of lipids and proteins in response to developmental and environmental signals in a process known as membrane remodeling that is critical for plant adaptation and survival. Sphingolipids are abundant lipids in plant membranes that contribute to development and signaling during stress conditions such as freezing, drought, and pathogen infection. This project aims to understand how changes in sphingolipids levels in Arabidopsis mutants, affect plasma membrane dynamics and trafficking processes such as, endocytosis and endosomal trafficking.

ORM proteins (ORM1 and ORM2) are negative regulators of sphingolipid biosynthesis in eukaryotes. Plants expressing an ORM1 variant with one amino acid deletion in the *orm2*$^{-/-}$ background are viable but do not progress beyond the seedling stage. This mutant is strongly compromised in the regulation of sphingolipid synthesis and hyperaccumulates ceramides.

Glucosylceramides consist of a glucose head group attached to a ceramide backbone. Glucosylceramides together with glycosylinositolphosphoceramides (GIPCs) are the two major classes of sphingolipids in plants. The glucosylceramide synthase mutant (*gcs-1*) has higher concentration of GIPCs, but lacks glucosylceramides. Analyses by high-pressure freezing/freeze substitution followed by transmission electron microscopy showed that accumulation of sphingolipids in *orm* mutants affects endosomal trafficking and plasma membrane dynamics. Compared to wild type, mutant cells that accumulate ceramides showed deep invagination in their plasma membrane, smaller multivesicular endosomes containing high density of abnormal intralumenal vesicles, and accumulation of extracellular membranes. In the case of the *gcs-1,* multivesicular endosomes showed larger intraluminal vesicles compared to wild type. These results suggest the abnormal sphingolipid composition affect membrane trafficking pathways.

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Characterization of Δ4-unsaturated sphingolipid long-chain bases in *Lotus japonicus*

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In plants, the variety and structure of sphingolipid long-chain bases (LCBs) in glucosylceramides (GlcCers) are more complicated than those in free ceramides (Cers) and glycosylinositolphosphoryl ceramides (GIPCs). One possibility to explain this complexity in the structure of sphingolipid LCBs of GlcCers is elevated amounts of 8-*cis* sphingolipid LCBs in GlcCers compared with 8-*trans* forms. We have previously reported that 8-*cis*-unsaturated isomers of 4-hydroxy-8-*cis*-sphingenine [*i.e.* t18:1(8c)] and 4-*trans*, 8-*cis*-unsaturated isomers of sphingadienine [*i.e.* d18:2(4t,8c)] were major components in GlcCers from the leaves of *Lotus japonicus* (*J. Plant Res.* 122: 415-419, 2009). In this study, we focused on the biosynthetic pathway of sphingadienine; the role of Δ4-unsaturated sphingolipid long-chain bases was investigated in *Lotus japonicus*. Sphingolipid profiles in *L. japonicus* mutants for the sphingolipid Δ4-desaturase (*LjSD4D*), *sd4d* were determined by liquid chromatography mass spectrometry (LC-MS/MS) analysis. GlcCers molecular species having d18:2(4t,8c) and d18:2(4t,8t) with hydroxy palmitic acid were detected in wild-type as major components. Conversely, the *sd4d* plants lacked these compounds, nevertheless the levels of GlcCer molecular species having 8-*cis/ trans*-unsaturated isomers of 8-sphingenine [*i.e.* d18:1(8c) and d18:1(8t)], t18:1(8c) and t18:1(8t) were substantially unchanged between wild-type and *sd4d* plants. As a results, *sd4d* lines had reduced GlcCer levels compared with the wild type. The levels of free Cers and GIPCs were substantially unchanged between wild-type and *sd4d* plants. The *sd4d* plants were found to be phenotypically normal. No difference in the response to Rhizobium inoculation was observed between wild-type plants and *sd4d* mutants.

Embracing Variability in Dynamic Metabolic Modeling: Novel Insights into *Arabidopsis thaliana's* Sphingolipid Biosynthesis

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Sphingolipids play a vital role in plant development and stress responses, driving a growing interest in the regulatory mechanisms of the sphingolipid pathway. However, the regulatory behavior of this pathway is not fully understood. This study investigates the regulatory dynamics of the sphingolipid pathway, focusing on *de novo* sphingolipid biosynthesis and homeostasis in *Arabidopsis thaliana* cell cultures. This aims to shed light on essential metabolic mechanisms. Nonetheless, detecting sphingolipids in metabolomics presents challenges due to temporal data variability, especially in plants. Initially, we utilized nitrogen-15 (^{15}N) isotope labeling to generate temporal dynamic metabolomic data with significant variations. To accurately measure the turnover fluxes of sphingolipids, we adopted a quantitative dynamic modeling approach, a **r**egularized and constraint-based **D**ynamic **M**etabolic **F**lux **A**nalysis (**r-DMFA**) framework. This innovative r-DMFA approach marks a significant progress in predicting time-course metabolic changes following enzymatic disruptions with precision, surpassing traditional methods. It efficiently handles variations between samples, thus improving the reliability and precision of our analyses. This method adeptly uses transient metabolomics data to unravel complex metabolic pathways, leading to experimentally testable hypotheses that could notably reduce the necessity for extensive future experimental efforts. Our study shows the delicate balance between the synthesis and breakdown of sphingolipids in cell cultures, highlighting their critical function in cellular homeostasis. We found a specific preference for *de novo* synthesis over recycling of sphingolipids before reaching the mid-exponential phase. Furthermore, despite the challenges of data variability, our analysis underscores the essential roles of enzymes like sphingoid-base hydroxylase, long-chain-base kinase, and glucosylceramide synthase. Interruptions in these enzymes significantly impact cell survival and programmed cell death, emphasizing their key role in maintaining the balance of sphingolipid metabolism. Therefore, this study not only deepens our understanding of sphingolipid metabolism but also illustrates the power of dynamic modeling in analyzing complex metabolic pathways.

Genome-wide characterization of plant CTP:phosphocholine cytidylyltransferases through evolutionary, biochemical and structural analyses

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Cytidine triphosphate:phosphocholine cytidylyltransferase (CCT) is a key regulatory enzyme in the *de novo* biosynthesis of phosphatidylcholine (PC), a major phospholipid class in most cell membranes. The biological and biochemical functions of CCTs have been extensively studied in mammals, however, in planta, a comprehensive examination of the evolutionary relationships of this important enzyme at the genome-wide level and its *in vivo* function in PC synthesis is still lacking. Also, a previous study has revealed that CCT1 from the model plant *Arabidopsis thaliana* (AthCCT1) has a major phosphorylation site serine 187 (Ser-187) and its enzyme activity can be reduced by 70% under phosphorylation at Ser-187. In the current study, we employed comprehensive phylogenetic analysis to disclose the evolutionary history, genetic and functional relationships, as well as structural variations among CCTs in the green lineage. Meanwhile, we combined biochemical and structural analyses to examine how the phosphorylation effect at Ser-187 affects the enzyme dynamics of AthCCT1. The results have shown that CCTs are widely present among plant groups including chlorophytes, bryophytes, lycophytes, monilophytes, gymnosperms, early-diverging angiosperms, monocots, and eudicots. Furthermore, two sites of AthCCT1 (Leucine 59 and Glutamine 156) have undergone positive selection, and they play an important role in maintaining its enzyme function and activity. In summary, the current study investigated the potential mechanism of how the phosphorylation site Ser-187 regulates the dynamics of AthCCT1, along with the evolutionary history and diversity of CCTs within the green lineage, which expands our knowledge of this important enzyme on a genome-wide scale.

3. Fatty acid and glycerolipid: biosynthesis, modification, biosynthetic evolution

Jyoti	Behera
Samuel	Decker
Yasmeen	Elharis
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Jordan	LaChance
Ida	Lager
Maneesh	Lingwan
Maneesh	Lingwan
Hui	Liu
Chunyang	Lu
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Rajeev	Ranjan
Noemí	Ruiz-López
Sarah	Salomon
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Jaruswan	Warakanont
Tingyuan	Xiao

ISPL abstract

Title: Genomic Resources to Understand Genetic Mechanisms of Oil Biosynthesis in *Camelina Sativa*

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Abstract Text:

Camelina sativa is an oilseed that has been cultivated for hundreds of years but has recently gained notice as a new crop for biofuels and as a source for nutritionally important omega-3 fatty acids such as linolenic acid. After assembling a new camelina genome using the spring cultivar Suneson, resequencing data from a 257-member recombinant inbred line (RIL) population and a 212-line diversity panel were used to search for quantitative trait loci (QTLs) associated with traits such as seed oil content and fatty acid composition. Out of a pool of over 5.6 million SNP and InDel markers, dozens have been discovered that are linked to a variety of genes, from transcription factors to retrotransposons and transport proteins. Such a large number of markers also enabled the calculation of a robust linkage decay statistic of ~50-100kb across the entire genome. When calculating local marker linkage using a smaller window during analysis with programs such as GAPIT, much smaller linkage decay windows of 4-20kb are obtained. Generating haplotypes using the resequencing data and the newly assembled genome, it is possible to search for alleles and further refine candidate gene selection without additional sequencing, therefore facilitating gene discovery and characterization. Altogether, these genomic tools should provide insight into camelina genetics and fatty acid production and enable the development of next-generation advanced varieties.

Elucidating Cyclic Fatty Acid Biosynthesis and Compartmentalization in Cotton

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Cotton is an important oilseed crop within the world economy. While cotton fiber for the manufacture of textiles is generally the economic driver for cotton, cottonseed value-added products provide an important source of calories for humans and livestock, as well as increased use for industrial bioproducts such as biofuels and lubricants. Cotton is an unusual crop in that it accumulates cyclopropane and cyclopropene fatty acids (CFAs). These CFAs accumulate to varying amounts in a range of tissues from seeds to roots, stems, petioles, and leaf blades. Notably, efforts to engineer and produce CFAs in model plants and other crop systems have been met with little to no success because of a lack of understanding within the biochemical pathways and their compartmentalization in cotton (despite evidence of these compounds for 60+ years). Here, we will present our progress on discovering the enzymes that contribute to CFA metabolism with structural insights into the evolution of specialized enzymes. Furthermore, we will detail the protein machinery required to package cyclic FAs into cytoplasmic lipid droplets facilitating the spatial distribution of CFA in cotton tissues. In turn, these results start to address knowledge gaps on how cotton (as opposed to most other plants) may produce these reactive chemicals, and reveal new strategies to enable metabolic engineering of these high-value compounds in both cotton and other plant systems.

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The Interplay of Diacylglycerol Acyltransferases and Phosphatidylcholine:diacylglycerol Cholinephosphotransferases Facilitates High Hydroxy Fatty Acyls Content in Triacylglycerols

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Castor bean has about 90% of the hydroxy fatty acid ricinoleic acid (Δ12-hydroxy-octadec-9-enoic acid, HFA), in its seed oil (triacylglycerol, TAG). HFA is formed from 18:1 while esterified to phosphatidylcholine (PC) by the enzyme castor bean oleate hydroxylase (FAH12). PC is a central molecule, both as a carrier of acyl groups exported from the plastid and as substrate for a large number of fatty acid modifications, but is also one of the major membrane lipids. Interestingly, membrane lipids in castor bean only constitute of 5% HFA, showing that the plants must have mechanisms for channeling HFA into TAG and excluding it from membrane lipids. Phosphatidylcholine:diacylglycerol cholinephosphotransferases (PDCT) regulate the fatty acid composition of seed oil by interconversion of diacylglycerols (DAG) and PC. We have recently biochemically characterized castor bean PDCT and showed that it utilized DAG with ricinoleoyl groups similarly to DAG with common acyl groups and show a 10-fold selectivity for DAG with one ricinoleoyl group over DAG with two ricinoleoyl groups. We extended our investigation of castor bean TAG involved enzymes by *in-vitro* characterize castor bean seed specific expressed PDAT (PDAT1.2) and showed that it was not capable to acylate DAG with no ricinoleoyl groups. However, the enzyme did not discriminate between DAG with one hydroxy group and two hydroxyl groups, either as single or mixed DAG species. Since castor bean DGAT2, although preferring DAG with 2 hydroxy groups, also acylated DAG with one hydroxy groups at a significant rate in assays with mixed DAG substrates, it further strengthened the hypothesis that castor bean PDCT play a crucial role in maximizing the amount of TAG with only hydroxy fatty acids. Further, we show that castor bean PDAT1.2 is involved in remodeling TAG by re-acylating DAG formed from microsomal TAG by castor bean TAG lipases.

Integrated Multi-omics and Cross-species Analytics for Sustainable Brassicaceae Biofuels and Bioproduct

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The "**B5: Bigger Better Brassicaceae Biofuels and Bioproducts**" initiative is at the forefront of advancing sustainable liquid fuels through the enhancement of non-food Brassicaceae oilseeds, notably camelina and pennycress. This project focuses on tailoring fatty acid structures, particularly aiming to produce oils abundant in medium-chain fatty acids (C8-C14), essential for **sustainable aviation fuel**. Employing a comprehensive systems biology approach, we delve into the metabolic specialization observed in Cuphea species, renowned for their accumulation of medium-chain fatty acid-rich oils. Furthermore, our investigation extends to analyzing engineered camelina and pennycress lines aimed at enhancing C10 oil production.

Our methodology integrates transcriptomics, lipidomics, proteomics, and phenotypes via a comprehensive **multi-omics** integrative analysis, coupled with **cross-species** comparisons. Through advanced computational tools, we explore the entire transcriptome of camelina, unraveling DEGs patterns crucial for fatty acid biosynthesis pathways. Additionally, we employ multiomics analysis, integrating transcriptomics, proteomics, lipidomics data, to elucidate the intricate relationships between **gene expression, lipid composition, protein profiles,** and **phenotype**. This holistic approach provides a comprehensive understanding of components and specific markers that are most relevant for medium-chain fatty acid synthesis, along with their directionality linkage to the phenotype and their functions and **pathways**, offering insights into key biological and molecular processes for targeted engineering. These insights will be accessible through our KBCommons, 3D Omics, and CCMT Tools, all of which serve as visualization and analysis portals.

By studying both **wild types** and **genetically modified lines**, we aim to decipher the biochemical intricacies driving optimal oilseed development. Our interdisciplinary approach not only addresses the urgent need for sustainable fuels and oils with defined chemical structures but also lays the groundwork for **next-generation oilseed engineering** principles. Ultimately, this research aims to provide diversified crop options

for US farmers and expanded feedstock choices for the bioeconomy, contributing to a more **sustainable and resilient energy future**.

This research is supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0023142.

Unraveling WRINKLED2: A Novel Player in Heart-Healthy Oil Synthesis in Avocado Mesocarp

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WRINKLED1 (WRI1) and its paralogs, except for WRI2, transcriptionally regulate *de novo* fatty acid synthesis in seed tissues. In contrast to *Arabidopsis* seeds, where WRI2 is not functional, the basal angiosperm avocado (*Persea americana*) mesocarp exhibits high expression levels of both *WRI1* and *WRI2*. Structural analyses revealed that *Pa*WRI2 possesses a single intact APETALA2 DNA-binding domain and exhibits relative stability due to the absence of a C-terminally located PEST motif and disordered region, distinguishing it from *At*WRI2. Through transient expression assays, we demonstrated the functionality of *Pa*WRI2, showing its ability to drive triacylglycerol (TAG) accumulation in *Nicotiana benthamiana* leaves. Moreover, co-infiltration of *PaWRI2*, alongside *PaWRI1*, *PaDGAT1*, and *PaPDAT1*, resulted in a substantial increase in total lipid levels (>4-fold) and TAG content (>260-fold) in 'benth' leaves, with an elevated proportion of oleic acid (C18:1). Further quantitative real-time PCR analyses involving over 46 glycolysis, fatty acid synthesis, and TAG assembly genes revealed the trans-regulatory roles of *Pa*WRI2, in conjunction with *Pa*WRI1, particularly enhancing the expression of plastidial glycolysis genes, such as *ENO1* (by >50-fold), revealing a synergistic effect on fatty acid biosynthesis. Despite the upregulation of plastidial fatty acid biosynthesis genes, low expression of *FATA* and *FATB* was observed in leaves, presenting a bottleneck for storage oil biosynthesis. Notably, yeast-one-hybrid assay demonstrated a unique characteristic of *Pa*WRI2, showcasing autoregulation and regulation by PaWRI1. Both transcription factors transactivated specific avocado lipid biosynthesis genes, exhibiting preferential and selective binding to the AW-box in their upstream proximal promoters. Further enhancement of trans-activation abilities for WRI1 and WRI2 was achieved through C-terminal domain deletions, resulting in improved oil accumulation in *N. benthamiana* leaves. Thus, our study elucidates the mechanistic role of *Pa*WRI2, a function potentially lost in modern angiosperms. These findings establish new candidates for heart-healthy oil enhancement in other plants and tissues through biotechnological approaches.

Improving plant cell type annotation for scRNA-seq data by utilizing deep-learning-model based approaches

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Cell type annotation is fundamental for interpreting scRNA-seq data in plant and biomedical research. It enables a detailed understanding of the cellular composition, function, and dynamics within tissues, leading to significant insights into biology, development, and responses to environmental changes. Current methods for cell type annotation employ dimension reduction technique such as PCA followed by unsupervised clustering algorithms like Louvain or Leiden to cluster cells to groups, then cell type for each group is determined according to its marker genes. However, due to pervasive dropout events causing expression matrix filled with many false zero counts, conventional clustering methods often fail to produce high quality clustering.

Recently a model-based deep learning approach called scDeepCluster [1] has been developed to improve this. It simultaneously learns feature representation and clustering via explicit modeling of scRNA-seq data generation. Extensive testing on human cell scRNA-seq data has demonstrated that it outperforms alternative methods. We have applied this technique to several human and mouse scRNA-seq datasets generated by our collaborators and have observed its advantages over alternative methods. In this study, we have applied scDeepCluster to several plant scRNA-seq datasets from scPlantDB [2] and compared it with alternative clustering approaches. For each clustering method, the same process to extract marker genes and determine cell types is used. We show that cell type annotation accuracy has been clearly improved by using scDeepCluster based clustering over other clustering methods.

Our future work to improve plant cell type annotation is by building plant scRNA-seq foundation model, inspired by scBERT and scGPT work done for human cells. We are going to address some unique challenges such as genome difference and data imbalances across diverse plant species. Also, in future the scRNA-seq clustering and annotations results will be available as new tools and capacity added into our in-house developed KBCommons framework.

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[2] Z. He, et. al. "scPlantDB: a comprehensive database for exploring cell types and markers of plant cell atlases", Nucleic Acids Research, Jan. 2024.

Lipid Analysis of *Physaria fendleri* for Improving Hydroxy Fatty Acid Production

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Hydroxy fatty acids (HFAs) are widely used in chemical, food, and cosmetic industries. The current commercial source of HFA is Castor oil which contains 90% ricinoleic acid (18:1OH). However, the United States banned the production of castor oil because of toxic compound in the seeds called “ricin”. Lesquerella (*Physaria fendleri*), native from the Southwest of the United States, is a promising alternative crop to Castor because its seeds do not have ricin and contain ~30% oil (w/w) of which 60% is lesquerolic acid (20:1OH). However, for lesquerella to become a more economically viable source of HFAs, its seed oil quantity must be further improved.

This study describes the workflow to identify and quantify different classes of lipids in mature seeds of two natural accessions of lesquerella with contrasting oil content—namely PI 596432 and W6—using state-of-the-art LC-MS/MS. A targeted lipid panel was optimized and included: i) a polar method that detects 12 glycerophospholipids classes (Phosphocoline, Phosphoethanolamine, Phoshoglycerol, Phosphatidylinositol, Phosphatidylserine, Lyso-phosphocoline, Lyso-phosphoethanolamine, Lyso-phoshoglycerol, Lyso -phosphatidylinositol, Lyso- phosphatidylserine, Monogalactosyldiacylglycerol, digalactosyldiacylglycerol) separated by their headgroup, and ii) a non-polar method that monitors three glycerolipid classes (Monoglycerides, Diglycerides, Triglycerides) separated based on their non-polar fatty acyl. Results from this study are helpful to improve our understanding of the metabolism underlying HFA synthesis in lesquerella seeds; the long-term goal of this research is to enhance the production of unusual fatty acids to meet human needs for industrial applications and biofuels.

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Biosynthetic mechanisms of omega-3 polyunsaturated fatty acids in microalgae

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Marine microalgae are the primary producers of ω3 polyunsaturated fatty acids (PUFAs), such as octadecapentaenoic acid (OPA, 18:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) for food chains. However, the biosynthetic mechanisms of these PUFAs in the algae remain elusive. To study how these fatty acids are synthesized in microalgae, a series of radiolabeled precursors were used to trace the biosynthetic process of PUFAs in *Emiliania huxleyi*. Feeding the alga with ^{14}C-labeled acetic acid in a time course showed that OPA was solely found in glycoglycerolipids such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) synthesized plastidically by sequential desaturations while DHA was exclusively found in phospholipids synthesized extraplastically. Feeding the alga with^{14}C-labeled α-linolenic acid (ALA), linoleic acid (LA) and oleic acid (OA) showed that DHA was synthesized extraplastidically from fed ALA and LA, but not from OA, implying that the aerobic pathway of DHA biosynthesis is incomplete with missing a Δ12 desaturation step. The in vitro enzymatic assays with ^{14}C-labeled malonyl-CoA showed that DHA was synthesized from acetic acid by a PUFA synthase. These results provide the first and conclusive biochemistry evidence that OPA is synthesized by a plastidic aerobic pathway through sequential desaturations with the last step of Δ3 desaturation, while DHA is synthesized by an extraplastidic anaerobic pathway catalyzed by a PUFA synthase in the microalga.

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Phase separation of a MYB transcription factor mediates seed oil biosynthesis in Arabidopsis

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The MYB family transcription factors play crucial roles in governing developmental processes, metabolic pathways, and responses to various stresses in plants. However, the involvement of MYBs in the regulation of fatty acid accumulation in seeds has remained largely unclear. Here, we identified a MYB transcription factor that regulated the expression of *FATTY ACID ELONGATION1* (*FAE1*) in Arabidopsis. Transgenic Arabidopsis overexpressing the *MYB* showed altered *FAE1* expression, seed oil content, and fatty acid composition. Transactivation assay indicated that the MYB repressed the activity of the *FAE1* promoter. Electrophoretic mobility shift assay (EMSA) further demonstrated that *FAE1* was a direct target of the MYB. Through RNA sequencing analysis, we found significant changes in diverse genes involved in fatty acid biosynthesis and triacylglycerol assembly. Intriguingly, we determined that the MYB exhibited hallmarks of intrinsic disorder and could form phase-separated condensates with liquid-like characteristics, which was vital for regulation of the expression of its targets. Removal of a domain in the MYB eliminated MYB phase separation and consequently, its repression activity. Together, our findings suggest that the MYB condensate formation constitutes a molecular basis underlying fine-tuning seed oil biosynthesis.

Unraveling Metabolic Patterns in Developing *Physaria fendleri* Embryos, a Promising Alternative Oilseed Crop Rich in Hydroxy Fatty Acids.

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Oils high in unsaturated hydroxy fatty acids (HFA), such as castor, are highly valued for use in cosmetics, greases, paints, soaps, and plastics. Domestic production of castor oil has been banned in the United States (US) due to the release of the toxic byproducts, ricin and ricinin during extraction. Castor stands as the only source of HFA in industry, leading to the import of approximately 80 million pounds of castor oil by the US each year.

Physaria fendleri, a member of the Brassicaceae family, is an alternative oilseed crop native to the southwestern United States with toxin-free embryos rich in HFA. The species has superior low-temperature properties and can be grown during winter in a double cropping system. However, to achieve widespread usage in industry, there is still a need for metabolic engineering to enhance the efficiency of oil production. To study the metabolic network of *P. fendleri*, comparison of two naturally occurring accessions that produce embryos with contrasting oil content was conducted, namely W6 20859 (HO, high oil ~ 35% embryo dry weight) and PI 596432 (LO, low oil ~ 29% embryo dry weight).

We hypothesize that accessions with higher oil content exhibit greater involvement of metabolites and pathways crucial for oil production. To test this hypothesis, HO and LO embryos were collected at five different stages of development between 28 and 42 days after pollination and processed for LC-MS/MS-based targeted metabolomic analyses. Analyzing the developing embryos of HO and LO through targeted metabolomics yields quantifiable dynamic metabolite profiles that may offer valuable insight into the observed contrast in oil content at maturity. We anticipate that the results generated through this study will identify metabolites and pathways that are important for fatty acid synthesis, which will ultimately guide metabolic engineering efforts aiming to improve oil content in *P. fendleri*.

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The role of TOR Sensor Kinase in the Regulation of Lipid Synthesis

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In eukaryotic cells, target of rapamycin (TOR) a conserved sensor kinase integrates a diverse set of environmental cues, such as growth factor signals, energy ability and nutritional status, to direct cell growth. In plants, TOR is strongly activated by light and sugars and regulates a wide range of cellular functions, including protein synthesis, and metabolic homeostasis in which TOR integrates signals from multiple pathways. Lipid metabolism is important for plant growth and development. Previous studies showed that lipid triacylglycerol (TAG) accumulated in microalgae and Arabidopsis thaliana after extended TOR inhibition either because TOR is a negative regulator of TAG, or as a more general stress response. To elucidate the primary regulatory roles of TOR in lipid metabolism, we track lipid changes in plants in which TOR protein levels or activity are changed for short time periods. We found that transient expression of TOR significantly elevated total fatty acids (TFAs) in *Nicotiana benthamiana*. On the other hand, TFAs were decreased when TOR was inhibited by the specific TOR inhibitor Torin2 in both Arabidopsis seedlings and *Brassica napus* suspension cells after as short as eight hours of treatment. Similarly, TFAs were significantly lower in a TOR estradiol-inducible gene silence line after one day of estradiol treatment relative to untreated controls. Taken together, we view TOR as playing a positive role in regulating lipid synthesis.

Interrogating *Camelina sativa* seed developmental transcriptomic data sets to decipher the impact of Omega-3 fatty acid metabolic engineering.

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This study presents a comparative analysis of two RNA-seq experiments aimed at elucidating the seed transcriptional dynamics between *C. sativa* wildtype (WT) and lines engineered (using seven desaturase and elongase elements) to produce novel long chain polyunsaturated fatty acids e.g., docosahexaenoic acid (C22:6 n-3). Specifically, we investigated transgene expression and identified significant transcript changes that could impact product characteristics and oil yield. Differential expression analysis was conducted across a range of seven developmental time points in two separate experiments with and without the seed coat. Our findings reveal several key insights:

Lipid metabolism: Trends in transcripts involved in lipid biosynthesis were more pronounced in later sampling stages, although early indications of these trends were evident.
Proteolysis Transcript Regulation: Early upregulation of proteolysis transcripts was identified, suggesting potential regulatory mechanisms.
Impact on Lipid Biosynthetic Network: Surprising effects on the wider lipid biosynthetic network were observed including changes in transcription factors, indicating complex regulatory interactions.

Overall, our study provides valuable insights into the transcriptional regulation of DHA1 Camelina lines compared to WT counterparts, such as a reduction in seed storage transcripts in the transgenic lines as the seed matures. The highlights the importance of considering other transcriptomic effects driven by the transgene expression. This data has utility in understanding metabolic pathways and optimizing desired traits.

Unveiling Novel Transcription Factors Orchestrating Lipid Biosynthesis in Seeds

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Plant oils, crucial for human consumption and biofuel production, are derived from fatty acids and stored as triacylglycerol (TAG). Despite extensive research on fatty acid biosynthesis and TAG assembly, the regulatory network driven by transcription factors (TFs) remains largely unknown. Using over 1500 mRNA sequencing data and computational tools, our study has constructed an Organ-Delimited Gene Regulatory Network (OD-GRN) for five Arabidopsis organs. We have inferred the Seed-GRN to predict TF regulators of lipid biosynthesis in Arabidopsis seeds. Experimental testing has validated the function of seven TFs, revealing that their manipulation can significantly alter seed lipid content. Knockouts of *MybS2*, *Div2*, *AGL18*, *SPL12*, and *TGA4* reduce seed lipid content, while overexpression of *MybS2*, *AGL18*, *SPL12, bHLH93*, *CESTA*, and *HB25* leads to increased total lipid content. Furthermore, mRNA sequencing of *MybS2* overexpression plants unveiled its role in inducing the expression of purple acid phosphatase (PAPs) genes in leaves and lipid biosynthesis genes in seeds. We propose that *MybS2*-induced PAPs gene expression may enhance carbon flux from leaves to seeds, stimulating the expression of FA biosynthesis genes in seeds and ultimately resulting in higher seed oil accumulation in *MybS2* overexpressing plants. Therefore, this study provides a new tool for gene discovery and significantly enhances our understanding of the regulation of fatty acid biosynthesis.

Characterizing SYT6, a lipid transfer protein at the secretory pathway.

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The SYT6 protein from *A. thaliana* has recently been identified as a lipid transfer protein localized at membrane contact sites (MCS). MCS are regions where membranes of two organelles closely approach without membrane fusing. Historically, research has focused on endoplasmic reticulum (ER) and plasma membrane MCS, but recently MCS involving the ER and other organelles have come to light. SYT6 is a plant-exclusive protein exhibiting a modular structure shared with mammalian Extended-Synaptotagmins and other plant synaptotagmins, such as SYT1.

Our ongoing experiments suggest that SYT6 anchors itself to the ER via its transmembrane domain, contains a lipid trafficking domain (named SMP) and attaches to specific trans-Golgi Network (TGN) vesicles through its C2 domains and coiled-coil domain. These observations make SYT6 particularly intriguing, as its physiological roles remain unclear.

Currently, our focus is on studying SYT6 to uncover its expression, subcellular localization and most importantly, its function. Confocal imaging, has confirmed SYT6 attachment to the ER and to vesicles in continuous motion, suggesting involvement in secretory trafficking. Co-Immunoprecipitation and BiFC experiments, have confirmed the interaction between SYT6 and specific TGN proteins linked to the independent Golgi TGN (GI-TGN). Preliminary findings indicate a correlation between SYT6, and exocytosis. Furthermore, *syt6* mutant displays altered negative gravitropism. Altogether, these findings suggest that SYT6 represents a novel ER-TGN CS protein that may play a role in secretory trafficking.

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Betaine Lipids production in higher plants

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During evolution, photosynthetic organisms have developed specific strategies to respond to the fluctuations of their environment. Among these various external stresses, nutrient scarcity like phosphate starvation is frequently observed and leads to drastic changes in the lipid metabolism. Phosphorus is a fundamental nutrient for cell physiology and is highly remobilized during such situation to maintain growth and development. During phosphate deprivation, phospholipids (PL) are degraded to provide phosphate resource for the cell. These lipids are then replaced by non-phosphorus lipids to maintain membrane integrity. In plants, digalactosyldiacylglycerol (DGDG) synthesized in chloroplast envelope is known to replace the phospholipid phosphatidylcholine (PC) in extra-plastidial membranes (Jouhet *et al*., 2004). Another remodelling mechanism can occur in algae, a diverse group of photosynthetic organisms living in ocean and freshwaters. In these organisms, the PC degraded during phosphate starvation is replaced by betaine lipids (BL), a poorly known class of glycerolipid present only in algae, some fungi and lower plants. It has been shown that BL can entirely replace PC in extra-plastidial membranes in marine algae (Abida *et al*., 2015) whereas DGDG can only replace 20% of the PC in terrestrial plants. Thus, BL seem to be a better substitute for PC during phosphate starvation and their disappearance in higher plant raises many questions. The goal of this project is to have a better understanding of BL roles and evolution in plant kingdom by overexpressing BL in plants. Results presented here will show the impact of DGTS (BL particular species) production in *Arabidopsis* transgenic lines and *Nicotiana benthamiana* wild type leaves.

Characterization of digalactosyldiacylglycerol synthases in the model diatom *Phaeodactylum tricornutum*

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Oceans are the "lungs of the Earth." They are characterized by a vast biodiversity of photosynthetic organisms. Diatoms, a large group of unicellular photosynthetic microalgae, are the primary producers in the ocean. Alone, they are responsible annually for approximately 40% of photosynthetically fixed CO_2 in the ocean and 20% of the oxygen produced on Earth. Diatoms originate from secondary endosymbiosis, and their plastid is bounded by four membranes instead of two. In recent decades, they have attracted increasing attention because of their ability to produce high-value biomolecules, mainly lipids. However, little is known about lipid synthesis in diatoms, and it has yet to be studied in detail. In photosynthetic organelles, galactolipids are highly abundant and crucial for maintaining optimal photosynthesis efficiency. They are composed of a glycerol backbone, two fatty acids (FAs), and one or two galactosyl moieties (MGDG or DGDG, respectively). In *Phaeodactylum tricornutum*, a model diatom, MGDG synthases (*PtMGDα, PtMGDβ, and PtMGDγ*) have been recently characterized (Gueguen et al., submitted). Yet, DGDG synthases need to be studied. In *Phaeodactylum tricornutum*, four isoforms were identified (*PtDGDα, PtDGDβ, PtDGDγ and PtDGDδ*). In this study, the four *DGD* genes were functionally characterized. After each gene had been subcellularly localized by eGFP fusions, they were knocked out using the CRISPR-Cas9 technology. The selected mutants were morphologically, physiologically, and lipidomically phenotyped under different culture conditions. A striking phenotype was observed for *PtDGDα* knock-out, with a consistent reduction of 20:5-containing DGDG species, showing potential substrate specificity. This study also highlights the first subcellular localization of a glycerolipid in diatoms through immunolabeling of DGDG and the evolutionary history of DGDG synthases in diatoms.

Nitrogen starvation leads to TOR kinase-mediated downregulation of fatty acid synthesis in the algae *Chlorella sorokiniana* and *Chlamydomonas reinhardtii*

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Abstract

The accumulation of triacylglycerol (TAG) as a storage compound in eukaryotic algae has been the subject of extensive studies over the last 50 years. The model industrial alga *Chlorella sorokiniana* accumulates TAG and other storage compounds under nitrogen (N)-limited growth. Previously we used transcriptomics to explore the regulation of TAG synthesis in *C. sorokiniana*. Surprisingly, our analysis showed that the expression of several key genes encoding enzymes involved in plastidic fatty acid synthesis are significantly repressed. Metabolic labeling with radiolabeled acetate showed that *de novo* fatty acid synthesis is indeed downregulated under N-limitation. Likewise, inhibition of the Target of Rapamycin kinase (TOR), a key regulator of metabolism and growth, decreased fatty acid synthesis. We compared the changes in proteins and phosphoprotein abundance using a proteomics and phosphoproteomics approach in *C. sorokiniana* cells under N-limitation or TOR inhibition and found extensive overlap between the N-limited and TOR-inhibited conditions. We also identified changes in the phosphorylation levels of TOR complex proteins, TOR-kinase and RAPTOR, under N-limitation, indicating that TOR signaling is altered. Our results indicate that under N-limitation there is significant metabolic remodeling, including fatty acid synthesis, mediated by TOR signaling. We find that TOR-mediated metabolic remodeling of fatty acid synthesis under N-limitation is conserved in the chlorophyte algae *Chlorella sorokiniana* and *Chlamydomonas reinhardtii.*

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SAD, FAD5a and FAD6 dependent fatty acid desaturation in Chlamydomonas grown under copper deficiency

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Copper is an essential micronutrient in photosynthetic organisms because it is a cofactor in essential proteins that are involved in many biological functions including plastocyanin in photosynthesis and cytochrome oxidase in respiration. In a microalga, *Chlamydomonas reinhardtii*, deprivation of copper leads to slightly reduced growth and changes in various cellular metabolisms. In contrast to land plants, Chlamydomonas replaces the Cuproprotein plastocyanin with the heme containing cytochrome c_6 when copper becomes limiting. Previous RNA sequencing data suggested upregulation of genes encoding proteins involved in fatty acid desaturation such as Δ-9 stearoyl-ACP-desaturase (*SAD*), MGDG-specific palmitate Δ-7 desaturase (*FAD5a*), and ω-6 fatty acid desaturase (*FAD6/DES6*), when cells are grown in copper deficiency. In this work, we aim to discover the function of SAD, FAD5a and FAD6 during copper deficiency using reverse genetics (CRISPR gene editing and inducible artificial mircoRNAs) combined with lipid profiling. Preliminary results reveal that under copper deficiency, Chlamydomonas accumulates lower levels of membrane lipids; monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), diacylglyceryl-trimethylhomoserine (DGTS) and phosphatidylethanolamine (PE). On the contrary, the level of triacylglycerol (TAG) is increased. The fatty acid profile of total lipids under these conditions shows a decrease in saturated and monounsaturated fatty acids and an increase in polyunsaturated fatty acids. The most striking change is the increase in $18{:}1^{\Delta 11}$. In summary, this work aims to understand how different fatty acid desaturases and nutrient availabilities shape the fatty acid profile in Chlamydomonas. We focus on cells that experience copper deficiency and suggest that perhaps subtle modifications to the bioenergetic membrane are necessitated by the replacement of the soluble electron carrier plastocyanin by cytochrome c_6.

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Identification of Seed Specific Promoters with A Range of Expression Strengths

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The relative expression levels of genes involved in primary or secondary metabolism can dramatically affect product profile and titer. To augment the suite of very strong seed specific promoters (*e.g.*, napin, glycinin) currently used for oil seed engineering, we have mined existing gene expression databases to identify 10 seed specific genes expressed at levels 0.1x and 0.01x relative to the strong seed specific promoters in these species (*e.g.*, napin orthologs). The promoter and terminator regions of these genes have been cloned to be compatible with Golden Braid 2.0 DNA assembly platform. We will evaluate the strength of this panel of promoters (including existing strong promoters) using by expressing *CpFatB2* sufficient for 14:0 synthesis and *EfDAcT* producing acetyl-TAG in camelina and pennycress. Quantifying the transgene's expression at four different stages of seed development of homozygous T3 plants with single transgene insertions using quantitative reverse transcription PCR (RT-qPCR) and measuring MCFA and acetyl-TAG accumulation in mature seed from these plants. As seed specific promoters typically function effectively across different species especially in Brassicaceae, we will initially only isolate camelina promoters but opportunistically extend to pennycress if warranted.

4. Lipid biotechnology: oilseeds, algae, vegetative organs, emerging platforms

Sania Zafar	Awan
Gavin	Chen
Grace	Chen
Agasthya Baby	Chenna Prakash
Kamil	Demski
Timothy	Durrett
Emma	Fitzgibbons
Dongxin	Huai
Daniela	Morales-Sanchez
Daniela	Morales-Sanchez
Thiya	Mukherjee
Cory	Nykiforuk
Prasad	Parchuri
Kiyoul	Park
John	Sedbrook
Dexter	White
Ross	Zirkle
Mi Chung	Suh
Yen Oh	Chan

Elucidating Fatty Acid Biosynthesis and Turnover in Camelina Seeds that Produce Medium Chain-Containing Lipids

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Biofuels that are environmentally friendly, with tailored compositions are needed to meet renewable fuel applications. Nature produces concentrated forms of diverse fatty acids that could meet this demand, if incorporated into crop plants. Previously, genes from Cuphea, that is comprised of fatty acids with a chain length of 90% C8-C12, were engineered into *Camelina sativa,* an emerging biofuel oilseed. A fatty acid thioesterase (CvFatB1), a lysophosphatidic acid acyltransferase (CvLPAAT2), and a diacylglycerol acyltransferase (CpuDGAT1) were incorporated, resulting in fatty acid compositions that contained up to 23% medium chain lengths at mid-development of seeds; however final levels were 13% of the total lipid. We hypothesize that the reduced quantity at maturity, either indicates a lower rate of medium chain biosynthesis in late development compared to other long chain fatty acids, or the plants may turn over some medium chains as a counter mechanism to the engineering efforts. Modified Camelina seeds and wild-type lines were collected over development at seven stages (10, 15, 20, 25, 30, and 35 days after flowering and maturity) and analyzed for fatty acid, protein, central metabolites, acyl carrier protein (ACP), and acyl-CoA content. Quantified ACP levels including fatty acid biosynthetic intermediates of the cycle differed over development and were not intuitive. The results likely reflect the kinetics of the CvFatB1 and the available substrates including nonacylated ACP-SH. ACP analysis combined with central metabolites and short to medium chain acyl-CoAs which can describe the dynamics of medium chain lipid metabolism will be presented.

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Photoprotective mechanism under high light intensity conducted by the up-regulation of the violaxanthin cycle and photopigment accumulation in the polar *Chlamydomonas* sp. RCC2488 *malina*

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Chlamydomonas sp. RCC2488 *malina* is a polar microalga with high PUFA and pigment production. This microalga can adapt to high light intensities (HLI) maintaining high growth rates and PUFA production. The violaxanthin cycle is known to have photoprotective effects at HLI in plants and algae by modulating the synthesis of violaxanthin and zeaxanthin. These pigments act as antioxidants in the lipid phase of the thylakoid membrane contributing to the dissipation of excess excitation energy (NPQ) in the antenna of PSII. In order to investigate the mechanisms for the HLI adaptation in *C. malina*, cultivations at 120 (LLI) and 750 μmol photons m^{-2} s^{-1} in photobioreactors were performed and compared to the model *C. reinhardtii*. *Chlamydomonas malina* maintained high growth rate at both light intensities while *C. reinhardtii* slowed it down at HLI. At HLI, the zeaxanthin/violaxanthin ratio in *C. malina* was higher than in *C. reinhardtii*. High PUFA synthesis was maintained in *C. malina* while high TAG production was observed in *C. reinhardtii*. High relative expression of violaxanthin de-epoxidase (VDR1) gene and low/basal relative expression of zeaxanthin epoxidase (ZEP/ZXE) genes were observed in *C. malina*. In contrast, there were no changes in the relative gene expression of those genes in *C. reinhardtii* at HLI as compared to LLI. The polar nature of *C. malina* allows it to adapt to HLI by favoring the synthesis of zeaxanthin, upregulating the expression of VDR1 with no stress, since the synthesis of PUFA was kept. On the contrary, *C. reinhardtti* was stressed at HLI demonstrated by its low growth rate, TAG accumulation and no pigment accumulation. Complementary studies of photosynthetic efficiency and NPQ are necessary to confirm our results. Having microalgae species with high tolerance to HLI like *C. malina* is essential in areas where such conditions limit the biomass and metabolite productivities.

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Polyunsaturated fatty acid production from the polar marine microalga *Chlamydomonas* sp. RCC2488 *malina* using potato peel hydrolysates in mixotrophic conditions

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Chlamydomonas sp. RCC2488 *malina* is a polar microalga with high PUFA and pigment production under phototrophic conditions. In order to investigate the feasibility of growing under mixotrophic conditions using an organic carbon source, cultivations of *C. malina* with glucose and potato peel hydrolysates (PPH) were performed in flasks at 120 µmol photons m^{-2} s^{-1} of light intensity. The potato peel was subjected to an acid-hydrothermal pretreatment. The product of this was separated in three treatments: PPH1, supernatant + paste; PPH2, only paste previous intensive wash step with water; PPH3, only supernatant. Then, each treatment was subjected to an enzymatic hydrolysis with amylase and amyloglucosidase. Transmembrane glucose transport, growth kinetics, macromolecular composition and lipid types and profile of *C. malina* were determined. The microalga was able to transport glucose at a rate of 0.015 μmol g^{-1} min^{-1}. Highest concentration of reducing sugar (glucose) was found in PPH1 and PPH3. However, *C. malina* was not able to grow on those carbon sources due mainly to the presence of furfural, Hydroxymethylfurfural (HMF) and acetic acid in high concentrations. In contrast, *C. malina* had the highest biomass productivity in glucose and PPH2. The PPHs promoted the lipid accumulation in *C. malina* but with different lipid composition. PPH1 and PPH3 promoted the synthesis of TAG while PPH2 allowed the accumulation of polar lipids with high linoleic and linolenic acid content. The presence of furfural, HMF and acetic acid in the pretreatment of the potato peel inhibited the growth and biomass productivity in *C. malina* but accumulated lipids. Therefore, optimization of the pretreatment and hydrolysis is necessary in order to use potato peel as an efficient carbon source without undesired by-products. Mixotrophic cultivation using *C. malina* is possible but not ideal since the microalga grow better under phototrophic conditions.

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Customizing carbon partitioning: A pathway to enhance soybean seed value and yield

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Soybeans (*Glycine max*) produce approximately 20% oil and 40% protein that are important for food, feed, and fuel. Previous research indicates a trade-off between oil, protein, and carbohydrates including Raffinose Family Oligosaccharides (RFOs), starch, and cell wall polysaccharides. This is economically unfavorable, as carbohydrates have less value, are less energy dense and RFOs cannot be properly digested by animals. The goal of this project is to develop strategies for altering carbon partitioning pathways that can overcome this trade-off and add value to the seed. Our working hypothesis is suppression of lipase encoding genes and/or direct inhibition of unfavorable carbohydrates biosynthesis would potentially alter biomass allocation across soybean seed development. Using a seed-specific RNAi approach we targeted Sugar Dependent Protein 1(SDP1), a patatin-like phospholipase that is responsible for oil turnover late in seed development. Detailed profiling of the lipid content in two subsequent generations resulted in oil increase (up to 5 %) with no protein loss and modest reduction in RFOs in the transgenic genotypes. *SDP1* suppression also enhanced seed size (12-27%) independent of number, primarily enhancing cell area significantly. Furthermore, additional lines were generated to reduce low value carbohydrates that represent up to 30% of seed biomass through seed-specific knock down of UDP-sugar pyrrophosphorylase (USPase) and UDP glucose dehydrogenase (UDPGDH). Homozygous transgenic lines with single or double knock down of the cell-wall biosynthetic genes resulted in significant oil increase (5-10%). A combination of biochemical, phenotypic, and transcriptomic studies associated with improved soybean seed value and yield will be discussed.
Funding source: United Soybean Board

Deciphering diacylglycerol enantiomer specificities of DGAT isoforms: Insights into TAG remodeling in lipid metabolism of different species

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Acyl-CoA: diacylglycerol acyltransferase (DGAT) isoenzymes catalyze the final step in triacylglycerol (TAG) biosynthesis by transferring a fatty acid from acyl-CoA to the *sn*-1 or *sn*-3 free hydroxyl of diacylglycerol (DAG). The molecular species of TAG produced depend on the selectivity of the enzymes for different acyl-CoA and DAG molecular species combinations. However, the specificities of these isoenzymes towards *sn*-1,2 and *sn*-2,3 stereoisomers of DAG and their biological significance are not fully explored. Recently, we have discovered a novel TAG biosynthetic pathway (coined TAG remodeling) in the Brassicaceae species *Physaria fendleri* where the differential specificities of DGAT1 and DGAT2 for *sn*-1,2 and *sn*-2,3 DAG stereoisomers are essential for the production of the unique *P. fendleri* oil composition. To further explore whether the DGAT DAG enantiomer specificities are species-specific, we carried out an enantiomer-specific DGAT assay using yeast microsomes heterologously expressing DGAT1 and DGAT2 isoenzymes from different species including plant, human, mouse, yeast, and algae. [^{14}C]18:1-CoA and *sn*-1,2-18:1-DAG or *sn*-1,2/*sn*-2,3-rac-DAG were used as acyl donors and acyl acceptors, respectively. The results demonstrated that the DGAT1 and DGAT2 isoenzymes from humans, mouse, and yeast can use both *sn*-1/2 and *sn*-2/3 stereoisomers of DAG. Interestingly, DGAT1 and DGAT2 isoenzymes from different plant species and microalgae are highly specific towards different DAG enantiomers. This indicates that plant and microalgal DGAT enzymes are highly selective and more complex in nature compared to other species, and is suggestive of different roles for TAG biosynthesis within the broader context of lipid metabolism in these organisms. Overall, these results expand our understanding of the novel specificities of DGAT isoenzymes and identify novel DGAT isoenzymes that can be exploited to create transgenic oilseed plants with value-added properties.

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Evaluation of the role of Camelina sativa SDP1 on the accumulation of total oil and MCFA in engineered lines

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Bioengineering efforts targeting medium-chain fatty acid (MCFA) biosynthesis in *Camelina sativa* have yielded transgenic lines capable of producing C10:0 (Capric acid; C10 lines) and C12:0 (Lauric acid; C12 lines) fatty acids, valuable for aviation fuel and oleochemical applications. However, the total seed oil was substantially reduced to up to ~10-20% in the engineered lines. Lipid analysis throughout seed development stages unveiled a declining trend in total lipid content, both in weight and molar basis, particularly during the middle and late stages, indicating triacylglycerol (TAG) turnover. The patatin-like lipase SUGAR-DEPENDENT1 (SDP1) has been identified as a major TAG lipase implicated in seed TAG turnover during maturation in various oilseed plants. Given the observed reduction in seed oil phenotype in C10 and C12 Camelina lines and the established role of SDP1 in TAG lipolysis across different plant species and bioengineered varieties, we hypothesized that SDP1 suppression could mitigate TAG turnover and thus rescue the reduced seed oil phenotype in MCFA-engineered lines. To investigate this hypothesis, we employed a seed-specific RNA interference (RNAi) knockdown strategy to suppress SDP1 expression. Subsequent analysis of T2 seeds revealed a significant increase in total oil content in C10 and C12 lines, restoring levels comparable to wild-type plants. However, independent T2 knockdown lines showed reduced MCFA levels and concurrent increases in polyunsaturated fatty acid (PUFA) levels, indicating the selectivity of SDP1 against MCFA. Results of T3 seed oil analysis and in vitro enzymatic assays of SDP1 TAG molecular species selectivity will be presented.

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FatPlants: A Comprehensive Information System for Lipid-Related Genes and Metabolic Pathways in Plants

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Increasing seed oil content for biofuels and bioproducts by breeding and biotechnology has resulted in trade-offs or penalties with respect to protein content, seed size, or seed fitness. The molecular basis for this impasse is mostly speculative. Use of current global profiling approaches to better understand both the metabolic consequences of altered oil content and composition and the basis for reduced yield must also deal with off-target genetic mutations, ultimately confounding cause-effect interpretations. We propose a diverse, integrated strategy to study the consequences of higher and tailored lipid production by studying transgenic plants specifically engineered to produce altered seed oil content and composition. As a continuation of a prior project, we are developing a “one-stop-shop” community web resource (www.fatplants.net) for all data pertaining to modifying oil composition and increasing oil content in plants, and to leverage data generated from this project with curated forms of public data from other funded websites, and the literature. The FatPlants framework and tools currently exist for a number of crop

and model oilseeds, including camelina (*Camelina sativa*). As part of the B5 project, we are expanding these resources to include pennycress (*Thlaspi arvense*) and *Cuphea viscosissima*, an “extreme” producer of seeds with medium-chain fatty-rich oils. We will present all the known fatty acid related proteins and genes in these species and overlay these data with lipidomic measurements from seeds of B5 target species. As a comparative analysis tool, FatPlants includes pathway viewer, protein structure viewer, BLAST, protein-protein interaction viewer, and GO enrichment viewer. To strengthen interactions among B5 investigators, a user authentication internal data-sharing space has been provided to all collaborative labs. Our website is publicly available as a community tool at www.fatplants.net.

G2PDeep-v2: Web-based Deep Learning Platform for phenotype prediction and Marker Discovery in Plants and Other Species.

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The research area of plant genotype to phenotype analysis can significantly benefit from the introduction of G2PDeep-v2, a web-based platform that utilizes a deep learning-based multi-CNN approach for phenotype prediction and marker discovery. In our latest development, we have thoroughly analyzed 1066 soybean accessions, handling the big data of millions of SNP and CNVs to address key issues in soybean oil quality traits such as fatty acid composition (including linoleic and oleic acids) and overall oil content. G2PDeep-v2 facilitates the prediction of these oil-related phenotypes, categorizing traits such as oil content percentage, linolenic, palmitic, and stearic acid levels, with detailed classifications. The platform extends its capabilities to quantitative traits like protein composition and other key agricultural traits in 1066 soybean accessions. The server's advanced hyperparameter tuning algorithm and comprehensive Gene Set Enrichment Analysis (GSEA) functionalities are designed to refine the predictions iteratively. Our dedicated multi-CNN model, in combination with well-known machine learning algorithms such as Logistic Regression, Support Vector Machine, Decision Trees, and Random Forest, ensures robust and reliable phenotype predictions. G2PDeep-v2 demonstrates the power of deep learning in revolutionizing the field of plant genomics and can also be applied for data from other species including human, mouse, bacteria, viruses and others.

With its comprehensive and user-friendly interface, researchers are empowered to undertake complex tasks with greater efficiency and accuracy. As we continue to refine and expand the capabilities of G2PDeep-v2, we look forward to contributing significantly to the global efforts in crop improvement and sustainable agricultural practices. The web portal is publicly available at: https://g2pdeep.org.

A Comprehensive Suite of Tools for Allele Discovery and Phenotype Improvement in SoyKB and KBCommons Frameworks for Crops

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Abstract

The whole-genome re-sequenced (WGRS) data availability has increased drastically as the sequencing technologies have improved over time. Nonetheless, conducting research with WGRS data without the enhancement of other data and tools is highly challenging. Hence, a comprehensive suite of tools, variant calling pipeline (SnakyVC), Allele Catalog pipeline (AlleleCatalog), Allele Catalog Tool, Genomic Variation Explorer (GenVarX), and Multiple Allele Discovery (MADis) Tool, have been developed for allelic variation explorations and phenotype distribution inferences. The Allele Catalog Tool is powered by Allele Catalog datasets generated using the SnakyVC and AlleleCatalog pipeline. The SnakyVC pipeline can parallelly process sequencing data to generate the Variant Call Format (VCF) files, and the AlleleCatalog pipeline takes VCF files to perform imputations, functional effect predictions, and assembles alleles for each gene to generate Allele Catalog datasets. The Allele Catalog Tool introduces a novel method to uniquely group alleles and annotations within coding regions and summarize accession into counts based on improvement status. Its capabilities also include connecting variant positions to phenotypes for phenotype distributions and associating accessions with metadata. Besides that, GenVarX offers exploration capabilities for allelic variations related to transcription factor binding in

promoter regions, structural variations in copy number variation regions, and phenotype distribution visualizations of those variations to understand the impact on gene expression and phenotype alterations. Furthermore, the MADis Tool conducts statistical scoring tests on variant positions within a candidate gene and a phenotypic trait to pinpoint the most effective combination of variant positions associated with causative mutations. This analysis provides potential insight into causative mutations that occur simultaneously to impact the phenotype of crops. All the tools are hosted on the SoyKB and KBCommons web portals. Researchers can use this tool to identify novel alleles of genes and select crop accessions to improve selective breeding strategies and agricultural traits of plants.

Towards Effective Production of Wax Esters with Medium-Chain Fatty Acyl and Fatty Alcohol Moieties in Seeds of Transgenic Crops

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Wax esters (WE) are widespread throughout global industry as an important source for the production of coatings, lubricants, candles, inks, polishes, pharmaceuticals and cosmetics. Nowadays WE are mostly obtained from the depleting and environmentally harmful fossil reserves in an energy-consuming process. Improving the sustainability of WE-producing industrial crops, might just be the much-needed substitute. Here, we focused on WE containing medium-chain (12C-14C) fatty acyl and fatty alcohol moieties. Possible applications of such WE are as follows: A) Replacement of the much-coveted spermaceti lubricant with high stability and low melting point, B) Generating a metabolic sink depository of WE, containing precursors of moth sex pheromones, specifically unsaturated derivatives of fatty acids and fatty alcohols; such pheromones can be used in crop protection by mating disruption for Lepidoptera pest control. Despite much effort, there is a limit to the amount of WE stored in plant seeds genetically modified for this purpose. Beyond a certain point, in GMO lines with higher WE content in seeds, the germination frequency decreases, as well as plant fitness and growth rate. Inability to properly pack WE into lipid droplets, due to absence of WE-specific lipid droplet proteins (LD), may cause disrupted oil packaging observed in seeds of WE-accumulating lines. Preliminary analysis by enhanced-resolution confocal fluorescence microscopy of *Nicotiana benthamiana* leaves, producing medium-chain WE, showed disrupted and swollen ER upon heterologous expression of medium-chain wax biosynthesis machinery. The addition of jojoba LDAP1 and LDIP, two proteins involved in packaging wax esters in jojoba seeds, restored normal ER organization and an apparent increase in cytoplasmic lipid droplets suggesting that LD packaging at the ER may be an important consideration of wax production in heterologous systems. Such considerations are likely to be important for future biotechnology strategies to produce medium-chain WEs in crop seeds.

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Complete Remodeling of TAG Composition to Generate Novel Oils in Transgenic Oilseeds

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Acetyl-TAG, a unique form of triacylglycerols (TAG) with an acetate group at the *sn*-3 position, possess valuable properties, such as reduced viscosity and freezing points. Previous attempts to engineer acetyl-TAG production in oilseed crops have failed to reach the levels occurring in *Euonymus* seeds, that naturally produce acetyl-TAG. Here, we report the successful generation of camelina and pennycress transgenic lines accumulating almost pure levels of acetyl-TAG, 93 mol% and 98 mol%, respectively, by expression of a diacylglycerol acetyltransferase (DAcT) with enhanced activity, combined with suppression of the endogenous TAG-synthesizing enzyme DGAT1 in *FATTY ACID ELONGASE1* (*FAE1*) mutants. These ultra-high levels of acetyl-TAG exceed those in earlier engineered oilseeds and are equivalent or greater than those in *Euonymus* seeds. MALDI-MS imaging provided insights into the DAG pool utilized by EfDAcT, and revealed that acetyl-TAG were synthesized in both the embryonic axis and the cotyledons, with the residual endogenous TAG localizing to the embryonic axis. Remarkably, the ultra-high production of acetyl-TAG in transgenic seeds exhibited minimal effects on seed properties, highlighting the potential for production of designer oils required for economical biofuel industries.

Characterizing and Manipulating Sorghum Kernel Wax as a Possible Replacement for Carnauba Wax

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Natural waxes, like carnauba wax, are high-value industrial plant products for which the United States has no domestic source. Literature from the past 50 years has proposed sorghum kernel wax as a replacement for carnauba wax, but currently it has too low of a melting point for it to be a viable substitute. This research aims to 1) characterize the melting point and chemical composition of sorghum wax and carnauba wax, and 2) increase the melting point of sorghum kernel wax. It has been reported that high molecular weight alkyl esters (C56+) are the main component of carnauba wax, giving it its high melting point and hardness. Two different sorghum waxes were used for analysis, sorghum bran and sorghum DDGS (distillers dried grains). GC-MS and direct-infusion APCI-MS revealed that sorghum DDGS wax contained mainly alcohols, sorghum bran wax contained mostly acids and alcohols, and carnauba wax contained mostly alkyl esters. A transesterification reaction was performed on carnauba wax, which provided further evidence for presence of alkyl esters. FTIR was used and showed the characteristic peaks corresponding to esters in carnauba and sorghum wax. The composition of sorghum wax differs between varieties and some are able to create esters. Our data suggests that a few varieties have similar absorbance values to carnauba wax at ester wavelengths. The melting points of the natural waxes and several of their derivatives were determined using DSC. Fractionation of carnauba wax was achieved by a colleague which gave an ester enriched fraction which was added to sorghum wax. This combination resulted in sorghum with a melting point that is comparable to carnauba wax. Overall, our results suggest that adding an ester rich fraction to sorghum wax in 1:1 or 2:1 ratios can increase the melting point to a comparable point to carnauba wax.

Identification of gene combinations for improving seed oil and protein contents

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Canola (*Brassica napus* L.) is one of the most important oilseed crops. The seed meal remaining after oil extraction contains around 40% protein, which is an excellent source of animal feed. However, the dominant black seed cultivars in Canada has approximately 7.9% of cellulose in the meal, which can inhibit digestion in monogastric animals. Thus, reallocating seed carbon from cellulose to protein and oil can enhance the feedstock's quality without compromising the oil content. In this study, we aimed to identify promising gene combinations in *Arabidopsis* to accelerate the genetic work needed to effectively manipulate seed carbon flow in canola. We firstly overexpressed *B. napus diacylglycerol acyltransferase 1* (*BnDGAT1*-OE) and its performance-enhanced variant (*BnDGAT1-L441P*-OE), respectively, with the downregulation of *cellulose synthase 1* (*Atcesa1*-RNAi) in *Arabidopsis*. The results indicated that the homozygous lines have equal or increased oil and protein and reduced cellulose. We then overexpressed different protein-associated genes, *amino acid permease 1* (*AtAAP1*), *alanine aminotransferase 1* (*AtAAT1*), and asparagine synthase 1 (*AtASN1*), in those lines, respectively. The combination of *Atcesa1*-RNAi/*BnDGAT1*-OE/*AtAAP1*-OE produced the most desirable seed composition outcomes, which is being used to develop canola germplasm with improved seed quality.

Arabidopsis SEIPIN1 reduces rubber particle size in guayule

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Current commercial source of natural rubber (NR) is from Hevea (Hevea. brasiliensis). Hevea is cultivated only in Southeast Asian countries and is vulnerable to leaf blight diseases. To secure NR production, guayule (Parthenium argentatum Gray) has been developed as an alternative crop. NR is synthesized in/on rubber particles (RPs). RPs resemble lipid droplets (LDs) as both of them are generated from endoplasmic reticulum and consist of a hydrophobic core and a phospholipid monolayer. A prominent LD protein SEIPIN plays an essential role in controlling LD biogenesis. Overexpression of AtSEIPIN1 in Arabidopsis increases LD size and consequently increases its core constituent triacylglycerol content in seed. To investigate if AtSEIPIN1 can also increase the rubber molecule cis-1,4-polyisoprene in RP, we generated transgenic guayule lines overexpressing AtSEIPIN1 (SEIoe). We found that SEIoe lines reduced RP size significantly. Compared with wild-type, fewer RPs were observed, and NR quantity and quality were also reduced in SEIoe lines. The mechanisms of how AtSEIPIN1 influences RP biogenesis and NR production in guayule are discussed.

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Enhancing peanut nutritional quality by editing *AhKCS* genes lacking natural variation

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Abstract

Peanut is a globally important source of vegetable oil, but contains some very long chain saturate fatty acids (VLCSFAs). As intake of high levels of VLCSFA in dietary would increase the cardiovascular disease risk, it is necessary to decrease the VLCSFA content in peanut. In our previous studies, *AhKCS1* and *AhKCS28* had been proven to be involved in regulating VLCFA content in peanut seed. Both of *AhKCS1* and *AhKCS28* were knocked out using CRISPR/Cas9 system in this study. Totally 66 T_0 plants were generated by *Agrobacterium tumefaciens* mediated transformation and screened with DsRed2. As a result, all of them were confirmed being successfully transformed based on red fluorescence observation. PCR identification showed that both *AhKCS1* and *AhKCS28* were knocked out in 61 T_0 plants, and the editing efficiency was as high as 92.4%. The VLCFA content in harvested seeds were detected by gas chromatography. The peaks of behenic acid and lignoceric acid in the edited seeds were absent, and the overall VLCFA content was significantly decreased from 6.8% to 0.4%. Interestingly, the VLCFAs in three elite lines were almost vanished. In summary, we provided an efficient method to knock-out genes in peanut and created novel genotypes with decreased VLCFA content, indicating a great potential for improving nutritional quality in peanut.

Key words: Peanut; very long chain fatty acid; transformation; gene knock-out

Core Factors: where lipid droplet biogenesis intersects biotechnology.

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Camelina sativa is an ancient crop, belonging to the *Brassicaceae* family, largely abandoned after World War II in lieu of more profitable oilseed crops. Agronomically it is attractive because of its fast growth cycle (~90 days), can be used in low-input agronomic systems, and is largely resilient to pathogens and disease. Today Camelina is grown as a cover crop, but interest as a new source of polyunsaturated fatty acids and proteins for feed, food and bio-based products is growing. For Core Biogenesis, the biogenesis of lipid droplets provides the carrier for high value recombinant proteins as oleosin fusions and/or the oil bodies (Core Factors®) derived upon aqueous extraction of the seeds. For companies and technologies focused on modifying the oil content, a new/old paradigm emerges to drive the development of complementary protein- and oil-bioengineering. When combined with farming infrastructure, the ability to scale high volumes for a sustainable approach to create novel products and applications can be exploited. At this intersection, we will discuss our “triangle” for Core Factor® technology, what opportunities exist for partnering technology on the platform, and how we are approaching the current bottlenecks to develop marketplaces ranging from concepts through commercialization.

Development and field evaluation of oil sorghum for sustainable bioenergy production

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Development of bioenergy crops that accumulate energy-dense triacylglycerol (TAG) in vegetative tissues has emerged to meet the increasing demand for sustainable bioenergy production. Since vegetative tissues, which account for the majority of plant biomass, are markedly lacking the ability to metabolize TAG, recent metabolic engineering strategies have focused on optimization of TAG metabolism. Understanding biosynthesis and degradation of lipids have enabled lipid production in vegetative tissues of plants. Sorghum (*Sorghum Bicolor*, L. Moench) has great potential as a TAG production platform because of its high productivity, drought and heat resistance, and germplasm diversity, in addition to its established biotechnology and functional genomics toolbox (e.g., smaller genome size, detailed genetic map, and transformation availability). Here, we applied metabolic engineering and synthetic biology tools to develop TAG-accumulating germplasm, using a genetic construct originally intended for medium-chain fatty acid TAG production. Through greenhouse study and field trials between 2021-2023, we identified TZ424-5-3a as our best-performing event. This event accumulates TAG to 3.5% DW and total fatty acids to 4.6% DW in its stems at the soft-dough stage, which are the optimal stage for oil accumulation. These concentrations are 50-fold and 15-fold higher than those of non-engineered controls, respectively. Despite the high TAG accumulation, this event did not display any notable reduction in growth. We found that the oil accumulation phenotype is correlated with expression of two transgenes: sesame oleosin and *Cuphea* diacylglycerol acyltransferase. The fluxomic approach and acyl-ACP thioesterase activity assay suggest that production of medium-chain fatty acid may play a role in oil accumulation. Current research is focused on phenotyping of field-scale agronomic traits at different locations and oil accumulation in different sorghum genotype backgrounds obtained through crosses. These collective efforts are advancing development, improvement, and utilization of biomass crop as viable feedstocks for renewable diesel and sustainable aviation fuel production.

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Title: Pennycress (*Thlaspi arvense* L.) seed size mutants affect seed oil and protein accumulation differently

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Pennycress (*Thlapsi arvense* L.) is being developed as an oilseed intermediate energy crop for the U.S. Midwest owing to its extreme cold tolerance, high seed yields, and short life cycle, fitting between corn and soybeans. Varieties have been bred to produce over 1,500 pounds of seed per acre, yielding 65 gallons of oil and 1,200 pounds of meal per acre. Seed compositional changes (reduced seed coat fiber and erucic acid content) have resulted in the domesticated variety named CoverCress™. Along with these and other compositional improvements, we have employed CRISPR gene editing to increase seed size for better grain handling and the ability to plant the seeds at greater depth, targeting *DA1*, *DA1-RELATED* (*DAR1*), and *UBIQUITIN PROTEIN LIGASE3* (*UPL3*). In Arabidopsis, *DA1* and the homologue, *DAR1*, encode ubiquitin receptors thought to set final seed and organ size by restricting the period of cell proliferation in the seed integuments. *UPL3* mediates proteasomal degradation of, among other targets, the transcription factor LEC2. LEC2 activates expression of seed maturation and seed lipid accumulation genes. We found that single *da1* mutants phenotypically grew like wild type and produced seeds about 14 to 20 percent larger than wild type. Double *da1dar1* mutants were found to have seed sizes about 40 percent larger than wild type and 50 percent heavier. Like *da1dar1* double mutants, *upl3* mutant seeds were bigger than wild type (17 to 32 percent larger) and plants were taller with bigger flowers, pods, and leaves and took relatively longer to flower under growth chamber conditions. Surprisingly, pennycress *upl3* mutant seeds had total oil content the same or less than wild type but higher protein content, differing from what was observed with Arabidopsis *upl3* mutant seeds. Omics analyses are underway to understand how these pennycress genes function relative to orthologues in other Brassica species.

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Genetic Research on Camelina: CRISPR/Cas9-mediated gene editing to enhance seed oil in cover crops

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Seed oils produced by cover crops offer a financial incentive for cover-cropping, and the value can be augmented by gene editing. The predominant constituent of seed oils, triacylglycerols (TAGs), can be altered by editing genes involved in lipid metabolic pathways. *Camelina sativa* and *Thlaspi arvense* L., two promising cover crops from the Brassicaceae family, are amenable to established methods of plant genetic modification. Four key enzyme-encoding genes were identified in both species from well-characterized orthologs in *Arabidopsis thaliana* and targeted for knockout using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR-associated enzyme 9) system. We hope to increase overall seed-oil content in mature seeds by individually knocking out two lipases, Sugar-dependent 1 (SDP1) and Oil-body lipase 1 (OBL1), which break down TAGs in late-stage seed development. We seek to individually knockout two enzymes that directly synthesize TAGs, Diacylglycerol O-acyltransferase 1 (DGAT1) and Phospholipid:diacylglycerol acyltransferase 1 (PDAT1), which will generate platform crop lines into which transgenic, TAG-synthesizing enzymes that produce specialty oils can be introduced. *C. sativa* presents a unique challenge for gene knockout due to its hexaploidy genome. The CRISPR/Cas9 system must generate a deleterious mutation in all six copies of each gene to achieve a full knockout. To facilitate screening for genetic mutations, CRISPR/Cas9 cloning constructs were designed with three guide RNAs (gRNAs) spaced >100 bp apart to direct Cas9 to cut DNA in multiple locations within the same gene. The purpose was to generate large DNA deletions in each gene for quick detection by PCR amplification and gel electrophoresis, which increases sample throughput and facilitates genetic screening. This research characterizes the efficiency of this method, and it shows preliminary results, providing a valuable tool for researchers working with CRISPR/Cas9 and other hexaploid crops, such as *Triticum aestivum* L. (wheat).

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Novel sources of docosapentaenoic acid n-3 DPA

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Currently, there is lack of a consistent and highly enriched source for docosapentaenoic acid (n-3 DPA, C22:5), and this work report the isolation of microorganism that naturally produces n-3 DPA. We screened microorganisms in our culture collections with the goal to isolate a strain with high levels of n-3 DPA. We isolated a strain of Sphaeroforma arctica that produces up to 11% n-3 DPA in total fatty acid and has a high n-3 DPA to DHA/EPA ratio. The cell growth of the isolated strain was characterized using microscopy imaging and flow cytometer technologies to confirm the coenocytic pattern of cell divisions previously described in S. arctica. Our novel isolate of S. arctica grew more robustly and produced significantly more n-3 DPA compared to previously isolated and described strains indicating the uniqueness of the discovered strain. Overall, this work reports a first isolate n-3 DPA producing microorganism and establishes the foundation for future strain improvement and elucidation of the physiological function of this LC-PUFA for human nutrition and health.

5. Lipid signaling: molecules, metabolism, mechanisms

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Communication Between Chloroplasts and Endoplasmic Reticulum Through Membrane Lipid Remodeling

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The methylerythritol phosphate (MEP)-pathway is responsible for the production of essential isoprenoid metabolites in gram-negative bacteria, and all plastid-bearing organisms. However, as the majority of the plastid genome, including the MEP pathway, has been relocated to the nucleus, establishing molecular communication pathways between these organelles becomes imperative. These retrograde signaling mechanisms, which relay stress and status information from plastid to the nucleus are required to maintain protein stoichiometry in organelle biochemical pathways, and to additionally adapt plastid functions to cellular requirements. Our lab has identified an MEP pathway intermediate methylerythritol cyclodiphosphate (MEcPP) which functions as a stress-inducible retrograde signaling metabolite in plants. MEcPP accumulation leads to robust alteration of the transcriptomic profile, followed by a notable restructuring of organism physiology to enhance stress resilience. Included in this restructuring is the activation of a cellular stress response pathway known as the unfolded protein response (UPR), which surveys endoplasmic reticulum (ER) homeostasis and activates nuclear responses to functional perturbations in ER protein folding or lipid stoichiometry (i.e., ER stress). Lipidomics analysis in an MEcPP-accumulating line establishes a connection between MEcPP and an increase in ER derived membrane phospholipids enriched in saturated fatty acids at the expense of plastid glycolipids. Subsequent genetic studies suggest that this cellular alteration in ER lipid composition may contribute to MEcPP-dependent induction of the UPR, and changes in ER network morphology.

In essence, our work fills crucial gaps in understanding the mechanisms behind MEcPP-dependent retrograde signaling. Moreover, we have demonstrated that shifts in lipid metabolism serve as a conduit linking plastidial stress perception to the regulation of broader cellular functions and organismal development.

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Structural and Functional Characteristics of Two Fatty Acid Amide Hydrolase Isoforms in the Legume Model, *Medicago truncatula* G.

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Fatty acid amide hydrolase (FAAH) utilizes a variety of acylamide substrates and is a known contributor to lipid signaling pathways in multicellular eukaryotes. Past studies have shown FAAH to inactivate *N*-acylethanolamine lipid mediators, but additional studies indicate hydrolysis of other acylamides like *N*-acyl homoserine lactones (AHLs). Analysis of FAAH sequences indicated that there are two conserved groups in angiosperms, termed FAAH1 and FAAH2, with different conserved residues in the substrate binding pocket. The single FAAH isoform in *Arabidopsis thaliana* has been characterized structurally and functionally, but similar data are lacking for FAAH2 from any plant species. In this study, we characterize and compare the biochemical properties of FAAH1 and FAAH2 isoforms from the legume *Medicago truncatula* (MtFAAH1 and MtFAAH2a). Polyhistidine-tagged, recombinant FAAH proteins were produced in *Escherichia coli*, and purified by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). FAAH purification was monitored by western blotting and enzymatic activity, and liquid chromatography with tandem mass spectrometry (LC/MS/MS) analysis confirmed the identity of the purified protein isoforms. Chromatograms from SEC suggested that purified MtFAAH1 and MtFAAH2a eluted as tetramers, different from the *A. thaliana* FAAH1 protein. FAAH in *A. thaliana*, as well as FAAH in animals and fungi are reported to be dimers, although various oligomeric structures have been reported in solution. The two MtFAAH isoforms have complementary substrate preferences for acylamide substrates, with MtFAAH1 preferring long-chain acylamides and the MtFAAH2 appearing to prefer shorter-chain or aromatic acylamides. Work is continuing to determine the structural and functional significance of these FAAH isoforms in Medicago and in angiosperms more broadly, especially given their propensity to hydrolyze microbial derived acylamides.

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Lipid phosphorylation by a diacylglycerol kinase suppresses ABA biosynthesis to regulate plant stress responses

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Lipid phosphorylation by diacylglycerol kinase (DGK) that produces phosphatidic acid (PA) plays important roles in various biological processes, including stress responses, but the underlying mechanisms remain elusive. Here, we show that DGK5 and its lipid product PA suppress ABA biosynthesis by interacting with ABA-DEFICIENT 2 (ABA2), a key ABA biosynthesis enzyme, to negatively modulate plant response to abiotic stress tested in Arabidopsis thaliana. Loss of *DGK5* function rendered plants less damaged, whereas overexpression (OE) of *DGK5* enhanced plant damage to water and salt stress. The *dgk5* mutant plants exhibited decreased total cellular and nuclear levels of PA with increased levels of diacylglycerol, whereas *DGK5*-OE plants displayed the opposite effect. Interestingly, we found that both DGK5 and PA bind to the ABA-synthesizing enzyme ABA2 and suppress its enzymatic activity. Consistently, the *dgk5* mutant plants exhibited increased levels of ABA, while *DGK5*-OE plants showed reduced ABA levels. In addition, we showed that both DGK5 and ABA2 are detected in and outside the nuclei, and loss of *DGK5* function decreased the nuclear association of ABA2. We found that both DGK5 activity and PA promote the nuclear association of ABA2. Taken together, these results indicate that both DGK5 and PA interact with ABA2 to inhibit its enzymatic activity and promote its nuclear sequestration, thereby suppressing ABA production in response to abiotic stress. Our study reveals a sophisticated mechanism by which DGK5 and PA regulate plant stress responses.

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Deciphering the mechanism of environmental regulation of Class IV HD-Zip transcription factors

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Transcription factors (TFs) of the Class IV Homeodomain Leucine-Zipper (HD-Zip IV) family, such as *Arabidopsis thaliana* MERISTEM LAYER1 (ATML1), PROTODERMAL FACTOR2 (PDF2), and HOMEODOMAIN GLABROUS11 (HDG11), intricately regulate gene expression, impacting cell-type differentiation and elongation growth in the epidermis, the outer layer at the interface to the environment. These TFs are highly conserved in crops, and ectopic expression of HDG11 was previously shown to increase biomass and drought tolerance. HD-Zip IV TFs contain a START lipid-binding domain, as well as a START-adjacent domain (STAD) of unknown function. We hypothesize that environmental signals, such as day-night switches and salinity stress, control HD-Zip IV TF activity via interaction with lipid and protein ligands. While metabolic lipids bind the START domain, we postulate that adaptor proteins regulate chromatin remodeling and gene expression by interaction with STAD. Our yeast two-hybrid experiments provide evidence that two candidate adaptor proteins, which share sequence similarity, physically interact with ATML1, PDF2 and HDG11. The EAR motif within the adaptor protein N-terminus is required for interaction with TOPLESS-related proteins to mediate chromatin remodeling, whereas cysteine-rich motifs in the C-terminus are required for HD-Zip IV interaction. Promotor analysis reveals light response elements in upstream regions of the corresponding genes. Loss-of-function phenotypes suggest that these adaptor proteins have roles in cell elongation and salinity tolerance. We propose a model in which the adaptor proteins transmit environmental signals to HD-Zip IV TFs to negatively regulate TF activity under daylight conditions, while metabolic lipids positively regulate the TFs in the dark. To further characterize the adaptor proteins as environmental sensors, multiomics experiments during salt stress and diurnal cycles will be complemented with proximity labeling using HD-Zip IV TFs as baits. The overall goal of this research is to decipher the mechanisms of protein and lipid interactions involved in modulating plant development.

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Identification of Chloroplast Localized Peroxidase Activity by Low Carbon Inducible Gene 2 (LCI2) Reveals Interface Between Photocatalyzed ROS Sensing and Chloroplast Lipid Metabolism

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The ability to compartmentalize is essential to all cellular life on this planet. Compartmentalization allows for the separation of cells from their environment but for eukaryotes, the ability to spatially separate processes within the cell. The chloroplast, an organelle uniquely equipped to facilitate conversion of light energy into metabolic substrates through carbon fixation. While complex organelles such as the chloroplast or the mitochondria maintain some degree of self-regulating abilities, segregation of specialize metabolic processes like photosynthesis have driven the evolution of equally specialized feedback loops communicating signals to the nucleus. Much work has gone into the mechanisms by which reduction/oxidation or REDOX biochemistry communicates physiological conditions to effect gene regulation. However, little is known about how the chloroplast communicates oxidative damage of photosynthetic membranes to initiate replacement, repair, or mobilization of damaged acyl chains. Through analysis of the *Chlamydomonas reinhardtii* genome, we have identified an incorrect annotation of the *LCI2* locus. Further analysis of this locus reveals a complex gene structure encoding two genes produced through a differential splicing event that share a start codon, localization sequence, and promoting region. Through *in vitro* enzyme activity assays and comprehensive fatty acid analysis of insertional and CRISPR generated mutants we have determined that the LCI2 protein encoded at the locus exhibits properties consistent with canonical thylakoid bound ascorbate peroxidases while the larger protein product encodes for fatty acid desaturase 4 (FAD4) in Chlamydomonas. This desaturase is responsible for the delta 3 trans 16:1 fatty acid found in phosphatidylglycerol (PG) pools of the chloroplast. While it is unclear how these two proteins activities specifically interoperate in a manner necessitating their co-regulation and co-transcription. The work presented in this study highlights two previously unknown gene functions of this locus and strongly suggests an interface of REDOX metabolism and specialized fatty acid production.

6. Plant lipids in abiotic and biotic stress resilience

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Galactolipids Regulate Systemic Immunity In Plants

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Systemic acquired resistance (SAR) is a unique form of long-lasting systemic immunity that provides protection against a broad-spectrum of pathogens in plants. SAR involves the generation of a mobile signal at the primary infected site, which upon translocation to the distal tissues, prepares the plant to better resist future infections. Multiple, chemically diverse SAR inducers that have been identified to-date. The phosphorylated sugar derivative, glycerol-3-phosphate (G3P) and C9 dicarboxylic acid, azelaic acid (AzA) are two such SAR inducers. AzA, which acts upstream of G3P and confers SAR by increasing G3P levels, is generated upon the hydrolysis of C18 unsaturated fatty acids present specifically on the galactolipids, mono- and di-galactosyl-diacylglycerol (MGDG and DGDG). MGDG and DGDG are major components of chloroplast membranes and therefore the most abundant lipids in plants. Previous studies have shown that mutants deficient in MGDG (*mgd1*) or DGDG (*dgd1*) synthesis, are impaired in SAR as well as photosynthetic activity. In addition, the *dgd1* mutant is defective in gene expression responsive to another important SAR activator, salicylic acid (SA). Notably, transgenic expression of a bacterial glucosyltransferase (GT) in the *dgd1* background, which introduces glucose instead of galactose into the membrane lipids partially restores photosynthetic functions, but not SAR functions. Using genetics, biochemical and molecular analyses, my work further elucidates the importance of the DGDG galactolipid in modulating various aspects of the plant's systemic immune response including generation and systemic movement of multiple SAR inducers.

Enhancing plant tolerance to drought and heat stresses: germplasm development for sustainable production of crop plants

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Wild tomato species, distributed from Ecuador to northern Chile and endemic species in the Galápagos Islands, represent a potential resource for abiotic stress screening due to their wide ecological and climate habits. It is well established that under various stress conditions, plant cells undergo substantial alterations to plastidic and extraplastidic membrane lipid composition via lipid remodelling, which aids in the maintenance of membrane fluidity, stability, and integrity. In this regard, triacylglycerol may act as a transitory pool to sequester some of these toxic intermediates, thus preventing cellular damage under stress conditions. Therefore, to gain insight into this matter, we sought to exploit the genetic variability available in wild tomato species and the knowledge of their genome sequences to generate crops with improved drought and heat stress tolerance as well as to elucidate the role of lipid remodelling in vegetative tissues under these stresses. For drought, plantlets were irrigated to a mild water content controlled with a moisture meter and then let dry for ten more days until reaching high stress levels. We observed that some of these varieties were less affected by the stress because of higher relative water content on leaves. In addition, preliminary data on root length under drought stress demonstrated that these tolerant plants were able to elongate roots faster than in control conditions. Under heat stress, some genotypes were more resistant and accumulated slightly higher amount of total lipids on leaves. With this information we will determine lipid-species amount and distribution at the subcellular level in vivo alongside an expression analysis to provide valuable information about the role of lipid remodeling, TAG accumulation and degradation on leaves under drought and heat stresses. Although tomato is the target of this research, the acquired knowledge will shed light on lipid signalling and/or membrane remodeling during plant stress responses which could be transferred to other commercial crops.

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Unveiling a Newly Characterized Plastoglobules-Localized Lipase 1 (PLL1) in *Arabidopsis thaliana*: Its Role in Abiotic Stress Tolerance, Male Sterility, and Jasmonic Acid Biosynthesis

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Plastoglobules (PGs), lipid reservoirs within chloroplasts, play a crucial role in lipid metabolism and stress responses in photosynthetic organisms. These structures dynamically respond to environmental stresses, impacting plant physiology and adaptation. Previous research has demonstrated that JA biosynthetic enzymes are recruited to plastoglobules under stress conditions, suggesting these sites are key for JA synthesis and regulation. Our study identified and characterized a lipase, *Plastoglobule-Localized Lipase 1* (*PLL1*), which increases significantly under high light stress. Phenotypic analyses of *pll1* mutants revealed growth impairment under environmental stress conditions. Under normal growth conditions, *pll1* exhibited male sterility, with partial fertility observed late in flowering. Localization studies confirmed that PLL1 is localized in PGs. Overexpression of PLL1 resulted in stunted growth and serration in leaf morphology. In vitro lipase assays confirmed PLL1 activity, showing a preference for MGDG as a substrate. Full-length PLL1 exhibited the highest activity, while catalytic site mutations abolished it, verifying PLL1 as a true lipase. The wounding experiment on *pll1* mutants revealed that the mutant showed no difference in JA level, suggesting *pll1*-specific response to abiotic stress, thus highlighting separate regulatory pathways of JA in abiotic and biotic stress. Our findings highlight PLL1's multifaceted role in stress response and male fertility, suggesting potential applications in crop enhancement strategies.

Key words: Plastoglobule, Lipase, Abiotic Stress, Jasmonic acid

Characterization of the maize membrane lipidome in a large collection of introgression lines derived from crosses between B73 and traditional maize varieties and teosintes.

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After domestication, maize was transported to the highlands of Mexico, where it crossed with a highland teosinte, *Zea mays ssp. mexicana*. This genomic introgression persists in modern maize inbreds and carries genes important for abiotic stress tolerance, particularly those involved in highland adaptation. Stresses typical of highland environments include high UV radiation, low temperature, and low phosphorus availability. Low-temperature stress, in particular, impacts the ability of tropical plants like maize to survive in colder environments. One way that plants adapt to low-temperature stress is through alterations in membrane lipids, which in turn impact membrane fluidity and structure. Using a biparental B73 x Palomero Toluqueño (a highland traditional maize variety from central Mexico) RIL population, we previously identified a gene, High Phosphatidylcholine 1 (*hpc1*), whose impaired function in highland maize impacts the amount of phosphatidylcholine, a key membrane lipid, and aids in highland adaptation. Here, we seek to quantify the extent of variation in membrane lipids and map the underlying genetic variation using a newly developed collection of introgression lines derived from crosses between B73 and hundreds of accessions of traditional maize varieties and teosintes from all across Latin America.

Deciphering the role of host lipids in sorghum-aphid interactions

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Abstract (231 words) for 2024 International Symposium on Plant Lipids meeting

Lipids form the building blocks of the cell membrane that shares a prime role in enhancing plant defense against a wide range of biotic and abiotic stresses. Sorghum, the world's fifth most important cereal crop, suffers severe yield losses by attack from the sugarcane aphid (SCA; *Melanaphis sacchari*). Here, we employed electrospray ionization mass spectrometry (ESI-MS) technique to unravel the lipidome profiling in SCA-resistant (SC265) and susceptible (SC1345) sorghum lines at early (1, 48 h) and late (7 dpi) time-points before and after SCA infestation. As opposed to the susceptible line, SCA feeding drastically reduced the overall lipids in the SCA-resistant sorghum line. RNA-sequencing revealed that a *Patatin-like protein* (*SbPLP*) gene with putative lipid acyl hydrolase activity was induced significantly in the resistant line (SC265) at 7 dpi. Furthermore, Virus Induced Gene Silencing (VIGS) was utilized to knockdown the transcript to validate its role in sorghum resistance to SCA. Our aphid bioassays show that *SbPLP*-silenced plants supported higher SCA counts compared to the control plants. Comparison of SCA feeding behavior using Electrical Penetration Graph (EPG) technique revealed that aphids spent significantly more time in the sieve elements of the *SbPLP*-silenced plants compared to the control plants. The present study would help us to unravel the role of lipids in modulating sorghum defense against aphids and our result provide valuable perspectives on harnessing plant lipid profiles for biotechnological applications and agricultural improvement.

Keywords (4): sorghum, *Melanaphis sacchari*, host lipids , RNA-sequencing.

Exploring the plant sphingolipidome: functions of the major enzymes and components in stress responses

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Plants often experience multiple stresses simultaneously and have complex, interconnected responses to acclimate to these stresses. In experiments combining abiotic and biotic stress, we recently found that in Arabidopsis, treatment with the ceramide synthase inhibitor Fumonisin B1 and the abiotic stress NaCl induced a signal that is transported over long distances and causes cell death in systemic leaves. The systemic signal regulated cell death and increased sphingolipid biosynthesis. Sphingolipids are vital components of biological membranes and bioactive molecules. We also found that pre-irrigation with chloride salts significantly limited the cell death caused by bacterial pathogen infection and inhibited bacterial multiplication. Further, chloride salt pre-irrigation improved plant defenses against a fungal pathogen. More interestingly, feeding on chloride salt–irrigated plants retarded the growth of herbivorous larvae of *Spodoptera exigua*. All these phenomena are potentially related to systemic sphingolipid homeostasis. To uncover the specific components during cell death by forward genetics, we recently found that mutations of *SPHINGOID BASE HYDROXYLASE 1* (*SBH1*) suppress the *acd5* ceramide kinase mutant and t18:0 ceramides were the key sphingolipid component mediating *acd5* cell death. We will discuss the importance of regulating intracellular sphingolipid components and systemic sphingolipid homeostasis in plants during stress responses.

Degradation of FATTY ACID EXPORT PROTEIN 1 by RHOMBOID-LIKE PROTEASE 11 contributes to cold tolerance in *Arabidopsis*

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Plants need to acclimate to different stresses to optimize growth under unfavorable conditions. In Arabidopsis thaliana, the abundance of the chloroplast envelope protein FATTY ACID EXPORT PROTEIN1 (FAX1) decreases after the onset of low temperatures (Trentmann et al., 2020, Plant Physiology, doi: 10.1104/pp.19.00947). However, how FAX1 degradation occurs and whether altered FAX1 abundance contributes to cold tolerance in plants remains unclear. The rapid cold-induced increase in RHOMBOID-LIKE PROTEASE11 (RBL11) transcript levels, the physical interaction of RBL11 with FAX1, the specific FAX1 degradation after RBL11 expression, and the absence of cold-induced FAX1 degradation in rbl11 loss-of-function mutants suggest that this enzyme is responsible for FAX1 degradation. Proteomic analyses showed that rbl11 mutants have higher levels of FAX1 and other proteins involved in membrane lipid homeostasis, suggesting that RBL11 is a key element in the remodeling of membrane properties during cold conditions. Consequently, in the cold, rbl11 mutants show a shift in lipid biosynthesis toward the eukaryotic pathway, which coincides with impaired cold tolerance. To test whether cold sensitivity is due to increased FAX1 levels, we analyzed FAX1 overexpressors. The rbl11 mutants and FAX1 overexpressor lines show superimposable phenotypic defects upon exposure to cold temperatures. Our results (John et al., 2024, Plant Cell, doi: 10.1093/plcell/koae011) show that the cold-induced degradation of FAX1 by RBL11 is critical for Arabidopsis to survive cold and freezing periods.

How Aquatic Plants Develop Tolerance to Heavy Metal Toxicity

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Unlike terrestrial angiosperm plants, the freshwater aquatic angiosperm duckweed (*Spirodela polyrhiza*) grows directly in water and has distinct responses to heavy-metal stress. Plantlets accumulate metabolites, including lipids and carbohydrates, under heavy-metal stress, but how they balance metabolite levels is unclear, and the gene networks that mediate heavy-metal stress responses remain unknown. Here, we show that heavy-metal stress induced by flue gas desulfurization (FGD) wastewater reduces chlorophyll contents, inhibits growth, reduces membrane lipid biosynthesis, and stimulates membrane lipid degradation in *S. polyrhiza*, leading to triacylglycerol and carbohydrate accumulation. In FGD wastewater–treated plantlets, the degraded products of monogalactosyldiacylglycerol, primarily polyunsaturated fatty acids (18:3), were incorporated into triacylglycerols. Genes involved in early fatty acid biosynthesis, β-oxidation, and lipid degradation were upregulated while genes involved in cuticular wax biosynthesis were downregulated by treatment. The transcription factor gene *WRINKLED3* (*SpWRI3*) was upregulated in FGD wastewater–treated plantlets, and its ectopic expression increased tolerance to FGD wastewater in transgenic Arabidopsis (*Arabidopsis thaliana*). Transgenic Arabidopsis plants showed enhanced glutathione

and lower malondialdehyde contents under stress, suggesting that SpWRI3 functions in *S. polyrhiza* tolerance of FGD wastewater–induced heavy-metal stress. These results provide a basis for improving heavy metal–stress tolerance in plants for industrial applications.

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Title: Plant-derived oxylipins facilitate infection by *Fusarium graminearum*, the causal agent of Fusarium head blight in wheat

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Abstract: Fusarium head blight is a fungal disease of wheat, barley, and other small grains that adversely impacts grain yield and quality. *Fusarium graminearum* is the primary cause of this disease. Mycotoxins that accumulate in the infested tissues contribute to fungal pathogenicity and also limit the utility of the grain for consumption by humans and livestock animals. Our studies with *Arabidopsis thaliana* and wheat (*Triticum aestivum*) have indicated the involvement of plant 9- and 13-lipoxygenase-derived oxylipins in facilitating *F. graminearum* infection. In particular we found that while jasmonic acid synthesis/accumulation in the host promoted disease, in part by suppressing the activation of plant defenses that target *F. graminearum* infection, jasmonic acid also promoted fungal spore germination. In comparison, 9-lipoxygenase-derived oxylipins stimulated expression of fungal mycotoxin biosynthesis genes and simultaneously promoted jasmonic acid accumulation in the host plant. These results suggest an iinteraction between plant 9- and 13-lipoxygenase-derived oxylipins in cross-kingdom communication between a plant and pathogenic fungus, which promotes fungal infection.

Non-Photochemical Quenching in PLASTOGLOBULAR PROTEIN 18 (PG18)

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Plastoglobules are ubiquitous chloroplast lipo-protein droplets physically bound to the thylakoid membrane where they harbor neutral lipids such as triacylglycerols and essential carotenoids for photosynthesis. Despite strong implications in stress response due to plastoglobule swelling and abundance, a role for the plastoglobule in photosynthetic maintenance has yet to be elucidated primarily due to unknown functions of plastoglobule-localized proteins. This poster will discuss a role for the plastoglobule in maintaining photosynthesis through the study of PLASTOGLOBULAR PROTEIN 18 (PG18), a small protein with large impacts to photosynthesis that is dependent on photoperiod, temperature, and a phosphorylation event. Current work is underway to determine how PG18 functions *in vivo* to maintain efficient light harvesting by acting as a negative regulator of non-photochemical quenching.

Biophysical Properties of Lipid Membranes from Barley Roots during Low-Temperature Exposure and Recovery

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Glycerolipid remodeling, a dynamic mechanism for plant subsistence under cold stress, has been posited to affect the biophysical properties of cell membranes. In barley roots, remodeling has been observed to take place upon exposure to chilling stress and to be partially reverted during stress relief. In this study, we explored the biophysical characteristics of membranes formed with lipids extracted from barley roots subjected to chilling stress, or during a subsequent short- or long-term recovery. Our aim was to determine to what extent barley roots were able to offset the adverse effects of temperature on their cell membranes. For this purpose, we analyzed the response of the probe Laurdan inserted in bilayers of different extracts, the zeta potential of liposomes, and the behavior of Langmuir monolayers upon compression. We found important changes in the order of water molecules, which is in agreement with the changes in the unsaturation index of lipids due to remodeling. Regarding Langmuir monolayers, we found that films from all the extracts showed a reorganization at a surface pressure that depends on temperature. This reorganization occurred with an increase in entropy for extracts from control plants and without entropy changes for extracts from acclimated plants. In summary, some membrane properties were recovered after the stress, while others were not, suggesting that the membrane biophysical properties play a role in the mechanism of plant acclimation to chilling. These findings contribute to our understanding of the impact of lipid remodeling on biophysical modifications in plant roots.

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A new cold tolerant germination rapeseed germplasm is associated with linolenic acid derived JA

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Winter oilseed (*Brassica napus* L.) is the main planted specie in China and largely grown under an intensive rice-oilseed rape cropping system along Yangtze River. Due to the delay of rice harvesting, gemination and growth of rape seedlings are adversely affected by cold temperature, seriously reducing rapeseed production. We identified a new rapeseed germplasm named *gec* (germination under cold), which can germinate under low temperature. The *gec* germplasm accumulates less reactive oxygen species (ROS). The lipidomics analysis of seeds revealed that fatty adid profile of *gec* is significantly changed. The unsaturated fatty acid content, especially 18:3 fatty acid is more the seeds and geminated seelings of *gec* mutant. During the germination, degradation of TAG and production of glycerol phospholipids including phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidyl ethanolamine (PE), phosphatidylcholine (PC) and sphingolipids such as Hex2cer are more quckily in *gec* after cold treatment. Transcriptome and quantitative PCR analysis showed that cold-resistant core transcriptional factor *DREB1s* genes and *MYC2* and VSP2, marker genes of JA, which is a key cold-defensive hormone, are expressed more in cold-tolerant plants. The *gec* germplasm cannot germinate under low temperature after the exogenous application of DIECA, the inhibitor of JA biosynthesis while MeJA supplement can accerlerate seed gemintation of *gec* and increase seed germination rates of other varietires under cold, suggesting that this germplasm relies on JA biosynthesis to regulate cold resistance. Consistently, JA accumulates more in *gec* than control after cold treatment. Genetics analysis indicates that the cold-tolerant phenotype of *gec* is controlled by two recessive loci and QTL-seq will be used to conduct genetic mapping and identify the candidate genes for the low-temperature germination loci. Therefore, a new germplasm that can germinate under cold is identified and the genes controlling the cold resistance and the mechanism are being explored.

Key words: Brassica napus; germination under cold; fatty acid profile; JA biosynthesis; candidate genes; mapping.

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7. Plastidic lipids: glycolipids, phospholipids, isoprenoids

Alyssa	Clews
Katharina	Guthbrod
Hyojin	Kim
Zolian Zoong	Lwe
Batoul	Moubarak
Akiko	Yoshihara

Uncovering a chloroplast lipase interactome in *Arabidopsis thaliana* via TurboID-proximity labelling

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Lipid metabolism is essential to plant growth and development, integral to many biological processes ranging from membrane homeostasis to storage lipid accumulation and turnover. Among the key enzymes involved in lipid metabolism are lipases, of which over 50 are annotated to be chloroplast localized in *Arabidopsis thaliana,* including DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1) and DAD-LIKE LIPASES (DALLs). Consisting of seven chloroplast phospholipid lipases A1 (PLA_1), several class I DALLs are putatively involved with jasmonic acid biosynthesis, but their other function(s) remain largely unknown. Notably, a DALL homolog in *Brassica napus*, CHLOROPLAST LIPASE PROTEIN (BnCLIP) was previously shown to localize to chloroplast-endoplasmic reticulum (ER) membrane contact sites, indicative of a novel BnCLIP function in inter-organellar lipid trafficking. Here, to gain further insight on the role of DALL lipases, TurboID-proximity labelling was employed with BnCLIP as a molecular 'bait' to uncover its local interactome in *A. thaliana*. Specifically, transgenic *A. thaliana* lines stably expressing BnCLIP fused to TurboID (a mutated biotin ligase) were developed. Proper subcellular localization of BnCLIP-TurboID to chloroplasts was confirmed in *Nicotiana benthamiana* leaf cells using fluorescence-based confocal laser-scanning microscopy (CLSM) and following protease digestion assays of BnCLIP from transgenic *A. thaliana* intact chloroplasts. From the biotinylated proteome of chloroplasts from *BnCLIP-TurboID*-expressing *A. thaliana,* several DALL-interactome candidates were identified via mass spectrometry. The co-localization and interaction of several top candidates are being investigated with CLSM-based intracellular localization assays in *N. benthamiana* leaves as well as bimolecular fluorescence complementation and membrane yeast-2-hybrid assays, respectively. Presented are results showcasing AtDALL3 as a representative BnCLIP interactor, and discussed are ongoing experiments aimed at elucidating the *in vitro* and *in planta* function of the AtDALL3 interactome, as well as the overarching significance of this project towards our understanding of lipid interplay between organelles in plant cells and potential future bioengineering strategies.

The relationship between two bilayer-forming glycolipids DGDG and SQDG in the thylakoid membrane

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Digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) are bilayer-forming glycolipids, accounting for ~30% and ~10% of total thylakoid membrane lipids, respectively. In the thylakoid membrane, ~90% of the DGDG and ~75% of the SQDG are estimated to be allocated to the inner leaflet of the bilayer. Despite these common features, little is known about the functional relationship between DGDG and SQDG. To reveal it, we crossed knockout mutants of DGDG synthase1 (*dgd1-2*) and UDP-sulfoquinovose synthase (*sqd1*) of *Arabidopsis thaliana* and examined the double mutant (*dgd1-2 sqd1*) in terms of growth and photosynthesis.

Previous studies revealed that *dgd1-2*, which largely lacks DGDG synthesis, shows hyperaccumulation of jasmonic acid (JA) and consequent growth impairment, whereas *sqd1*, which completely lacks SQDG, has no obvious defective phenotypes. Although the *dgd1-2 sqd1* double mutant showed stronger growth impairment than *dgd1-2*, our transcriptome analysis revealed that the double mutant has increased JA-responsive gene expression similar to *dgd1-2*, suggesting that the loss of SQDG neither enhances nor attenuates the JA overproduction caused by DGDG deficiency. While no global downregulation of photosynthesis-related genes was observed in *dgd1-2 sqd1*, the double mutant showed greater decreases in chlorophyll content and photosynthetic activity than *dgd1-2*, with the thylakoid membrane structure being intensely curled.

Our results indicate that SQDG partially supports the roles of DGDG in maintaining the thylakoid architecture, chlorophyll accumulation, and photosynthesis, presumably by locating preferentially in the inner leaflet of the thylakoid membrane with DGDG.

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Structural basis for donor sugar specificity in a plant galactolipid synthase

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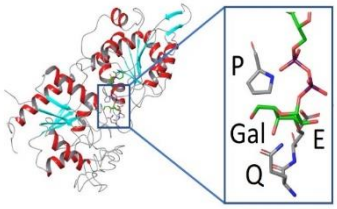
One of chloroplasts' essential and unique features is their high content of the galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Galactolipids constitute up to 80% of the total lipid content in chloroplastic membranes and are thought to be essential for the integrity and stability of membrane systems. However, it remains unclear why galactose (and not the more abundant glucose) has been selected and conserved during the evolution of photosynthetic organisms, from cyanobacteria to plants.

The key enzyme for the synthesis of the majority of galactolipids in Arabidopsis is MGD1, a monotopic membrane protein localized in the inner envelope membrane (iem). Plastid MGDs appear to differ from the two-step cyanobacterial pathway which produces MGDG from a monoglucosyldiacylglycerol substrate. MGD1 is a galactosyltransferase that transfers a galactose (Gal) residue from UDP-Gal to diacylglycerol (DAG) to form MGDG. This study aimed to comprehend the molecular determinants of MGD1 specificity, and possibly change its donor substrate from UDP-Gal to UDP-Glucose. The crystal structure of MGD1 was previously solved [1], identifying three key residues that determine donor specificity: P433, Q455 and E456, also named the PQE motif. Several mutants were engineered using rational protein design based on MGD1 structure and comparing the equivalent 'PQE' motif in closely related glucosyltransferases. The P433 residue and its environment were found to be the key for donor specificity. The presence of a $GP^{433}G$ motif seems to be a signature of a galactosyltransferase activity, whereas a $GG^{433}X$ (X being an aliphatic residue) is indicative of a glucosyltransferase activity. MGD enzyme engineering will be discussed.



[1] Rocha J. et al. Plant J. 2016, 85, 622-633

Mass Spectrometry-Based Identification of Triacylglycerol and Isoprenoid Esters Produced by Cyanobacterial Acyltransferases

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According to the endosymbiont theory, plant chloroplasts originated from an ancient cyanobacterium through endosymbiosis. As a consequence, many of the molecular and structural features of chloroplasts were inherited from and are therefore similar to cyanobacteria. Chloroplasts and cyanobacteria are the site for the production of a variety of lipids essential for photosynthesis and other metabolic processes. In plants, plastoglobules store non-polar lipids including fatty acid phytyl esters which are produced by phytyl ester synthases 1 and 2 (PES1, PES2), members of the family of esterase-lipase-thioesterase (ELT) proteins, during stress. Several cyanobacteria harbor ELT-like acyltransferase genes that might carry out similar functions as PES1 and PES2 in plants. It was previously demonstrated that the ELT-like acyltransferase slr2103 from *Synechocystis* sp. PCC6803 is responsible for the synthesis of triacylglycerol (TAG), fatty acid phytyl esters (Aizouq *et al*., 2020, PNAS), as well as plastoquinone-C esters and plastoquinol esters (Mori-Moriyama et al., 2023, Biochem Biophys Res Comm; Kondo et al., 2023, Front Plant Res; Ishikawa et al., 2023, PNAS nexus). We have established a strategy for the isolation of glycerolipids (including TAG), fatty acid phytyl esters as well as plastoquinol and their esters via solid-phase-extraction and subsequent analysis from one single sample. This procedure enables maximal recovery of minor lipid analytes from cyanobacteria and plants and protects the analytes from oxidation. The analytes are quantified using direct infusion nanospray quadrupole-time-of-flight mass spectrometry (Q-TOF MS) (Gutbrod et al., 2021, Plant Lipids: Meth Prot) as well as liquid chromatography (LC) coupled to Q-TOF MS, by comparison to internal standards. Here we present the workflow for the identification and quantification of the products synthesized by the *Synechocystis* slr2103 acyltransferase and its homologous proteins from other cyanobacteria, to study their broad range of activity.

Nature-Guided Strategies to Maximize Astaxanthin Production and Purity in Camelina Oil for Aquaculture Feed and High-Value Food Applications

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Astaxanthin (3,3'-dihydroxy-β, β'-carotene-4,4'-dione) is a red lipophilic pigment derived from β-carotene and is distinguished by keto groups on each ionone ring ("ketocarotenoid"). Recent research has focused on the discovery of sustainable sources and cost-effective production of natural astaxanthin for use in aquaculture feedstocks to confer red color to products such as salmon and shrimp. Flower petals of *Adonis aestivalis* are one of the few plant sources of astaxanthin, which have among the highest astaxanthin concentrations in land plants (~1.5% DW). Our research has focused on the transfer of the *Adonis* astaxanthin biosynthetic pathway to *Camelina sativa* (camelina) seeds for cost-effective and oilseed-based production. In our first prototype, we introduced and expressed transgenes of maize phytoene synthase, *A. aestivalis* carotenoid β-ring 4-dehydrogenase (CBFD2) and carotenoid 4-hydroxy-β-ring 4-dehydrogenase (HBFD1) under seed-specific promoters. While this strategy was effective at generating seeds rich in astaxanthin, the seeds also contained ketocarotenoid intermediates and had delayed germination. To identify additional genes to maximize astaxanthin production and purity, we conducted transcriptome profiling of *A. aestivalis* flower petals. Candidate genes from the transcriptome were initially characterized using *Agrobacterium*-infiltration of *Nicotiana benthamiana* leaves for transient astaxanthin production. To improve quantity and quality of astaxanthin in camelina seed, we used seed-specific expression of transgenes which encode astaxanthin biosynthetic enzymes (CBFD1/2 and HBFD1/2), candidate for astaxanthin esterifying enzyme and maize phytoene synthase. Engineered camelina accumulated the high purity of astaxanthin in seed and displayed normal seed germination and seedling establishment compared to the first prototype. We will describe the use of the top candidate genes for generating high astaxanthin concentrations while maintaining uncompromised seed fitness.

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AT1G78690 mutants display altered lipid profiles and early leaf senescence

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Lipids are known to have structural and developmental roles in plants, such as the requirement for specific phospholipids in the initiation of flowering. At the *AT1G78690* locus, our lab identified two T-DNA insertion mutants that exhibit earlier leaf senescence and an altered lipid profile in comparison to wild type (WT, Col-0). An *in vitro* study by Bulat and Garrett (2011, JBC, doi:10.1074/jbc.M111.269779) showed that the *AT1G78690* gene product has lysoglycerophospholipid acyltransferase activity when incubated with lipid extract from *E. coli*, but little else is known about its activity and/or biological role. Initial results indicate that both mutant lines have higher numbers of senescing leaves and perturbed lipid profiles compared to WT. The leaf lipid profiles of both mutants, analyzed by direct-infusion electrospray ionization triple quadrupole mass spectrometry and compared to those of WT, reveal higher levels of polyunsaturated lipids such as PE_36:6, lower levels of less unsaturated lipids such as PE_36:2, lower levels of the major cardiolipin species (CL_72:12, CL_72:11, CL_72:10, and CL_72:9), and higher levels of lysocardiolipin. The AT1G78690 predicted protein exhibits amino acid similarity (42%) and shares the NHXXXXD acyltransferase motif with TAFAZZIN, a lysocardiolipin acyltransferase in humans whose mutation results in lower cardiolipin levels and disease. Integrated analysis of lipidomics and transcriptomics data identified the lipid molecular species that contribute most to distinguishing the mutants from WT, which included the previously identified lipids. The integrated analysis also indicates genes that are highly correlated, positively or negatively, with the identified lipids. Several of the identified genes are highly expressed in senescent leaves and/or later stages of development. Further experiments are underway to determine how the altered lipid composition and functions of correlated genes contribute to early senescence.

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AT1G78690 mutants display altered cardiolipin remodeling akin to mutations in TAFAZZIN

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Lipid profiling under control and wounded conditions of 364 confirmed SALK T-DNA lines, all in putative lipid metabolism genes, identified the *AT1G78690* mutant among those with the most altered profile from wild type (WT, Columbia-0; Lusk, Neumann et al., 2022, doi: 10.1093/pcp/pcac088). An *in vitro* study by Bulat and Garrett (2011, JBC, doi:10.1074/jbc.M111.269779) showed that the *AT1G78690* gene product has lysoglycerophospholipid acyltransferase activity when incubated with lipid extract from *E. coli*, but little else is known about its activity and/or biological role. The leaf lipid profiles of two different T-DNA lines, analyzed by direct-infusion electrospray ionization triple quadrupole mass spectrometry and compared to those of WT, revealed higher levels of polyunsaturated lipids such as PE_36:6, lower levels of less unsaturated lipids such as PE_36:2, lower levels of the major cardiolipin species (CL_72:12, CL_72:11, CL_72:10, and CL_72:9), and higher levels of lysocardiolipin. Analysis of *AT1G78690* mRNA levels using RT-qPCR showed that one of the mutants is a knockout and the other a knockdown. Multiple independent complementation lines in the knockout mutant background displayed WT levels for both cardiolipin profile and *AT1G78690* mRNA. The AT1G78690 predicted protein exhibits amino acid similarity (42%) and shares the NHXXXXD acyltransferase motif with TAFAZZIN, a human lysocardiolipin acyltransferase whose mutation results in lower cardiolipin levels and disease. *AT3G05510* is another gene that is also predicted to be the TAFAZZIN homolog in Arabidopsis. Lipid profiling of *AT3G05510* T-DNA mutants showed no difference in their cardiolipin profiles compared to WT. So far, our results indicate that *AT1G78690* is the only plant homolog of TAFAZZIN. Interestingly, we also observed a clear early senescence phenotype in the knockout mutant of *AT1G78690* and in another T-DNA line. However, our attempted complementation of the knockout mutant did not restore WT-like senescence phenotype. Analysis of F_2 plants from a cross between WT and the knockout mutant is underway to determine if both the cardiolipin and early senescence phenotypes co-segregate with mutations in *AT1G78690*. Future experiments will assess whether alterations in cardiolipin composition affect respiration.

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8. Surface lipids: biosynthesis and regulation of plant protection

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Mi Chung Suh
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Liu Huazhen
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Characterization of the gene networks underlying cuticular wax production in maize silks via systems' biology approaches

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The plant cuticle is comprised of a cutin polymer matrix infused with and coated by cuticular waxes that together form a protective layer against environmental stresses on aerial organs. Maize (*Zea mays*) silks are female floral organs responsible for pollen reception and hence essential for crop production. The cuticular waxes on maize silks are predominantly comprised of very-long-chain fatty acids (VLCFAs) and VLC-hydrocarbons. Herein, we collected the silks from two agronomically important inbreds B73 and Mo17, and queried the cuticular wax metabolomes and transcriptomes along the silk length to capture its developmental progression and environmental transition as silks emerge from the husks into the external environment. Cuticular wax biosynthesis was most impacted by the transition of silk micro-environment and genetic background, reflected by varying wax compositions between the husk-encased and emerged silk portions, and between the inbreds. Joint statistical analysis of wax metabolomes and companion transcriptomes was then employed to dissect the gene networks underlying the observed metabolome variations, and identified ~300 genes associated with cuticular wax variation between genotypes and/or silk microenvironments. These cuticular wax-associated genes include those confirmed to participate in wax biosynthesis as well as genes from pathways that directly or indirectly interact with wax deposition, including cell wall biogenesis, proteasome-mediated protein degradation, and vesicle trafficking. In a synergistic approach to dissect the regulation of cuticle biosynthesis, a small-scale expression-quantitative trait loci (eQTL) experiment identified two transcription factors potentially regulating the genes with documented roles in cuticular wax biosynthesis as well as the cuticular wax-associated genes

identified by the joint transcriptome-metabolome statistical analysis. Collectively, these quantitative genetic and systems' biology approaches reveal complex cuticular wax-associated gene networks that that determine cuticle deposition.

Identification of a receptor-like kinase, which positively regulates the biosynthesis of extracellular lipids in Arabidopsis

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Receptor-like kinase (RLK) is the largest single-transmembrane receptor family in plants. They are widely involved in plant development and stress responses, being one of the most important protein families. However, not much is known about the function of RLKs in biosynthesis of extracellular lipids in plants. Here, we investigated the role of RLKx in the formation of suberin and cuticle. Knockout of *RLKx* resulted in a significant reduction in 4- to 5-day-old root suberin, which was observed by the fluorescent signal from FY088 staining and suberin polyester analysis. RNA-seq and RT-qPCR analysis revealed that numbers of genes responsible for suberin formation were down-regulated in *rlkx* roots compared to the wild type. Similar results were observed in seed coats as well. In addition, knockout of *RLKx* also led to defective cuticle formation. Toluidine blue-O (TBO) staining showed that the *rlkx* leaf was highly permeable to TBO compared to the wild type. This phenotype was accompanied by the decreased expression of wax biosynthetic genes. These results suggest that RLKx plays a significant role in the formation of suberin and cuticle.

Exploring Chemical Diversity in *Brassica carinata* Epicuticular Wax

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Epicuticular wax plays an important role in water conservation, pathogen and insect defense, and UV protection of land plants. It is present on the outside of aerial plant surfaces, serving as the outermost boundary layer of the cuticle. Highly variable in chemical composition and structure, the epicuticular wax is generally made up of a mixture of C_{20}-C_{40} alkanes, alcohols, ketones, aldehydes and very long chain fatty acids. Less abundant constituents include alkyl esters and triterpenes. In an effort to enhance drought tolerance of plants, many studies have engineered increased wax production. While these studies were successful, the biosynthesis of wax is metabolically expensive and could result in yield losses. As an alternative, the alteration of wax composition may provide an avenue to improve water conservation without a huge metabolic expense. However, the role individual components of epicuticular wax perform has not been studied in great detail. To properly investigate this, there is a need to identify chemical diversity in wax composition. Surveying natural diversity may offer altered wax profiles for investigation without the need for genome editing and may uncover novel aspects of the wax biosynthetic pathways. Here, we present *Brassica carinata* as a model system to further the understanding of the role of wax in drought conditions. Three unique lines with distinctly different wax profiles were discovered. Wax of various plant tissues, such as leaf, stem, pod, and petal was extracted and analyzed by gas chromatography. This poster will present a chemical analysis of the epicuticular wax of these lines, and preliminary results of physiological studies designed to assess the role of wax.

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Systemic signaling in plants; from chemicals to cuticle

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Systemic acquired resistance (SAR) is a form of broad-spectrum resistance induced in response to local infection that protects uninfected parts against subsequent secondary infections. Several diverse chemical signals contributing to SAR have been isolated and characterized, including glycerol-3-phosphate (G3P) and pipecolic acid (Pip), both of which play an important role in human and plant disease physiologies. In addition to these, salicylic acid (SA) also regulates SAR and normal transport of SA and G3P is required for *de novo* biosynthesis of Pip in the distal leaves. Pip is catabolized via multiple pathways, and this in turn regulates vitamin homeostasis. A normal SAR also requires a normal cuticle, which in turn regulates water potential and thereby apoplastic transport of SA. Interestingly, cellular pH also plays an important role in SA transport and this was established using pH sensor lines as well as analysis of mutants affected in pH homeostasis. Both SA and G3P regulate the stability of trans-acting small interfering RNA (tasi-RNA), which function as an early mobile signal in SAR. Conversely, knock-out mutations in tasi-RNA or RNA silencing components required for tasi-RNA biogenesis compromise SAR without altering levels of SA or G3P. Together, these results highlight a novel relationship between plant cuticle, SA, G3P, Pip and RNA-mediated signaling in SAR.

9. Trafficking of lipids among cells and tissues

Evan LaBrant
Cailin Smith

Candidate Membrane Lipid Trafficking Proteins Located at Membrane Contact Sites Between Chloroplast Inner Envelope and Thylakoid Membranes

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Chloroplasts perform a variety of functions necessary for plant viability, particularly the capture and conversion of light energy into chemical potential by thylakoid membranes. Thylakoid membranes lack *de novo* glycerolipid synthesis, relying on lipid trafficking from the inner envelope membrane (IEM). However, mechanisms by which thylakoid lipids are exchanged with the IEM are largely unknown. Other membrane systems with high lipid flux typically traffic lipids through membrane contact sites (MCS) or vesicles, and both have been observed in chloroplasts. Research into thylakoid/IEM lipid trafficking and the proteins facilitating the movement is largely hampered by a lack of robust function/targeting prediction and a paucity of bait proteins available for probing the physical gap between thylakoids and IEM. To address this, we compiled a set of candidate proteins that may participate in chloroplast lipid trafficking using three metrics. First, we interrogated public datasets for kingdom-wide functional homologs predicted to localize in chloroplasts. We supplemented this literature-based approach by using proteomic detection in double-fractionated chloroplast membranes, as well as enrichment in protein populations residing at MCS using a split-biotin ligase system. Candidate coding sequences were then cloned and fluorescently tagged for confocal laser scanning microscopy using transient expression in *Nicotiana benthamiana* by agroinfiltration. Of the 30 candidates screened thus far, six localized to chloroplasts, while several others localized to compartments near the chloroplast. These include proteins with domains predicted to confer lipid binding and/or MCS tethering functions, as well as proteins lacking known functions. Candidates are currently being assessed by co-localization with fluorescent protein MCS markers, co-fractionation with known chloroplast membrane proteins, and effects of knock-out/over-expression on chloroplast membrane morphology during low temperature de-etiolation. By experimentally determining constituents of lipid trafficking within the chloroplast, we aim to gain a better understanding of thylakoid membrane lipid dynamics.

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Intraorganellar Membrane Contact Sites May Facilitate Synthesis of Photosynthetic Membranes

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The lipids that make up the photosynthetic thylakoid membrane are synthesized in the chloroplast envelope membranes and must be transported to the thylakoid to generate and repair it. The role of membrane contact sites is well established in many lipid transport systems; however, their presence, role, and components are unknown between the thylakoid and inner envelope membrane. So far, we have identified putative membrane contact site proteins located in the chloroplast which affect thylakoid-inner envelope contact site ultrastructure. We are following up on this finding to determine the precise sub-organellar location of these proteins. Our current work probes the role of these proteins in facilitating lipid transfer from the chloroplast envelope to the thylakoid. We developed a genetic cross with a known stromal vesicle mutant to determine how lipid transport and the photosynthetic capacity of the thylakoid membrane may be affected by the absence of our putative contact site proteins. The characterization of these contact sites will strengthen our understanding of thylakoid membrane synthesis and inform further research into photosynthetic membrane remodeling and repair.

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10. Triacylglycerol: metabolism, biosynthetic regulation and storage

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Marta Carletti
Jillian Fan
Matthew Garrison Garneau
Tomoko Hatanaka
Evelyn Kulesza
Jose Manual Martínez-Rivas
Sean McGuire
Stewart Morley
Jorg Schwender
Jay Shockey
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Deborah Ighalo
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The first intron of DGAT1 regulates temporal expression and enables complementation of the lethal *dgat1/pdat1* mutant with foreign DGATs

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The accumulation of triacylglycerols (TAGs) is crucial during plant development. Two main enzymes share overlapping functions to produce TAGs, namely acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) and phospholipid:diacylglycerol acyltransferase 1 (PDAT1). The absence of both genes in a *dgat1-1/pdat1-2* double mutant is pollen and embryo lethal. To rescue the lethality, a *dgat1-1/PDAT1/pdat1-2* parent was transformed with transgenic constructs containing the *AtDGAT1* promoter fused to the *AtDGAT1* open reading frame (ORF) either with or without the first intron. Triple homozygous mutant/transgenic lines were obtained, however, in the absence of the first intron anthers fail to fill with pollen, total seed yield is ~10% of wild-type, seed oil content remains reduced (like the *dgat1-1* mutant), and non-Mendelian segregation of the *PDAT1/pdat1-2* locus occurs. In contrast, plants expressing the *DGAT1pro:AtDGAT1* transgene with the first intron recover to near wild-type phenotypes. Alignment of plant *DGAT1*s and motif analysis indicated that the first intron of various plant species share common transcription factor binding sites, suggesting that first intron functionality may be shared among diverged plant lineages. Furthermore, the regulatory elements were validated by transforming the *dgat1-1* mutant with the *DGAT1* ORFs of *Camelina sativa*, *Physaria fendleri*, and *Ricinus communis* with the *DGAT1Pro* and the *Arabidopsis DGAT1* first intron. The *Camelina* and *Physaria* transgenics complemented the *dgat1-1* phenotype back to wild-type. In contrast, the *Ricinus* DGAT1 had similar, but unique seed oil contents, with increased erucic acid (~4%), and decreased linolenic acid (~28%) content when compared to *dgat1-1*. We hypothesize that *PDAT1* can be mutated in these lines, resulting in the foreign DGATs being the main acyltransferase for TAG synthesis. Altogether, we demonstrate that the combination of the *DGAT1pro* with the first intron regulates DGAT1 activity in developing pollen and embryos and that these regulatory elements can be useful genetic tools to functionally replace TAG biosynthetic enzymes.

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Title: Fueling the future: Evaluating carbon conversion efficiency and biosynthetic pathways in *fae1-3* pennycress (*Thlaspi arvense*) embryos

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Abstract: The aviation industry's growing interest in renewable jet-fuel has encouraged the exploration of alternative oilseed crops. Replacing traditional fossil fuels with a sustainable, domestically sourced crop can significantly reduce carbon emissions, thus mitigating global climate instability. *Thlaspi arvense*, commonly known as pennycress, is an emerging oilseed cover crop that can be grown during the offseason of corn and soybean to produce renewable biofuel. Pennycress is widely undomesticated and can be genetically modified, providing opportunities for trait enhancement. To bolster its economic competitiveness, metabolic engineering was employed to enhance favorable traits such as seed oil composition. FATTY ACID ELONGATION-1 (*FAE1*) was targeted using CRISPR-Cas9 to eliminate erucic acid from pennycress seed oil thereby enhancing its cold flow properties. This project investigated the impact of modifying fatty acid composition on carbon conversion efficiency (CCE) and metabolism. Findings revealed that WT embryos are 21.8% more efficient at converting carbon substrates into biomass components than *fae1-3* embryos. Additionally, ^{13}C labeling experiments in developing embryos uncovered unique metabolic patterns useful in understanding key pathways contributing to fatty acid synthesis and elongation. Overall, this research provides crucial insights for optimizing pennycress seed oil while preserving essential traits for biofuel applications.

Transcriptional Regulation of TAG Degradation by PHL7-PA Interaction in Seed Development

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Unraveling the molecular mechanisms governing seed development is crucial for advancing our understanding of broader plant biology. Triacylglycerol (TAG) degradation plays a pivotal role in regulating seed oil content, yet its transcriptional regulation of this process remains poorly understood. Here, we identify PHL7 as a transcription factor and validate its interaction with phosphatidic acid (PA), conducting a comprehensive functional analysis in *Arabidopsis thaliana*. Remarkably, knockout (KO) of *PHL7* results in an approximate 10% increase in seed oil content, whereas overexpression (OE) leads to a reduction of about 7.7-13.1% in oil content, highlighting PHL7's regulatory role in seed oil accumulation. PHL7-OE lines exhibit increased expression of genes involved in TAG degradation, whereas PHL7-KO shows decreased expression levels. Further analysis reveals that the SDP1 gene promoter region contains a binding site of PHL7, P1BS motif. This interaction is confirmed in vivo by EMSA. Additionally, PA inhibited the binding of PHL7 to SDP1 promoter P1BS motif. Taken together, our findings elucidate the crucial involvement of the PHL7-PA interaction in mediating TAG degradation during seed development.

Exploring Fatty Acid Synthesis in Alternative Crops via ^{13}C-Labeling Approaches

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To address global issues related to food and energy security, my research group is studying alternative crops, historically considered as weeds until recently being identified as promising sources of renewable specialty fuels and other industrially-relevant chemicals. Indeed, *Physaria fendleri* (aka. lesquerella) and *Thlaspi arvense* (aka. field pennycress), winter annuals closely related to *Arabidopsis thaliana*, produce and store in their seeds unusual fatty acids (FAs) that can replace several petrochemicals currently used in industry. Our long-term goal is to advance these plants—that can be grown off-season—as dedicated bioenergy and industrial crops. However, for these oilseed crops to become economically viable sources of unusual FAs, oil synthesis needs to be improved. A lack of knowledge of the metabolic pathways underlying FA synthesis in *Physaria* and pennycress seeds presents a major constraint.
Free of toxins and rich in hydroxy fatty acids, *Physaria* is a promising alternative to imported castor oil and is on the verge of being commercialized. This study aims to identify important biochemical step(s) for oil synthesis in *Physaria*, which may serve as target(s) for future crop improvement. To advance towards this goal, the endosperm composition was analyzed by LC-MS/MS to develop and validate culture conditions that mimic the development of the embryos in planta. Using developing *Physaria* embryos in culture, we were able i) to determine the efficiency with which embryos convert substrates into biomass components, and ii) to replace the substrates by ^{13}C-labeled ones and monitor the flow of ^{13}C-carbon in central metabolic pathways leading to oil synthesis. Finally, different *Physaria* accessions with contrasting seed oil content are currently under investigation and compared to other plant embryos, including pennycress.

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The Seipin protein of *Phaeodactylum tricornutum*: structure and function specificities

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Microalgae are a diverse group of photosynthetic unicellular organisms found in a wide variety of habitats. In response to abiotic and biotic stresses, microalgae produce lipid droplets (LD), dynamic organelles involved in the storage of carbon- and energy-rich molecules and remodeling of membrane lipids.

The biogenesis of LD is a complex mechanism in which the Seipin protein is a key player. Seipin has been studied in a variety of organisms. It is a transmembrane protein forming an oligomer embedded in the ER membrane, and is functionally involved in various aspects of LD biogenesis: orientation of LD budding, control of LD size and control of triacylglycerols (TAG) flux into the LD.

In the oleaginous diatom *Phaeodactylum tricornutum*, a Seipin isoform (PtSeipin) has been identified, and has been shown to be involved in LD biogenesis. The KO of PtSeipin revealed a strong phenotype with oversized lipid droplets remarkably coupled to accumulation of TAG, in particular in response to high light stress. Such a strong effect on TAG accumulation has not been observed in other organisms, suggesting that it has specific molecular functions. In order to understand these specificities and better understand LD biogenesis and functions in diatoms, we are exploring the structure of PtSeipin and its interactions with other proteins.

Exploring lipid metabolism through isotropic tracing of cultured embryos: Implications for the engineering of Camelina and Related Crop Plants

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Camelina sativa and many of its relatives in *Brassicaceae* represent promising crop species for sustainable agriculture due to their resilience to diverse environmental conditions, ease of genetic manipulation, and potential for producing high-quality oils suitable for food, biofuel, and industrial applications. However, understanding the mechanisms governing lipid accumulation, especially in plant lines with engineered lipid metabolism is crucial for optimizing their seed oil content and composition. Isotopic tracing of lipids in developing embryos provides a powerful tool to elucidate these changing dynamics in lipid metabolism. By monitoring the flux of labeled substrates through various metabolic pathways, including *de novo* fatty acid synthesis, lipid biosynthesis, and triacylglycerol assembly and turnover, insights can be gained into the regulatory mechanisms governing lipid accumulation. We have traced metabolism in developing Camelina seeds engineered to accumulate both medium chain fatty acids and healthy fatty acids. The results demonstrate plant adaptations to bioengineering approaches which alter acyl flux through lipid metabolism. These results provide insights for the development of more robust engineering strategies and can be a useful tool in the "Design-Build-Test-Learn" engineering cycle.

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A Specific Amino Acid Residue Increases Activity of Higher Plant DGAT1

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Acyl-CoA:diacylglycerol acyltransferase (DGAT) catalyzes the final step of triacylglycerol (TAG) synthesis and plays an important role in plant oil accumulation. We previously reported that the TAG synthesis activity of Vernonia (*Vernonia galamensis*) DGAT1 was significantly higher than that of Arabidopsis and soybean DGAT1 in yeast microsome assays. We also introduced *DGAT1* cDNAs of Arabidopsis, Vernonia, soybean, and castor bean into Arabidopsis to compare their activities in planta. All Vernonia *DGAT1*-expressing lines showed significantly higher seed oil contents (an average increase of 49% compared to the wild type). All other Arabidopsis *DGAT1*-overexpressing lines did not show a significant seed oil increase. In addition to these four *DGAT1*s, sunflower, Jatropha, and sesame *DGAT1* genes were introduced into yeast mutants defective in TAG biosynthesis. The yeasts harboring Arabidopsis, castor, and soybean *DGAT1*s had only slightly increased TAG content, while the yeasts harboring Vernonia, sunflower, Jatropha, and sesame *DGAT1*s accumulated 10 times higher levels of TAG compared to the former three strains. When the amino acid sequences of the former three strains (‘Low’ group) and the latter four strains (‘High’ group) were compared, three amino acid residues were different. When these three amino acid residues of soybean *DGAT1* (‘Low’ group) were replaced with the corresponding amino acids of the ‘High’ group, TAG accumulation increased 2.1 to 3.7 times higher than the native *DGAT1*. The effects of these amino acid replacements in Arabidopsis *DGAT1* were relatively greater than such replacements in the native soybean *DGAT1* and the highest TAG

accumulation level of the A286V variant was 91% of that of sunflower *DGAT1* ('High' group).

DGAT1 of Camelina (*Camelina sativa*), of the Brassicaceae family as for Arabidopsis, has a similar (93%) amino acid sequence to that of Arabidopsis. Camelina *DGAT1* is being improved using "base editing" technology.

***Reference**: Hatanaka T. et al. (2022) Different acyl-CoA:diacylglycerol acylgransferases vary widely in function and a targeted amino acid substitution enhances oil accumulation. **J. Exp. Bot.** 73: 3030-3043.*

Transcriptional Regulation of Oil Biosynthesis genes in Arabidopsis Seed tissue Induced by Avocado WRINKLED2

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Triacylglycerol (TAG), a primary storage lipid found in plants' seed and non-seed tissues, is the primary component of vegetable oils. The transcription factor WRINKLED1 (WRI1) directly or indirectly controls its synthesis. *AtWRI2* is not functional. In avocado (*Persea americana*) mesocarp, which constitutes 60-70% oil by dry weight, three paralogs of the WRI1 are highly expressed. We previously demonstrated that *PaWRI1* and *PaWRI2* can interact with AW-box present in the promoters of target genes and transactivate them. Recognizing the importance of enhancing seed oil content to meet the global demand for vegetable oil, we hypothesize that *PaWRI1* and *PaWRI2* can induce oil accumulation in seed tissue through trans-activating the genes associated with fatty acid (FA) biosynthesis. We propose performing functional complementation of Arabidopsis mutant seeds, *wri1-1* (CS69538) and *wri2* (SALK_111105C) with *PaWRI1* and *PaWRI2*. The strategy involves introducing the cDNA sequence of *PaWRI2* and *PaWRI1*, cloned under a seed-specific promoter (Napin), into Arabidopsis using a floral dip method. Transformed T0 seeds will be selected, grown in selective kanamycin media, and further cultivated for T1, and T2 generations to harvest homozygous transformants. Further, Seeds will be collected at maturation stages from T3 plants to analyze total lipid, FA, and TAG using Gas Chromatography-Flame Ionization Detection (GC-FID). A comparison will be made between the T3 plants and the control group. Quantitative Reverse Transcription PCR (qRT-PCR) will be done using mRNA extracted from the seeds to measure the relative expression of oil biosynthesis-related genes. The outcome of this study will provide insights into the possible functional role of *PaWRI2* in seed tissue, which is otherwise considered non-functional in other oilseed crops, and how it can be harnessed to increase oil production in other oil-rich plants.

Metabolic Engineering of *Physcomitrium patens* (moss) for Oleic Acid-rich Vegetable Oil Production

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Triacylglycerols (TAGs), the predominant storage lipids in plants, are integral to vegetable oil composition. The anticipated surge in vegetable oil demand by 2030 necessitates sustainable production strategies. Oleic acid, a vital omega-9 monounsaturated fatty acid with diverse industrial applications, faces challenges in current production methods using high oleic plant oils. Extended growth cycles and climatic limitations highlight the need for sustainable alternatives. Preliminary results in *Nicotiana benthamiana* leaves show a significant increase in TAG content, particularly oleic acid, through co-expression of avocado (*Persea americana*) WRINKLED genes (*PaWRI1* and *PaWRI2*) and enzymes diacylglycerol acyltransferase 1 (*Pa*DGAT1) and phospholipid: diacylglycerol acyltransferase 1(*Pa*PDAT1). This study aims on metabolically engineering *Physcomitrium patens* (moss) to improve oil production in moss, mostly TAG, which has a higher proportion of oleic acid through stable expression of avocado WRINKLED transcription factors and enzymes *Pa*WRI1, *Pa*WRI2, *Pa*DGAT1, and *Pa*PDAT1. *P. patens*, a non-seed plant model organism, offers advantages like climatic tolerance, short life cycle, efficient gene targeting via homologous recombination and transgene integration . We hypothesize that expressing avocado genes in moss will enhance oleic acid production, providing a sustainable alternative for the oleochemical industry. The experimental approach involves cloning the avocado gene cDNA into an expression vector, via polyethylene glycol (PEG)-mediated protoplast transformation, transformant screening, and subsequent lipid analysis via TLC followed by gas chromatography for TAG quantification, and fatty acid composition analysis. Nile Red staining followed by confocal fluorescence microscopy will be done to visualize and quantify the lipid droplets. We expect to demonstrate a substantial increase in oleic acid production in *P. patens*. The transgenic *P. patens* will provide an eco-friendly solution for the oleochemical industry, addressing the growing demand for oleic acid in an environmentally conscious manner.

Natural variation in triglyceride and diglyceride composition impacts thermal traits of cocoa butter

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The seeds of Theobroma cacao L., or the chocolate tree, are highly valuable due to their rich cocoa butter content, prized for its diverse applications in chocolate making, cosmetics, and pharmaceuticals. Cocoa butter is primarily composed of triglycerides (TAGs) which contribute to its unique melting properties. Minor lipid components such as diglycerides (DAGs) may also affect its thermal characteristics. Factors such as genotype, origin, and cultivation environment influence the lipid content and composition of cocoa butter, yet its natural variation remains insufficiently explored, requiring further research to comprehensively characterize its diversity. My work involves characterizing total fat content, TAG and DAG composition, and thermal traits across multiple cacao genotypes to categorize this natural variation. Results have indicated significant differences across many of these traits, offering insights for selecting optimal genotypes or breeding programs targeting specific cocoa butter traits. Furthermore, utilizing the cacao transcriptome atlas, I have investigated the expression of key lipid genes in cacao seeds and tissues across development stages, identifying potential genes influencing fat content and composition. These findings may advance cacao breeding for lipid traits and future innovations in transgenic cacao.

Increasing Triacylglycerol and Vitamin E Content to Improve the Production and Nutritional Quality of the Virgin Olive Oil

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Olive is the second most important oil fruit crop cultivated worldwide. One of the primary objectives of olive breeding programs is the generation of new cultivars with a higher oil content in the olive fruit or improved functional quality in their oils, such as increased vitamin E content. Understanding the molecular basis of triacylglycerol accumulation and α-tocopherol biosynthesis will allow the development of associated molecular markers to improve the efficiency of those breeding programs.

Lipid droplets (LDs) are subcellular organelles which consist of a neutral lipid core surrounded by a phospholipid monolayer and a diversity of surface-associated proteins, such as the lipid droplet-associated protein (LDAP). Although the original identification of LDAP was conducted in avocado mesocarp, studies on the regulation and physiological role of LDAPs in oil-rich fruit mesocarp are scarce. Olive mesocarp represents an interesting tissue to study the regulation of LDAP since triacylglycerol accumulation and response to environmental stresses occur simultaneously in the olive fruit. In the present study, the isolation and characterization of five olive *LDAP* genes have been carried out, as well as the subcellular immunolocalization of LDAP2 isoform in the LDs of mesocarp cells. In addition, expression analysis in two different olive cultivars during fruit development and ripening was performed to investigate the physiological function of each *LDAP* gene regarding to its specific participation in LD formation and their potential implication in the response to abiotic stresses.

On the other hand, cDNA sequences encoding the four enzymes that catalyze the committed steps of tocopherol biosynthesis have been cloned from olive. Sequence analysis indicated that they code for the corresponding enzymes and transcriptional analysis in distinct olive tissues and cultivars indicated that their expression levels are spatially and temporally regulated in a cultivar-dependent manner.

Expression of malic enzyme reveals subcellular carbon partitioning for storage reserve production in soybeans

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The market value for soybeans is derived from high-quality protein and oil seed reserves. Soybeans contain ~40% protein and ~20% oil by weight, whereas other legumes produce a greater percentage of starch. Though protein-enriched meal is the primary market driver for soybeans, oil has greater value on a per pound basis because of its utility for cooking and as a raw material for polymers, plastics, surfactants, and biofuel. With such large demands, small improvements in seed protein and oil content have significant impacts on agriculture, export markets, and the US and other global economies.

Biosynthesis of storage reserves occurs in multiple organelles that exchange central intermediates including two essential metabolites linked by malic enzyme; malate, and pyruvate. Prior studies based on isotopic labeling and steady-state metabolic flux analyses indicated malic enzyme provides carbon for fatty acid biosynthesis in plants, though genetic evidence confirming this role is lacking. We hypothesized that increasing malic enzyme flux would alter carbon partitioning and result in increased lipid levels in soybeans.

To examine this theory, transgenic soybean plants expressing Arabidopsis malic enzyme targeted inside or outside the plastid were generated. Protein, oil, central metabolites, cofactors, and acyl-acyl carrier protein (ACPs) were quantified over development. Amino and fatty acid levels were altered resulting in an increase in lipids by 0.5–2% of seed biomass (i.e. 2–9% change in oil) with no measurable impact on seed mass or protein levels.

Lipid Remodeling as a Heat Tolerance Mechanism in Soybean

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Soybean is the most important oilseed and a major affordable protein source worldwide. High temperature is a major factor limiting soybean yield. Incomplete knowledge of the molecular changes underlying soybean heat tolerance has hindered progress in developing heat-resilient varieties for sustainable production. This study aimed at discovering specific lipid metabolic pathways regulating heat tolerance in soybeans using a recombinant inbred line population (F6-derived population of 200 lines developed from a cross between the heat-tolerant genotype DS25-1 and heat-susceptible genotype DT97-4290), under heat stress (38-42°C) imposed under field conditions. Leaf samples were collected on the final day of heat stress, lipids were extracted, and ESI-MS/MS was utilized for lipid profiling. Using LipidSig software, over 100 differentially expressed lipids (based on a cut-off log2FC ±0.5 and padj 0.05) were identified across the genotypes between ambient and high temperatures. Among these, phospholipids including PA, PC, PE, PG, and PI accounted for >50% of the differentially expressed lipids. When ranked using random forest based on heat responsiveness, PG, PC, MGDG, and TG lipids were top ranked. Glycolipids including DGDG and MGDG and phospholipids including PG, PA, and PC displayed decreased unsaturation levels under heat stress as lipid species rich in unsaturated fatty acids (such as 36:6, 34:6, and 36:4) decreased, and those with saturated and less unsaturated fatty acids (such as 18:0, 34:0, 32:0, 18:3, 18:2, 16:0, and 18:1) increased. Pathway analysis using Plant Metabolic Network confirmed the glycolipid desaturation pathway as the major lipid remodeling mechanism in both up- and down-regulated lipids for altering the lipid unsaturation levels and maintaining membrane stability and integrity. Additionally, phospholipid remodeling pathway was identified for up-regulated lipids, emphasizing the role of the Lands cycle. This cycle, responsible for the deacylation and reacylation of phospholipids, plays a crucial role in maintaining phospholipid homeostasis under stress conditions.

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Co-expression of *WRI1* and *DGAT1* Increases Seed Oil Content in Pennycress

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Thlaspi arvense (field pennycress) is an emerging oilseed crop closely related to *Arabidopsis thaliana*. Prior research on oilseed species has demonstrated that seed specific upregulation of *WRINKLED1* (*WRI1*), the key regulator of triacylglycerol (TAG) biosynthesis, can augment seed oil content. This phenomenon extends to the TAG biosynthetic enzyme diglyceride acyltransferase 1 (DGAT1). In this study, we show that stable co-expression of *WRI1* and *DGAT1* in pennycress during seed development significantly elevates oil content in mature seeds. Utilizing *in vitro* cultured developing embryos, we thoroughly characterized the alterations in seed composition resulting from *WRI1*/*DGAT1* overexpression, employing a combination of transcriptomic analysis, targeted metabolomics, and metabolic flux analysis. The increase in conversion of glucose into TAG in the *WRI1*/*DGAT1* overexpression line is mostly accomplished by increased flux through RubisCO, bypassing a section of glycolysis. The increase in pyruvate formation is achieved by increased flux through cytosolic pyruvate kinase.

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Beyond FAD2: Towards a Better Understanding of the Genetics of Non-GMO High-Oleate Cottonseed Oil.

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Cottonseed fiber is a multibillion-dollar global commodity used for production of fabrics, textiles, and nonwovens. Cottonseed oil is an extremely valuable coproduct in its own right but has recently lost frying oil market share due to unfavorable polyunsaturated/monounsaturated acid ratios that require the now-banned partial hydrogenation process, which also creates unhealthy trans fats. These issues have created high demand for a naturally occurring cottonseed oil with high oleate levels. Rare South American Pima (*Gossypium barbadense*) cotton varieties contain a mutant allele of *fatty acid desaturase-2* (*FAD2-1D*); breeding these lines to standard upland cotton (*G. hirsutum*) resulted in public release of medium oleate lines called HOa1-HOa4. The HOa lines contain 33-35% oleate, approximately twice that found in upland cottonseed oil. Ongoing breeding strategies created four new lines with 52-60% oleate, substantially exceeding the HOa levels and the ~42% oleate levels in the wild Pima parent. The overall fatty acid profiles of these oils will also be ideal for deep fat frying. However, to assist in future breeding of the high oleate trait into elite fiber production lines, marker assisted selection would be greatly aided by identification of the genes responsible for this trait. The current results focus on the genetic and biochemical characterization of novel alleles of candidate type A and type B fatty acid thioesterase (*FATA* and *FATB*) genes that may exert influence over the saturate/monounsaturate fatty acid pools during seed filling.

Identifying the genes associated with high levels of medium-chain fatty acids (MCFAs) in Chinese elm (*Ulmus parvifolia*)

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Chinese elm (*Ulmus parvofolia*) is a large ornamental deciduous shade tree native to Eastern Asia. Various parts of this tree are utilized in traditional medicine, and its samaras are edible. Interestingly, Chinese elm seed has a high content of medium-chain fatty acids (MCFAs,) with about 60 mole% capric acid (FA 10:0) and over 20 mole% caprylic acid (FA 8:0) during the mid-stages of development. We aim to explore MCFA production during seed development and identify genes involved in the synthesis of MCFA and its incorporation into triacylglycerols (TAGs.) Preliminary findings from gas chromatography and electrospray ionization tandem mass spectrometry indicate an accumulation of MCFAs during seed development, alongside consistently low levels of common fatty acids. Ongoing research is focusing on gene discovery. This will generate transgenic plants with potential candidate genes, ultimately enhancing our understanding of MCFA synthesis and metabolism.

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Isolation and characterization of Arabidopsis Mutants affecting Lipid Droplet accumulation in leaves

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Lipid droplets (LDs) serve as crucial organelles within cells, contributing to energy storage, lipid metabolism, and overall cellular equilibrium in eukaryotic organisms. Understanding LD biology holds significant importance for fields such as agriculture, bioenergy, and human health. However, despite their significance, the complex processes governing LD formation, growth, maturation, and utilization remain largely elusive. To gain deeper insights into the molecular factors and regulatory mechanisms influencing LD abundance in Arabidopsis, we conducted a forward genetic mutant screening for LD mutants. Through this approach, we identified a diverse array of mutants exhibiting variations in LD number and neutral lipid composition in leaves. These mutants can be categorized into three groups based on LD count and triacylglycerol (TAG) content: those with increases in LD count and TAG levels (Type 1), those with decreases in LD count and TAG content (Type 2), and those with increased LD presence but diminished TAG levels (Type 3). Here, we present our findings from the initial biochemical and genetic analysis of some of these mutants. One Type 3 mutant predominantly accumulates sterol esters as an LD component. We anticipate that identifying the disrupted genes in these mutants will offer insights into the molecular components involved in LD assembly and the mechanisms regulating lipid metabolism and homeostasis.

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Other Category

Kaitlin	Bachle

Cowpea as a Model Organism to Study Plastoglobule Function

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Plastoglobules are ubiquitous chloroplast lipo-protein droplets physically bound to the thylakoid membrane where they harbor neutral lipids such as triacylglycerols and essential carotenoids for photosynthesis. Despite strong implications in stress response due to plastoglobule swelling and abundance, functional elucidation has been widely understudied primarily due to the large abundance of tissue required for study. To this end, we have discovered that the stress tolerant legume crop, *Vigna unguiculata,* or cowpea, accumulates large amounts of plastoglobules under seemingly unstressed conditions. Owing to the natural resilience of cowpeas and other legumes, Initial studies are focused on determining if the degree of vegetative oil accumulation in cowpea is the response of an unintentional stressor or if abundance underlies a natural mechanism for stress tolerance. Overall, the poster and ongoing project aims to determine plastoglobule inducing and reducing conditions in a developing model species so that future work may better study plastoglobular function.