Multistate Research Project NC1168

Project No. and Title:	NC1168 Regulation of Photosynthetic Processes
Period Covered:	November 2007 to November 2008
Date of Report:	December 2, 2009
Annual Meeting Dates:	November 21, 2008 to November 23, 2008

Participants:

Robert Aiken (Kansas State University), Fred Below (University of Illinois), Christoph Benning (Michigan State University), Hans Bohnert (University of Illinois), John Cushman (University of Nevada), Gerald Edwards (Washington State University), Glenda E. Gillaspy (Virginia Tech), Irwin Goldman (University of Wisconsin, *Administrative Advisor*), Mark Guiltinan (Pennsylvania State University), Jeff Harper (University of Nevada), Robert Houtz (University of Kentucky), Steve Huber (USDA-ARS, University of Illinois), Jyan-Chyun Jang (Ohio State University), Robert Jones (University of Minnesota), Karen Koch (University of Florida), Wayne Loescher (Michigan State University), Stephen Long (University of Illinois), Ron Mittler (University of Nevada), Brandon Moore (Clemson University), Thomas Okita (Washington State University), Jack Preiss (Michigan AES), Vara Prasad (Kansas State University), Steven Rodermel (Iowa State University), Mary E. Rumpho (University of Maine), Michael E. Salvucci (USDA-ARS, Arizona), Martin Spalding (Iowa State University), Robert Spreitzer (University of Nebraska), Donald P. Weeks (University of Nebraska)

Brief Summary of Minutes of Annual Meeting:

This was the second meeting of Multistate Research Project NC1168 (Regulation of Photosynthetic Processes), which is the approved renewal of NC1142. The meeting was hosted by the USDA-ARS, Arid-Land Agricultural Research Center, Maricopa, Arizona and held at the Fiesta Inn Resort in Tempe, Arizona on November 22, 2008.

Attending members included Christoph Benning, Irwin Goldman, Jeffery Harper, Jyan-Chyun Jang, Brandon Moore, Mary Rumpho, Michael Salvucci, Robert Spreitzer and Martin Spalding Attending guests included Csengele Barta and Elizabete Carmo-Silva (USDA-ARS, Maricopa).

The meeting convened at 8:30 AM in the Encantada I Room in the Fiesta Inn Resort. Mike Salvucci (Arizona ARS) provided opening remarks and introduced the guests. Dr. Irwin Goldman, Administrative Advisor (University of Wisconsin) welcomed the group and discussed the purpose of the project and his role as Administrative Advisor.

JC Jang (Ohio AES) gave the first presentation on the identification of the CCCH TZF gene that appears to be a positive regulator of ABA responses and a negative regulator of GA responses. Jeff Harper (Nevada AES) then presented his research findings on the targets of 14-3-3 proteins. Jeff also discussed his research on the role of lipid flippases in stress tolerance. Next, Marty Spalding (Iowa AES) presented his findings on the induction of the CO_2 concentrating mechanism in Chlamydomonas. At 10:30 AM the group took a break and resumed work at 11 AM with a presentation by Bob Spreitzer (Nebraska, AES). Bob Spreitzer's presentation focused on new information about Rubisco structure/function, including the properties of hybrid enzymes. Mike Salvucci (Arizona ARS) gave the next presentation, describing his research findings on the regulatory mechanisms controlling Rubisco activase, particularly under heat stress. Lunch occurred 12:00 PM at the Fiesta Inn dining room. The meeting reconvened at 1:30.

After lunch, Mary Rumpho (Maine AES) presented her findings on the plastid genome in *Vaucheria litorea*. Brandon Moore (South Carolina AES) followed with a presentation on his findings with hexokinase and hexokinase-like proteins. Christoph Benning (Michigan AES) spoke next and discussed his research to develop rutabaga tubers as oil storage organs. Christoph also mentioned that Jack Preiss, a long-time member of the project group, would be retiring in January. The final formal presentation was given by Vera Prassad (Kansas AES), a new member of the group. Vera highlighted his research on the effects of high temperature stress on photosynthesis.

At 3:30 PM the business meeting convened. Mike Salvucci brought up the next and future-meeting dates and posed the question, should the annual meeting be held on the second weekend in November or switch to the third and should it rotate among sites or establish a single location. After some discussion, the group decided to retain the second weekend in November as the meeting time for future meetings and keep the location rotating. Bob Spreitzer reminded the group that Christoph Benning was nominated and elected to serve as host of the 2011 meeting and would be in charge of the project renewal. The renewal will be due in the Spring of 2011.

The schedule for future meetings was determined:

2009	Ohio AES	J. C. Jang
2010	Virginia AES	Glenda Gillaspy
2011	Michigan AES	Christoph Benning

The meeting was adjourned at 5:00 PM.

Accomplishments:

A. Plastid Function and Intracellular Communication.

IA-AES has continued to investigate the role of the *var2* gene of *Arabidopsis* (which encodes a chloroplast AtFtsH metalloprotease) in the repair of photodamaged photosystem II.

Selection of suppressors has now identified a chloroplast-localized homolog of pseudouridine (Ψ) synthase, whose physical presence is necessary for proper chloroplast rRNA processing.

ME-AES has continued to study the endosymbiotic relationship between the marine mollusk *Elysia chlorotica* (sea slug) and chloroplasts of the heterokont alga *Vaucheria litorea*. Sequencing of the plastid genome of *Vaucheria litorea* and the transcriptome of *E. chlorotica* confirmed that the algal genome lacks the full complement of genes required for autonomous photosynthesis and that horizontal gene transfer may have occurred as a means for maintaining photosynthesis in the endosymbiotic algal chloroplasts.

B. Photosynthetic Capture and Photorespiratory Release of CO_2.

NE-AES continued research on the structure/function relationships of Rubisco. A strategy for expressing foreign *rbcS* cDNA in Chlamydomonas was developed and used to produce hybrid Rubisco enzymes containing *Arabidopsis*, spinach, or sunflower small subunits. The *Arabidopsis* small subunit causes an increase in CO_2/O_2 specificity.

IA-AES has investigated whether the limiting-CO_2-inducible, putative ABC-type transporter, HLA3 might function as a HCO_3^- transporter by assaying the effect of pH on growth, photosynthetic Ci affinity and Ci uptake in VLC conditions following RNA interference (RNAi) knockdown of *HLA3* mRNA levels in wild-type and mutant cells. The combination of nearly complete knockdown of *HLA3* mRNA with mutations in *LCIB* and/or simultaneous, apparently off-target knockdown of *LCIA* mRNA provide compelling evidence that HLA3 is directly or indirectly involved in HCO_3^- transport and provide additional evidence for a role of LCIA in chloroplast envelope HCO_3^- transport.

WA-AES is studying unique species in the family Chenopodiaceae that perform C_4 photosynthesis without Kranz anatomy. Visualization with fluorescent dyes targeted to membranes or to specific organelles, showed the formation of two cytoplasmic compartments (one functioning to capture atmospheric CO_2 in the C_4 cycle, the other to accept CO_2 from the C_4 cycle and assimilate it by Rubisco/C_3 cycle), which are interconnected by cytoplasmic channels, is dependent on unique development of the vacuole.

AZ-ARS has continued to examine the regulation of Rubisco activase, focusing on regulation in plant species that express only the non-redox regulated β-isoform of activase. The potential involvement of CP-12 in the redox modulation of tobacco activase was investigated but no evidence was uncovered for an interaction between CP12 alone or as a complex with GAPDH and/or PRK and activase.

KY-AES has characterized alternative substrates for Rubisco large subunit methyltransferase (LSMT) as well as structural determinants that define the interaction between Rubisco and Rubisco LSMT. A high resolution crystal structure was obtained for the essential chloroplast-localized enzyme peptide deformylase, a region of the protein which may be a determinant for polypeptide substrate specificity was identified.

C. Mechanisms Regulating Photosynthate Partitioning.

FL-AES has continued to investigate the role for sorbitol metabolism and/or shuttling within developing kernels, identifying a maize mutant deficient in the sole gene for sorbitol dehydrogenase (Sdh1). The effects of this mutation suggest a central role for sorbitol in the sugar balance of developing kernels indicate that SDH may not be the only sorbitol-handling enzyme in the maize kernel. FL-AES has continued to refine a method for 3'-UTR profiling that can be used to dissect the functional roles of genes involved in photosynthate partitioning or other processes.

IL-AES/ARS has elucidated details about the involvement of 14-3-3 proteins as essential, positive regulators of brassinosteroids (BRs) by identifying the likely binding sites for 14-3-3 proteins on BRI1 as Ser-858 and Thr-872. Using an affinity tagged 14-3-3 protein, NV-AES identified more than 124 14-3-3 clients, 103 of which have not previously been reported. Many of the newly identified clients are involved directly in metabolism, such as phosphoenol pyruvate (PEP) carboxylase, ion transport (e.g. glutamate receptors), transcription (e.g. multiple WRKY transcription factors), vesicle trafficking (e.g. dynamin), lipid signaling (e.g. phospholipase D), and hormone signaling (e.g. proteins implicated in ethylene and branssinolide signaling).

IL-AES/ARS has continued to investigate the role of tyrosine phosphorylation in BR signaling. The results indicated that tyrosine phosphorylation may be an important and previously unrecognized component of plant receptor kinases.

In an effort to globally identify glucose responsive genes in Arabidopsis, OH-AES identified a CCCH TZF gene that appears to shuffle between nucleus and P-bodies. Microarray analyses reveal that AtTZF1 affects ABA/GA responses via the changes on ABA/GA responsive genes, and overexpression of AtTZF1 affects sugar repressible genes, implicating AtTZF1 as a positive regulator of sugar responses.

SC-AES has established the primary function of Arabidopsis hexokinase-like1 (HKL1) protein in mediating cross-talk with plant ethylene signaling. Gene expression studies showed that HKL1 is required for ethylene-dependent regulation of some ethylene response genes and for glucose-dependent regulation of some ethylene biosynthesis genes.

To increase accumulation of oil in developing embryos or in tissues normally not producing oil, MI-AES generated transgenic canola lines (*Brassica napus var. napus*) expressing an Arabidopsis WRINKLED1(*WRI1)* cDNA that are now at the T2 stage and being analyzed. This same gene was inserted into rutabaga, starchy root storage organ, to reengineer carbon partitioning in this organ towards the accumulation of oil. Proof of concept for this approach was obtained by expressing the *WRI1* cDNA in hairy roots and showing that the transgenic produce small amounts of triacylglycerol, while control roots dis not.

MI-AES and WA-AES continue to define the structure-function relationships of ADP-glucose pyrophosphorylase, which catalyzes the first step in starch synthesis. To determine the relevance of the subunit activities *in planta* a T- DNA mutant of *APS1* (*aps1*) was used to show that the large subunits (APL1 and APL2) are not only regulatory but also have catalytic activity that may

contribute to ADP-Glucose synthesis *in planta*. Complementary studies with directed mutants showed that, when assembled with catalytically silenced small subunits, the resulting enzyme shows significantly elevated catalytic activity and is activated by 3-PGA.

WA-AES also examined the role of plastidic phosphorylase, Pho, in starch synthesis in rice. These results indicate that Pho1 is essential for in starch initiation at low temperatures and that one or more other factors can complement the function of Pho1 at high temperatures.

PA-AES continued the functional dissection of the maize Starch Branching Enzyme family (SBE), demonstrating that each of the SBE isoforms (SBEI, SBEIIa and SBEIIb) play specific roles in the plant life cycle.

D. Developmental and Environmental Limitations to Photosynthesis.

AZ-ARS has continued to investigate the role of Rubisco activase in thermotolerance and thermal acclimation. Previously, an involvement of cpn60β in protecting activase from denaturation was identified and a reconstituted system using recombinant activase and cpn60β. has been developed to characterize the nature of the interaction between cpn60β and activase.

IL-AES investigated the N response of single leaf CER and initial Rubisco activity in maize. The results suggest that maize CER, and potentially grain yield, could be improved by increasing the partitioning of N into Rubisco.

VA-AES is studying the role of *myo*-inositol signaling in abiotic stress. Characterization of one Class B enzyme, 5PTase13, has shown that the WD40 repeats in the 5PTase13 protein allow for complex formation with the SnRK1.1 protein, a Sucrose Nonfermenting Related Kinase. To understand how 5PTase13 and SnRK interact to alter nutrient and/or stress signaling, SnRK activity was measured in wildtype and *5ptase13* mutants exposed to various nutrient conditions. The results support a model of 5PTase13 as a positive regulator of SnRK signaling.

NV-AES has continued to use microarray transcript profiling, quantitative RT-PCR, and metabolite profiling to show that metabolite differences found under long-term salinity or water deficit stress are linked to differences in transcript abundance of many genes involved in energy metabolism and nitrogen assimilation, particularly photosynthesis, gluconeogenesis, and photorespiration. Since genetic dissection of regulatory and metabolic attributes of CAM has been limited by the difficulty of identifying a reliable phenotype for mutant screening, a novel method to screen for CAM-deficiency was developed and used to screen fast neutron-mutagenized populations of common ice plant (*Mesembryanthemum crystallinum* L.). The CAM-deficient mutants were deficient in leaf starch and lacked plastidic phosphoglucomutase, an enzyme critical for gluconeogenesis and starch formation, resulting in substrate limitation of nocturnal C_4 acid formation.

NV-AES continued to investigate the role of calcium signals in plant stress responses. Cyclic nucleotide signaling was implicated in plant abiotic stress responses by showing that a calcium

permeable cyclic nucleotide gated channel (CNGC16) is critical to the success of plant reproduction under conditions of hot days and cold nights.

KS-AES conducted controlled environment and field studies with grain sorghum to identify mechanisms underlying differences in transpiration efficiency (TE). Irrigation requirement and crop productivity were related to the duration of canopy temperature in excess of a stress threshold—an irrigation decision criterion.

Impacts:

Further insight into the function of VAR2 FtsH metalloprotease in photosynthesis, plant development and plant stress responses might lead to the design of strategies to manipulate the photosynthetic capacity and quality of crop plants.

Characterization of the endosymbiotic association in the *Vaucheria-Elysia* system may provide a further understanding of the requirements for nuclear-cytosolic interactions to sustain chloroplast structure and function. Ultimately, this may add to the information necessary for maintaining these energy-capturing organelles in culture or foreign hosts for long periods of time, thus leading to breakthroughs in artificial photosynthesis.

Understanding the mechanisms that allow *Chlamydomonas* to acclimate to such low CO_2 concentrations and identifying the genes involved is important for evaluating the potential for transfer of all or part of this CCM into higher plants, as well as to increase our understanding of CO_2 assimilation and its regulation in this key group of photosynthetic organisms.

Understanding the precise role of Rubisco activase in the inhibition of photosynthesis under moderate heat stress provides information essential for developing strategies that improve the thermotolerance of plants.

Defining the pattern of development in single cell C4 photosynthesis is important in designing approaches to genetically modify important C_3 crops, like rice, to improve carbon acquisition in source tissue.

Hybrid Rubiscos containing foreign subunits were produced. Since regions in either the large or small subunit far from the active site can influence carboxylation catalytic efficiency and CO_2/O_2 specificity, these regions may serve as targets for either the design of an improved Rubisco, or for genetic selection following random mutagenesis or DNA shuffling.

A structure for the peptide deformylase was resolved. Resolution of the structure of this enzyme provides novel insights into polypeptide substrate specificity and provides unique opportunities for the development of specific inhibitors capable of acting as broad spectrum.

A 3'UTR 454 profiling method was developed that provides a less-expensive and more specific means of expression profiling. The capacity to individually quantify expression of gene-family

members can be invaluable to efforts to dissect their functional roles (in photosynthate partitioning or other processes).

Information about the *sdh1* mutant offers insights into potential roles of sorbitol in grain development. It also has potential as a new sweetcorn, and may be useful in sweetcorn breeding efforts.

The recognition that HKL proteins are not merely compromised in catalytic activity, but have specialized non-catalytic functions is important for our understanding of sugar-sensing.

Evidence for a role for $InsP_3$ signaling in regulation of SnRK1.1 and nutrient sensing suggests that this signaling could be targeted for increasing plant health and/or biomass.

Developing rutabaga as a biofuel crop might provide mid-west sugar beet farmers with an alternative biofuel crop that could be handled, harvested, and processed using methods established for sugar beets.

ADP-glucose pyrophosphorylase has been shown to be a useful target for engineering increases in the production of starch and biomass.

Increase the capacity for starch synthesis is an important strategy for improving rice yields.

New knowledge about the mechanisms of starch branch-point formation and its role in starch granule formation will lead to the development of novel starch types for industrial, medical, and food-processing applications.

Understanding how N is used in the establishment and maintenance of the photosynthetic apparatus is paramount to developing maize genotypes that yield well under low N conditions.

The involvement of cyclic nucleotide signaling in plant abiotic stress tolerance represents a new pathway that can be modified for improving stress tolerance.

CAM-deficient mutants were generated. The availability of mutant in CAM could improve our understanding of the complex day-night metabolic and circadian regulatory processes that govern CAM.

The demonstration that P-bodies exist in plants and the role of cytplasmic mRNPs in development and stress responses is expected to advance our understanding in sugar and hormone signaling in plants.

Information about how signaling pathways regulate the expression and activity of important cellular proteins and enzymes provides the necessary fundamental knowledge for understanding how hormonal signaling pathways control expression of genes involved in assimilate production and utilization, and hence plant growth and development.

Knowledge of stomatal regulation of assimilation and transpiration can guide effective irrigation management as well as cultivar development. Enhanced transpiration efficiency could boost land productivity of grain as well as biofuel feedstock.

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