Final Technical Report

Award No. DE-SC0009885

Applicant/Institution: University of Southern California

Project Principal Investigator: Steve A. Kay, Biological Sciences, University of Southern California

Project Title: Systems Level Regulation of Rhythmic Growth Rate and Biomass Accumulation in Grasses

Objectives: Several breakthroughs have been recently made in our understanding of plant growth and biomass accumulation. It was found that plant growth is rhythmically controlled throughout the day by the circadian clock throught a compelx interplay of light and phytohormone signaling pathways. While plants such as the C4 energy crop sorghum (*Sorghum bicolor* (L.) Moench) and possibly the C3 grass *Brachypodium distachyon* also exhibit daily rhythms in growth rate, the molecular details of its regulation remain to be explored. A better understanding of diurnally regulated growth behavior in grasses may lead to species-specific mechanisms highly relevant to future strategies to optimize energy crop biomass yield. Here we propose to devise a systems approach to identify, in parallel, regulatory hubs associated with rhythmic growth in C3 and C4 plants. We propose to use rhythmicity in daily growth patterns to drive the discovery of regulatory network modules controling biomass accumulation.

Description: The project is divided in three main parts: **1)** Performing time-lapse imaging and growth measurement in *B. distachyon* and *S. bicolor* to determine growth rate dynamic during the day/night cycle. Identifying growth-associated genes whose expression patterns follow the observed growth dynamics using deep sequencing technology, **2)** identifying regulators of these genes by screening for DNA-binding proteins interacting with the growth-associated gene promoters identified in Aim 1. Screens will be performed using a validated yeast-one hybrid strategy paired with a specifically designed *B. distachyon* and *S. bicolor* transcription factor libraries (1000 clones each), and **3)** Selecting 50 potential growth regulators from the screen for downstream characterization. The selection will be made by using a sytems biology approach by calculating the connectivity between growth rate, rhythmic gene expression profiles and TF expression profile and determine which TF is likely part of a hub and a potential regulator of plant growth. We will confirme the 50 DNA-protein interactions using transient transcriptional assays in *B. distachyon* and *S. bicolor*, and perform further testing *in vivo* by measuring growth parameters in transgenic loss- or gain-of-function lines for the selected transcription factors (25 *B. distachyon* and 3 *S. bicolor* lines).

In 2016 the Principal Investigator relocated the laboratory to The Scripps Research Institute (TSRI) where the project has continued with an end date of 08/31/17.

Accomplishments:
We successfully collected large datasets of gene expression form the model grass Brachypodium. We used and developed bioinformatics analysis tools to investigate the structure, dynamics and robustness of circadian regulated gene expression in Brachypodium.

Relevant Discoveries:
We were able to determine that the endogenous circadian clock appears to play a much more subdued role in growth regulation in Brachypodium, that has been demonstrated in either Arabidopsis, or crop plants like Rice, Corn and Soybean. This led to our conclusion that Brachypodium unfortunately is unlikely to serve as an informative model for understanding how

growth regulation in plants is under the control of circadian network circuitry. However, we were able to leverage our datasets in Brachypodium to inform us and reinforce a large collaborative study on gene networks governing cell wall deposition in Arabidopsis. This led to a major and highly cited publication relevant to improving biomass production.

Major Participants: Steve A. Kay, PI, USC is a leader in the field of circadian biology. By using innovative approaches combining genetics and real-time monitoring of bioluminescent reporters, he uncovered a large number of the animal and plant clocks genes. Recently he took the lead in constructing an expanded *A. thaliana* transcription factor collection for functional genomic studies. This toolset has already proven to be instrumental in the identification of novel clock genes and growth regulators in *A. thaliana*.

John E. Mullet, PI, Texas A&M University is using cutting edge genomics technology to identify genes in sorghum involved in plant drought tolerance, flowering time regulation, biomass composition, and yield. The information gained through these studies is being utilized to develop grain sorghum with improved drought tolerance and energy sorghum with increased yield and improved composition.

Samuel P. Hazen, PI, University of Massachusetts, Amherst is an expert in cell wall biosynthesis and transcriptional regulation in *Brachypodium distachyon*. He is co-PI of multiple *B. distachyon* sequencing projects and the Hazen laboratory has established numerous molecular biology techniques in this system including stable and transient transformation, genetic linkage mapping, and transcription profiling.