**178. Nutrient Cycling for Biomass: A multi-omics approach to model ectomycorrhizal regulatory networks**

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**Project Goals**: This project is focused on developing system-scale models at the symbiotic interface between ectomycorrhizal fungi (such as Laccaria bicolor) and tree species (such as poplar) in response to environmental nutrient availability/biochemistry. A multiple "omics" approach is implemented that integrates next generation sequencing transcriptomics, proteomics, biochemical analyses and ChIP-Seq analyses to construct ectomycorrhizal regulatory networks and computational modeling approaches to predict how atmospheric carbon is sequestered as plant and/or subsurface fungal biomass.

**Poster Abstract**: Recent advancement in high-throughput ‘omics’ approaches has led to the precise quantification of all classes of biological molecules, from genes through mRNA to proteins and metabolites. Though each class of ‘omics’ data provides important information, no single analytical approach provides comprehensive insight into the molecular mechanisms of biological phenomena. Therefore, multi-‘omics’ approaches (i.e. integration of multiple layers of information) allow a better understanding of the complex molecular interactions that orchestrate biological processes.

Our lab has been using such approaches to build a predictive understanding of the molecular mechanisms underlying symbiotic plant-fungal associations called ectomycorrhiza (ECM). Such interactions play an essential part in the regulation of soil nutrient cycling and subsequent carbon management in a variety of tree species. However, the complexity and variation of such systems in natural environments has hindered the accurate assessment of the factors that signal, establish and maintain these interactions. Consequently, our project employs a controlled laboratory Populus tremuloides X Laccaria bicolor in vitro ECM model system to explore nitrogen utilization and carbon management under nutrient limiting conditions.

RNA sequencing (RNA-Seq) and ChIP sequencing (ChIP-Seq) were employed to produce global transcript abundance data along with increased resolution on transcriptional regulation. Our developed methods are among the first to successfully use such techniques for an ectomycorrhizal system. The transcriptomics data generated a mycorrhizal metabolome model that predicts the synthesis of specific nitrogen rich compounds by Laccaria in response to nitrogen limitation. Such compounds or their metabolites may in turn be used for the growth of tremuloides in exchange for photosynthetically derived sugars. The data also identified regulated metabolic pathways, transporter proteins and transcription factors (TFs) that are implicated in nutrient assimilation and carbon management. Sixteen TFs having potential roles in mycorrhiza-specific carbon and nitrogen flux metabolism were selected for ChIP-Seq. The results from ChIP-Seq identified several enriched binding motifs that may be indicative of new classes of the transcription factor target genes. For example, 961 loci were identified as potential targets of the selected MADS-Box regulators, and the expected CC[A/T]6GG motif (termed the CArG-box) was present in the promoters of many of these target loci, thus validating the ChIP-Seq pipeline in P. tremuloides. However, other equally well-represented motifs were also identified in the promoters of other possible target genes, including some genes that are involved in biochemical and metabolic pathways associated with cellulose and lignin biosynthesis during plant growth and development.

As a means to further interrogate the sequencing data, biochemical analyses were performed to quantify key C, N and P metabolites in the leaves, stems and roots of P. tremuloides subjected to nutrient limiting
conditions during interaction with Laccaria. Metabolite levels were then correlated with the transcriptomics data to delineate ~200 biochemical pathways that play roles in symbiosis-linked nutrient uptake and resulting biomass development. The pathways involved in starch and sucrose biosynthesis as well as nitrate reduction were especially sensitive to mycorrhizal interaction under varying nutrient stresses. Here, optimum nitrogen availability produced trees that directed more carbohydrates in form of soluble sugars towards the mycorrhizal roots, leading to healthier colonization and higher tree biomass. However during nitrogen limiting conditions, the carbohydrate supply to the roots was reduced as the trees put more sugar resources into the production of sucrose and starch within the shoot tissues. Further correlations of these biochemical pathways with the ChIP-seq data and quantitative proteomics are currently underway.

As transcript expression levels do not always correlate well with protein expression levels, our project is using nanoLC-ESI-SWATH-MS to fill the gap in our understanding of protein activities during mycorrhizal interaction under nutrient limiting conditions. Sequential Windowed Acquisition of all THeoretical MS (SWATH-MS) is a quantitative proteomics approach that detects large numbers of peptides with high sensitivity, quantitative accuracy and reproducibility. In order to implement this technology for P. tremuloides, we developed a P. tremuloides protein database with 35,328 unique peptide sequences using our transcriptomics data (>780 million RNA-Seq short reads), significantly improving the protein identifications for this tree species by 48% over the P. trichocarpa database alone. This subsequently allowed the identification and quantification of proteins within crude protein extracts from the various tissues of P. tremuloides. For example, SWATH-MS analysis of the leaf proteome successfully detected and quantified 6,250 peptides, representing the abundance levels of ~2,000 proteins under each fungal and nutrient condition. Our analysis revealed that 626 of these proteins have differential abundance in response to different nitrogen availability, whereas only 38 proteins show differential abundance in response to mycorrhizal interaction. This highlights the importance of nitrogen availability on the overall system. In addition, using NonLinear Dynamics software, 2D gel electrophoresis (IEF/SDS-PAGE) detected both post-translational modifications and/or changes in protein abundance for a total of 62 proteins that were significantly different in each of the experimental groups. Thus, the new technologies and continuing computational approaches that have been implemented during the course of this project have not only identified novel biochemical and regulatory pathways but also implicate specific protein expression and post-translational modifications as playing important roles in the molecular mechanisms of how plants, including trees, respond to changes in environmental nutrient conditions.