3. PLANT & ENVIRONMENTAL BIOTECHNOLOGY

3.5. OMICS

Oral

Systems response to environmental conditions as studied by metabolome analysis

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The metabolome comprises the entity of small molecules present in a given biological system. Due to its vast chemical diversity resulting in major differences concerning extraction, purification or detection, the development of metabolomics has lagged behind the development of tools for genome-wide analysis on the RNA or protein level.

A number of years ago we have started to develop mass spectrometry based methods aiming at the high-throughput characterization of the metabolome.

In the presentation examples will be discussed showing the application of metabolomics in systems biology.

More specifically the kinetic response of *A. thaliana* towards changing environmental conditions will be presented.

Correlation matrices will be evaluated using a variety of graph tools and resulting hypothesis concerning the importance of certain metabolites and pathways with respect to adjustment of the biological system will be discussed.

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Plant metabolomics: a basis for plant functional genomics and biotechnology

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Metabolomics, which deals with all metabolites of an organism, is one of the major components of omics research. It plays significant roles in a variety of fields from medicines to agriculture and holds a fundamental position in functional genomics and biotechnology. Metabolomics research is particularly important in the plant field, because plants collectively produce a huge variety of chemical compounds, far more than animals and even microorganisms. In addition, most of the human-beneficial properties of plants — foods, medicinal resources, or industrial raw materials — are ascribed to plant metabolites.

Using *Arabidopsis thaliana*, an integrated analysis of metabolome and transcriptome led to prediction of gene-to-metabolite relations. Co-regulation framework models of genes and metabolites in the pathways of flavonoids, sulfur compounds and lipids, suggested the specific involvement of co-expressed genes in the synthesis and accumulation of the metabolites in the pathways. Reverse genetic and reverse biochemistry confirmed those delimited genes' functions in the pathways.

This strategy was further applicable to decipher the genes' functions of medicinal plants rich with a variety of phytochemicals, such as *Perilla frutescens* producing anthocyanins, *Ophiorrhiza pumila* root cultures producing an anti-cancer alkaloid camptothecin and *Glycyrrhiza uralensis* (licorice) producing natural sweetener glycyrrhizin. These results suggest the versatility of integration of metabolomics and transcriptomics for decoding the genome basis underlying secondary metabolism.

For biotechnology application of metabolomics, the substantial equivalence of GMO has been evaluated by a platform comprising multiple mass spectrometers established in RIKEN. Metabolomics

on rice genetic resources led to the prediction of agronomical and food traits of rice by regression analysis.

In this presentation, the crucial roles of metabolomics in plant functional genomics and biotechnology will be discussed.

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Functional proteomics: a cornerstone in plant systems biology

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We have assembled a proteome map for *Arabidopsis thaliana* from high-density, organ-specific proteome catalogs. We matched 95,988 unique peptides to 14,179 proteins and provide expression evidence for a number of gene models that are not represented in the TAIR8 protein database. Analysis of the proteome identified organ-specific biomarkers and allowed us to compile an organ-specific set of proteotypic peptides for 4105 proteins to facilitate targeted quantitative proteomics surveys. On the basis of proteome maps for cell organelles, we compared the quantitative proteome composition of different cell organelles in the different organs, and generated flux-balance models of plastid metabolism in seeds, roots and leaves. Current organellar proteome maps are not exhaustive, and we therefore analyzed the proteome of two plastid protein import mutants, *ppi1* and *ppi2*, lacking components of the plastid protein import machinery. These plastids are depleted of abundant photosynthetic proteins and therefore provide an improved dynamic range for the characterization of low abundance proteins. More than 1500 different proteins were identified and quantified from isolated plastids. Overall, the protein accumulation in the different mutants was surprisingly similar suggesting basic robustness principles and limited plasticity for the assembly of organellar proteomes. In order to further characterize chloroplast protein import in the different mutants, we systematically searched for N-terminal acetylated peptides in genome-scale WT, ppi1 and ppi2 proteomics data. These analyses revealed the accumulation of precursor proteins in the TOC159-deficient mutants (ppi2), probably as a result of the impaired import reaction. We discuss these observations in the context of protein import specificity and signaling pathways for retrograde communication. In order to expand our grasp on the dynamic regulation of the chloroplast proteome, we analyzed the chloroplast phosphoproteome and its dynamics during a circadian cycle. Motif-X analysis of the phosphorylation sites in chloroplast proteins identified three significantly enriched kinase motifs, which include known casein kinase II and proline-directed kinase motifs. To identify the kinases responsible for the regulation of chloroplast phosphoproteome dynamics, we characterized the phosphoproteome of T-DNA insertion mutants for different chloroplast kinases in comparison to wildtype in order to establish their *in vivo* substrate spectrum. We present here our data obtained with the thylakoid associated kinase STN8.

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Gene regulatory networks and transcription factor transcriptomics

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Transcription factors (TFs) are regulatory proteins that turn on and off the activity of the genes they control (target genes), by binding to short DNA elements (*cis*-regulatory elements; CREs) in their promoter sequences, constituting important components of gene regulatory networks (GRNs). TFs play important roles in almost all biological processes and thus it is perhaps not surprising that a large percentage of the genome encodes for these regulatory proteins. Our lab studies the function of TFs that control GRNs during development and biomass accumulation of photosynthetic organisms, including higher plants and photosynthetic micro-algae. Due to rapid progress in sequencing technologies, more and more genome sequences are rapidly becoming assessable providing an excellent resource for the analysis the evolutionary and functional relationships of transcriptional regulators across species. To speed up the discovery of TF genes we have established a bio-computational pipeline and established the Plant Transcription Factor Database (http://www.plntfdb.bio.uni-potsdam.de), which includes TF genes from micro-algae to higher plants. At the experimental level we use multi-parallel quantitative real-time polymerase chain reaction (qRT-PCR) to identify TFs that are functionally important for leaf development, control responses to changes in light and nutrient availability in the alga *Chlamydomonas reinhardtii*, and affect abiotic stress tolerance in rice and Arabidopsis. The available qRT-PCR platforms allow the facile expression analyses of ~2.000 TF genes in both rice and Arabidopsis, and the known set of ~240 transcriptional regulatory genes in Chlamydomonas. To assist the development of further qPCR platforms, we have established QuantPrime, a user-friendly, fully automated tool for primer pair design (available at www.quantprime.de). qRT-PCR has been demonstrated to be superior to micro-array-based technologies in expression analyses (providing higher sensitivity), enabling us to discover TF transcriptome responses that remained undetected before. We are currently developing a new experimental pipeline to support the discovery of TF—TF control cascades in plants. Examples will be presented.

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