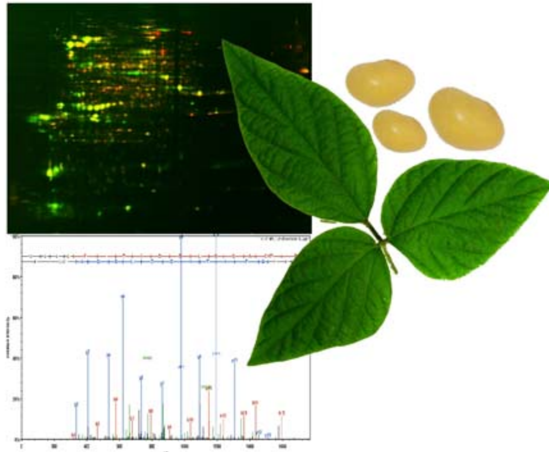
Plant Proteomics Symposium
June 8-9, 2009

Christopher S. Bond Life Sciences Center

University of Missouri-Columbia, MO USA



Program/Abstracts

Hosted by

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Schedule

Monday, June 8, 2009

8:00 - Registration begins – Main floor - MU Life Sciences Center

8:30 - Coffee/pastry breakfast

8:50 - Meeting introduction – Monsanto Auditorium

9:00 - Sacha Baginsky – "Chloroplast functional proteomics: A cornerstone in plant systems biology"
9:50 - Frances DuPont – "Proteomic analysis of the wheat endosperm, a major source of starch and protein for human nutrition"

10:40 - Break

11:00 - Susanne Hoffmann-Benning – "Proteomics and Beyond - Identification and Characterization of Novel Chloroplast Envelope Proteins"

11:50 - Lunch *Map and restaurant guide at the end of the booklet*

1:20 - Michael Goshe – "Data-Independent Methods for Quantitative Proteomics"
2:10 - Sophie Alvarez – "Changes in protein phosphorylation in a drought sensitive rice MAP Kinase mutant using a gel-based proteomics approach"
2:25 - Norma Houston – "Temporal Analysis of Protein Phosphorylation Changes in the Soybean Seed Proteome after Pod Detachment"
2:40 - Dong Xu – "Prediction of Protein Phosphorylation Sites in Plants"

3:30 - Break

3:50 - Steve Huber - "An emerging role for tyrosine phosphorylation in plant receptor kinase signaling"
4:40 - Carmen Giglione – "Cotranslational proteolysis dominates Glutathione homeostasis"
4:55 - Jane Robb – "Proteomic analyses of defence gene expression in a model tomato-Verticillium pathosystem"

5:10 - Reception

Tuesday, June 9, 2009

8:30 - Coffee/pastry breakfast

9:00 - Julian Whitelegge – “The subunits of a large integral membrane protein complex characterized by top-down fourier-transform mass spectrometry”
9:50 - Thierry Meinnel – “Proteomics of post-translational modifications at plant proteins ends: focus on acylation and related modifications”

10:40 - Break

11:00 - Bill Plaxton – “Regulatory monoubiquitination of castor bean phosphoenolpyruvate carboxylase”

11:50 - Lunch *Map and restaurant guide at the end of the booklet*

1:20 - Laurent Brechenmacher – “Soybean root hair proteome: establishment of the reference map and identification of proteins significantly regulated after Bradyrhizobium japonicum inoculation”
1:35 - Dave Emerich – “Proteomic analysis of B. japonicum bacteroids: Isolation and validation of periplasmic proteins”
1:50 - Christoph Borchers – “Novel mass spectrometric based approaches in metabolomics and quantitative proteomics applied to plant research”

2:40 - “Round-table” discussion

Chloroplast functional proteomics: A cornerstone in plant systems biology

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We analyzed the proteome of two plastid protein import mutants, ppi1 and ppi2, lacking components of the plastid protein import machinery. More than 1500 different proteins were identified and quantified from isolated plastids. Groups of co-regulated genes were assembled by K-means clustering and the transit peptide composition of the members in each cluster analyzed. 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. The plastid precursor proteins enter into the two-step cytosolic "methionine removal/acetylation pathway". Interestingly, the import of many other plastid proteins was not affected by the mutation and the proteins accumulated in their mature, processed form in plastids. We discuss our observations in the context of protein import specificity. In order to expand our grasp on the dynamic regulation of the chloroplast proteome in response to environmental signals, we analyzed the chloroplast phosphoproteome and its dynamics during a circadian cycle (1). 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 as well as one unknown motif at the C-terminus of ribosomal proteins. To establish the substrate spectrum of specific chloroplast kinases, we characterized the phosphoproteome of T-DNA insertion mutants for chloroplast kinases, and present here our data obtained with the STN8 kinase, a homolog of the state transition kinase STN7.

(1) Reiland et al. (2009) Plant Physiol, in press.

Proteomic analysis of the wheat endosperm, a major source of starch and protein for human nutrition

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There are major challenges to proteomic identification of wheat endosperm proteins, as illustrated by our studies of amyloplast and storage proteins. Although wheat DNA databases include over 1 million ESTs, there are less than 20,000 cDNA and genomic sequences, many of which are incomplete. Amyloplast proteins are of low abundance and must be obtained from fractionated organelles while storage proteins are abundant but include many similar proteins with unusual amino acid compositions and repetitive sequences. Amyloplasts were isolated from developing grain, proteins were separated by 2-DE and identified by MS/MS analysis of tryptic digests (Balmer et al). Since most matches were to wheat contigs, the peptides were used to interrogate the NCBI veridiplantae database to identify the closest homologs with complete cDNA or genomic sequences. At least 135 enzymes were predicted to be located in the amyloplast, based on Target P predictions and existing literature. The arrangement of these enzymes into 18 metabolic pathways suggested that amyloplasts play a central role in production of carbohydrates, amino acids, nucleic acids, fatty acids and secondary products. The storage protein proteome was analyzed by 2DE and three sets of MS/MS data were generated from separate digestions of each spot with chymotrypsin, thermolysin and trypsin. Spectral data were used to interrogate a specialized protein database that included sequences of gliadins and low-molecular-weight glutenins assembled from ESTs from the cultivar under study. Addition of cultivar-specific sequences to the database significantly improved identification of gliadins and glutenins and made it possible to use 2DE to analyze the effects of a fertilization regimen on individual storage proteins. Changes in starch-to-protein ratios and in the balance of S-rich and S-poor proteins may be mediated in part by regulation of biosynthetic pathways for synthesis of starch and amino acids that are compartmented within the amyloplast.

Proteomics and beyond - identification and characterization of novel chloroplast envelope proteins

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The chloroplast envelope represents the interface between the cytosol and the plastid. Yet only a few metabolite transport proteins have been characterized at the molecular level. These would be of particular importance in the case of the C4 plant maize where most transport proteins necessary for the CO2 accumulation in the bundle sheath as well as other C4-specific metabolic fluxes are still unknown. We hypothesized that a comparative proteomics of Zea mays mesophyll and bundle sheath chloroplasts will reveal proteins that are differentially expressed in BS and MS cells, including candidate proteins that control metabolite fluxes between and within the cells. We were able to identify over 200 proteins from maize chloroplast envelopes. 70% of those contain transmembrane regions and 45% are known chloroplast envelope proteins. However, 25% have not been previously characterized. YFP labelling has so far confirmed the chloroplast localization of eight of these novel proteins. A semi-quantitative analysis based on peptide counts of the proteome of Z. m. mesophyll and bundle sheath chloroplast envelopes has revealed 23 differentially expressed proteins. RT-PCR from mRNA from bundle sheath and mesophyll protoplasts has so far corroborated the expression patterns identified through proteomics. We are continuing to confirm localization and expression patterns of those novel proteins throughout the plants. We are also analyzing Arabidopsis knock-out mutant of some of the candidate genes.

One of the candidates is Mep1 (Maize envelope protein 1), one of the most abundant proteins in maize mesophyll chloroplast envelopes. Metabolite analysis suggests a possible role for Mep1 as monocarboxylate transporter.

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Data-independent methods for quantitative proteomics

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Bottom-up quantitative proteomic analysis using liquid chromatography-tandem mass spectrometry (LC/MS/MS) involves isotope coding strategies and intensity driven data-dependent acquisition (DDA) analysis to identify and quantify peptides which are then equated to the proteins present in a given sample. Although these methods are useful for the discovery and characterization of proteins and their post-translation modification (PTM) sites, such as phosphorylation, they often lack the dynamic range and the quantitative aspects required for studying temporal changes across various experimental conditions. Data-independent acquisition (DIA) methods represent a relatively new approach for simultaneous qualitative and quantitative sample analysis which holds promise for providing a single platform that permits the use of both isotope-coded and label-free protocols to more effectively quantify protein abundance and PTM changes. To that end, a DIA parallel fragmentation approach using precursor and product ion mass measurement accuracy and high resolution separations (LC/MSE) alongside traditional serial, intensity-driven DDA LC/MS/MS methods were evaluated to assess their analytical capabilities to both identify and quantify protein abundance changes and their site-specific modifications. The data-independent LC/MSE approach provided significant improvements in protein identification and sequence coverage, particularly for lower abundance species, while promoting hypothesis-driven interrogation of the data. When applied to proteomic studies involving plant apoplastic defense mechanisms, hormonal signaling, and phosphorylation site analysis of leucine-rich repeat receptor-like kinases, LC/MSE demonstrated that it is a powerful method for obtaining both relative and absolute proteome-scale quantification that can be applied to complex, time- and dose-dependent experimental designs occurring in a meaningful biological context.

Prediction of protein phosphorylation sites in plants

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Protein phosphorylation is a crucial regulatory mechanism in various organisms. With recent improvements in mass spectrometry, phosphorylation site data are rapidly accumulating. We developed P3DB (http://www.p3db.org/), a comprehensive resource of protein phosphorylation data from multiple plants. With a web-based user interface, the database is browsable, downloadable and searchable by protein accession number, description and sequence. Despite this wealth of data, computational prediction of phosphorylation sites remains a challenging task. This is particularly true in plants, due to the limited information on substrate specificities of protein kinases in plants and the fact that current phosphorylation prediction tools are mostly trained with kinase-specific phosphorylation data from non-plant organisms. To address these issues, we proposed a new machine learning approach for phosphorylation site prediction. We incorporate features of amino acid frequencies, protein disorder information, k-nearest neighbors (KNN) using support vector machines (SVM) with supervised or semi-supervised learning for predicting phosphorylation sites. Test results on the PhosPhAt dataset of phosphoserines in Arabidopsis and the TAIR7 non-redundant protein database show good performance of our proposed phosphorylation site prediction method. Our method combined both KNN to take advantage of potential similarities to known phosphopeptides and SVM to account for generic sequence features. As more phosphorylation sites are experimentally identified, the accuracy of our method is expected to increase automatically.

An emerging role for tyrosine phosphorylation in plant receptor kinase signaling

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Both plant and animal cells have receptor kinases that span the plasma membrane and function to transfer extracellular signals to the interior of the cell. In response to binding of the ligand to the extracellular domain, the cytoplasmic protein kinase domain of the receptor is activated via autophosphorylation, and the generalization is that animal receptor kinases often autophosphorylate on tyrosine residues while plant receptors do not (they autophosphorylate on serine and threonine residues). However, recent results indicate that several tyrosine residues in the brassinosteroid receptor kinase, BRI1, are essential for signaling and at least two of these (Tyr-831 and Tyr-956) are phosphorylated in vivo. Of particular interest is Tyr-831, which affects BR signaling that controls leaf shape and size, biomass accumulation, and flowering time. Transgenic plants expressing BRI1 substituted with an acidic residue (either aspartate or glutamate) at position 831 suggest that phosphorylation of Tyr-831 suppresses flowering time and inhibits overall growth. Several other plant receptor kinases also autophosphorylate on tyrosine residues, suggesting that in contrast to current dogma, tyrosine phosphorylation may be a prominent feature of many plant receptor kinases. The occurrence of tyrosine phosphorylation opens a new level of potential regulation of plant receptor kinases that may ultimately lead to strategies to control signaling pathways with beneficial impact on agriculture.

The subunits of a large integral membrane protein complex characterized by top-down fourier-transform mass spectrometry

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Top-down proteomics uses high-resolution Fourier-transform mass spectrometry (FT-MS) to define proteins by their intact masses in combination with dissociation experiments for unambiguous primary structure determination. It is vital that top-down mass spectrometry address all segments of the proteome, including the integral membrane proteins of biological bilayer membranes that compartmentalize living cells and make up around one third of the proteome and a greater proportion of drug targets. Integral membrane proteins present many technical challenges for efficient mass spectrometry in a large part due to hydrophobic transmembrane domains that limit their solubility in the aqueous solvents usually used for MS analysis. High-resolution top-down mass spectrometry is here used to characterize twenty integral and five peripheral subunits of the 2M Da Photosystem II complex from *Galdieria sulphuraria*. Preparations of Photosystem II complex from *Galdieria sulphuraria* were analyzed by reverse-phase liquid chromatography with online electrospray-ionization mass spectrometry and concommitant fraction collection (LC-MS+). Selected fractions were transferred to static nanospray tips for nano-electrospray ionization on either a hybrid linear ion-trap/Fourier-transform ion-cyclotron resonance mass spectrometer (LTQ-FT Ultra; ThermoFisher) or hybrid linear ion-trap/Fourier-transform orbitrap mass spectrometer (LTQ Orbitrap; ThermoFisher). Collision-activated dissociation (CAD) was achieved in the ion-trap whereas electron-capture dissociation (ECD), IRMPD and activated-ion electron-capture dissociation (aiECD) were performed in the cyclotron cell using an infra-red laser and an electron source (LTQ-FT). Top-down datasets were deconvoluted using Xtract and matched to a *Galdieria* proteome database using Prosight PC (Thermo Fisher). The primary LC-MS+ separation yielded thirty-nine intact mass tags (IMTs) with 100 ppm mass accuracy on a low-resolution instrument. The large subunits PsbA, PsbB, PsbC and PsbD measured 38184, 56521, 50927 and 39346 Da respectively consistent with their gene sequences and known N- and C-terminal post-translational modifications. These subunits form the bulk of the Photosystem II reaction center and account for a twenty-two of a total of a least thirty-six transmembrane alpha helices. The major challenge of this experiment was to determine exactly how many other subunits assemble into the *Galdieria* Photosystem II complex and to define their primary structure. Selected fractions collected during the primary LC-MS+ separation were analyzed by manual top-down high-resolution mass spectrometry targeting predefined ions. Top-down using both CAD and ECD of the smaller (< 10 kD) integral subunits has so far confirmed the presence of PsbE, PsbF, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbN, PsbT, PsbX and PsbZ all having a single transmembrane helix except PsbF that has two. PsbJ, for example, showed two isoforms with and without Met1 but both N-terminally acetylated. As well as the integral subunits, the Photosystem II complex has a collection of peripheral polypeptide subunits that are bound in association with Manganese and Calcium ions to assemble a fully active complex. These peripheral subunits are less conserved than the integral ones and *Galdieria* is believed to have potentially unique structures in this part of the complex. The peripheral subunits were less retained in reverse-phase chromatography and five polypeptides were identified of mass 10587, 12820, 16556, 18036 and 28821 Da provisionally assigned as PsbU, PsbV, PsbP, PsbQ, PsbO respectively. The identity of PsbU, for example, was confirmed by top-down CAD and ECD experiments that demonstrated that the mature product had a signal peptide of 81 amino-acid residues removed post-translationally.

Proteomics of post-translational modifications at plant proteins ends: focus on acylation and related modifications

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The proteins of Higher Eukaryotes are featured by a vast number of modifications events occurring at their N- and C-terminal ends. A proteomic and functional overview of such modifications is now feasible with plants being one of the best characterized systems. Modifications at the N-end usually occur co-translationally in the three compartments where protein synthesis occurs. N-modifications types are most diverse but the most frequent and conserved modification deals with N-Methionine excision (NME). The plastid proteome is very well characterized in this regard. In the cytosol , acylation events - i.e. addition of an N-acetyl or of lipids - play a central and crucial role and often strictly rely on NME. Focus will be made on acylation in plants. The data of recent investigation of structure-function relationships of such modifications will be discussed on some most interesting examples. Lipidation by saturated or unsaturated fatty acids results in protein targeting to the membrane, with the protein behaving as a peripheral component and being oriented toward the inside of the cell. Addition of a fatty acid is not thought to be sufficient for stable membrane targeting and a second signal, often involving another lipidation, is anticipated to be required. The methods available for predicting lipid modifications to proteins highlight the importance of identifying short protein motifs in a field in which few data are currently available from large-scale proteomic analyses, due to the complex, hydrophobic nature of the modification. Full proteome annotation is already feasible with dedicated predictive tools. Using Arabidopsis thaliana as a reference proteome, it is expected that lipidation by N- or S-acylation may affect as much as 3% of all open reading frames in plants. N-acetylation is a very widespread modification and its significance in plants will be discussed in the frame of protein accumulation.

Regulatory monoubiquitination of castor bean phosphoenolpyruvate carboxylase

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Ubiquitin (UB) is a small, conserved eukaryotic protein that modifies a lysine residue of target proteins. Polyubiquitination tags numerous proteins for their 26S proteosomal degradation. However, monoubiquitination has emerged as a nondestructive and reversible PTM that mediates protein-protein interactions and localization to control diverse processes such as gene expression, signal transduction, and endocytosis. This presentation will discuss the first precedent for regulatory monoubiquitination of any metabolic enzyme, namely phosphoenolpyruvate carboxylase (PEPC), a tightly regulated anaplerotic enzyme situated at a major branch point of plant C-metabolism. PEPC's control by allosteric effectors, reversible phosphorylation, and oligomerization has been well documented in developing castor oil seeds (COS). However, all that was known about germinating COS PEPC was that germination is accompanied by increased PEPC activity and accumulation of immunoreactive 110- and 107-kDa PEPC polypeptides (p110 & p107). A 440-kDa PEPC heterotetramer composed of a 1:1 ratio of p110:p107 subunits was purified from germinated COS. MS, N-terminal microsequencing, and immunoblotting established that p110 is a monoubiquitinated form of p107, and that both subunits originated from the same gene (RcPpc3) encoding the p107 subunit of a phosphorylated 410-kDa PEPC homotetramer of developing COS. Incubation with a human deubiquitinating enzyme (USP-2 core) converted the p110:p107 PEPC heterotetramer into a p107 homotetramer while significantly reducing the enzyme's Km(PEP) and allosteric effector sensitivity. MS/MS sequencing of a diglycinated tryptic peptide identified Lys-628 as p110's monoubiquitination site. Lys-628 is conserved in plant PEPCs and is proximal to a PEP binding/catalytic domain. Although high-throughput proteomic screens have identified numerous ubiquitinated metabolic (including glycolytic) enzymes in Arabidopsis, it is unknown whether they were poly- or monoubiquitinated. Thus, an important research objective will be the identification of monoubiquitinated enzymes, the UB-binding domain proteins that they interact with, and the influence of this PTM on their subcellular localization and kinetic/regulatory properties.

Novel mass spectrometric based approaches in metabolomics and quantitative proteomics applied to plant research

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The University of Victoria – Genome British Columbia Proteomics Centre has developed numerous mass spectrometric approaches in metabolomics and quantitative proteomics, in particular in targeted and absolute quantitation of proteins. For metabolomics, special focus is given in developing approaches using high field Fourier Transform Ion Cyclotron Resonance mass spectrometry (FTICR-MS) since this technology provides unprecedented accuracy and resolution. These unique features of FTICR-MS enable direct measurement of metabolites extracted from biological samples without chromatography prior the MS analysis and therefore provides high sample throughput capabilities while detecting hundreds to thousands of metabolites per experiment. Ultra-high accuracy allows determination of elemental composition which can be used for identification of metabolites. This approach has been successful applied to characterize different canola strains including single gene knock-out strains and analyzing the change of the metabolome in spruce cell lines involved in the defense mechanism.

The Centre has developed quantitative approaches using iTRAQ for unbiased and multi-reaction monitoring (MRM) for targeted and absolute quantitative analysis of proteins. Both techniques have been applied to study proteins implicated in defense mechanism of spruce cells. The MRM technology combined with enrichment steps allows the accurate and precise determination of changes in the expression level of specific enzymes even those which belong to the same enzyme class and which are highly similar in protein sequence and therefore not distinguishable by common ELISA. These results not only demonstrate the power of novel MS based approaches in analyzing plants but it also brought already new insights in plant biology.

POSTER ABSTRACTS

Poster 1

Cadmium induced changes on proteome of Catharanthus roseus (L.) G.Don

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Introduction

Cadmium is a non-essential element that negatively affects plant growth and development. It is released into the environment by power stations, heating systems, metal-working industries or urban traffic. A large number of genes in plants are induced after exposure to various abiotic stresses such as heavy metals and also initiate the synthesis of different classes of proteins including transcription factors, enzymes, molecular chaperones and ion channels to alter their activities. Most of these molecules display an essential function either in the regulation of the response or in the adaptation process, allowing plants to recover and survive in the stress condition.

Methods

Total soluble proteins were extracted from leaves of Catharanthus roseus and separated by 2-D gel electrophoresis. Gels were stained and images analyzed by Decodon Delta-2D software. Differentially expressed proteins spots were excised and In-gel digested with Trypsin. Tryptic peptides were analyzed by MALDI-TOF-MS and MS/MS. Then data were searched on database for protein identification.

Results

2-D protein patterns obtained from control and Cd-treated leaves samples after 48 h of treatment displayed significant changes in their expression levels in Cd-treated plants compared with control plants. A total of hundred and twenty two proteins spots showed an altered expression pattern following Cd-treatment. The number of up-regulated spots largely exceeded that of down-regulated ones. Among the total differentially expressed proteins 65 proteins were identified by MALDI-TOF-MS and MS/MS, these proteins coming under nine functional categories such as signaling proteins, HSPs, metallothionein, transcription and replication, metabolism, protein degradation, cell defense and rescue, binding and transport and biotransformation. In conclusion, cadmium induced stress altered the many functional groups protein expressions.

Poster 2

Analysis of the abundant zinc-binding proteins from developing soybean seeds

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A total soluble protein fraction was prepared from developing soybean (Glycine max (L) Merr., cv, Jack) cotyledons, then dialyzed at 4°C first against 2 mM EDTA then against deionized water. The sample was clarified by centrifugation, then loaded onto a 2.5 mL bed-volume column of NTI-agarose previously loaded with Zn. The sample was cycled through the column several times, followed by a buffer wash. The zinc-binding proteins were eluted with the zinc-chelator Zincov. The eluted fractions were combined, reduced in volume, then subjected to SDS-PAGE. Protein bands were cut from the gels, destained, dehydrated, and subjected to in gel trypsin digestion. The resultant tryptic peptides were analyzed by MALDI-TOF mass spectrometry. The most prominent gel band was identified as lipoxygenase, a known soybean zinc-binding protein, validating the methods. Other proteins were tentatively identified, including RNA-directed RNA polymerase, NDP-kinase, and several unnamed/unidentified/unknown function proteins. An abundant LMW protein is being analyzed without tryptic digestion using the LTQ Orbitrap hybrid mass spectrometer.

Poster 3

Chromium toxicity study in fenugreek seedlings

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Fenugreek is an important minor spice crop of Gujarat, crop is cultivated from seeds, seed germination and biochemical events are important in growing seedlings of such crops. Chromium is phytotoxic in nature. It was of interest to study the chromium effects on growth of fenugreek seedlings and to find out the changes in peroxidase, polyphenol oxidase and soluble protein level in sprouting seeds. Seeds (Trigonella foenumgraceum L) were germinated in DW and 200, 400, 600, 800 and 1000 µg/ml of sodium dichromate in petri plates under lab conditions, experimental period was 96h. Root elongation, shoot elongation, fresh weight and dry weight of seedlings were gradually lowered by gradual increase in sodium dichromate concentration, root was target organ. Moderate concentration of Cr increased soluble protein during first 8h of germination, but later on it was lowered, while severe concentration significantly lowered it. Peroxidase activity was lowered by Cr during 8h then it was stimulated. Cr enhanced polyphenol oxidase activity, severe concentration was more effective. Biochemical symptoms of Cr toxicity were lowering in soluble protein and peroxidase activity, stimulation in polyphenol oxidase activity in sprouting seeds, significant changes were found in peroxidase activity. Peroxidase activity may be selected as a biochemical marker for study on Cr toxicity in fenugreek seedlings.

Poster 4 – Selected for short talk

Temporal analysis of protein phosphorylation changes in the soybean seed proteome after pod detachment

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Partitioning of photosynthate among carbohydrates, oils, and proteins within plant seed is a highly regulated process. To systematically characterize the role of protein phosphorylation in response to photosynthate supply, global quantitative phosphoproteomics was performed on developing seed from detached soybean pods. Soybean pods, 28 days after flowering, were detached from the stem in biological triplicate and seeds were harvested at 0, 6, 24, and 48 hours after pod excision. Whole seed proteins for each harvested time point were isolated under denaturing conditions, and fractionated by high-resolution two-dimensional gel electrophoresis. Phosphoproteins were detected in-gel using Pro-Q Diamond stain followed by laser imaging. Gels were then stained with colloidal Coomassie to visualize total protein. Spot detection and quantitation was performed using ImageMaster software. Differential expression (p-value <0.01) was established for 108 phosphoproteins and 326 non-phosphoproteins based on statistical significance of protein expression changes between the control (0 hours) and at least two experimental (6, 24, 48 hours) samples. To date, 75 phosphoproteins and 129 non-phosphoproteins have been identified by liquid chromatography-tandem mass spectrometry. Among the differential proteins identified was the well-characterized phosphoprotein, mitochondrial pyruvate dehydrogenase alpha subunit, which increased eight-fold by 48 hours. Novel phosphoproteins were also identified including cytosolic triose-phosphate isomerase, which showed a six-fold increase six hours after pod excision and decreased to twice the zero time point level by 24 hours. Many of the differential phosphoproteins are involved in metabolism suggesting the regulation of carbon assimilation and partitioning in seed may be more complex than previously thought.

Poster 5

System analysis of an Arabidopsis mutant altered in *de novo* fatty acid synthesis reveals diverse changes in seed composition and metabolism

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Embryo-specific overexpression of biotin carboxyl carrier protein 2 (BCCP2) inhibited plastid acetyl-CoA carboxylase (ACCase) and altered oil, protein, and carbohydrate composition in mature seed. To characterize the gene and protein regulatory consequences of this mutation, global microarray, two-dimensional difference gel electrophoresis, iTRAQ, and quantitative immunoblotting approaches were performed in parallel. These analyses revealed: 1) transgenic overexpression of BCCP2, did not affect expression of other ACCase subunits; 2) four subunits to plastid pyruvate dehydrogenase complex (PDC) were 25-70% down-regulated at protein but not transcript levels; 3) key glycolysis and de novo fatty acid/lipid synthesis enzymes were induced; 4) multiple storage proteins, but not cognate transcripts, were up-regulated; and 5) biotin synthesis pathway was up-regulated at both transcript and protein levels. Biotin production appears closely matched to endogenous BCCP levels, since overexpression of BCCP2 produced mostly apo-BCCP2 and the resulting ACCase-compromised, low-oil phenotype. Differential expression of glycolysis, plastid PDC, fatty acid, and lipid synthesis activities indicate multiple, complex regulatory responses including feedback as well as futile “feed-forward” elicitation in the case of fatty acid and lipid biosynthetic enzymes. Induction of storage proteins reveals oil and protein synthesis share carbon intermediate(s) and that reducing malonyl-CoA flow into fatty acids diverts carbon into amino acid and protein synthesis.

Poster 6

Proteome analysis of cold stress response in Arabidopsis thaliana: A label-free quantitative proteomic study

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The effect of cold shock on rosette leaves from Arabidopsis thaliana has been studied using a label free exact mass LC-MS technique. Protein extracts were digested with trypsin, to enable a bottom-up proteomics approach. Relative response in protein expression was observed by comparison to appropriate controls.

Poster 7

Late embryogenesis-abundant proteins are involved in soybean salt tolerance

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Late embryogenesis-abundant (LEA) proteins accumulate at high concentrations in plant embryos during the late stages of seed development before desiccation. These proteins also accumulate in vegetative tissues exposed to exogenous abscisic acid as well as dehydration, osmotic stress, and low temperature. To determine which proteins are involved in responses of soybean to salt stress, the related changes in protein expression were investigated using the proteomic approach. Soybean plants were exposed to 0, 50, 100, and 200 mM NaCl. Especially at 200 mM, the length and fresh weight of the hypocotyl and root reduced under salt stress, while the proline content increased. Proteins from the hypocotyl and root treated with 100 mM NaCl were extracted and separated by two-dimensional polyacrylamide gel electrophoresis; 321 protein spots were detected. In response to salt stress, seven proteins were reproducibly found to be up- or down-regulated by two to sevenfold: late embryogenesis-abundant -conglycinin, elicitor peptide three precursors, andβprotein, basic/helix-loop-helix protein were up-regulated, while protease inhibitor, lectin, and stem 31-kDa glycoprotein precursor were down-regulated. These results indicate that salinity can increase the expression level of LEA proteins -conglycinin, elicitor peptide, and basic/helix-loop-helix proteinβas well as in hypocotyls and roots of soybean that may in turn play a role in its adaptation to saline conditions.
Keywords: LEA proteins, Proteome, Soybean, Salt stress

Poster 8 – Selected for short talk

Soybean root hair proteome: establishment of the reference map and identification of proteins significantly regulated after Bradyrhizobium japonicum inoculation

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Root hairs are single tubular cells formed from the differentiation of epidermal cells, called trichoblasts, on primary and secondary roots. They are involved in water and nutrient uptake, anchorage of the plant into the soil and represent the colonization site of leguminous roots by rhizobia, soil bacteria that establish a nitrogen fixing symbiosis. Root hairs develop by polar cell expansion or tip growth, a unique mode of plant growth shared only with pollen tubes. We decided to comprehensively examine soybean root hair proteins by 2D gel electrophoresis and shotgun proteomic analyses. Soybean was selected for this study due to its agronomic importance and its root size, which permits isolation of root hairs in sufficient amount for proteomic studies. Through this effort, we established the first soybean root hair proteome reference map by identifying 1492 proteins. Only 169 proteins were identified by both approaches. The proteins identified are involved in basic cell metabolism (primary metabolism, protein synthesis and processing), but also in functions more specific to the single root hair cell, including water and nutrient uptake, vesicle trafficking, hormone and secondary metabolism. The data presented provide useful insight into the metabolic activities of a single, differentiated plant cell type.
We decided to identify soybean root hair proteins significantly regulated in response to B. japonicum. Proteins were extracted from root hairs 0, 12 h, 18 h, 24 h, 36 h and 48 h after inoculation with B. japonicum and from corresponding mock-inoculated controls. Differential In Gel Electrophoresis (DIGE) was performed and identified 178 spots significantly regulated after B. japonicum inoculation ($p<0.05$). These proteins are currently being identified by mass spectrometry.

Poster 9

Monitoring changes in the proteome of transgenic peanuts engineered for reduced allergen content using linear ion trap and triple quadrupole mass spectrometry

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Plant seeds provide a significant portion of the protein present in the human diet, but are also the major contributors of allergenic proteins that cause a majority of the reported cases of food-induced anaphylaxis in the U.S. New varieties of oilseeds, cereal grains and tree nuts could potentially be screened for their relative allergen content before they are introduced as cultivars for food production using contemporary mass spectrometry-based quantitation approaches. Mass spectrometers are valuable tools for research and development purposes, and with improvements on throughput also have the potential to be used for analytical screening or protein phenotyping for the seed industry. With all the available MS options, it is important to use the correct tool for the job at hand. Spectral counting with a fast scanning linear ion trap quadrupole (LTQ) mass spectrometer can provide a rapid and indiscriminant preview of proteomes, allowing for the discovery of differing protein abundances. This analysis, when coupled with targeted AQUA (absolute quantitation) analysis using a triple quadrupole (QQQ) mass spectrometer, can accurately elucidate the actual quantities of allergens and proteins of interest. Here we present a unique application of spectral counting and AQUA for screening transgenic seed crops for their relative and absolute allergen content, and show the advantages of using a QQQ versus an LTQ for reaction monitoring.

Poster 10 – Selected for short talk

Changes in protein phosphorylation in a drought sensitive rice MAP Kinase mutant using a gel-based proteomics approach

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Mitogen-activated protein kinases (MAPK) are key intermediates in signaling pathways in all eukaryotes. In rice the m40 mutant is more drought sensitive. The m40 MAP Kinase is similar to the Raf family (MAPKKK). MAPKKKs are serine/threonine kinases, activated by physical interaction and/or phosphorylation either by the receptor itself or intermediate bridging factors. MAPKKK are the first players of the MAPK cascade which will phosphorylate a variety of substrates including transcription factors, proteins kinases and cytoskeleton-associated proteins. We used a proteomics approach to identify the downstream substrates phosphorylated directly or indirectly by M40 using 2D gel electrophoresis and a phosphoprotein specific stain (ProQDiamond). Rice plants (4 weeks old) from wild type (WT) and from the MAPKKK mutant (m40-2) were drought stressed and roots and leaves were collected. By comparing protein abundance and phosphorylation under different conditions (drought stressed and control plants from wild type and m40-2 mutant), it was possible to identify differentially phosphorylated proteins involved in drought responses in the wild type and the m40 mutant. Some possible downstream targets (mRNA binding protein, ribonuclease, translational factors) phosphorylated directly or not by M40 MAP kinase cascade in response to drought were identified using this proteomics approach. In addition to this data, the comparison between the different conditions allowed determination of a general list of proteins differentially phosphorylated involved in drought response signaling. Finally the protein abundance data also led to the identification of protein differentially expressed in response to drought.

Poster 11 – Selected for short talk

Proteomic analyses of defence gene expression in a model tomato-verticillium pathosystem

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Relatively little is known about the molecular mechanisms utilized by plants to defend themselves against fungal vascular pathogens. Based on DNA microarray analyses, in past studies we used a model tomato-Verticillium pathosystem to compare the global expression of genes in compatible and incompatible interactions. While very significant patterns of mRNA changes were defined, how these are translated at the protein level remained unclear. In the present study, 2D gel electrophoresis and proteomic analyses were applied to evaluate the actual changes in protein levels. Whole cell protein was extracted simultaneously with RNA to permit parallel comparisons of both mRNA and proteins from the same samples. Proteins representing 13 of the most intense changes were identified using mass spectrometry. Three were products of commonly identified pathogenesis related genes, three were peroxidase related and two were osmotin related proteins, all products of genes commonly reported to be involved in genetic responses to stress or pathogens. The levels of induction relative to other cellular proteins were particularly striking, an observation which underlines the plant's heroic systemic response to a vascular disease such as Verticillium wilt. Supported by the Natural Sciences and Engineering Research Council of Canada.

Poster 12

A quantitative mass spectrometry-based approach for discovering and assaying protein kinase substrates

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Protein phosphorylation is a key mechanism for metabolic regulation and cell signaling. The Arabidopsis genome encodes over 1000 putative kinases. The clients of most of these kinases remain unknown. Therefore a quantitative, high-throughput approach to assay synthetic kinase substrates *en masse* would be useful for kinase client identification. We have developed a method that utilizes spectral counting on a LTQ-XL linear ion trap mass spectrometer, which we have verified produces a linear response range of four orders of magnitude with low-complexity samples. Ser-containing peptides originating from *A. thaliana* pyruvate dehydrogenase (PDH) E1α subunit were synthesized and employed in the *in vitro* kinase assay with recombinant pyruvate dehydrogenase kinase (rPDK). Phosphopeptides were analyzed by liquid chromatography coupled to tandem mass spectrometry. To optimize for phosphopeptide detection, initial assays were conducted with rPDK and the PDH peptide substrate YHGHS^{292}MSDPGSTYR (peptide 61) which contains the known Ser phosphorylation site. The correctly phosphorylated Ser was detected by tandem MS and reaction kinetics were optimized and quantified by spectral counting. When mixed with 45 other synthetic peptides covering every Ser residue of PDH, rPDK phosphorylated only the known peptide, demonstrating assay specificity. It was previously shown for some phosphoproteins that phosphorylation can be abrogated by oxidation of nearby Met residues. To investigate whether oxidation of Met^{293} to methionine sulfoxide affects Ser^{292} phosphorylation, peptide 61 was pre-treated with hydrogen peroxide. Analysis by MS revealed 96% of peptide 61 was Met oxidized after H_2O_2 treatment, and phosphorylation of Ser^{292} was completely inhibited. Results from assaying two peptides where Met^{293} was replaced by Ala or Gln revealed Ser^{292} phosphorylation was also inhibited. In summary, our results indicate that a quantitative MS-based assay can be applied to assay kinase client peptide specificity in a high-throughput context, and will thus be a useful method for both small and large-scale study of protein phosphorylation.

Poster 13 – Selected for short talk

Cotranslational proteolysis dominates glutathione homeostasis

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The earliest proteolytic event occurring on most proteins corresponds to N-terminal methionine excision (NME), an essential and ubiquitous process. The effects of NME on unknown complex cellular networks and the ways in which its inhibition leads to developmental defects and cell growth arrest remain poorly understood. To depict fully this process, we have used a strategy based on the combined use of defined genetic contexts and small molecules to overcome the difficulty cause by lethality and gene redundancy. We also have designed a genetic system with modulation of chemical dosage to study the process under non lethal conditions. The pertinence of such a system was checked using other genetic tools (RNAi) which clearly –because of epigenetic adaptation - are however less prone to robust biological analysis than chemical biology investigation. We present and discuss the considerable body of data that we have amassed relating to this question and the novel model which emerges from this approach by combining various proteomic and metabolomic tools. We demonstrate that NME process plays a crucial role in maintaining appropriate status of the key cellular redox Glutathione homeostasis and that this occurs via a tight control of the proteolytic activity of the cell. Finally, we show that these effects are universal in Higher Eukaryotes, Fungi and Archaea, thus permitting us to describe a novel model in which NME, glutathione and proteolysis operate together in a ubiquitously conserved, indispensable crosstalk mechanism to ensure growth and correct development.
The discovery of this crosstalk will provide the mechanistic framework for further studies aimed at identifying its importance in the molecular mechanism(s) regulating cell behavior during development, tumorigenesis and/or aging. Our work is another example showing how Arabidopsis studies do have a crucial impact in the understanding of ubiquitous and basic process with direct relevance to human health.

Poster 14

Brain MS imaging after ischemic stroke in mice – insight for brain injury by abnormal proteolysis

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Stroke, a devastating disease, is of lacking blood supplies to brain. We have demonstrated that abnormal metalloproteinase proteolysis causes degradation of its substrates that links to stroke. MALDI imaging mass spectrometry (MALDI-IMS) is a high-content assay for determining both relative abundance and spatial distribution of large amount of molecules in tissue. In this study, we employed a focal cerebral ischemia model in mice that representing human stroke condition and MALDI-IMS to examine the molecular profiling and to investigate the linkage to brain proteolysis. We determined the differences between the ischemic versus non-ischemic regions and identified mass signatures that are correlated with disease states. The brain was harvested and processed into a serial of sections, one set for histology assessment, and the adjacent sections subject to ion mass analysis using MALDI-TOF mass spectrometer. Selected ions were then displayed as pseudo color images in which the color intensity correspond to ion signal abundance. MALDI spectral were collected at 100-micron spatial resolution on the coronal section of the mouse brain after ischemia. In response to the ischemia, the brain showed swelling due to the traumatic injuries to the brain. The optical image stained with cresyl violet indicates that ischemia induced brain injuries within the red line (Panel A). The total averaged spectra showed rich features through out the measured mass range. The overall anatomical brain structure was intact evidenced by selective localization of a number of molecular ions (Panel B). Interestingly, some molecular ions co-localize well with the injury area. Further study of these molecules that correlate with the brain architecture and proteolysis may identify novel targets for stroke therapy.

Poster 15 – Selected for short talk

Proteomic analysis of Bradyrhizobium japonicum bacteroids: Isolation and validation of periplasmic proteins

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The periplasm was isolated from B. japonicum bacteroids and analyzed by LC/MS/MS. A global proteomic analysis of the periplasm in rhizobium has not yet been reported. A total of 365 proteins were identified with 92 being found in the periplasm, 124 in the cytoplasm, and 149 proteins found in both compartments as determined by spectral count analysis. Fifty proteins identified as plant proteins were also found in the periplasmic fraction. On hundred and twenty-five of the 365 periplasm proteins were predicted to be periplasmic by the localization programs pSORT, Cello, AdaBoost, TatP and LipoP. The distribution of the proteins identified in the periplasm by functional category was different from those of the cytosol and the overall genome distribution. Analysis of the periplasmic proteins revealed a number of metabolic pathways, one of which was the catabolism of benzoates. Proteins in the benzoate metabolic pathway were explored further and the presence of 3,4-dihydroxybenzoate dioxygenase was found in the periplasm by enzymatic measurement.

Poster 16

Methionine oxidation as redox switch linking ROS to protein phosphorylation

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The mechanisms involved in sensing oxidative signaling molecules such as H2O2 in plant and animal cells are not completely understood. An emerging concept in our lab is that oxidation of methionine (Met) to Met sulfoxide (MetSO) is one mechanism to couple oxidative signals to changes in protein phosphorylation. We found that when a Met residue functions as a hydrophobic recognition element within a phosphorylation motif, its oxidation can strongly inhibit phosphorylation in vitro. This is shown to occur with recombinant soybean calcium dependent protein kinases (CDPKs) and human AMPK phosphorylating synthetic peptides conforming to several motifs. To determine whether this effect occurs in vivo, we monitored the phosphorylation status of Arabidopsis leaf nitrate reductase (NR) Ser534 using specific antibodies. NR was a candidate protein for this mechanism because Met538, located at the P + 4 position, serves as a hydrophobic recognition element for phosphorylation of Ser534 and hence its oxidation strongly inhibits phosphorylation by CDPKs in vitro. Feeding exogenous H2O2 to darkened Arabidopsis seedlings in liquid culture increased phosphorylation of NR at the Ser534 site at low H2O2 concentrations and progressively decreased phosphorylation at higher concentrations, while overall phosphorylation of the proteome remained relatively constant as monitored by ProQ Diamond staining. These results are consistent with the notion that oxidation of surface-exposed Met residues in kinase substrate proteins can inhibit the phosphorylation of nearby sites and thereby couple oxidative signals to changes in protein phosphorylation. NR may be one candidate protein regulated by this mechanism, which may contribute to activation of the enzyme in response to some stresses, e.g., hypoxia. Bioinformatic analysis suggests that numerous plant proteins could be regulated by this mechanism.

Poster 17

Label-free quantitative proteomics in biomarker discovery during viral-infected systemic recombination signaling events in Arabidopsis thaliana

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Biotic and abiotic stresses induce somatic recombination events resulting in inheritable genome rearrangements. This process may be the basis for induced, adaptive evolution. Pathogen infection in a local leaf activates a systemic recombination signal (SRS) that moves through the plant, triggering somatic intra-chromosomal recombination events in leaves never ‘seeing’ the pathogen; but the nature of this signal is not known. To identify potential early biomarkers for this systemic response, we performed a quantitative proteomic study of systemic leaves following a viral infection of Arabidopsis.
Non-infected distal leaf tissue from wild type Col-0 Arabidopsis was collected 0, 72, or 96 hours after a mock or ORMV (virus) treatment of a local leaf. Total protein were extracted from these leaves and separated by 1D SDS-PAGE prior to in-gel digestion with trypsin. The resulting peptide mixture was subjected to on-line nanospray-ESI-LC-MS/MS analysis using the LTQ-Orbitrap. Quantitation of proteins was performed using spectral-counting method. From three biological repetitions, thirteen proteins showed reproducible changes. Of these, the levels of nine increased and one decreased following virus infection versus the mock treated control. We are presently repeating these experiments using an alternative quantitative method, iTRAQ, to obtain further quantitative information about possible changes in less abundant proteins.

Poster 18

Workflow optimization for gel-free quantitative mass spectrometry analysis of soybean seed proteins

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Gel-free quantitative proteomics is gaining momentum as an unbiased, high-throughput alternative to 2-D gel and GeLC-based quantitative proteomics. However, the majority of method development and optimization for quantitative proteomics pertains to gel-based techniques. We discuss the development and optimization of a workflow for gel-free quantitative proteomics, with the objective of identifying methods that reproducibly deliver the highest yield and proteome coverage. Beginning with dry soybean seed we compared four protein extraction methods, TCA/acetone, urea, urea/thiourea, and phenol. Soybean proteins were extracted by each method in quadruplicate, quantified and visualized by Coomassie-SDS-PAGE. Of the four methods, the phenol extraction method yielded 2-, 5-, and 7-fold higher protein than TCA/acetone, urea, and urea/thiourea extractions, respectively. To further investigate the efficiency of phenol extractions, we performed the first phenol extraction plus two successive back extractions, which produced approximately 80, 15, and 5% of total protein, respectively. We also compared in-solution digestion procedures to determine optimal resuspension and digestion conditions. Resuspension media containing simply 50 mM Tris-HCl pH 8.0 and 7 M urea showed the highest spectral counts. Addition of thiourea, CHAPS, or n-octylglucoside dramatically reduced proteome coverage and spectral counts. Comparison of trypsin to protein ratio (1:25, 1:50, 1:75, and 1:100) showed a near linear increase in spectral counts with increasing trypsin levels and revealed the 1:25 ratio produced the highest spectral counts. Overall, this study shows the time-honored phenol extraction method consistently and unequivocally yielded the highest amounts of protein from dry soybean seed. Resuspension of precipitated protein in buffered urea is sufficient for maximum protein retrieval and digestion efficiency. Elimination of all detergents post protein isolation improved the frequency of assigned tandem mass spectra as much as 3-fold, presumably by reducing ion suppression.

Poster 19

NitroDIGE: A new method to investigate the subproteome of the s-nitrosylated proteins in neurological diseases

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Introduction

Nitric oxide (NO) is a signal molecule that plays diverse roles in regulating biological processes ranging from development to disease. We have recently demonstrated that NO regulates important signal pathways linked to neurodegenerative disorders. Pathophysiological NO-mediated protein modification is predominately affected by S-nitrosylation, that is, the coupling of an NO moiety to a reactive cysteine thiol to form an S-nitrosothiol. Previously, these S-nitrosylated proteins were individually identified using a method called biotin switch assay. Evolved from this assay and 2D-DIGE, we developed a gel-based quantitative proteomic strategy – named as NitroDIGE, attempting to investigate a subset of proteome of the S-nitrosylated proteins in neurological diseases.

Methods

Neuroblastoma SH-SY5Y cells were lysated and exposured to a physiological NO donor, S-nitrosocysteine (SNOC), and then blocked the free thiols with MMTS. Incubation with ascorbate selectively released NO from the S-nitrosylated protein cysteine thiols. After reduction, the fluorescence-tagged differential thiol linkers allow to binding to the NO-released thiols to form stable fluorescence-tagged complexes. The differential fluorescence-tagged labeled samples (control vs. the samples exposed to NO) were subjected to 2-D electrophoresis, and imaged by fluorescence scanner. Furthermore, the total proteins were visualized by silver staining. The differential fluorescence-intensity spots (S-nitrosylated proteins) were subject to MALDI-TOF/TOF. And S-nitrosylation of such proteins was further confirmed by biotin switch assay.

Results

Here we introduce a new gel-based proteomic strategy, termed – NitroDIGE. The in vitro results from the SH-SY5Y lysates exposed to SNOC reveal that this method allows to directly detection of a subset of proteome for protein S-nitrosylation. Controls by omitting NO donor, thiol blocker and reducing reagent were introduced to verify the specificity of labeling for NitroDIGE. Equal amount of total proteins per lane was confirmed in the silver staining. These results demonstrated a high specificity for NitroDIGE to detect protein S-nitrosylation.

Poster 20

Developing a label-free method for membrane protein quantification: measuring the gibberellin-induced changes in the arabidopsis membrane subproteome

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Plant integral membrane proteins are involved in a myriad of cellular processes and play a major role in the cell signaling. Our interest in cell signaling centers around a group of membrane bound protein kinases called leucine-rich repeat receptor-like kinases (LRR RLKs). Typically detergents are used to promote membrane protein solubility, but these reagents require multiple LC processing steps to remove them prior to liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis which can result in sample losses. These losses can complicate quantitative proteomic analysis when performed prior to the isotope-coded labeling step using reagents such as ICAT and iTRAQ. In an effort to develop a suitable method for identification and quantification of LRR RLKs, we used a label-free gel-based approach. Our new method was applied to studying abundance changes of Arabidopsis membrane proteins in response to gibberellin (GA) treatments using a novel parallel fragmentation mode during LC/MS/MS analysis termed MSE. In our study of Arabidopsis seedlings treated with 1 uM of GA, an average of 146 and 174 proteins were identified by LC/MSE using an in-solution and in-gel approach, respectively. Analysis between the GA-treated and untreated control seedlings involved a total of 9 injections for each treatment group compromised of 3 biological replicates and 3 technical replicates. Analysis of the peptide datasets revealed at least a 1.5 to 2.0 fold change for some of the integral membrane proteins such as plasma membrane intrinsic proteins (PIPs) and Arabidopsis H+-ATPases (AHAs). Several LRR RLKs were identified, however no significant change in abundance was observed in response to GA.

Poster 21

Label-free, quantitative proteomic comparison of purified plastids from oilseed rape leaves and embryos reveals targeted expression differences in multiple metabolic pathways

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Comparative label free quantification of two plastids types from *Brassica napus* (oilseed rape) was performed. Intact plastids from three week-old leaves (chloroplasts) and three week-after-flowering embryos (embryoplasts), were isolated and verified for purity. Purified plastid preparations (5 biological replicates for each plastid type) were then pre-fractionated by 12% SDS-PAGE. Each SDS-PAGE lane was then sectioned into twelve bands according to the molecular weight standards and incubated with trypsin to digest the intact proteins prior to mass spectrometry, a technique referred to as GeLC-MS. Tryptic peptides from each band of each biological replicate were analyzed by nanospray ionization-liquid chromatography tandem mass spectrometry (240 total analyses), and acquired MS^2 spectra (1,277,144 total) were then searched against a comprehensive plant database, using the SEQUEST algorithm within BioWorks 3.2 software. For quantification of protein expression, MS^2 spectral counting was performed on the BioWorks output files using Scaffold 2.0. Over 500 proteins were identified from each plastid type using this approach and quantitative data was obtained for most of these proteins. Functional classification of identified proteins revealed that both plastid types possess a high percentage of energy-related (ATP synthesis, photosynthesis, electron transport), and metabolic proteins (lipid, amino acid, and sugar metabolism). Prominent embryoplast proteins are mostly related to lipid metabolism and glycolysis; proteins related to sugar and amino acid metabolism are among those in highest abundance based upon spectral counts. Prominent chloroplast proteins are involved in photosynthetic and electron transport processes. We conclude that with sufficient biological and technical replicates label-free, GeLC comparative proteomics is a useful discovery approach for in-depth quantitative analysis of protein expression.

Downtown — Campus Area Restaurants

1. Addison's - Variety
2. Bambino's - Italian
3. Booche's - Cheeseburgers
4. Boone Tavern - Variety
5. Brady Commons - Fast Food
6. Bread Basket Cafe
7. Broadway Diner - Open Late
8. CC's City Broiler - Fine Dining
9. Candy Factory - Chocolates/Coffee
10. Cherry Street Wine Cellar - Variety
11. Cherry Street Artisan - Cafe/Breakfast
12. Chipotle - Mexican Style Fast Food
13. CJ's - Wings and Variety
14. Coffee Zone - Variety
15. Das Kaffehaus - Middle Eastern
16. El Rancho - Mexican
17. Ernie's Steak House - Diner/Breakfast
18. Felinis - Variety
19. Flat Branch Pub & Brewing - Variety
20. Formosa - Chinese
21. Grill-1-5 - Variety
22. Hamid's - Middle Eastern
23. Harpo's Bar & Grill - Variety
24. Heidelberg - Sandwiches
25. India's House
26. International Cafe - Middle Eastern/Greek
27. Jimmy John's Gourmet Subs - Sandwiches
28. Lakota Coffee Company - Pastry/Coffee
29. Main Squeeze - Vegetarian/Smoothies
30. Memorial Union - Sandwich/Pasta
31. Moe's - Mexican Style Fast Food
32. Ninth Street Deli - Sandwiches/Salad
33. Panera Bread - Sandwiches/ Salad
34. Pasta Factory - Italian
35. Quinton's - Sandwich/Salad
36. Quizno's - Sandwiches/Salad
37. Shakespeare's - Locals Favorite Pizza
38. Sake - Sushi
39. Starbuck's
40. Sub Shop - Sandwich/Soup
41. Subway - Sandwiches/Salad
42. Tellers - Sandwich/Soup/Salad
43. The Forge & Vine - Upscale Variety
44. Uprise Bakery
45. W. G. Grinders - Sandwich/Salad
46. Willie's - Variety Bar Food
47. Wing Zone - Chicken Wings

5th Street
6th Street
7th Street
8th Street
9th Street
10th Street
Walnut
Broadway
Tiger Hotel
Cherry Street
Locust Street
Elm Street
University Avenue
Hitt Street
College Avenue
Jesse Hall
Conley Avenue
5 - Brady Commons
30 - Memorial Union
Rollins Road