Journées du PROTEOME VERT

12 et 13 Juin 2023

IDEEV, Université Paris-Saclay

Gif-sur-Yvette

Organisé avec le soutien de :

université PARIS-SACLAY
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Programme

Lundi 12 juin

13h45-14h00 **Accueil** - présentation des journées

14h00-14h40 **Guillaume Tcherkez** (IRHS ; INRAE, L'institut agro Rennes Angers, Université d'Angers)
Mitochondrial complex I disruption causes broad reorchestration of plant metabolism, as revealed by lipidomics and proteomics

14h40-15h00 **Alix Boulouis** (UMR 7141; CNRS, Sorbonne Université)
Time-resolved redox proteomics based on differential labelling of redox forms gives access to kinetics of redox activation of proteins during photosynthesis induction in tobacco

15h00-15h20 **Antoine Davière** (IJPB ; INRAE, AgroParisTech, Université Paris-Saclay)
Early changes in A. thaliana protein apoplastic content following infection by the broad range fungus B. cinerea

15h20-15h40 **Thierry Balliau** (GQE ; INRAE, Université Paris-Saclay, CNRS, AgroParisTech)
Evaluation of the efficiency of the SPEED protein extraction protocol on plant samples

15h40-16h00 Pause

16h00-16h40 **Céline Masclaux** (IJPB ; INRAE, AgroParisTech, Université Paris-Saclay)
How proteomics help us to undestand autophagy in plant ?

16h40-17h00 **Willy Bienvenut** (GQE ; INRAE, Université Paris-Saclay, CNRS, AgroParisTech)
Success and bottlenecks of large scale plant protein turnover measurement: a tomato fruit maturation case study

17h00-17h20 **Chloé Beaumont** (BFP ; INRAE, Université de Bordeaux)
Proteomics and transcriptomics analyses to study protein turnover of developing fruits

17h20-17h40 **Véronique Santoni** (IPSiM ; CNRS, INRAE, Institut Agro, Université Montpellier)
Deubiquitination of plasma membrane proteins

17h40-18h00 **Norbert Rolland** (LPCV; CNRS, CEA, UGA, INRAE) Chloroplast biogenesis: towards the role of localized translation in Arabidopsis

18h00-18h20 **Michel Zivy/Loïc Rajjou/Karine Gallardo**
Hommage Dominique Job

18h20-19h20 : **Apéritif**

Mardi 13 juin

9h00-9h40 **Julie Neveu** (LRSV; Université de Toulouse, CNRS)
The root iron uptake machinery: organization, regulation, and beyond

9h40-10h00 **Aurélie Dupriez** (LRSV ; Université de Toulouse, CNRS)
Développement de stratégies pour maximiser la couverture du peptidome extracellulaire

10h00-10h20 **Elisabeth Jamet** (LRSV ; Université de Toulouse, CNRS)
Toward a better understanding of the molecular mechanisms underlying the heterophylly of Potamogeton nodosus

10h20-10h40 Pause

10h40-11h00 **Chiara Guerrera** (Plateforme de protéomique Necker ; Institut Imagine de Paris)
Data Independent Acquisition (DIA) MS for ultradeep proteomics: principles and applications

11h00-11h20 **Elodie Marchadier** (GQE ; INRAE, Université Paris-Saclay, CNRS, AgroParisTech)
Proteome-based analysis of floral transitions in maize inbred lines divergently selected for early and late flowering

11h20-12h00 **Antoine Danon** (LCQB ; CNRS, Sorbonne Université)
Les comportements collectifs en réponse au stress chez Chlamydomonas reinhardtii

12h00-12h20 Discussion générale

Mitochondrial complex I disruption causes broad reorchestration of plant metabolism, as revealed by lipidomics and proteomics

Jean-Baptiste Domergue[1], Cinzia Bocca[2], Rosine de Paepe[3], Guy Lenaers[2,4], Anis M. Limami[1] and **Guillaume Tcherkez[1,5*]**

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Plant mutants with alteration in mitochondrial function have been used for a long time because it can cause cytoplasmic male sterility, which can be useful for genetic crossing and breeding. Amongst them, mutants affected in respiratory proteins are particularly interesting. In fact, unlike animals where respiratory mutations lead to serious symptoms or premature death, plant mutants are generally not lethal and exhibit only mild symptoms. This allows us to assess consequences of the mutation and elucidate the role of mitochondria in controlling metabolic pathways. Here, we examined the effects of nad7 (complex I protein) knock-out in Nicotiana sylvestris, using the well-characterised mutant cytoplasmic male sterile II (CMSII) which is devoid of mitochondrial complex I activity. Lipidomics show multiple changes in cellular lipids including galactolipids (chloroplastic), sphingolipids and ceramides (synthesised by ER), suggesting that mitochondrial homeostasis is essential for the regulation of whole cellular lipidome via specific signalling. In particular, we observed modifications in both phospholipid and sphingolipid/ceramide molecular species. Proteomics analyses during germination and post-germination stages indicate changes in mitochondrial and non-mitochondrial proteins, such as enzymes of the glyoxylic cycle and branched chain amino acid catabolism. Also, changes in kinetics of protein abundance were observed, including in the biosynthesis of the photosynthetic machinery. Our results demonstrate that mitochondrial function has an important role in the regulation of metabolism in various cellular compartments (chloroplast, peroxisome) and not only in the mitochondrion.

Time-resolved redox proteomics based on differential labelling of redox forms gives access to kinetics of redox activation of proteins during photosynthesis induction in tobacco

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Modifications of cysteine residues are common posttranslational modifications of proteins. In plant cells, cysteines are mostly reduced, harboring a thiol group. Cysteine oxidation as disulfide bonds or via gluthationylation or nitrosylation can regulate protein function and be studied by proteomics. Indeed, thousands of proteins have oxidizable cysteine residues, and a dozen of chloroplast enzymes change kinetic properties when their disulfide bonds are reduced by the thioredoxin system in response to light. While some modifications are very labile, disulfide bonds are stable enough to allow extraction of the proteins from *in vivo* leaf material and differential labelling of the reduced and oxidised cysteines by alkylating agents. Using this labelling technique, we separated the redox forms by gel migration and measured them with mass spectrometry coupled to nanoHPLC. Data analysis was designed to identify the gel fractions containing the redox forms of regulated proteins. We used this method to study the reduction kinetics of tobacco leaf proteins at the dark–to–low light transition. We show that redox states of some thioredoxins follow the photosynthetic linear electron transport rate. While some redox targets have kinetics compatible with an equilibrium with one thioredoxin (TRXf), reduction of other proteins shows specific kinetic limitations, allowing fine-tuning of each redox-regulated step of chloroplast metabolism.

Early changes in *A. thaliana* protein apoplastic content following infection by the broad range fungus *B. cinerea*

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Plant responses to pathogen invasion leads to the production of defense related metabolites, hormones and proteins. Since the apoplastic space represents the first layer of defense, important changes are taking place in this compartment. These changes have not been described yet in the case of necrotrophic fungal pathogens that kill host cells rapidly and proliferate on dead tissues. However, the initial phase of the interaction is critical since these pathogens have to cope directly with plant defenses, in particular in the apoplastic space. In this study we describe for the first time the modifications that occur in the apoplast of *Arabidopsis thaliana* in this initial phase of the *Botrytis cinerea* infectious process. Our results showed that important changes are triggered in the first hours of infection at the proteomic level. Numerous identified proteins were already described as important in defense or susceptibility of the plant against this fungus but the majority were still undescribed in this pathosystem or others. Furthermore, a metabolomic analysis also revealed the strong production of specialised metabolites in the apoplast upon infection. By confronting our results with previously obtained transcriptomic data we found that four major plant processes were prominently affected by the early interaction with *B. cinerea*: plant cell wall metabolism, carbohydrate metabolism, proteolysis and redox metabolism. Significant proteins related to these processes were for the large majority depleted in the infected apoplast while only few specific proteins were strongly accumulated. Some fungal proteins were also identified and more than half of them have undescribed role in pathogenicity. This approach also allowed us to identify putative targets of a fungal secreted protease by comparing wt and mutant infected apoplastic extracts. Taken together, this cartography of the early apoplastic molecular dialogue between the two organisms revealed the presence of new putative defense, susceptibility and virulence factors in this compartment.

Keywords : *Arabidopsis thaliana*, *Botrytis cinerea*, defense, apoplast.

Evaluation of the efficiency of the SPEED protein extraction protocol on plant samples

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In bottom-up proteomics, sample preparation is a critical step that strongly influences the quality of the results. In particular, protein extraction should enable efficient protein digestion for subsequent mass spectrometric analysis. Recently, Doellinger et al. [1] reported a detergent-free method called Sample Preparation by Easy Extraction and Digestion (SPEED), which consists of three mandatory steps: acidification, neutralization, and digestion. SPEED is an attractive method that holds the potential to dramatically simplify and standardize sample preparation while improving the depth of proteome coverage, especially for challenging samples. In addition, its authors report it as universal because it uses pure trifluoroacetic acid, which is supposed to extract protein from any sample by complete dissolution. However, this method has not been tested on plant samples. Yet, plants have specific compounds (e.g., polyphenols, chlorophyll, or major proteins) that can interfere with sample preparation. Here, we aim to evaluate if the SPEED method is suitable for preparing plant protein extracts. To this end, we sampled leaves, roots, and seeds from Arabidopsis Col-0 plants. We ground each tissue in liquid nitrogen and divided the resulting powders into nine aliquots that we subsequently used to extract proteins by three different protocols in three replicates: the SPEED method, the TCA-acetone-based, and the phenol-based extraction methods. We routinely use the latter two protocols to extract proteins from leaves and seeds, respectively. Mass spectrometry analysis for the whole experimental design is currently in progress. Preliminary results obtained on leaf samples indicate that the SPEED method provides good extraction yields and mass spectrometry signals.

References

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How proteomic studies help us to understand essential roles of autophagy in plants

Marien Havé[1], Jie Luo[1], Mégane Mahieu[1], Fabien Chardon [1], Fabienne Soulay [1], Anne Marmagne[1] And Céline **Masclaux-Daubresse[1*]**

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Macroautophagy is involved in cell homeostasis and organelle quality control in eukaryotic cells. Induced in plant in response to many stresses and in parallel with leaf senescence, it works at maintaining leaf longevity. Contributing to the degradation of macromolecules as protein aggregates and damaged cell components, autophagy also contributes to nutrient recycling. Nutrients released through autophagy are either reused in the cell or exported to growing organs. Our group showed that autophagy is responsible of 50% of the nutrient remobilization from source leaves to sink organs, especially under nutrient restriction. Using proteomics, we identified several proteins that abnormally accumulate in the leaf tissues of autophagy mutants under stress conditions. These proteins that could be specific autophagy cargoes or autophagy partners are now under study. Their roles in autophagy process is under study.

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Success and bottlenecks of large-scale plant protein turnover measurement: a tomato fruit maturation case study

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Proteostasis is a key element among the molecular mechanisms required to maintain the equilibrium between protein synthesis and degradation during organism's development. The protein turnover rate (PTR) is the parameter to survey to better understand proteostasis mechanisms which are associated to protein synthesis (K_s) and degradation (K_d) constants. Presently, PTR measurement based on pulse SILAC labelling are not compatible with autotroph species. Then, we developed a novel large scale proteomics strategy based on suboptimal ^{15}N metabolic labelling to calculate K_s and K_d constants in plants. Our approach essentially requires to determine the "protein fold change" (PFC) and the "protein labelled fraction" (PLF) at several labelling kinetics time points. These two values are calculated from mass spectrometry data after adapting MassChroQ, a peptide quantification software and MCQR, an R package dedicated to the statistical analysis of proteomics quantification. First, our developments take into account the peptide ^{15}N-modified isotopic distribution to perform robust label-free quantitation to determine the PFC for each characterised peptide/protein. Second, we used the variation of the peptide isotopic distribution to estimate the LPF and its evolution during the ^{15}N kinetic assay. Finally, we filtered the calculated K_d constants using statistical approaches. We tested this processing pipeline in a project dedicated to study tomato fruit growth and maturation. To this end, we first setup a methodology for tomato fruit ^{15}N labelling. Then we prepared and analysed the collected samples at different time points after the ^{15}N starting pulse. Finally, we used the raw mass spectrometry data to test our processing pipeline. This initial application provides an excellent opportunity to improve our processing pipeline and the results, successes and bottlenecks will be detailed more extensively during this presentation.

Keywords: Protein synthesis, Protein degradation, metabolic labeling, ^{15}N stable isotope, bioinformatics, MassChroQ, MCQR

Acknowledgment: Work supported by the INRAE BAP department (PROOFER grant). The proteomics analysis were performed on the PAPPSO facility which is supported by INRAE, the Ile-de-France regional council, IBiSA and CNRS.

Proteomics and transcriptomics analyses to study protein turnover of developing fruits

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Protein turnover is the result of both synthesis, which integrates transcription and translation, and degradation by various molecular processes. While these mechanisms are well known, experimental determinations of turnover are tedious and expensive so they are very rarely performed at the genome-scale level. The turnover of thousands of proteins has recently been estimated in tomato fruit from time-series data of transcriptomics and proteomics [1] by solving a simple mathematical model involving synthesis (ks) and degradation (kd) rate constants [2]. To better understand protein stability during fruit development, a complex process from anthesis to ripeness, we applied the same strategy to estimate and compare the turnover of nine fruit species. For that, transcriptomic and proteomic datasets were generated in absolute quantification for eight new fruit species: pepper, eggplant, kiwifruit, cucumber, apple, strawberry, peach, and grapes. As for tomato fruit, mRNA and protein levels were poorly correlated throughout development. Thus, with the new transcriptomic and proteomic datasets, we solved the same mathematical model to calculate synthesis (ks) and degradation (kd) rate constants of more than two thousand of proteins per fruit species. The comparison of protein turnover shows that, while fruit development extends from 30 to more than 200 days, both synthesis and degradation rate constants are of the same order of magnitude for all fruit species, suggesting that protein stability could be an intrinsic property encoded in its sequence.

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Deubiquitination of plasma membrane proteins

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In recent times, the knowledge of the roles ubiquitin plays in plant cellular processes has expanded in plants, with one example being the role of ubiquitin in receptor endocytosis and trafficking (1). In mammals several deubiquitinating enzymes (DUBs) have been implicated either in facilitating receptor endocytosis and degradation or in rescuing receptor levels by preventing endocytosis and/or promoting recycling to the PM (2). By contrast, in plants, the role of DUBs, a small family of 57 members, in deubiquitinating and regulating PM protein abundance and trafficking is by far less known (3).

In order to extend our knowledge of membrane transporters deubiquitinated by the DUB UBP13, a quantitative proteomics strategy was used to compare the proteomes and ubiquitinomes (4) of wild-type (WT) plants to that of transgenic plants over-expressing UBP13 (35S::UBP13). This study reveals that UBP13 preferentially targets K11 poly-ubiquitin linkages and modulates the ubiquitination of about 500 peptides among 7000 identified ubiquitinated peptides. UBP13 also exerts a dual role by inducing i) the accumulation of 500 proteins enriched in channels, transporters and protein kinases, and ii) the degradation of 400 proteins. A low correlation between the decreased ubiquitination of proteins and the abundance of their cognate proteins was observed and will be discussed. However, among 7 aquaporin PIP isoforms that double their abundance in 35S::UBP13, 4 of them showed a decreased ubiquitination profile at specific lysine residues. An immuno-precipitation assay coupled to MS allowed to confirm the interaction between UBP13 and PIP2;1 aquaporin. More interestingly, the accumulation of PIP aquaporins in 35S::UBP13 plants resulted in a huge increase of the root hydraulic conductivity, due to an increased PIP aquaporin activity. The molecular mechanisms involved in PIPs stabilization by UBP13 are under investigations as well as the behavior of 35S::UBP13 plants in osmotic challenging environments.

Such a global approach has pinpointed several nutrient transporters as putatively targeted by UBP13 and thus offers promising insights in the regulatory mechanisms of plant mineral nutrition.

Keywords: *Arabidopsis thaliana*, proteomics, ubiquitinome, deubiquitinase, aquaporin

Acknowledgment: financial support by INRAE.

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Chloroplast biogenesis: towards the role of localized translation in Arabidopsis

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Chloroplasts are a major component of plant cells. Until recently, all nuclear-encoded proteins destined to chloroplast were believed to possess an N-terminal and cleavable chloroplast targeting peptide, and to engage the TOC/TIC machinery. However, recent studies have revealed that alternative routes also exists and identified a series of nuclear-encoded proteins imported via such pathways. Recent proteomic studies, conducted by our team (Bouchnak et al., Mol Cell Proteomics 2019), identified a list of cyto-ribosomal subunits associated to chloroplasts, thus suggesting that localized translation might occur at the chloroplast surface. We were recently able to isolate plastid-associated cyto-ribosomes and to decipher their composition when compared to purified whole cell cyto-ribosomes. Interestingly, these plastid-associated cyto-ribosomes contain a few non cyto-ribosomal proteins which might participate to the control of localized translation at the chloroplast surface. These non cyto-ribosomal proteins were first fused to GFP to analyze their subcellular location. Then, we isolated Arabidopsis knock-out mutants affected in the expression of two of these specific proteins and initiated their phenotypical characterization. Finally, with the aim to identify the nature of the nuclear-encoded mRNAs that are translated by these chloroplast-associated cyto-ribosomes, the identification of mRNAs trapped within these plastid-associated cyto-ribosomes was performed. Surprisingly, very few of these mRNAs code for chloroplast proteins.

Keywords: *Arabidopsis thaliana*, proteomics, localized translation, chloroplast, cytoribosomes.

Acknowledgment: Work supported by the Agence Nationale de la Recherche (ANR) Grants PolyGlot (PRC ANR-18-CE12-0021) and C-TRAP (PRC ANR-22-CE12-0012).

The root iron uptake machinery: organization, regulation, and beyond

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In plants, iron is an essential metal for optimal growth and development. Iron uptake by dicotyledonous plants such as the model plant Arabidopsis thaliana occurs through a process that requires three successive steps: rhizosphere acidification, reduction of iron and transport of ferrous iron into root epidermal cells by the membrane transporter IRT1 (Iron-Regulated transporter1) (Vert et al., 2002). In addition to iron, IRT1 also transports zinc (Zn), manganese (Mn), cobalt (Co), and cadmium (Cd), that are non-iron metals which are toxic when present in excess in plant cells. In the team, several post-translational regulatory mechanisms of IRT1 have been identified allowing to limit uptake of these non-iron metals and to protect plants from their harmful effects (Cointry and Vert, 2019). Indeed, in addition to its role as a transporter, IRT1 also acts as a receptor able to sense non-iron metal excess and initiate a response resulting in its degradation (Dubeaux et al., 2018 ; Ivanov and Vert, 2021 ; Spielmann and Vert, 2021). Direct metal binding to a histidine-rich cytoplasmic loop in IRT1 induces its phosphorylation by the CIPK23 kinase (Calcineurin B-like-Interacting Protein Kinase23) and facilitates the subsequent recruitment of the E3 ubiquitin ligase IDF1 (IRT1 Degradation Factor1) (Rodenas and Vert 2021). IDF1-mediated polyubiquitination of IRT1 then triggers IRT1 endocytosis and its vacuolar degradation. More recently, characterization of the IRT1 interactome by Co-IP/MS has revealed the existence of a complex composed of IRT1 and two other proteins, the H+-ATPase AHA2 and the reductase FRO2 (Ferric Reduction Oxidase2) (Martin-Barranco et al., 2020). This complex gathers together the different actors of the acidification-reduction-transport mechanism allowing plant iron uptake. Furthermore, it has been shown that in response to non-iron metal excess, phosphorylation of IRT1 causes its selective detachment from the complex.

In order to identify new actors involved in the regulation of IRT1 activity and in its endocytosis and degradation in response to an excess of non-ferrous metals, we have developed proximity labeling approaches by Bio-ID. This technique allows the identification of weak and transient protein-protein interactions, and is based on the fusion of IRT1 in its first cytosolic loop and a biotin ligase (TurboID). Transgenic lines expressing this functional fusion between IRT1 and TurboID were generated and characterized. We also determined the optimal conditions for biotinylation and subsequent affinity purification steps and were able to establish reliable protocols for the BioID experiment with a membrane transporter and under metal excess conditions. This work allowed us to identify new candidates interacting with IRT1 by mass spectrometry analysis and which are involved in its endocytosis and degradation.

Keywords: Metal nutrition, Cell signaling, Post-translational modifications, Bio-ID.

Développement de stratégies pour maximiser la couverture du peptidome extracellulaire

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La paroi des végétaux joue un rôle critique pendant le développement et en réponse aux stress environnementaux. En particulier, elle est impliquée dans la signalisation entre cellules et certaines molécules provenant de parois peuvent agir en tant que signaux. En se basant sur notre expertise en protéomique de parois [Int J Mol Sci 2022, 23: 4273], nous avons élaboré des stratégies visant à identifier et caractériser des peptides extracellulaires ou des petites protéines (<10 kDa) potentiellement impliqués dans des mécanismes de défense. Deux stratégies ont été élaborées : (i) l'analyse de fluides extracellulaires extraits en utilisant une centrifugation à basse vitesse ou (ii) l'extraction de peptides totaux avec différents solvants. Dans les deux cas, nous avons prêté attention à limiter la protéolyse en limitant le temps d'extraction ou en ajoutant des inhibiteurs de protéases dès les étapes initiales d'extraction. La sélection des peptides de taille inférieure à 10 kDa a été réalisée via une étape de *cut-off* et les échantillons n'ont pas été systématiquement digérés à la trypsine pour accéder à leur(s) formes natives. Les résultats nous montrent que les deux méthodes sont complémentaires, certains peptides n'étant identifiés que dans un cas. Une grande quantité de peptides/petites protéines ont été identifiées dans les feuilles d'*Arabidopsis thaliana* par spectrométrie de masse et bioinformatique. De plus, nous avons pu déterminer les formes natives de certains d'entre eux ainsi que des modifications post-traductionnelles telles que l'hydroxylation de la proline en hydroxyproline (Hyp) [Plants(Base l) 2022, 11: 3554]. Dans le cas de SCOOP10, un peptide jouant un rôle lors de la floraison, la variabilité du pattern d'hydroxyprolination a de nouveau été démontrée, suggérant un rôle dans la régulation de l'activité biologique des peptides via la *O*-glycosylation [Front Plant Sci 2017, 8: 1802].

Mots clés : *Arabidopsis thaliana*, fluides extracellulaires, paroi, peptidomique

Remerciements : Ce travail a été financé par le projet ANR STRESS-PEPT (contrat ANR-20-CE20-0025), le CNRS et l'Université Paul Sabatier de Toulouse.

Toward a better understanding of the molecular mechanisms underlying the heterophylly of *Potamogeton nodosus*

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Potamogeton nodosus is an angiosperm belonging to one of the plant families which has returned to an aquatic life by implementing new strategies. For instance, the underwater low O_2 availability has required the emergence of specialized aerated tissues (aerenchyma) and mechanisms of cell wall acidification have occurred to facilitate the conversion of CO_2 to diffusible HCO_3^-. The cell wall has therefore played a major role in the adaptation to the physico-chemical constraints of the aquatic environment. This project aims at identifying cell wall-related changes that allowed the aquatic lifestyle. The genome of *P. nodosus* has been sequenced, revealing the presence of 57637 predicted open reading frames of more than 80 amino acids with the Braker 3 software, and, as expected, pairs of homeologues genes resulting from its allopolyploïdy. As a test case, the whole set of class III peroxidase genes has been analyzed. Transcriptomics and cell wall proteomics approaches have been conducted to better understand the molecular mechanisms underlying the heterophylly of *P. nodosus* exhibiting two different types of leaves depending on their position, either floating or submerged. The mass spectrometry data have allowed to distinguish homeologue proteins for about 75% of the identified proteins despite their high level of homology, and to refine the structural annotation of some genes. About 30% of the identified proteins have been predicted to be CWPs, *i.e.* about 400 different CWPs corresponding to a coverage of about one fourth of the expected cell wall proteome. The cell wall proteomics results point at a different distribution of three functional classes of cell wall proteins between the two leaf types with a predominance of proteins related to cell wall carbohydrates in the floating leaves, and of oxido-reductases and proteases in the submerged leaves.

Keywords : cell wall, heterophylly, *Potamogeton nodosus*, proteomics

Acknowledgements: This work has been supported by the TULIP LabEx project (ANR-10-LABX-41), the TULIP-GS (ANR-18-EURE-0019), the CNRS and the Paul Sabatier-Toulouse 3 University (UT3).

Data-Independent Acquisition Mass Spectrometry (DIA-MS): Principles and Application to Senescence and the Effects of *Oenothera biennis* Hydrophilic Extract

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Data-Independent Acquisition Mass Spectrometry (DIA-MS) has emerged as a powerful tool for comprehensive proteomic analysis, offering distinct advantages over Data-Dependent Acquisition Mass Spectrometry (DDA-MS). In this presentation, we elucidate the principles of DIA-MS, highlight its advantages compared to DDA-MS, and present a specific application involving the investigation of the effects of *Oenothera biennis* hydrophilic extract (ObHEx) on senescence in normal human dermal fibroblasts subjected to stress-induced premature senescence (SIPS).

DIA-MS overcomes the limitations of DDA-MS by systematically acquiring fragment ion spectra for all detectable ions in a given m/z range. This untargeted approach enables comprehensive proteomic profiling and ensures that fewer peptides are missed during analysis. DIA-MS also provides higher reproducibility, enhanced quantification accuracy, and increased depth of proteome coverage.

Utilizing DIA-MS, we implemented an ultra-deep proteomic analysis to investigate senescence-associated changes in normal human dermal fibroblasts under SIPS conditions and we explored the effects of ObHEx, derived from Oenothera biennis cell cultures, on senescent cells.

In conclusion, DIA-MS provides a robust platform for in-depth proteomic analysis, surpassing the limitations of DDA-MS. Our application of DIA-MS in investigating the effects of *Oenothera biennis* hydrophilic extract on senescence revealed promising insights into the restoration of critical mitotic proteins.

Keywords: Data-Independent Acquisition Mass Spectrometry, DIA-MS, Data-Dependent Acquisition Mass Spectrometry, DDA-MS, proteomic analysis, senescence, Oenothera biennis, hydrophilic extract, stress-induced premature senescence, SIPS.

Etude protéomique de la transition florale chez des lignées de maïs issues d'une experience de sélection divergente pour la date de floraison.

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La période de floraison est une étape de développement critique pour le rendement du maïs et qui est souvent soumise à des conditions de sécheresse. L'expérience de sélection divergente de Saclay* commencée il y a 25 ans a permis de générer des lignées précoces et tardives dérivées de lignées ancestrales consanguines. Les études de développement de ces lignées démontrent que ce décalage observé de date de floraison s'explique principalement par un décalage de la transition florale. Afin de mieux comprendre les bases moléculaires de ce processus adaptatif, une analyse protéomique a été réalisée deux années de suite sur des génotypes précoces et tardifs dérivés de la lignée MBS. Les tissus des feuilles immatures et matures ont été échantillonnés avant, pendant et après la transition florale. Sans surprise, des changements dans l'abondance des protéines ont été détectés entre les années (en particulier pour les tissus matures) et entre les stades de développement (en particulier pour les tissus immatures). Malgré la forte parenté des génotypes, certains changements spécifiques au génotype ont également été identifiés entre les stades de développement. Notre intérêt s'est porté sur ces protéines candidates. Des connaissances externes (résumées dans la base de données STRING) ont été comparées à cette liste de protéines et ont permis d'établir qu'une grande partie des protéines candidates sont fonctionnellement connectées sans nécessairement avoir été préalablement identifiées comme impliquées dans la transition florale.

Flowering time is a critical developmental stage for maize yield and is often subjected to drought conditions. Saclay's Divergent Selection Experiment* started 25 years ago allowed to generate early and late lines derived for inbred ancestral lines. Developmental studies of these lines demonstrate that this observed flowering time shift is underlined by a shift of the floral transitions. To better understand the molecular bases of this adaptative process, a proteome analysis has been performed two successive years on early and late MBS derived genotypes.

Immature and mature leaves tissues were sampled before, during and after the floral transition. Unsurprinsingly, changes in protein abundances were detected between years (especially for mature tissues) and between the developemental stages (especially for immature tissues). Despite the strong relatness of the genotypes, some genotype-specific changes were also identified between the developmental stages. Our interest has been focused on these candidates. External knowledge (summarized in STRING database) were compared to this list of proteins and allowed to establish that a large part of the candidate proteins are functionally connected without necessarly been previously reported as involved in floral transition.

Remerciements aux plateformes PAPPSO (GQE) et PMM (IPS2) qui ont réalisé les analyses protéomique et métabolomiques.

Keywords : maïs, transition florale, adaptation, protéomique

Réference

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Les comportements collectifs en réponse au stress chez Chlamydomonas reinhardtii

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Chlamydomonas reinhardtii est une algue unicellulaire modèle, autant pour la recherche fondamentale que pour les applications qui peuvent en déboucher. Nous utilisons Chlamydomonas pour essayer de comprendre comment un organisme unicellulaire s'adapte pour survivre dans un environnement hostile. Nous avons montré que selon le niveau de stress, Chlamydomonas peut adopter différents types de comportements collectifs *[1]*. Si le stress est trop intense, une partie des cellules vont déclencher un processus de mort cellulaire programmée (PCD), visant à permettre à la population de survivre. En cas de stress intermédiaire, les algues vont former de larges structures multicellulaires protectrices, pouvant contenir plusieurs milliers de cellules. Nous avons identifié une famille de mutants, qui forment spontanément des structures multicellulaires, les mutants socializer (saz). Afin de comprendre comment ces deux comportements collectifs sont contrôlés, nous avons analysé les protéomes de 6 mutants saz ainsi que le protéome de cellules en cours de PCD. Nos analyses de protéomique couplées à des stratégies de transcriptomique et de génétique inverse nous ont permis d'identifier les premiers régulateurs de l'agrégation chez Chlamydomonas *[2]*. Ces mêmes stratégies nous ont permis de découvrir des caractéristiques fondamentales de l'exécution de la PCD chez Chlamydomonas. Tout d'abord, ce processus est dépendant de la lumière et utilise probablement la voie de synthèse de la chlorophylle pour produire des ROS. Ensuite, nous avons montré pour la première fois chez Chlamydomonas que la PCD est dépendant des métacaspases de type II.

Réference

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