Résumés des communications aux journées du protéome vert, 3-4 juin 2019

Invités lundi

Stéphane Lemaire (Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes) : "Protéomique redox et biologie synthétique de la fixation du carbone et des réponses au stress chez Chlamydomonas"

Alexandre Giuliani (Synchrotron SOLEIL) : "L'analyse des protéines au synchrotron SOLEIL"

Invités mardi

Jean-Michel Camadro (Institut Jacques Monod, unité Mitochondries, Métaux et Stress Oxydant) : "SLIM-Labeling: principle, data processing workflows and applications in quantitative proteomics"

Denis Chéreau (Institut Mutualisé pour les Protéines Végétales IMPROVE, http://www.improve-innov.com, rue Fond Lagache, 80480 Dury) : « Les défis liés à l'industrialisation de la production des protéines alternatives »

Les protéines sont au centre de plusieurs enjeux mondiaux : nourrir les bientôt 10 Mds d'humains, équilibrer la part animal vs végétales, développer une agriculture plus durables, répondre aux nouvelles attentes des consommateurs…
Face à ces enjeux un grand nombre d'acteurs travaillent sur des projets visant à développer de nouveaux ingrédients ou de nouveaux produits finis riches en protéines. Nous essayerons de dégager les facteurs clés de succès pour ce type de projet.

Soutenu par

Résumés dans l’ordre du programme

Novel regulatory mechanisms of plant aquaporins revealed by interactomics

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The absorption of soil water by roots allows plants to maintain their water status. Water transport is regulated by the function of aquaporins (1) and can be affected at the endodermis by initial formation of a Casparian strip and further deposition of suberin lamellas (2). Proteins that molecularly interact with two majors root aquaporins (PIP1;2 and PIP2;1) were searched to get new insights into regulatory mechanisms of root water transport using a immuno-purification strategy coupled to protein identification and quantification by mass spectrometry. Such interactome revealed PIPs to behave as a platform for recruitment of a wide range of transport activities and provided novel insights into regulation of PIP cellular trafficking by osmotic and oxidative treatments. We also show that members of the receptor-like kinase (RLK) family can modulate PIP activity (3). Interestingly, 4 Casparian strip membrane domain proteinlike (CASPL) also co-purified with PIP2;1. We showed that 3 of them (CASPL1B1, CASPL1B2, and CASPL1D2) are exclusively expressed in suberized endodermal cells, suggesting a cellspecific role in suberization and/or water transport regulation. None of the mutants showed root hydraulic conductivity (Lp) rphenotype, whether in control or stress conditions. However, the data suggest a slight negative role for CASPL1D1 and CASPL1D2 in suberization under control or salt stress conditions. At the molecular level, CASPL1B1 was able to physically interact with PIP2;1 and potentially could influence the regulation of aquaporins by acting on their phosphorylated form (4). The overall work opens novel perspectives in understanding PIP regulatory mechanisms and their role in adjustment of plant water status.

1. Maurel C, et al. (2015) Aquaporins in Plants. Physiological reviews 95(4):1321-1358.
2. Geldner N (2013) Casparian strips. Current Biology23(23):R1025-R1026 .
3. Bellati J, et al. (2016) Novel aquaporin regulatory mechanisms revealed by interactomics. Molecular and Cellular Proteomics 15:3473-3487.
4. Champeyroux C, et al. (2019) Regulation of a plant aquaporin by a Casparian strip membrane domain protein-like. Plant Cell Environ. doi: 10.1111/pce.13537.

Is there a core plant cell wall proteome?

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Cell wall proteins (CWPs) are minor components of the plant cell wall, representing 5-10% of its mass, compared to polysaccharides (90-95%). However, they play critical roles during plant development and in response to biotic and abiotic stresses. Proteomics has revealed the great diversity of CWPs and has provided an overview of the protein families present in cell walls [1,2]. The best described cell wall proteome is that of Arabidopsis thaliana since it covers the main plant organs, roots, stems, leaves, inflorescences and seeds, as well as cell suspension cultures [2]. About half of the expected CWPs have been identified so far in at least one organ. Other cell wall proteomes have also been described. Among them are those of Populus sp., Solanum tuberosum, S. lycopersicum, Medicago sativa and Linum usitatissimum as dicots and Brachypodium distachyon, Oryza sativa, Saccharum officinarum as monocots [3]. Despite the fact that these proteomes have been established following diverse protocols, it is possible to combine the results to search for the presence of similar protein families. A few families have thus been identified. It allows defining a core cell wall proteome which however does not pretend to be exhaustive yet. Indeed, all the plants have not been studied in detail and some proteins are still recalcitrant to extraction from cell walls (e.g. covalently linked structural proteins) and/or to identification by mass spectrometry and bioinformatics (e.g. small size arabinogalactan proteins). Among the protein families shared by all the cell wall proteomes described so far, there are (i) proteins acting on cell wall polysaccharides (e.g. glycoside hydrolases GH3, GH17, GH38 and pectin methyl esterases),(ii) class III peroxidases, (iii)) proteases (Asp proteases and Cys proteases), (iv) lipid transfer proteins, (v) fasciclin arabinogalactan proteins, (vi) purple acid phosphatases and (vii) thaumatins. In addition, some protein families are present in at least two thirds of the presently known cell wall proteomes, among which two families of proteins of unknown function, the DUF642 proteins [4] and the PAC domain proteins [5]. Despite the difference in cell wall structure and composition between monocots and dicots, it was not possible to highlight any CWP families specific for either of them. All these CWP families could represent a set of house-keeping proteins critical for the maintenance of the cell wall functions.

[1] Jamet et al. (2008) Proteomics 8: 893
[2] Albenne et al. (2013) Front Plant Sci 4: 111
[3] WallProtDB (www.polebio.lrsv.ups-tlse.fr/WallProtDB), San Clemente and Jamet (2015) Plant Methods 11: 2
[4] Vásquez-Lobo et al. (2012) Mol Phylogenet Evol 63: 510
[5] Hijazi et al. (2014) Ann Bot 114: 1087

Une nouvelle sous-classe d'anhydrase carbonique dans le plancton marin

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Nous avons montré récemment, en utilisant diverses approches, que la diatomée marine Thalassiosira pseudonana n'effectue pas une photosynthèse de type C4 et que les anhydrases carbonique (CA) sont des acteurs majeurs de l'adaptation de cette diatomée à de faibles concentrations de CO 2. Elles contribuent ainsi au mécanisme de concentration du CO2 (CCM)[1]. Par des expériences de 2D DIGE et une analyse protéomique, nous avons montré qu'une protéine (sous-unité d'environ 63 kDa) était induite à faible concentration de CO , et nous l'avons appelée LCIP63 pour « low CO inducible protein of 63 kDa »[2]. Le gène codant la LCIP63 a été cloné, la protéine exprimée et purifiée. LCIP63 a une activité CA et une activité estérase ; elle est inhibée par un inhibiteur classique de CA. La LCIP63 est localisée près de l'enveloppe interne des chloroplastes et joue un rôle important pour le CCM chez T. pseudonana puisqu'une augmentation de l'affinité pour le carbone minéral est observée lorsque la LCIP63 est surexprimée. Son activité semble être modulée par le Mn^{2+} et non par le cofacteur typique de nombreuses CAs, à savoir le Zn^{2+}. De plus, comme la LCIP63 a une faible identité de séquence avec d'autres sous-classes de CA connues, nous avons proposé qu'elle appartienne à une nouvelle sous-classe de CA que nous avons désignée -CA (iota-CA). Des homologues de LCIP63 sont largement répandus sur les plans géographique et taxonomique chez les eucaryotes et les procaryotes photosynthétiques, ainsi que chez les bactéries et les archées. Cette protéine pourrait donc jouer un rôle important dans le cycle global du carbone.

[1]Clement R, Dimnet L, Maberly SC, & Gontero B (2016) The nature ofthe CO_2-concentrating mechanisms in a marine diatom, Thalassiosirapseudonana. New Phytol.209(4):1417-27. doi: 10.1111/nph.13728.
[2]Clement, R., Lignon, S., Mansuelle, P., Jensen, E., Pophillat, M.,Lebrun, R., Denis, Y., Puppo, C., Maberly, S. C., and Gontero, B. (2017) Responses of the marine diatom Thalassiosira pseudonana to changes inCO_2 concentration: a proteomic approach, Sci Rep 7, 42333.

Analyse protéomique du dialogue moléculaire responsable de la fusariose de l’épi chez le blé tendre

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La fusariose de l’épi est une maladie du blé principalement causée par l’agent phytopathogène Fusarium graminearum. Cette maladie est contrôlée par un dialogue moléculaire complexe impliquant des effecteurs fongiques capables de manipuler les facteurs de sensibilité du blé dès les premiers stades de l’interaction. L’analyse des stades précoces de la maladie a permis la mise en évidence d’ajustements simultanés dans les protéomes fongique et végétal, au cours des 96 premières heures de l’interaction. Bien qu'aucun symptôme ne soit encore détectable lors de ces stades précoces, les variations d’abondance d’un ensemble extrêmement diversifié de protéines sécrétées et d'effecteurs fongiques candidats censés cibler les organites végétaux ont pu être observés chez F. graminearum. Certaines de ces protéines se sont révélées être accumulées rapidement au cours de l'interaction tandis que d’autres étaient déjà stockées dans les spores ou dans les spores en germination avant même le premier contact avec l’hôte. Les protéines de blé régulées étaient étroitement liées aux processus cellulaires basaux intervenant au cours de l'ontogenèse de l'épillet, et des profils de co-régulation spécifiques ont été mis en évidence entre les protéines chloroplastiques du blé et les protéines fongiques prédites comme étant adressées au chloroplaste. Les réponses coordonnées identifiées chez la plante et F. graminearum fournissent un ensemble de données original et élargissent notre compréhension des mécanismes moléculaires déterminant le devenir la maladie.

Fabre, F., Vignassa, M., Urbach, S., Langin, T., and Bonhomme, L. (2019). Time-resolved dissection of the molecular crosstalk driving Fusarium head blight in wheat provides new insights into host susceptibility determinism. Plant. Cell Environ. 0. doi:10.1111/pce.13549.

Chetouhi, C., Bonhomme, L., Lecomte, P., Cambon, F., Merlino, M., Biron, D. G., et al. (2015). A proteomics survey on wheat susceptibility to Fusarium head blight during grain development. Eur. J. Plant Pathol. 141, 407–418. doi:10.1007/s10658-014-0552-0.

Studying the interplay between sulfur nutrition and water stress tolerance in pea by proteomics: a focus on seed development and composition

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Water stress and sulfur-deficiency are two constraints increasingly faced by crops due to climate change and low-input practices. To investigate their interplay in the grain legume pea (*Pisum sativum* L.), sulfate was depleted at mid-vegetative stage and a moderate 9-day water stress period was imposed during the early reproductive phase. The combined stress accelerated seed production, lowering yield, one-seed weight and seed number per plant, but rebalanced seed protein composition. In fact, the moderate water stress mitigated the negative effect of sulfur-deficiency on the accumulation of sulfur-rich proteins in seeds, probably due to a lower seed sink strength for nitrogen, enabling a readjustment of the sulfur-poor/sulfur-rich globulin ratio. These results uncovered the importance of sulfur for stabilizing seed yield in pea facing short and moderate water stress episodes, and showed that the adaptive response of sulfur-deprived plants to water stress is much more complicated than a simple additive response. Developing seeds were subjected to shotgun proteomics and transcriptomics to advance our knowledge of the seed response to the single or double stresses. Among the results that will be presented is the building of a protein network together with a differential analysis, highlighting hubs potentially implicated in seed development and stress response.

Proteomic and lipidomic analyses of the Arabidopsis *atg5* autophagy mutant reveal major changes in ER and peroxisome metabolisms and in lipid composition

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Autophagy is a universal mechanism in eukaryotic cells that facilitates the degradation of unwanted cell constituents and is essential for cell homeostasis and nutrient recycling.
Large scale proteomic and lipidomic analyses of *atg5* and *atg5.sid2* mutants facilitated the determine the salicylic-acid independent effects of autophagy defects in leaf tissues under various N and S nutritive conditions.
Results revealed that irrespective of the growth conditions, plants carrying the *atg5* mutation presented all the characteristics of endoplasmic reticulum (ER) stress. Increases in peroxisome and ER proteins involved in very long chain fatty acid synthesis and b-oxidation indicated strong modifications of lipid metabolism. Lipidomic analyses revealed changes in sphingolipid, phospholipid and galactolipid contents. Significant accumulations of phospholipids and ceramides and changes in GIPCs (glycosyl-inositol-phosphoryl-ceramides) in *atg5* mutants indicated large modifications in endomembrane -and especially plasma membrane- lipid composition. Decreases in chloroplast proteins and galactolipids in *atg5* under low nutrient conditions, indicated that chloroplasts were used as lipid reservoirs for b-oxidation in *atg5* mutants.
In conclusion, this report demonstrates the strong impact of autophagy defect on ER stress and reveals the role of autophagy in the control of plant lipid metabolism and catabolism, influencing both lipid homeostasis and endomembrane composition.

Label-free quantitative proteomics reveals the involvement of the N-end rule pathway in Arabidopsis thaliana seed responsiveness to ethylene

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Ethylene, an important gaseous hormone, participates in the alleviation of seed dormancy in numerous species including Arabidopsis thaliana. Using mutant of the N-end rule pathway, lacking the E3 ligase PROTEOLYSIS (PRT6), it has been demonstrated that this proteolysis pathway that targeted the protein by its N-terminal residues, is involved in seed responsiveness to ethylene. In the present work, we carried out shotgun label-free quantitative proteomics (LC-MS/MS) of wild type Col-0 and prt6 seeds treated by ethylene. Dormant seeds of both lines were imbibed in water at 25 °C for 16 and 30 h in the presence of exogenous ethylene (100 ppm), the control seeds being incubated without ethylene. After 16h, 1737 proteins were identified, and then quantified by extracted ion currents (XIC) based method, but none was significant after two factors (genotype and treatment) ANOVA analysis with adjusted P value 0.05. After extending the ethylene treatment up to 30 h, 2552 proteins were identified, and 619 proteins have significant interaction effects with adjusted P value 0.05. Turkey test (P=0.05) and fold change ratio (<0.7 or >1.3) allowed to select 587 and 30 proteins in the group Col+/Col- and prt6+/prt6-, respectively. These results indicated that ethylene did not have a marked effect on the global proteome of prt6 seeds and suggested that the N-end rule pathway might be an important hub in ethylene response. Over representation analysis of the significant proteins in Col+/Col- showed that the down-regulated proteins were enriched in biological process of seed development, response to ABA, lipid, water, H O , light, heat and second metabolism; while the 2 2up-regulated proteins were enriched in the categories of amino acid biosynthesis, RNA modification, gene expression and protein metabolism and lipid catabolism. All the changes observed in the Col+/Col- group were summarized as signals reception and transduction, reserve mobilization and new material generation, which could contribute to seed germination. Twenty nine common proteins were overlapped in the two groups, while 5 proteins (LDAP2, EXPANSIN2, GDSL LIPASE-LIKE PROTEIN, JACALIN-RELATED LECTIN and BETA-GLUCOSIDASE) presented opposite changes. These proteins, could act as good markers of ethylene response in Col and prt6 mutant, it would be then important to use mutants to prove whether these proteins are involved in the ethylene effects on breaking of dormancy.

Sunflower hybrids and inbred lines adopt different molecular and physiological strategies to respond to water deficit

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The domesticated sunflower, *Helianthus annuus* L., is the fourth most important oilseed crop in the world, is cultivated as hybrids and is promising for agriculture adaptation because it can maintain acceptable yields across a wide variety of environmental conditions, especially during drought stress.

To highlight the molecular processes related to the drought adaptation strategies, we studied the responses of eight parental lines (four males and four females) and their 16 hybrids cultivated on the outdoor high-throughput automated phenotyping platform Heliaphen [1], where ecophysiological traits were measured [2]. Leaf samples from 144 plants were collected for multi-omics analyses including label-free shotgun proteomics analysis.

Statistical learning methods without a priori allowed us to reduce this complex molecular system and to model proteomic and phenomic relationships to identify molecular players of heterotic behaviours and its role during drought stress.

We demonstrate strong behavioral differences between hybrids and parental lines with solid interaction with water deficit. They will be exemplified by interesting candidate proteins that seem to play an important role in regulatory networks. Selecting for the expression of these proteins could be interesting to improve heterotic groups and to breed for tolerance to drought.

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[2] N. Blanchet, P. Casadebaig, P. Debaeke, H. Duruflé, L. Gody, F. Gosseau, N.B. Langlade, P. Maury, Data describing the eco-physiological responses of twenty-four sunflower genotypes to water deficit, Data Br. (2018). doi:10.1016/J.DIB.2018.10.045.

Améliorations de X!TandemPipeline pour la protéomique haut débit et la métaprotéomique

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L'évolution rapide des spectromètres de masse en fréquence d'acquisition et précision de mesure permet l'identification et la quantification de plus en plus de protéines et de peptides. Il est aussi plus simple d'analyser de grandes cohortes d'échantillons au sein d'une expérience. Cela représente un défi en bioinformatique pour analyser ces grands jeux de données. X!TandemPipeline (Langella et al, 2017) a été modifié pour permettre l'analyse de ces très grands jeux de données, tout en apportant une grande qualité de résultats.

X!TandemPipeline est une application graphique conçue pour filtrer, analyser et visualiser les données de protéomique quantitative (Windows or Linux). Il prend en charge les fichiers d'identification (Mascot, X!Tandem, pepXML ou le nouveau standard mzIdentML), permet la validation automatique ou manuelle des résultats, dispose de son propre mécanisme d'inférence de protéines, et maintenant permet la visualisation des XICs (MS1), avec vérification des massifs isotopiques. Il permet la prise en charge d'expériences avec ou sans marquage isotopique. Une vue dédiée permet la visualisation des phosphoprotéines qui est maintenant généralisée à toute autre modification post traductionnelle. Cette nouvelle version, totalement réécrite en C++, est aussi beaucoup plus rapide et moins gourmande en mémoire vive. Elle permet de calculer 10 fois plus vite qu'avec l'ancienne version en Java et de manipuler des milliers d'échantillons au lieu de centaines d'échantillons.

Cela a permis l'analyse de très grands jeux de données. Nous vous présenterons quelques résultats obtenus en protéomique à haut débit (1103 échantillons Q-Exactive, ANR Amaizing, article en cours de soumission), ainsi qu'en métaprotéomique avec l'analyse du microbiote intestinale (250 échantillons Lumos, ANR Proteocardis, article en cours de soumission).

Ces analyses peuvent être effectuées sur de simples ordinateurs de bureau (Windows ou Linux), avec une configuration minimale.