
# IRN-FJFPB

## Fourth Webinar « Photosynthesis and Metabolism »

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**Abstracts**

**Dr Benjamin Bailleul**, IBPC, Paris, France

Website of the lab: http://www.ibpc.fr/UMR7141/

**Evaluating Cyclic Electron Flow around PSI in various microalgae**

It is commonly assumed that in plants and green algae, Cyclic Electron Flow (CEF) around photosystem I (PSI) plays a crucial role in optimizing photosynthesis by regulating the photosynthetic control and non-photochemical quenching in photosystem II (PSII), and by providing the extra ATP required for carbon fixation. However, several decades of research did not provide a clear-cut answer about the mechanism, extent, regulation and conservation of CEF among different photosynthetic clades. This is mostly due to the absence of a consensus method to estimate this flow in physiological conditions. The most accepted approach, which compares the quantum yields of PSII (by chlorophyll *a* fluorescence) and PSI (accessible through absorption changes associated to variations of the redox state of P700), can lead to aberrant conclusions regarding CEF. We propose an alternative method based on the electrochromic shift of photosynthetic pigments, to test for the presence of CEF and, where appropriate, describe the relationship between LEF and CEF. We could highlight 3 behaviors: in some photosynthetic species, CEF is not occurring under steady state illumination (e.g. in the dinoflagellate *Amphidinium carterae*). In the ones where CEF exists, it can be independent from LEF (*Symbiodinium sp.*) or dependent on LEF (*Chlamydomonas reinhardtii*). This method can be used to explore how CEF depends on physiological and environmental conditions.

**Dr Kaori Yoneyama**, Ehime University, Japan

Website: https://www.ehime-u.ac.jp/english/

**Strigolactones, how are they synthesized to regulate plant growth and development?**

Strigolactones (SLs) are multifunctional plant metabolites working not only as allelochemicals in the rhizosphere, but also as a novel class of plant hormones regulating growth and development *in planta*. To date, more than 30 SLs have been characterized and recent studies using transcriptomics and reverse genetic techniques have paved the way to clarify the entire biosynthetic pathway of structurally diverse SLs. Our group mainly investigates how strigolactones are synthesized and their production and exudation are regulated. I would like to introduce our recent results.

**Dr Tania Tibiletti**, I2BC-UMR 9198, Dept. B3S, CEA Paris-Saclay, France

tania.tibiletti@cea.fr

Website of the lab: https://www.i2bc.paris-saclay.fr/equipe-photosystem-ii/

**Probing photosynthesis by EPR spectroscopy**

Photosynthesis has enormous potential to meet the great challenges of this century concerning alternative energy, food, sustainable agriculture and climate change. One promising approach is to enhance the efficiency of photosynthesis that aims to increase the conversion of light energy into biomass. Understanding in detail photosynthesis and its regulation will as well stimulate the development of efficient photo-catalysts to use in artificial photosynthesis.

The conversion of solar energy into chemical energy of photosynthesis occurs in thylakoid membranes through the concerted actions of several protein complexes. Of these complexes, Photosystem I and II, Cytochrome b6f and Plastocyanin contain organic free radicals and metal transition ions that are responsible of electron transfer reactions and membrane bioenergetics. Together with triplet states, organic free radicals and metal transition are paramagnetic species that can be monitored by Electron Paramagnetic Resonance (EPR) spectroscopy providing detailed information on the structure of key biological molecules and their interaction with the surrounding environment. Here, I will show you how we use EPR spectroscopy to investigate photosynthesis, particularly the water oxidation mechanism in Photosystem II.

**Dr Keisuke Yoshida**, Associate Professor, Lab. Chem. Life Sci., Tokyo Tech., Japan
kyoshida@res.titech.ac.jp
Website of the lab: http://www.res.titech.ac.jp/~biores/

**Thioredoxin-based redox regulatory network in chloroplasts**

Redox regulation is a posttranslational protein modification that plays a key role in adjusting chloroplast functions under varying light conditions. Redox-sensitive target proteins are activated in the light by receiving reducing power derived from photosynthetic electron transport reactions. A redox cascade via thioredoxin (Trx) has been classically recognized as the key system for transmitting the light-induced reductive signal to target proteins. However, owing to recent identification of multiple Trx isoforms and their potential target enzymes, it has become increasingly apparent that the redox regulatory system in chloroplasts is organized as a more complicated network. My goal is to elucidate its whole organization and biological significance comprehensively. In this webinar, I briefly introduce recent advances of our group.

**Marine Messant**, PhD student, CEA, CNRS, Université Paris-Saclay, Photobiology, Photocatalysis (3P), Gif-sur-Yvette, France
marine.messant@cea.fr
Website of the lab:
https://www.i2bc.paris-saclay.fr/equipe-photobiology-photosynthesis-photocatalysis/

**Manganese excess and deficiency study in *Marchantia polymorpha* : effect on Photosynthesis**

Manganese (Mn) is a fundamental element for plant growth, in particular for its involvement in photosynthesis. It forms, at the photosystem II donor side, the oxygen evolving complex (OEC) catalyzing water oxidation. Mn deficiency and excess are known factors affecting crop yields mainly because of their impact on photosynthetic activity. The use of *Marchantia polymorpha*, a liverwort, made it possible to study the impact of the two stresses on a single organism. Its simple anatomy compared to higher plants allowed the use of super-resolution microscopy. We have developed a culture technique on a solid medium to study excess and deficiency in wild-type plants. We have shown that both stresses affect the shape of chloroplasts as well as their thylakoids structure. Non-favourable Mn concentrations disrupt photosynthetic activity by changing the PSI/PSII ratio. Deficiency results in increased dissipation of excess light energy as heat most likely due to an increase of the activity of cyclic electron flow. Finally, we have performed for the first time a metabolomics analysis on *Marchantia polymorpha*. Mn excess leads to an increase in certain metabolites allowing protection against heavy metals. Mn deficiency causes a sharp decrease in 10 essential amino acids. Both conditions strongly affect the activity of antioxidant enzymes.

**Dr Yusuke Kobayashi**, Assistant professor, Plant molecular biology, Ibaraki University, Japan
yusuke.kobayashi.botany@vc.ibaraki.ac.jp

**Holliday junction resolvase MOC1 mediates plastid and mitochondrial genome segregation**

When DNA double-strand breaks occur, four-stranded DNA structures called Holliday junctions (HJs) form during homologous recombination. Because HJs connect homologous DNA by a covalent link, resolution of HJ is crucial to terminate homologous recombination and segregate the pair of DNA molecules faithfully. We recently identified Monokaryotic Chloroplast1 (MOC1) as a plastid DNA HJ resolvase in algae and plants. Although Cruciform cutting endonuclease1 (CCE1) was identified as a mitochondrial DNA HJ resolvase in yeasts, homologs or other mitochondrial HJ resolvases have not been identified in other eukaryotes. We found that MOC1 dually targeted to plastids and mitochondria in some land plant lineages, such as the moss Physcomitrella patens, a liverwort and a fern. Moreover, mitochondrial targeting of MOC1 was also predicted in charophyte algae and some land plant species. Taken together, we propose that MOC1 resolves HJs in mitochondria of some lineages of algae and plants as well as in plastids in algae and plants.

**Ousmane Dao**, PhD student, Institute of Biosciences and Biotechnologies Aix-Marseille, CEA Cadarache, France
Ousmane.dao@cea.fr
Website of the lab: https://www.cite-des-energies.fr/biam/recherche/ebm/

**Crosstalk between photosynthetic electron management pathways and carbon metabolism under fluctuating nutrient conditions in Chlamydomonas**

Photosynthetic organisms constantly need to adjust their energy production to meet the metabolic demand varying largely in their natural environment. Any imbalance between the energy production and the utilization could potentially lead to over-reduction and ROS generation. In the past 10 years, electron management pathways (such as PGRL1/PGR5 mediated cyclic electron flow (CEF) and O2 photoreduction by the Flavodiiron -FLV- protein) have been extensively studied to understand the regulation of photosynthetic energy production. However, most of those studies focused on understanding the function of PGRL1 and FLV under different light regimes such as high light or fluctuating light but their role during nitrogen (N) deficiency have been barely investigated. I will present our latest results on the interplay between carbon metabolism and electron management pathways in the model microalga Chlamydomonas reinhardtii by using mutants impaired in PGRL1 and FLV grown under N replete and deprived conditions. By measuring the O2 exchange rate, photosystem II quantum yield and reduced carbon accumulation, we have showed that the O2 photoreduction and TAG biosynthesis compensate for the deficiency of PGRL1 during N limitation while the lack of FLV results in high accumulation of starch under N replete condition.

**Dr Ryosuke Munakata**, assistant professor, Laboratory of Plant Gene Expression, Research Institute for Sustainable Humanosphere, Kyoto University

ryosuke_munakata@rish.kyoto-u.ac.jp
Website of the lab: https://www.rish.kyoto-u.ac.jp/lpge/

**Repeated evolution of furanocoumarin biosynthesis in plants**

Furanocoumarins (FCs) are phenolic allelochemicals and show a scattered distribution in plants with a chemotaxonomical tendency toward Apiaceae, Fabaceae, Rutaceae, and Moraceae. Even though these four plant families are phylogenetically distant each other, they use the common precursor and intermediates to produce the FC backbone. This raises a question how the FC biosynthetic pathway has emerged and spread in plants. Here, we have carried out molecular identification of FC biosynthetic enzymes from different plant taxa and these FC biosynthetic genes/enzymes were subjected to phylogenetic analysis. Firstly, umbelliferone dimethylallyltransferase (UDT) genes, which are located at the first committed reaction step, were isolated from Apiaceae and Moraceae. Phylogenetic tree analysis and comparison of exon-intron structures strongly suggested that Apiaceous and Moraceous UDTs are derived from different ancestors each other. Moreover, in Moraceae, we identified CYP71F112 as a marmesin synthase gene at the biosynthetic step next to UDT. Phylogenetic analysis demonstrated that CYP76F112 has been acquired in Moraceae through a taxon-specific molecular evolution. These results support that the FC biosynthetic pathway has been emerged repeatedly in plants. We will also discuss factors which might have promoted the repeated emergence of FCs.

**Samuel Knosp**, PhD student, Evolution and diversity of plant metabolism, Institute of Plant Molecular Biology, Strasbourg
samuel.knosp@etu.unistra.fr
Website of the lab:
http://www.ibmp.cnrs.fr/teams/evolution-and-diversity-of-plant-metabolism/?lang=en

**Cutin biosynthesis in bryophytes through the characterization of cutin synthase genes in *Physcomitrium patens***

About 500 million years ago, first embryophytes emerged on land, thus bringing about the huge diversification on land plants and dramatic geo-climatic changes. The transition from water to land exposed plants to numerous specific constraints such as drought, UV radiation and new pathogens. Most of these challenges are faced with the biosynthesis of apoplastic hydrophobic polymers, which shape plant tissues and provide impermeability, rigidity and protection. Among them, cutin is the main component of the cuticle, a lipidic layer that covers plant aerial organs and prevents water loss. Most of the work on cutin biosynthesis has been carried out on vascular plant species. By investigating this metabolic pathway in bryophytes, we aim to shed light on how this pathway is conserved in embryophytes, and how it has evolved. I will present my work conducted in the model moss *P. patens*, about the characterization of the last step: the polymerization of cutin monomers by cutin synthases (CUS). This reveals that CUS activity and function are conserved in all embryophytes, therefore suggesting that the emergence of CUS family was crucial in the adaptation of plants on land. Moreover, other polymerization mechanisms remain to be discovered, maybe providing new clues on the evolution of plant apoplastic barriers.

**Dr Fanny Bellegarde**, Designated assistant professor Nagoya University, Laboratory for plant signaling
fannyb@nuagr1.agr.nagoya-u.ac.jp
Website of the lab: https://www.agr.nagoya-u.ac.jp/~ck/en/sakakibara.html

**Exploring the role of histone modifications in response to nitrate variation for cytokinin biosynthesis**

In soil, nitrate concentration fluctuates and is often a limiting factor of crop growth and development. Cytokinin (CK), a class of hormone promoting cell division, is necessary for the long-distance nitrate signaling, especially an intermediate form of *trans*-zeatin (tZ): the tZ-riboside (tZR). Interestingly, genes encoding for the enzymes permitting the production of this tZR, IPT3 and *CYP735A2* are strongly induced by nitrate and repressed by a starvation. However, the underlying mechanisms of these regulations remain poorly understood. Our research aims to understand how variation in nitrate availability impacts the transcriptional regulation of CK biosynthesis genes through chromatin modification. By the analysis of post-translational modifications of the histone H3, and expression profile during a kinetic of nitrate variation (nitrate-starvation-nitrate resupply), we observed that the chromatin profiles at CK biosynthesis genes change dynamically during nitrate variation in correlation with the transcripts profile. To better understand the role of this chromatin dynamic and effectors involved, we are now analyzing mutants impaired for different chromatin regulators (deposition or removal of histone marks). Our recent results suggest that the chromatin dynamic constitutes a new regulatory mechanism in response to nitrate availability for CK biosynthesis genes regulation. I will illustrate these results by presenting the characterization of *IPT3* chromatin profiles during the nitrate variation.