Defining small molecule-protein interactions to reveal novel components of sugar signalling in plant growth and metabolism.

PI 1 Dr Mike Haydon, School of BioSciences, University of Melbourne (Primary Institution)
http://blogs.unimelb.edu.au/haydonlab/

The Haydon lab is interested in plant cell signalling, with a particular interest in integration of external and internal signals and their impact on plant physiology. For example, Dr Haydon's research has shown a role for daily rhythms of sugars produced from photosynthesis in setting the pace of the circadian clock in Arabidopsis (Haydon et al., *Nature*, 2013). Presently, the primary research focus in the lab is to identify novel components of sugar signalling that act independently of light input. We use genetic and small molecule screens, transcriptomics and molecular genetic tools to better understand the integration of sugar and light signals in plant cells.

PI 2 Dr Aleksandra Skirycz/Prof Lothar Willmitzer, MPIMP
http://www.mpimp-golm.mpg.de/2031673/Protein-metabolite_interactome_to_unravel_small-molecule_signaling

The Willmitzer group is interested in small molecule signalling and regulation. We aim to understand how diverse metabolites regulate plant growth and development by interacting with their protein receptors. We have developed a methodological pipeline to study protein-metabolite interactions in a cell-wide manner. We exploit classical biochemical methods, state of the art metabolomics and proteomics platforms and bio-physical tools to assess the binding. Importantly, our experimental approach is suitable not only for endogenous small molecules, but also for the identification of protein partners of exogenous compounds, such as drugs and herbicides. At present, the small molecule signalling group (established in February 2015) comprises two doctoral students and six post-doctoral researchers from Germany, Poland, Brazil and Columbia.

Proposed project

Carbohydrate metabolism is fundamental to plant growth and biomass production. Sugars provide the building blocks and energy for life, but also act as modulators of key developmental and physiological processes. Presently, we have a limited understanding of the precise molecular pathways that underpin these signalling processes in plant cells. To define key components of plant sugar signalling, a sensitive luciferase-based assay has been developed to report dynamic transcriptional responses to sugars in living Arabidopsis seedlings. A high-throughput small molecule screen based on this assay identified ~80 drug-like compounds with significant, reproducible effects on the transcriptional output. These compounds are predicted to target signalling proteins including kinases, phosphatases and receptor proteins, but the specific identity of many of the targets in plant cells is unknown.

The aim of this project is to define the specificity of a subset of these compounds on transcriptional networks using a combination of luciferase-reporter assays, qRT-PCR and RNA-Seq to investigate their impact on transcripts associated with the circadian clock, known sugar signalling pathways and novel markers defined by in-house transcriptomics datasets. The project will also investigate the effects of these compounds on growth (pre- and post-germination, developmental transitions, circadian rhythms) and metabolism (sugar quantification, chlorophyll content, chlorophyll fluorescence). A combined strategy of size exclusion chromatography (SEC) and affinity chromatography (Veyel et al. in revision) will be used to detect *in vivo* small molecule-protein interactions and identify the specific target of effector compounds. Molecular genetic tools will then be applied to confirm the role and mechanism of the target protein in sugar signalling towards defining the contribution to physiology and development.

Timeline

Project aims	Year 1		Year 2		Year 3	
1. Define transcriptional and physiological effects of selected compounds - Luciferase reporter assays, qRT-PCR, RNA-Seq - Germination assays, plant growth assays, circadian clock experiments - Photosynthesis measurements by chlorophyll fluorescence						
2. Identify and validate compound-protein interactions - SEC, Affinity chromatography - Recombinant protein production for validation of interaction						
3. Molecular genetic characterization of target protein - characterization of mutant or knock-down Arabidopsis plants - generate transgenic Arabidopsis lines for *in vivo* protein characterization						

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