SACLAY PLANT SCIENCES

SPS Meeting 2015

ABSTRACT BOOK

March 20, 2015

Orsay, France

www.saclayplantsciences.fr

PROGRAMME

9:00 am	**Dirk Inze**	*"Plant growth beyond limits"*
9:45 am	**Cécile Raynaud**	*"Interplay between DNA replication and maintenance of genome integrity in Arabidopsis"*
10:30 am	**Coffee Break**	
11:00 am	**Cathie Martin**	*"Reprogramming of primary metabolism facilitates metabolic engineering of bioactive compounds in tomato fruit"*
11:45 am	**Herman Höfte**	*"Perception of cell wall integrity by Receptor Like Kinases"*
12:30 am	**Lunch (offered by SPS)**	
2:00 pm	**Karin Schumacher**	*"pH in the endomembrane system - an import and export business"*
2:45 pm	**Romain Le Bars**	*"Autophagosome formation in plants"*
3:30 pm	**Coffee Break**	
4:00 pm	**Raphaël Mercier**	*"Multiple mechanisms limit meiotic crossovers"*
4:45 pm	**Bernd Weisshaar**	*"The sugar beet genome sequence, steps to its improvement and a use case"*
5:30 pm	**End of the meeting**	

Plant growth beyond limits

Dirk Inze

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Plant and plant organ growth are regulated by an exceedingly complex interplay of many genes and their interactions with the ever changing environment. Numerous genes of which the modified expression enhances plant organ growth have now been identified, and a detailed study of these genes provided novel insights in the molecular machines driving growth. Furthermore, evidence obtained both in the model plant Arabidopsis and in maize, demonstrated that the combination of multiple growth enhancing genes can have very profound effects on organ sizes. I will discuss how our insights open up new perspectives for the identification of optimal growth regulatory networks that can be selected by advanced breeding, or for which more robust variants can be obtained through genetic engineering.

Interplay between DNA replication and maintenance of genome integrity in Arabidopsis

Cécile Raynaud

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Plant development relies on the proliferation of meristematic cells that retain the ability to divide throughout the life cycle of the organism. In addition, gametes do not originate from cells specialised during embryo development but are produced throughout the plant's life from floral meristems. However, despite direct exposure to UV or ionising radiation, gametes from ten thousand years old trees, are still able to produce viable seeds. This intriguing observation may result from unique mechanisms involved in the regulation of DNA replication or sensing of replication errors in plants. To gain insight into the specificities of plant DNA replication, we have analysed the function of the plant homologues of CDT1 proteins which are involved in the initiation of DNA replication in other eukaryotes. We have found that in Arabidopsis, CDT1 is involved in the maintenance of genome integrity, and that it interacts with a replicative DNA polymerase. This interaction appears unique to plants and may be required for faithful transmission of the genome in proliferating cells.

Reprogramming of primary metabolism facilitates metabolic engineering of bioactive compounds in tomato fruit

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Phenylpropanoids are derived from phenylalanine and comprise an important class of plant secondary metabolites that include specialized bioactives with medicinal properties and important phytonutrients, that promote human health. A number of transcription factors have been used to upregulate specific branches of phenylpropanoid metabolism, but by far the most effective has been the fruit-specific expression of *AtMYB12* in tomato, which resulted in an astonishing 10% of fruit dry weight accumulating as flavonoids and hydroxycinnamates. AtMYB12 not only increases the demand of flavonoid biosynthesis, but also increases the supply of carbon from primary metabolism, and the supply of energy and reducing power, by upregulating glycolysis, the TCA cycle, the oxidative pentose phosphate pathway, fuelling the shikimate and phenylalanine biosynthetic pathways to supply more aromatic amino acids for secondary metabolism. AtMYB12 directly activates at least some genes encoding enzymes of primary metabolism. The enhanced supply of precursors, energy and reducing power achieved by AtMYB12 expression can be harnessed to engineer high levels of novel metabolites in tomato fruit, offering an effective production system for high value polyphenols, and foods fortified in health-promoting phytonutrients.

Perception of cell wall integrity by Receptor Like Kinases

Rodnay Sormany[1], Samantha Vernhettes[1], Martine Gonneau[1], Thierry Desprez[1], Marjolaine Martin[1], Kian Hematy[1], Gregory Mouille[1], Alexis Peaucelle[2,3] and **Herman Höfte**[1].

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The cell wall of growing plant cells accomplishes a remarkable feat, resisting to very high tensional stresses imposed by the high turgor pressure of up to 10 bars, while maintaining the ability to extend. Cell wall strength is determined by the cross links between cell wall polymers, whereas cell wall extensibility reflects the ability to remove and recreate these crosslinks. Recent studies have revealed the existence of feedback signalling networks that control the chemorheological processes underlying the mechanical homeostasis of the walls of growing cells. In this presentation I will first discuss some recent insights into the architecture of plant cell walls in particular the role of pectins as part of a load-bearing network. I will then discuss how receptor kinases of the CrRLKL1 family are part of a regulatory module involved in a feedback regulatory loop that senses cell wall properties and triggers wall stiffening upon wall damage. These modules play a role in normal growth and development and in the response to abiotic and biotic stresses.

pH in the endomembrane system - an import and export business

Karin Schumacher

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pH homeostasis is an essential process in all plant cells and the maintenance of correct luminal pH in the compartments of the endomembrane system is important not only for secondary active transport but also for a variety of cellular functions including protein modification, sorting, and trafficking. Due to their electrogenicity primary H^+-pumps cannot establish and control the often large proton-gradients single-handedly but require the co-action of other ion transporters that serve as either shunt conductances or proton-leaks. Here, I will thus focus on recent results that highlight the interplay of proton-pumps and proton-coupled transporters in controlling pH in the compartments of the plant endomembrane system.

Autophagosome formation in plants

Romain Le Bars[1,2], Béatrice Satiat Jeunemaitre[1,2] and Michele Wolfe Bianchi[1, 3]

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Understanding compartment formation represents a fundamental challenge in cell biology. Autophagosomes are responsible, amongst other functions, for non-selective macroautophagy (autophagy hereafter), and arise, in yeast and animals, from the sealing of a cup-shaped double-membrane precursor, the phagophore. Elements of the associated protein machinery, first identified with the yeast Atg genes, are well conserved in eucaryotes. How the phagophore is generated and grows into a sealed autophagosome is still not clear in detail, and completely unknown in plants, due in part to the scarcity of structurally informative, real-time imaging data of ATG proteins at the phagophore site. The ATG5 complex directs anchoring of ATG8 to the phagophore, an event required for membrane expansion. We find that, in intact living Arabidopsis tissue, ATG5 is present at the phagophore site from early sub-resolution stages, and later defines a torus-shaped domain in a flat cisternal early phagophore anchored to the underlying ER membrane. Detailed real-time and 3D imaging of the growing phagophore are leveraged upon to propose a model for autophagosome formation in plants.

Multiple mechanisms limit meiotic crossovers

Raphael Mercier

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Meiotic crossovers (COs) have two important roles, shuffling genetic information and ensuring proper chromosome segregation. Despite their importance and a large excess of precursors (i.e DNA double strand-breaks, DSBs), the number of meiotic COs is tightly regulated, typically one to three per chromosome pair. Nevertheless, the mechanisms that ensure DSBs repair mostly as non-crossovers, and the evolutionary forces that impose this constraint, are poorly understood. Following a specific genetic screen, we identified several proteins that antagonize crossover formation in *Arabidopsis Thaliana*. This includes the helicase FANCM and its two co-factors MHF1 and MHF2, the BLM-like helicase RECQ4, the TOPOISOMERASE3α (TOP3α) and the AAA-ATPase FIDGETIN-LIKE1. Strikingly, the concomitant disruption of several of these activities led to a nine fold increase in CO frequency, without affecting chromosome segregation and fertility. This shows that several parallel pathways actively limit CO formation and supports the idea that crossover number is restricted not because of mechanical constraints but likely because of long-term costs of recombination. Furthermore, this demonstrates how manipulating a few genes holds great promise for increasing recombination frequency in plant breeding programs.

The sugar beet genome sequence, steps to its improvement and a use case

Bernd Weisshaar

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Sugar beet (Beta vulgaris ssp. vulgaris) is a diploid plant with n=9 chromosomes and an estimated genome size of 714-758 Mbp. The taxonomical position of the species is within the Amaranthaceae family and in the order Caryophyllales. The plant is an important crop in Europe and the US, accounting for 30% of the yearly global sugar production. While beet varieties cultivated for their leaves have been known since Roman times, sugar beet is one of the most recently domesticated crops. First efforts of breeding sugar beets date back to the end of the 18th century, following the discovery that the sugar accumulating within the beet's storage root is chemically identical to sugar extracted from sugar cane.

We sequenced the sugar beet genome and generated an assembly that encompasses 567 Mbp. This genome sequence is the first representative from the Caryophyllales clade, a taxon comprising 11,500 species. In addition to Beta vulgaris and Spinacia oleracea, this clade also includes species such as the cacti, stone plants, and several carnivorous taxa. We constructed phylogenetic trees for each sugar beet gene (collectively referred to as "phylome") and used the data to infer accurate phylogenies among eudicot plants. Our analysis revealed the separation of Caryophyllales prior to the split of rosids and asterids.

In order to further develop Beta vulgaris genomics and to promote the value of its genome sequence for plant biotechnology and academic research, we (i) improved the assignment of unanchored contigs to pseudochromosome sequences by 'genotyping by sequencing' using low coverage NGS data from F2 plants of a mapping population, (ii) and extended the genetic map. Also, we applied mapping-by-sequencing in a proof of concept experiment to map th RED gene of sugar beet.