MistralMontpellier
Montpellier International School on Ion and water TRAnsport in pLants

July 11 - 22, 2016

Campus Montpellier SupAgro / INRA

Theoretical training program

July 11 - 15
After a general introduction (1h), this session will tackle 5 series of topics, from biophysics to whole plant physiology:

- Concepts in ion and water membrane transport
- Methods for experimental studies of ion and water transport in plants
- Membrane transport proteins, structure-function relationship and regulation
- From molecular analyses to whole plant physiology
- Plant nutrition and agroecology

Each series (7-8h) will include lectures questions/discussions (most often, 45 min lecture and 15 min questions/discussions).

Speakers

Monica Calvo, Alex Costa, Léon Dirick, Toru Fujiwara, Alain Gojon, Philippe Hinsinger, Tomoaki Horie, Laurence Lejay, Nathalie Leonhardt, Antoine Martin, Alexandre Martinière, Christophe Maurel, Jean-Benoît Peltier, Hatem Rouached, Véronique Santoni, Hervé Sentenac, Mitsunori Seo, Jean-Baptiste Thibaud, Lionel Verdoucq, Anne-Aliénor Véry & Sabine Zimmermann.

Challenge

As challenge during the thematic school, all participants are invited to create a draft for a poster that presents the MISTRAL Summer School. Working in small groups, the participants should use all the scientific information from the two weeks school and their creativity to make an attractive poster describing MISTRAL for the next 2018 session and indicating the scientific content and organization. These poster proposals, their scientific and pedagogical contents, will be presented by the groups during the second week. The organisation committee will retain the best proposal for advertising the next MISTRAL Summer School.

Lectures & conferences

Session 1: Concepts in ion and water membrane transport

History of concepts and approaches in Plant Membrane transport and mineral nutrition
Hervé Sentenac, BPMP
This lecture will provide a historical survey of the evolution of concepts in plant mineral nutrition and membrane transport. Major sections will be as follows: identification of essential mineral

nutrients, kinetics approaches of ion transport, thermodynamics analyses underlying the notions of active and passive transport, molecular identification of transport systems (pumps, channels and transporters), and integrated studies of the roles and regulation of these systems at the cell and whole plant levels, ending with present perspectives in this field of plant biology.

Biophysics of ion diffusion in water and across a membrane

Jean-Baptiste Thibaud, Institut des biomolécules Max Mousseron, UMR 5247 CNRS-UM-ENSCM, Université de Montpellier, France

This lecture (4 h in total) will aim at providing a basic presentation of biophysical concepts used to describe ion diffusion in water and across membranes: energy of an ion in water, Fick's law of diffusion, forces that drive ion diffusion in water and across membranes, the notion of thermodynamical equilibrium of an ion in a model system consisting in a membrane separating two electrolytic solutions, the Nernst potential. The notion of diffusion potential across such a membrane will be presented based on the Donnan potential model. The Goldman-Hodgkin-Katz model that describes the electrical current resulting from diffusion of a given permeable ion across a membrane will be introduced. Then, considering electroneutrality of ion exchanges, it will be discussed how the membrane potential that settles across the membrane is related to: (i) the Nernst potential of permeable ions (*i.e.* the concentrations of the different permeable ionic species present at both faces of the membrane) and (ii) membrane conductance to each of these ion species. Finally, the crucial role, especially in plant cells, of electrogenic pumps (mostly H+-ATPases) in the cell electrical polarisation and ion exchange energising, and the relevance of electrical equivalent circuit representations in cell electrophysiology will be emphasised.

Biophysics of water transport

Christophe Maurel, BPMP

This lecture will address the physical principles that govern water transport in plant cells and tissues. The driving forces involved and resulting flow equations will be presented, with a special emphasis on the relations between water and solute transport. This knowledge will be applied to understanding the non-steady state water status of plant cells, in the context of osmotic and turgor adjustments, or expansion growth. Finally, theoretical evidence for membrane water channels will be discussed.

Session 2: Methods for experimental studies of ion and water transport in plants

Tools and techniques used in electrophysiology

Anne-Aliénor Véry, BPMP

The principle of Ag/AgCl micro-electrodes broadly used in electrophysiology will be presented. Then, the theory of voltage-clamp and the principle of operation of voltage-clamp and patch-clamp systems will be outlined. Finally, analysis of single-channel currents (patch-clamp) and of macroscopic currents (voltage-clamp) will be introduced.

Heterologous expression systems

Anne-Aliénor Véry, BPMP & Jean-Benoît Peltier, BPMP

Heterologous expression of plant membrane transport systems can be obtained in a wide range of

cellular systems. The most frequently used ones (*Xenopus* oocyte, *S. cerevisiae*, *P. pastoris*, *E. coli*, *Lactococcus lactis*, animal cell lines, protoplasts) will be introduced with their benefits and drawbacks. In summary: how and why using heterologous expression systems and which one for what.

Quantitative imaging of the plant cell

Alexandre Martinière-Delaunay, BPMP

Applications of microscopy and of fluorescent probes in plant membrane transport studies will be addressed. Especially the use of GFP and its derivatives and the use of ion specific probes will be detailed. Experimental strategies will be described to characterize subcellular localization, targeting/traffic/recycling and protein-protein interactions of plant membrane transport systems.

***In vivo* analyses of Ca2+ transport across plant cellular membranes**

Alex Costa, University of Milan, Department of Biosciences, Milan, Italy

The lecture will provide information about the *in vivo* molecular imaging strategies adopted to study Ca2+ dynamics in the cytosol and in different subcellular compartments of plant cells, including mitochondria, endoplasmic reticulum, peroxisome and plastids/chloroplast by using the genetically encoded probe Cameleon (FRET based probe). During the lecture particular attention will be paid to provide feedback on the technical aspects of the strategies adopted (type of imaging solution employed: wide-field *vs* confocal) as well as to point out the importance of using a ratiometric probe for the study of Ca2+ dynamic in moving organelles. The use of new imaging technologies (light sheet microscopy coupled with FRET detection) will be also presented with the description of advantages/limitations.

Yeast as a versatile heterologous expression system for the characterization of transporters functions and regulations

Léon Dirick, BPMP

As a unicellular eukaryotic organism, budding yeast is an ideal model to study general biological functions, often highly conserved. With numerous and handy tools, yeast can be modified to gain insights into gene functions from heterologous organisms, including from plants. Thus, we will present this model organism and see how it can be used to address questions related to plants' transporters, their functions and regulations.

SESSION 3: MEMBRANE TRANSPORT PROTEINS, STRUCTURE-FUNCTION RELATIONSHIP AND REGULATION

Structure/function relationship of transport systems

Lionel Verdoucq, BPMP

This lecture will address how the functional properties of a transport system can be accounted for by protein structure. Topics will include the structural modelling of membrane proteins involved in transport, and how studies of engineered mutants can help in understanding the molecular bases of the functional features of transport systems (with examples of aquaporins and K+ channels). Interest and limitations of homology modelling approaches will also be discussed.

Transceptors
Laurence Lejay, BPMP & Alain Gojon, BPMP
The aim of this lecture will be to introduce a new class of transport proteins that actually fulfill a dual transport/signalling function. This class is mostly documented in yeast, where several nutrient transporters have been shown to also act as nutrient sensors, and were therefore called 'transceptors' (transporter-receptors). Increasing evidence suggests that higher plants also rely on transceptors for nutrient sensing. This evidence will be reviewed, and the mechanisms by which a membrane transport protein can activate signalling transduction pathways will be discussed.

Regulation of membrane transport by post-translational modifications
Laurence Lejay, BPMP & Véronique Santoni, BPMP
This talk will review proteomic methodologies for the study of post-translational modifications acting on membrane transport proteins in plants (phosphorylation, ubiquitination,...).
The mechanisms of these modifications will be detailed, and their physiological relevance for regulation of transport protein tracking or activity will be analyzed, using specific examples selected within water and ion transport proteins.

The use of genomics to understand plant adaptation to nutrient and water fluctuations
Antoine Martin, BPMP
Large-scale genomic approaches have recently emerged as efficient methods to identify molecular bases of plant adaptation to variable environments. This lecture will give an overview of the main techniques used in genomics, including transcriptome and epigenome profiling, genome-wide association, and identification of regulatory elements, as well as more complex analysis of systems biology. We will provide in the same time recent illustrations of how these approaches have led to original mechanisms of adaptation to nutritional or water constraints in several plant models.

CONFERENCE

***In vivo* analysis of Ca2+ dynamics in *Arabidopsis*: a tool to study molecular components of its transport**
Alex Costa, University of Milan, Department of Biosciences, Milan, Italy
The use of Cameleon coupled to a genetic approach (its expression in isolated mutants) has permitted to reveal molecular components of Ca2+ transport regulation in plant. Two examples, one related to Ca2+ transport across plant mitochondrial inner membrane and one in the plasma membrane will be presented.

SESSION 4: FROM MOLECULAR ANALYSES TO WHOLE PLANT PHYSIOLOGY

Water transport: from aquaporins to whole plant hydraulics
Christophe Maurel, BPMP
The mechanisms that underlie the regulation of root and leaf hydraulics in response to environmental stimuli will be presented. The significance of these regulations with respect to water and nutrient uptake, transpiration and tissue expansion will be discussed.

Nutrient exchange and adaptation to environmental stress in plant-fungal interactions

Sabine Zimmermann, BPMP & Monica Calvo, BPMP
Mycorrhizal interaction between fungi and their host plants involves a series of membrane transport systems that will be discussed. Furthermore, we will focus on molecular plant-fungi responses to salt and drought and the different stress tolerance mechanisms involved in ion and water balance.

CONFERENCES

Boron transport in plants (1): Mechanisms of B-dependent regulation of transporter expression and localization
Pr. Toru Fujiwara, Laboratory of Plant Nutrition and Fertilizers, Graduate School of Agricultural and Life Sciences, University of Tokyo, Japan
Boron (B) is an essential element for plants and for plants it is essential to maintain B homeostasis. For this transport processes need to be regulated in accordance with the B condition in soils/media. Many members of two major types of boron transporters, BORs and NIPs, are regulated in B-dependent manners, but the mechanisms of regulation different. In this talk, I would like to introduce mechanisms of regulating accumulation of BORs and NIPs in response to B conditions in the media. Regulation of BOR1 mainly occurs at the levels of protein degradation, while NIP5;1 is regulated at the level of mRNA accumulation. We recently found a unique role of ribosome in regulating NIP5;1 mRNA accumulation in a B dependent manner.

Boron transport in plants (2): Mathematical modelling and boron distribution in roots
Pr. Toru Fujiwara, Laboratory of Plant Nutrition and Fertilizers, Graduate School of Agricultural and Life Sciences, University of Tokyo, Japan
For the understanding of B homeostasis in plants, understanding of the regulation of each members of boron transporter is not sufficient. Plants have several different boron transporters whose accumulation patterns and regulation mechanisms are different. For example, NIP5;1 is a boric acid channel, which facilitate boron permeation across plasma membrane, while. BOR1 and BOR2 are is boric acid exporters. NIP5;1 and BORs have "outer" and "inner" localization patterns, respectively. It is likely that B concentration in cells are affecting BOR and NIP5;1 accumulation and *vice versa*. I would like to present our trial to comprehensively understand the overall B transport/distribution in *A. thaliana* roots through mathematical modelling, in combination with B distribution analysis using Laser-Ablation ICP-MS.

Functional screening of plant hormone transporters using modified yeast two-hybrid systems with the plant hormone receptor complexes
Mitsunori Seo, RIKEN Center for Sustainable Resource Science, Tsurumi-ku, Yokohama, Kanagawa, Japan
Plant hormones are naturally occurring small compounds that regulate various physiological processes at low concentrations. Although studies have indicated that most plant hormones are mobile, it remains largely unknown how the transport of hormones is regulated. In this presentation, I will introduce our strategy to functionally identify potential plant hormone transporters based on their plant hormone transport activities.

Guard cell movements: roles of ion transport systems
Nathalie Leonhardt, Laboratoire de Biologie du Développement des Plantes, CEA Cadarache,

France
The guard cell can be taken as a paradigm to study the coupled transport of anions and cations across the tonoplast and plasma membrane. The integration of transport systems within cell signalling cascades will be discussed.

Session 5: Plant nutrition and agroecology

Root-soil interactions: ecological relevance of phosphate acquisition in the rhizosphere
Philippe Hinsinger, UMR Eco&Sols CIRAD-INRA-IRD-SupAgro Montpellier, France
Phosphorus scarcity is a major issue in many environments, which becomes increasingly at stake. The aim of this lecture is to show that besides phosphate uptake at the root/soil interface, higher plants use a number of tricks to acquire soil-bound phosphorus, implying a number of ion transport processes (uptake of other nutrients and release of protons and carboxylates) that ultimately alter phosphate availability in the vicinity of the roots, *i.e.* in the rhizosphere. The ecological relevance of phosphate uptake versus other root/soil interactions will be discussed in the context of various types of ecosystems and agroecosystems.

Modern agriculture: genomics for better agri-productivity
Hatem Rouached, BPMP
The ultimate goal of plant biotechnology is to increase biomass production and grain yield in crops. This involves improving plant responses to abiotic stresses such as nutrient deficiency or drought. Attempts to improve crops through conventional breeding and/or genetic engineering will be presented and discussed in the context of the preceding lectures.

Conference

Plant salt tolerance ~ water and Na+ homeostasis upon salinity stress
Tomoaki Horie, Shinshu University, Tokida, Ueda, Nagano 386-8567, Japan
Plants suffer a reduction in water absorption and excessive invasion of toxic ions such as Na+ upon salinity stress, which cause serious damages in growth and productivity. In this seminar, a part of water and Na+ transport mechanisms in crops in response to saline environments will be presented with the reference to important transport systems for plant salt tolerance, for which the genes are yet to be identified.