

# **IRN-FJFPB**

### Webinar « Cell biology & signal transduction »

April 8th, 2021 Abstracts



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https://www.biomemb.cnrs.fr/thematique/communication-intercellulaire-mediee-par-les-plasmodesmes/

### Staying tight: controlling plant cell-to-cell communication through plasmodesmata membrane contacts

#### Abstract

Our group investigates the question of how plasmodesmata-mediated cell-to-cell communication benefits from organelle tethering. We integrate modelling methods and super-high resolution 3D imaging into molecular cell biology of plant cell-to-cell communication. Current objectives are to 1) Identify the mechanisms of PD membrane-tethering at the molecular level 2) Elucidate the dynamics and 3D architecture of ER-PM contact sites at PD 3) Uncover the function of ER-PM apposition for plant intercellular communication.



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Arabidopsis BOR1 is a borate transceptor that senses the boron concentration and promotes its own ubiquitination and degradation.

#### Abstract

Plants take up and translocate nutrients through transporters. In *Arabidopsis thaliana*, the borate exporter BOR1 acts as a key transporter under boron (B) limitation in the soil. Upon sufficient-B supply, BOR1 undergoes polyubiquitination and is transported from the plasma membrane to the vacuole for degradation, to avoid overaccumulation of B. Our study to elucidate the B sensing mechanism suggests that polyubiquitination of BOR1 relies on its conformational transition during the transport cycle. We propose that BOR1 is a transporter-receptor, "transceptor", directly senses the B concentration and promotes its own polyubiquitination and vacuolar sorting for quick and precise maintenance of B homeostasis.



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#### Signaling peptides acting in the systemic regulation of root system architecture in legumes

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#### Abstract

Plant growth is limited by soil nutrient availability and symbiotic nitrogen-fixing nodulation allows legume plants to use the atmospheric nitrogen source as an alternative. Legumes tightly regulate nodule number to balance the cost of supporting symbiotic rhizobial nitrogenfixing bacteria growth with nitrogen fixation's benefits. Two antagonistic pathways involving signalling peptides regulate nodule numbers systemically from shoots: under low nitrogen conditions, CEPs (C-terminally Encoded Peptides) are produced in roots and promote rhizobial infection and nodule formation through the CRA2 (Compact Root Architecture 2) Leucine-Rich Repeats Receptor-Like Kinase (LRR-RLK) acting in shoots; and after symbiotic rhizobia have initiated nodulation, CLE (CLAVATA3-like) peptides are produced to limit the energetically costly nodulation in relation to the plant's needs, through the SUNN (Super Numeric Nodules) LRR-RLK receptor acting in shoots. Molecular mechanisms explaining how these two signalling peptide hormonal pathways are coordinated to fine-tune nodule number remains however poorly understood. Progress will be reported on different aspects of these peptide systemic signalling pathways, notably in relation with their interaction with other types of hormonal regulations and with the characterization of potential common downstream shoot-to-root signals.



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#### **Molecular regulation of plant-unique membrane trafficking pathway in Arabidopsis** Abstract:

Membrane traffic, which is also known as vesicular transport, distributes proteins and lipids among organelles, or to the plasma membrane and the extracellular space. Since membrane traffic is a pivotal process for all eukaryotic cells, the system is well conserved among eukaryotic species. Yet, neofunctionalization of the key regulators have allowed the cells to cultivate species-specific trafficking routes during the course of evolution. RAB5, a member of Rab GTPase family, is known to coordinate membrane traffic routes at endosomes. The genome analysis had shown that RAB5 is conserved in plants, however, land plants and some green algae species possess plant-unique RAB5 member (called ARA6/RABF1 in Arabidopsis) in addition to the canonical RAB5s. Our studies, aiming at elucidating the trafficking route regulated by this plant-unique RAB5 member, indicated that ARA6 promotes trafficking route that counteracts to the vacuolar transport regulated by canonical RAB5s. Next, we performed molecular analysis, and discovered that ARA6 competitively bounds to plant-unique RAB5 effector protein called PUF2 that promotes activation of canonical RAB5s. We also found that subpopulation of PUF2 is located near the trans-Golgi network, while other effector of ARA6, named PUF3, predominantly localized to the endosomes. Our results suggest that ARA6 acts at multiple steps during vacuolar transport by interacting with different effector proteins at different steps.



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#### Plant Membrane Nano-organization and cell signaling

#### Abstract

In a crowded environment, establishing interactions between different molecular partners can take a long time. Biological membranes have solved this issue, as they simultaneously are fluid and possess compartmentalized domains. This nanoscale organization of the membrane is often based on weak, local and multivalent interactions between lipids and proteins. However, from local interactions at the nanoscale, different functional properties emerge at the higher scale, and these are critical to regulate and integrate cellular signaling. Taking the plant osmotic signaling pathway as an example, we show that a single isoform of Plant Rho GTPase is recruited in membrane nanodomain and is sufficient to trigger secondary messenger that convey plant response. In addition, we found that Rho GTPase nanodomain differ in their composition depending of the upstream signaling events, suggesting that descript organization of membrane can encode for signal specificity. We are now trying to determine what type of molecular actor are controlling such structures.



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#### **Mechanisms underlying outer/inner lateral polar-localization of membrane proteins** Abstract

Cell polarization is essential for plant development, growth and reproduction. Plants have multiple cell polarity axes in the root cells: e.g. apical (shootward)/basal (rootward) and inner lateral (stele-side)/outer lateral (soil-side). Many membrane proteins have been shown to localize to the specific polarity domains in the plant cells, while molecular mechanisms which generate cell polarity and maintain polar localization of membrane proteins remain to be elucidated. The borate transporter BOR1 and the boric acid channel NIP5;1 are polarly localized in the inner and outer lateral plasma membrane domains, respectively. We revealed that the polar localization of the boron transport proteins is maintained by clathrin-mediated endocytosis and intracellular membrane trafficking. More recently, we identified a receptor-like kinase localized in the distinct polarity domains in a tissue-specific manner. At the present, we are trying to reveal comprehensive mechanisms underlying cell polarization and inner/outer polar localization of proteins by using these polarly-localized membrane proteins as models.



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## Suppressor of actin 9 (*sac9*) mutant underlines the interplay between membrane composition and cytoskeleton dynamics during plant cytokinesis Abstract

Cell division is a common mechanism to eukaryotic species important for cell renewal, growing and tissue expansions. This highly regulated mechanism ends with a complete physical separation of the mother cell into two daughter cells. In plant cells, cytokinesis is characterized as a dynamic process of membrane trafficking and cytoskeletal rearrangement involving a membrane precursor, the cell plate, and a microtubules and actin bipolar structure, the phragmoplast. Here we aim to characterize SAC9, a lipid converting enzyme, as a new class of cytokinetic mutant underlying the interplay between cell plate composition and the phragmoplast dynamic in *Arabidopsis thaliana* dividing root cells. Unlike all reported defects in cytokinesis mutants (abortive cell plate and miss-guidance of phragmoplast), we uncover in *sac9* mutant the formation of unique enclose "pyramidal" compartments derived from an over-functional cytokinesis process and we link these defects to an abnormal lipid composition of the cell plate.



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#### Distribution of two phospholipids defines a dynamic plasma membrane domain for reorientation of root hair tip growth

Abstract:

Many types of plant cell elongate along the cell polarity axis, and the polarized growth is crucial for morphogenesis and function of specialized cell types. Root hair and pollen tube cells undergo typical polarized growth, in which they extend their tip part forward. Cellular components are actively transported to the tip part along the cytoskeleton. That is highly regulated by many proteins such as small GTPases and lipid metabolic enzymes. In yeast, nematodes and mammals, atypical protein kinase C (aPKC), which belongs to the AGC group of serine/threonine protein kinases, regulates polarity of cells. While there is no plant homolog of aPKC, several members of *Arabidopsis* AGC VIII family, including PID/WAGs and D6PKs, are known to control cell polarity and its related processes such as polar auxin transport. To reveal a relationship between plant AGC kinases and polarized cell growth, we have focused on the molecular species expressed in root hairs. We demonstrate that these kinases localize on dynamic domains of the plasma membrane at the tips of growing root hairs, and that two types of phospholipid are crucial for the localization. We will discuss how these kinases contribute to root hair growth.



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#### Distinctive roles of E2F transcription factors during plant replicative stress response

(collaboration with Masaki Ito at Kanazawa University)

#### Abstract

Survival of living organism is fully dependent on the maintenance of genome integrity. Due to their sessile lifestyle, plants are constantly exposed to biotic and abiotic stresses, that could lead to DNA damage. Studies show that compared to animals, plants have both shared and unique mechanisms that control the DNA Damage Responses (DDR). One central integrator of the plant DDR is the SOG1 transcription factor, but there is accumulating evidence that other pathways function independently of SOG1, notably in the context of replicative stress. Replicative stress is one of the most common threats to genome integrity as it occurs in all proliferating cells. Core cell cycle regulators, that are highly expressed in proliferating cells, are thus good candidates to contribute to the signaling pathways activated by replicative stress. Among those, the E2F transcription factors, are well characterized for their role at the G1/S transition, and one of them, EF2a has been recently shown to contribute to the cellular response to double strand breaks. However, there are still loopholes that require extensive study to clearly understand the role of E2Fs in the plant DDR signaling pathways. In order to test the involvement of E2Fs in replicative stress response in plants, we used genetic approaches, taking advantage of a mutant deficient for a replicative DNA polymerase, that displays constitutive replicative stress. Our results indicate that E2Fb may function in parallel of SOG1 in replicative stress response, providing evidence for the existence of a novel pathway in plants' DDR signaling, and suggesting that the two closely-related transcription factors E2Fa and E2Fb may play distinctive roles in this process.



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#### Phosphate starvation induces ER degradation via selective autophagy

#### Abstract

Phosphate (Pi) is one of the essential nutrients for plant growth. However, Pi is often limited in soils, thus, plants have several mechanisms of response to Pi starvation. These mechanisms can be divided into "Enhance Pi uptake" and "Pi recycle" broadly. Here, we focused on the later.

Autophagy is one of the intracellular degradation systems. The study of Naumann et al. (2019) showed that autophagy is induced by ER stress under Pi starvation in roots. However, the relationship between autophagy and Pi recycle is still unclear.

Recently, we discovered that autophagy deficient mutants showed severe growth phenotype under Pi starvation. Interestingly, autophagy is important for not long-term Pi-depleted conditions but early Pi-depleted conditions and ER-phagy, a type of selective autophagy which selectively degrades ER, was induced under the early Pi-depleted conditions. This ER-phagy was induced by iron mediated ER stress and contributed to the Pi recycle under early Pi-depleted conditions. The mechanism of response to early Pi starvation might delay the triggering of response to long-term Pi starvation. Thus, plants must be cleverly utilizing two different phase of response to Pi starvation to survive under non-uniform Pi concentration conditions.

