総合研究院イメージングフロンティアセンター講演会

平成 **29** 年 **7** 月 **7** 日**(**金**) 9:00-10:30**　野田キャンパス講義棟 **K701** 教室

REACTIVE OXYGEN SPECIES AS KEY REGULATORS OF POLAR GROWTH AND SYMBIOSIS

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The symbiotic interaction between rhizobia and legumes requires a molecular dialogue that involves the exchange of specific signal molecules. The legume plants secrete particular flavonoids compounds that are specifically recognized by the Rhizobium. These flavonoids induce the expression of specific genes, which encodes for proteins involved in the synthesis and secretion of the Nod factors (NFs), which are lipochin-oligosacharides. Thereafter, NFs are specifically recognized by the root hair cells of the root and induce several responses at the cellular level, such as: ionic changes, membrane depolarization, cytoskeleton rearrangements, reactive oxygen species (ROS) and gene expression. Calcium and ROS have a special importance. First, it has been described to increase few seconds after NFs addition, secondly, it is well accepted that these changes are important to induce gene expression. These gene expression is required for expressing all the proteins involved in nodule development, and therefore they are referred as nodulins. During the early responses, the root hair experiments a root tip curling response which allow to trap few bacteria inside the curling (infection chamber), which further induce the invagination of the plasma membrane and the cell wall to induce a tunnel like structure in a NF-dependent way. This is the infection threat (IT). This IT allow the bacteria to get in through the root hair and then through the cortical cell to reach some cells that have started to divide, also in a NF- dependent manner in order to form the nodule primordia. When the IT reach this primordium, the bacteria are released from the IT and taken up by some cells from the nodule primordia. The bacteria then differentiate to bacteroids and start fixing the nitrogen. In response, the plant provides a good source of carbon. However, this developmental program for nodule development is also followed by nodule senescence, which also involve a programed cell death process (Estrada-Navarrete et al., 2016).

In plant cells calcium (Ca^{2+}) and ROS accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. In guard cells from *Vicia faba* regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS levels generate and maintain an apical calcium gradient. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes and other tip growing cells. Therefore, the enzymes generating ROS such as the NADPH oxidases, plays a key role during these processes, including the pathogenic and mutualistic interactions.

The finding that ROS respond to the FNs from rhizobia (Cárdenas et al., 2008)(Cárdenas et al., 2008)in a different way to pathogenic signals such as elicitors (Cárdenas et al., 2008; Cárdenas and Quinto, 2008; Cárdenas, 2009) suggest that plant cells have a way to differentiate the symbiotic from the pathogenic signals. Our consortium has analyzed most of the NADPH oxidases (Rboh) from *Phaseoulus vulgaris*. From the nine identified genes, some of them are mainly expressed in root or root hairs. The silencing or overexpression of the Rboh result in increased nodulation or reduced mycorrhization, but silencing resulted in antagonic responses. This suggest that ROS could be important player for the mutualistic interactions. For instance, silencing of them (RbohB) resulted in reduced nodulation, but increased mycorrhization (Montiel et al., 2012; Arthikala et al., 2013; Arthikala et al., 2014). The IT, a key structure that result from the invagination of the plasma membrane and the cell wall from the root hairs, can be arrested when we silenced one Rboh. This suggest that the polar growth of the root hairs and the infection thread, require ROS as a key component for a successful infection.

Herein I will also present several approaches to study the mutualistic interactions at the cellular level. From the cytoskeleton to ionic changes and ROS. For instance, cameleon and Hyper are genetically expressed sensors that can be used to monitor intracellular calcium and hydrogen peroxide, respectively. This molecular probe can be expressed in root hair and then by using high resolution microscopy we can depict an apical H_2O_2 gradient at the tip dome where the polar growth occurs, furthermore we were able to visualize dynamic ROS oscillations in root hair cells, which are couple to growth. The implication for the rhizobia-legume interaction will be also discussed.

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Cárdenas L (2009) New findings in the mechanisms regulating polar growth in root hair cells. Plant Signal Behav **4:** 4-8

Cárdenas L, Martinez A, Sanchez F, Quinto C (2008) Fast, transient and specific intracellular ROS changes in living root hair cells responding to Nod factors (NFs). Plant J **56:** 802-813

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Recommended literature:

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