

DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di assegnazione di una borsa di Dottorato ad una linea di ricerca

Research title: The role of plant cell wall-derived damage-associated molecular patterns (DAMPs) in development and immunity

Supervisor: Prof.ssa Giulia De Lorenzo

State of the art

Plants are continually threatened by biotic and abiotic stresses and rely on an innate immune system for their survival. Immunity is costly and is accompanied by inhibition of growth, referred to as growth-defense trade off; thereby it is activated only upon sensing of danger signals (elicitors). These are Microbe-Associated Molecular Patterns (MAMPs), which activate the so-called pattern-triggered immunity (PTI) (Bigeard et al., 2015), and microbial effectors, which are recognized by Resistance (R) proteins and activate the robust effector-triggered immunity (ETI). Most of the R proteins are nucleotide-binding leucine-rich-repeat (NB-LRR) proteins (Jones et al., 2016). Plant endogenous molecules that are released upon pathogen invasion or wounding [Damage-Associated Molecular Patterns (DAMPs)] also activate PTI (Schaefer, 2014; Gust et al., 2017).

PTI includes very early responses [ion fluxes, generation of reactive oxygen species (ROS), mainly mediated by the NADPH oxidase RbohD, activation of phosphorylation cascades], early responses [stomatal closure, up-regulation of defense response genes and down-regulation of growth-related genes] and late responses (cell wall reinforcement and priming, i.e. the ability to respond promptly to a new pathogen attack) (Bigeard et al., 2015). Salicylic acid (SA), jasmonate and its methyl-derivative (JA and Me-JA) and ethylene are the major hormones involved in immunity (Shigenaga and Argueso, 2016). On the other hand, ETI includes the so-called hypersensitive response (HR) (Yu et al., 2017).

This project will focus on oligogalacturonides (OGs), signal molecules that derive from the plant extracellular matrix (ECM), hereon indicated as cell wall, and act as DAMPs. OGs are released from the pectic component homogalacturonan (HGA) by pathogen- or plant-secreted enzymes [e.g. polygalacturonases (PGs)], upon infection or wounding (Savatin et al., 2011; Ferrari et al., 2013; Savatin et al., 2014).

In plants, like in animals, the ECM is a complex and dynamic array of polysaccharides, proteoglycans and (glyco)-proteins, the function of which goes far beyond the mechanical/structural support. The ECM mediates cell-cell adhesion and communication, providing the spatial context for many signaling events that govern cell functions and differentiation, developmental patterning, growth and immunity. It is also a repository of both “physiological” and “stress” indicator signals, reversibly sequestered through associations with proteins or other components (Albersheim et al., 2011; Neill et al., 2014; Tarbell and Cancel, 2016).

Animal and plants share remarkably similar regulatory mechanisms involving the ECM, some of which are mediated by evolutionary conserved elements, whereas others derive from a convergent evolution. Examples of the latter are the two “most simply designed, yet most versatile biological molecule(s) in nature” (Cyphert et al., 2015), the

vertebrate hyaluronic acid (HA) and the plant HGA. Both molecules are linear and acidic high molecular weight polysaccharides. The structure of HA and HGA is crucial for health and survival of animals and plants, respectively, and is continuously and strictly monitored to sense alterations of the ECM integrity, often caused by mechanical injury or infection by pathogens secreting ECM-degrading enzymes (Ferrari et al., 2013), but also by dysregulation of its synthesis and remodeling during growth, development and formation of organs (Hamann, 2015a, b).

Both animals and plants have evolved mechanisms to sense the status of HA and HGA and counteract the consequences of loss of their structural integrity. Breakage products of these macromolecules, for example, are specifically perceived as indicators of a “damaged self”, i.e. they behave as damage-associated molecular patterns (DAMPs) capable of activating immune responses. For both HA and HGA, size is a main factor dictating their biological action. HGA fragments, the OGs, with a degree of polymerization (DP) between 10 and 16 show the highest immunity-triggering activity (Ferrari et al., 2013). Similarly, HA fragments with a DP of 10–18 show better binding to the receptor CD44 as well as angiogenic and cytokine release activity (Cyphert et al., 2015).

OG formation is favored by plant PG-inhibiting proteins (PGIPs) [13], and the transgenic expression of a PG-PGIP fusion (OG-machine or OGM) leads to overproduction of OGs, resistance to pathogens, affected growth and eventually death (Benedetti et al., 2015), which is temperature-sensitive, similar to HR mediated by the class of Toll Interleukin-1 receptor (TIR)-NB-LRR proteins (Hammoudi et al., 2018).

The OGM is a tool that is boosting the study of the OG biology and led us to the discovery of a mechanism that likely regulates OG homeostasis and controls the deleterious effects of their hyper-accumulation. Indeed, homeostatic mechanisms that prevent a deleterious overzealous response to both PAMPs and DAMPs are crucial for survival (Rothlin et al., 2015). In *Arabidopsis*, OG homeostasis may rely on at least 4 paralogous FAD-dependent specific oxidases (OGOX1-4) that produce H₂O₂ and oxidize OGs, abolishing elicitor activity (Benedetti et al., 2018). OGOXs belong to the largely uncharacterized berberine bridge enzyme-like (BBE1) family, which in *Arabidopsis* comprises 27 members (Daniel et al., 2015; Rajniak et al., 2015).

Biotechnology aims at enhancing plant immunity, maintaining normal growth; however, disentangling the interplay between immune and developmental processes is a daunting task, due to pleiotropy, cross-talk antagonism and synergy of many players, which may also show high genetic complexity, with members showing redundancy/synergy/antagonism.

Main Objective of the Research

This project aims at elucidating whether DAMPs, beside acting in immunity, also play a role in development, when they are released, usually at low levels, during the physiological remodeling of the ECM. The OGM will be used, along with other biological tools, to unravel this key aspect that is still much debated in both animal and plant biology. Moreover, the project aims at uncoupling immune responses induced by OGs from the negative effects on growth, for biotechnological purposes.

Specific aims

A β -estradiol-inducible OGM (β -OGM) is a unique tool to test the hypothesis that OGs are DAMPs when released at high levels, whereas they are regulatory signal during development, when released during the physiological cell wall remodeling. We have generated transgenic plants expressing the β -OGM in specific cells/tissues that are strategic for defense (leaf, stem and root epidermis, stomata) (Laplaze et al., 2005).

This project is focused on the following points:

- 1) The effect of the release of OGs in specific root cells on root development will be examined, also in plants that express fluorescent sensors for ROS and Ca²⁺ to correlate phenotypic alteration with

- intracellular redox status and Ca²⁺ dynamics. In addition these studies will be performed in plants that have altered expression of the OG-oxidizing enzymes (null mutants and overexpressing plants; some already available in the laboratory), in order to uncouple signaling mediated by OGs from signaling mediated by cell wall rupture.
- 2) The effect of the release of OGs in specific root cells on local and systemic protection against pathogens and aphids will be also investigated.
 - 3) The pathways involved in the hyper-immunity response caused by high OG levels will be dissected using plant that express the β -OGM in backgrounds mutated in immune-related transduction/response elements (already available in the laboratory). Preliminary data show an involvement of RBOHD, SA, and EDS1, which is required for HR mediated by R proteins of the TIR-NB-LRR type (Zhang et al., 2003). The cellular and molecular basis of the rescue of phenotype in the mutants will be analyzed. We aim at obtaining increased resistance to pathogens induced by OGs with no major effect on growth.
 - 4) The role of the OG-oxidizing enzymes will be elucidated to demonstrate their role as a homeostatic mechanism for the regulation of DAMP signaling at the local level (infection site) or at the systemic level. This novel research area will address the mechanisms that control DAMP-triggered immunity to prevent deleterious effects.

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International Collaborations

Jonathan D. Jones, The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK.

Maria J. Pozo, Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain. -

John Mundy, Department of Biology, University of Copenhagen. Copenhagen, Denmark.

Saskia A. Hogenhout, Department of Crop Genetics, The John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK.